G&I Genomics & Informatics

eISSN 2234-0742
Genomics Inform 2018;16(2):36-41
https://doi.org/10.5808/GI.2018.16.2.36

ORIGINAL ARTICLE

Identification of *LEF1* as a Susceptibility Locus for Kawasaki Disease in Patients Younger than 6 Months of Age

Hea-Ji Kim[1], Sin Weon Yun[2], Jeong Jin Yu[3], Kyung Lim Yoon[4], Kyung-Yil Lee[5], Hong-Ryang Kil[6], Gi Beom Kim[7], Myung-Ki Han[8], Min Seob Song[9], Hyoung Doo Lee[10], Kee Soo Ha[11], Sejung Sohn[12], Ryota Ebata[13], Hiromichi Hamada[14], Hiroyuki Suzuki[15], Yoichiro Kamatani[16], Michiaki Kubo[17], Kaoru Ito[18], Yoshihiro Onouchi[18§], Young Mi Hong[12§], Gi Young Jang[11§], Jong-Keuk Lee[1§*], The Korean Kawasaki Disease Genetics Consortium

[1]Asan Institute for Life Sciences, University of Ulsan College of Medicine, Seoul 05505, Korea, [2]Department of Pediatrics, Chung-Ang University Hospital, Seoul 06973, Korea, [3]Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul 05505, Korea, [4]Department of Pediatrics, Kyung Hee University Hospital at Gangdong, Seoul 05278, Korea, [5]Department of Pediatrics, Daejeon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Daejeon 34943, Korea, [6]Department of Pediatrics, Chungnam National University Hospital, Daejeon 35015, Korea, [7]Department of Pediatrics, Seoul National University Children's Hospital, Seoul 03080, Korea, [8]Department of Pediatrics, Gangneung Asan Hospital, University of Ulsan College of Medicine, Gangneung 25440, Korea, [9]Department of Pediatrics, Inje University Busan Paik Hospital, Busan 47392, Korea, [10]Department of Pediatrics, Pusan National University Hospital, Busan 49241, Korea, [11]Department of Pediatrics, Korea University Ansan Hospital, Ansan 15355, Korea, [12]Department of Pediatrics, Ewha Womans University Hospital, Seoul 07985, Korea, [13]Department of Pediatrics, Chiba-University Graduate School of Medicine, Chiba 260-8670, Japan, [14]Department of Pediatrics, Tokyo Women's Medical University Yachivo Medical Center, Yachivo 276-8524, Japan, [15]Department of Pediatrics, Wakayama Medical University, Wakayama 641-8509, Japan, [16]Laboratory for Statistical Analysis, RIKEN Center for Integrative Medical Sciences, Yokohama 230-0045, Japan, [17]Laboratory for Genotyping Development, RIKEN Center for Integrative Medical Sciences, Yokohama 230-0045, Japan, [18]Laboratory for Cardiovascular Diseases, RIKEN Center for Integrative Medical Sciences, Yokohama 230-0045, Japan

Kawasaki disease (KD) is an acute febrile vasculitis predominately affecting infants and children. The dominant incidence age of KD is from 6 months to 5 years of age, and the incidence is unusual in those younger than 6 months and older than 5 years of age. We tried to identify genetic variants specifically associated with KD in patients younger than 6 months or older than 5 years of age. We performed an age-stratified genome-wide association study using the Illumina HumanOmni1-Quad BeadChip data (296 cases vs. 1,000 controls) and a replication study (1,360 cases vs. 3,553 controls) in the Korean population. Among 26 candidate single nucleotide polymorphisms (SNPs) tested in replication study, only a rare nonsynonymous SNP (rs4365796: c.1106C>T, p.Thr369Met) in the lymphoid enhancer binding factor 1 (*LEF1*) gene was very significantly associated with KD in patients younger than 6 months of age (odds ratio [OR], 3.07; $p_{combined} = 1.10 \times 10^{-5}$), whereas no association of the same SNP was observed in any other age group of KD patients. The same SNP (rs4365796) in the *LEF1* gene showed the same direction of risk effect in Japanese KD patients younger than 6 months of age, although the effect was not statistically significant (OR, 1.42; p = 0.397). This result indicates that the *LEF1* gene may play an important role as a susceptibility gene specifically affecting KD patients younger than 6 months of age.

Keywords: genome-wide association study, Kawasaki disease, lymphoid enhancer binding factor1 (*LEF1*), single nucleotide polymorphism

Received March 23, 2018; Revised April 11, 2018; Accepted April 11, 2018

***Corresponding author:** Tel: +82-2-3010-4142, Fax: +82-2-488-3312, E-mail: cookie_jklee@hotmail.com
[§]These authors contributed equally to this work.

Introduction

Kawasaki disease (KD) is an acute, self-limited vasculitis that predominantly occurs in children between the ages of 6 months and 5 years old. Approximately half of all KD patients are between 6 months to 2 years of age, which is the peak incidence age of KD [1, 2]. The etiology of KD is not known, and it has no specific diagnostic test. As such, KD diagnosis is based solely on six clinical symptoms: prolonged fever, bilateral conjunctival injection, erythema of the oral mucosa, lips, and tongue, polymorphous rash, erythema of the palms and soles, and cervical lymphadenopathy [3]. Complete KD is diagnosed when patients have at least five of the above six clinical symptoms, and incomplete KD is diagnosed when patients have less than four of the six clinical symptoms. The standard treatment of KD is a high-dose intravenous immunoglobulin (IVIG), which is derived from pooled plasma of healthy donors, reducing the duration of fever and the incidence of coronary artery abnormalities [4, 5].

KD is considered an abnormal immunological reaction to an infection or unknown immunological triggers in genetically susceptible individuals [6, 7]. B cell–related genes including *BLK*, *CD40*, and *FCGR2A* were also identified as KD susceptibility genes by genome-wide association studies (GWAS) [8-10]. In particular, a reduced level of *BLK* expression in blood B cells may be a crucial reason for dysfunction of B cells and a pathogenesis of KD [10, 11]. Our previous study identified that a risk allele in *FCGR2A* was only susceptible for KD patients younger than 1 year of age, whereas the KD susceptible allele in *BLK* affected all ages of KD patients, except those older than 5 years of age. This result revealed a possibility that there are other genetic variants affecting specific age subgroups of KD patients. In this study, to further identify age-specific susceptibility genes of KD in KD patients younger than 6 months or older than 5 years of age, we performed an age-stratified GWAS and a replication study and identified lymphoid enhancer binding factor 1 (*LEF1*) as a KD susceptibility gene specifically affecting KD patients younger than 6 months of age.

Methods

Study subjects and genotype data

KD patients in this study were collected from 10 hospitals in Korea. The diagnosis of all KD patients was determined by the diagnostic criteria of the American Heart Association [12, 13]. All laboratory test data were performed before the initial IVIG treatment, including white blood cell count, neutrophil count, platelet count, erythrocyte sedimentation rate (ESR), hemoglobin (Hb), C-reactive protein (CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total protein. A total of 1,699 KD patients in Korea were used in this study. Of them, 118 KD patients were younger than 6 months of age and 231 KD patients were older than 5 years of age. A total of 4,553 controls with no history of KD were obtained from the adult health cohort of the general population in Korea, which included 1,000 controls used in the initial GWAS and 3,500 controls used in the replication study. The GWAS was initially performed using our previous Illumina HumanOmni1-Quad BeadChip data (296 KD patients and 1,000 healthy controls) [14]. From the age-stratified GWAS analysis (19 cases; younger than 6 months of age KD patients and 45 cases; older than 5 years of age KD patients), a total of 12 single nucleotide polymorphisms (SNPs) and 14 SNPs were chosen as age-specific KD susceptibility loci for patients younger than 6 months and older than 5 years of age, respectively. Genotyping for the replication study in the 1,403 KD patients, including 99 KD patients younger than 6 months and 186 KD patients older than 5 years of age, were performed using TaqMan assays and analyzed using an Applied Biosystems 7900HT Fast Real-Time PCR system (Thermo Fisher Scientific, Waltham, MA, USA). The genotypes of the control subjects in Korea were provided by the Biobank for Health Sciences at the Center for Genome Sciences in Cheongwon, Korea. The second replication study using a Japanese cohort was comprised of 1,306 KD cases, including 120 KD cases younger than 6 months of age, and 6,893 controls. Genotype data of the Japanese cohort was generated using the Illumina HumanOmniExpressExome BeadChip (Illumina, San Diego, CA, USA). Informed consent was obtained from the parents of all KD patients in this study.

Statistical analysis

Statistical analyses for the genetic associations and meta-analysis of the SNPs were performed using PLINK (ver. 1.07) [15]. To test the association with KD, we performed the chi-square test to compare allele and genotype frequencies between cases and controls. To analyze the significance of differences in the distribution of variables of clinical characteristics in each genotype group, we used SPSS ver. 18 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to test for normality of the continuous variables. The continuous variables with a non-normal distribution were described by median and interquartile range. The Mann-Whitney U test was used in the continuous variables and the chi-square test was used in the categorical variables to contrast the genotype groups depending on the distribution of the data. The functional prediction of nonsynonymous SNP (rs4365796) was performed by PolyPhen and SIFT programs.

Results

Identification of *LEF1* as a KD susceptibility gene specifically associated with KD in patients younger than 6 months of age.

To identify the genetic variants affecting two extreme age subgroups of KD patients (younger than 6 months and older than 5 years of age), we performed an age-stratified GWAS using 19 KD cases younger than 6 months or 45 KD cases older than 5 years of age, respectively, compared to 1,000 controls. A total of 12 SNPs for patients younger than 6 months and 14 SNPs for those older than 5 years of age were chosen for the replication study on the basis of our arbitrary threshold ($p < 1\times10^{-4}$ and genes related to immune functions) for each subgroup. Among 26 candidate SNPs tested in the replication study (Supplementary Tables 1 and 2), only a nonsynonymous SNP (rs4365796: c.1106C>T, p.Thr369Met) in the *LEF1* gene was validated in Korean KD patients younger than 6 months of age (odds ratio [OR], 5.92; p = 0.000268 in GWAS and OR, 2.60; p = 0.00126 in the replication study) (Table 1). The combined analysis of the Korean GWAS and replication for *LEF1* SNP (rs4365796) showed very significant association with KD in patients younger than 6 months of age (118 cases vs. 4,553 controls; OR, 3.07; $p_{combined} = 1.10 \times 10^{-5}$), whereas no association of the same SNP was observed in any other age group of KD patients (Fig. 1, Supplementary Table 3). To further validate our findings in another population, we also performed a replication study in the Japanese cohort comprised of 1,306 KD cases and 6,893 controls. The SNP rs4365796 in the *LEF1* gene showed the same direction of risk effect in Japanese KD patients younger than 6 months of age, although the effect was statistically not significant (OR, 1.42; p = 0.397) (Table 1). In a meta-analysis of Korean and Japanese data, a significant association was observed in KD patients younger than 6 months of age (OR, 2.50; $p = 5.01 \times 10^{-5}$), whereas no association was detected in KD patients older than 6 months of age (OR, 1.10; p = 0.342) (Table 1). This result indicates that the *LEF1* gene is a novel susceptibility gene specifically affecting KD patients younger than 6 months of age.

To determine the effect of the *LEF1* risk allele on clinical features of KD patients, we examined the clinical data classified by the *LEF1* genotypes (rs4365796, risk allele: T)

Table 1. Age-stratified association results for *LEF1* (rs4365796; risk allele: T) in Korean and Japanese populations

Country	Collection	KD (age ≤0.5 y)				KD (age >0.5 y)			
		No. (case/control)	RAF (case/control)	OR (95% CI)	p-value	No. (case/control)	RAF (case/control)	OR (95% CI)	p-value
Korea	GWAS	19/1,000	0.105/0.020	5.92 (2.00–17.48)	**2.68×10^{-4}**	277/1,000	0.032/0.020	1.69 (0.96–2.98)	0.067
	Replication	99/3,553	0.061/0.024	2.60 (1.42–4.75)	**1.26×10^{-3}**	1,261/3,553	0.025/0.024	1.05 (0.78–1.40)	0.744
	Combined	118/4,553	0.068/0.023	3.07 (1.81–5.19)	**1.10×10^{-5}**	1,538/4,553	0.026/0.023	1.15 (0.89–1.50)	0.275
Japan	Replication	120/6,893	0.025/0.018	1.42 (0.63–3.23)	0.397	1,186/6,893	0.018/0.018	1.03 (0.74–1.42)	0.884
Meta	Korea+ Japan	238/11,446		2.50 (1.61–3.90)	**5.01×10^{-5}**	2,724/11,446		1.10 (0.9–1.35)	0.342

A meta-analysis was performed using 2 patient populations (Korea-combined and Japan-replication) with 2,962 cases of KD and 11,446 control subjects.
These statistical values are for the allelic model, and significant p-values ($p < 0.05$) are shown in bold.
KD, Kawasaki disease; RAF, risk allele frequency; OR, odds ratio; 95% CI, 95% confidence interval; GWAS, genome-wide association studies.

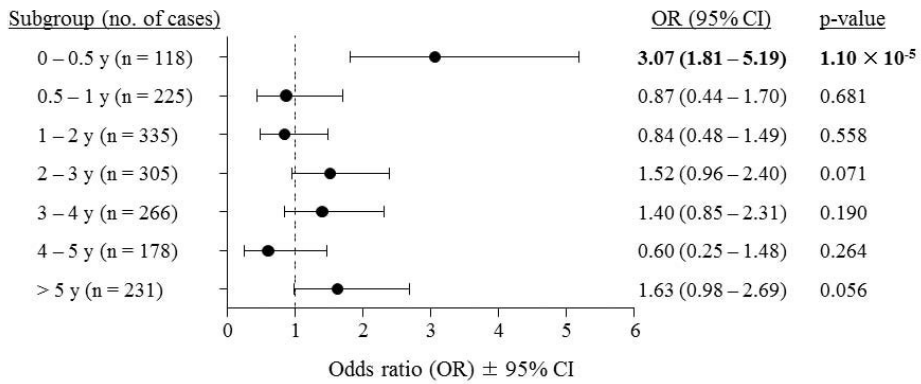

Fig. 1. Odds ratios (ORs) and confidence intervals (CIs) of the *LEF1* (rs4365796) association with Kawasaki disease (KD) according to age of Korean KD patients (total number of KD patients, 1,658). Each horizontal bar is a 95% CI. A total of 4,553 controls were used in the genetic association analysis for each age subgroup of KD patients. Significant p-values ($p < 0.05$) are shown in bold.

in 1,656 Korean KD patients (number of genotype: CC = 1,559, CT/TT = 96/1). When we investigated the effect of genotype of the *LEF1* risk allele in two KD subgroups (younger than 6 months and older than 6 months of age), the risk allele did not show any significant effect on any clinical variables in KD patients with one exception; the risk genotypes (either CT or TT) of the *LEF1* gene (rs4365796) had slightly increased CRP levels (median, 8.48 mg/L; p = 0.032) in KD patients older than 6 months of age compared to the non-risk genotype (CC) of the *LEF1* gene (median, 6.50 mg/L) (Supplementary Table 4).

Discussion

The incidence of KD in patients younger than 6 months and older than 5 years of age was comparatively lower than other age groups. The peak incidence of KD was in the age group between 6 months and 2 years of age. KD has no diagnostic test and the diagnosis depends on clinical symptoms. In particular, KD patients younger than 6 months of age can be difficult to diagnose because they show fewer clinical symptoms, called incomplete KD, which leads to delayed diagnosis or misdiagnosis in infants. Consequently, cardiac complications are more common in KD patients younger than 6 months than in older children [16-18]. Therefore, early and accurate diagnosis of infantile KD is important to reduce the risk of cardiac complications. In this study, we analyzed GWAS data of patients younger than 6 months and older than 5 years of age to identify new risk loci for KD susceptibility in these age groups. We identified a new KD susceptibility locus in the *LEF1* gene (rs4365796: c.1106C>T, p.Thr369Met) on chromosome 4. The associated amino acid-altering SNP (rs4365796) in the *LEF1* gene had an unusually strong effect size, a 3.07-fold increased risk for incidence of KD, in Korean KD patients younger than 6 months of age. This result indicates that the *LEF1* gene plays a crucial role in the pathogenesis of KD in very young children and this amino acid-altering variant can be used as a candidate marker to identify high risk KD patients in infant patients in a clinical setting.

The *LEF1* gene encodes a transcription factor that is expressed in developing B and T cells and at multiple sites of organogenesis during embryonic development [19-21]. *LEF1* is a central mediator of the Wnt signaling pathway through recruiting β-catenin and plays crucial roles during development, including normal hematopoiesis [22, 23]. Abnormal protein expression of *LEF1* has been detected in chronic lymphocytic leukemia (CLL) cells and monoclonal B-cell lymphocytosis, indicating that *LEF1* plays an early role in B-cell development and CLL leukemogenesis [24]. Transplantation of *LEF1*-transduced bone marrow also developed acute myeloid leukemia and B-precursor acute lymphoblastic leukemia in mouse models [25]. Additionally, *LEF1* contributes to the survival and proliferation of pro-B cells during early B cell development [26]. GWAS also identified the *LEF1* gene as a susceptibility locus for systemic lupus erythematosus and CLL [27-29]. These previous results suggest that the dysfunction of the *LEF1* gene is involved in early B lymphocyte development, which is involved in the pathogenesis of KD. In our study, we found that a nonsynonymous SNP (rs4365796: c.1106C>T, p.Thr369Met) was significantly associated with KD in patients younger than 6 months of age. This SNP was predicted as probably damaging and deleterious by PolyPhen and SIFT, respectively, suggesting that this amino acid-altering variant can change the biological functions of *LEF1* protein, probably during the early development of B cells.

Although we found that a nonsynonymous SNP (rs4365796: c.1106C>T, p.Thr369Met) in the *LEF1* gene is significantly associated with KD in Korean patients younger than 6 months of age, the biological role of the *LEF1* variant is still unknown. Therefore, we should investigate how the amino acid-altering *LEF1* variant specifically affects the immune response in infants and subsequently the potential mechanism of *LEF1*-mediated pathogenesis in KD. Additionally, the significantly associated SNP (rs4365796) in the *LEF1* gene in Korean KD patients was not replicated in the Japanese samples, although the same direction of risk effect was detected (Table 1). As shown in Table 1, the risk allele of the *LEF1* gene is a rare variant with lower allele frequency in the Japanese population (risk allele frequency = 0.023 in Korean vs. 0.018 in Japanese control samples). Furthermore, the portions of KD patients younger than 6 months of age was higher in Japan (9.19% in Japan vs. 7.13% in Korea) (Table 1), suggesting that Korean KD patients younger than 6 months of age are a genetically enriched and more homogeneous case population. A previous study also reported that the Japanese population has a higher incidence of KD in patients younger than 6 months of age compared to the Korean population (11.2% in Japanese KD patients vs. 7.7% in Korean KD patients) [30]. Therefore, we assume that no replication of the *LEF1* gene in the Japanese samples may be due to the lower frequency of the risk allele in the *LEF1* gene and/or higher KD incidence in those younger than 6 months of age compared to the Korean population. Conversely, another limitation of our study is the low statistical power due to the small sample size resulting from the selection of a rare variant with an allele frequency less than 2.5% and of age subgroups of KD patients, particularly younger than 6 months of age (less than 10% of total KD cases). Therefore, to support our findings, further replication studies are necessary in more independent sample sets with larger

sample size.

In conclusion, we identified that a nonsynonymous SNP (rs4365796: c.1106C>T, p.Thr369Met) in the *LEF1* gene is significantly associated with KD in children younger than 6 months of age. This amino acid-altering variant in the *LEF1* gene will be useful to identify high risk KD patients younger than 6 months of age because this SNP had a strong effect size (OR, 3.07). This result will provide new insight into the pathogenesis of the KD in infants.

ORCID: Hea-Ji Kim: https://orcid.org/0000-0002-6285-1121; Sin Weon Yun: https://orcid.org/0000-0001-8947-703X; Jeong Jin Yu: https://orcid.org/0000-0003-1601-3685; Kyung Lim Yoon: https://orcid.org/0000-0003-4734-900X; Kyung-Yil Lee: https://orcid.org/0000-0001-6510-1580; Hong-Ryang Kil: https://orcid.org/0000-0003-4925-8240; Gi Beom Kim: https://orcid.org/0000-0002-7880-280X; Myung-Ki Han: https://orcid.org/0000-0003-1817-8088; Min Seob Song: https://orcid.org/0000-0003-3426-6803; Hyoung Doo Lee: https://orcid.org/0000-0002-6125-187X; Kee Soo Ha: https://orcid.org/0000-0001-6753-5411; Sejung Sohn: https://orcid.org/0000-0001-9960-7774; Ryota Ebata: https://orcid.org/0000-0001-8471-1042; Hiromichi Hamada: https://orcid.org/0000-0002-8990-5265; Hiroyuki Suzuki: https://orcid.org/0000-0003-0420-9146; Yoichiro Kamatani: https://orcid.org/0000-0001-8748-5597; Michiaki Kubo: https://orcid.org/0000-0002-0095-2322; Kaoru Ito: https://orcid.org/0000-0003-1843-773X; Yoshihiro Onouchi: https://orcid.org/0000-0002-9272-2689; Young Mi Hong: https://orcid.org/0000-0002-6600-7876; Gi Young Jang: https://orcid.org/0000-0002-4831-1188; Jong-Keuk Lee: 0000-0003-1125-4017

Authors' contribution

Conceptualization: JKL
Data curation: SWY, JJY, KLY, KYL, HRK, GBK, MKH, MSS, HDL, KSH, SS, RE, HH, HS, YK, MK, KI, YO, YMH, GYJ
Formal analysis: HJK
Funding acquisition: JKL
Methodology: HJK
Validation: YO
Supervision: JKL, YMH, GYJ, YO
Writing - original draft: HJK
Writing - review & editing: JKL, YMH, GYJ, YO

Acknowledgments

We thank all the patients with Kawasaki disease and their families for participating in this study. This work was supported by a grant from the Ministry of Health & Welfare of the Republic of Korea (HI15C1575).

Supplementary materials

Supplementary data including four tables can be found with this article online at https://doi.org/10.5808/GI.2018.16.2.36

References

1. Melish ME. Kawasaki syndrome. *Pediatr Rev* 1996;17:153-162.
2. Rowley AH, Shulman ST. Kawasaki syndrome. *Pediatr Clin North Am* 1999;46:313-329.
3. Burns JC, Glode MP. Kawasaki syndrome. *Lancet* 2004;364:533-544.
4. Wu MH, Chen HC, Yeh SJ, Lin MT, Huang SC, Huang SK. Prevalence and the long-term coronary risks of patients with Kawasaki disease in a general population <40 years: a national database study. *Circ Cardiovasc Qual Outcomes* 2012;5:566-570.
5. Gürcan HM, Keskin DB, Ahmed AR. Information for healthcare providers on general features of IGIV with emphasis on differences between commercially available products. *Autoimmun Rev* 2010;9:553-559.
6. Newburger JW, Fulton DR. Kawasaki disease. *Curr Opin Pediatr* 2004;16:508-514.
7. Lee KY, Han JW, Lee JS. Kawasaki disease may be a hyperimmune reaction of genetically susceptible children to variants of normal environmental flora. *Med Hypotheses* 2007;69:642-651.
8. Onouchi Y, Ozaki K, Burns JC, Shimizu C, Terai M, Hamada H, *et al*. A genome-wide association study identifies three new risk loci for Kawasaki disease. *Nat Genet* 2012;44:517-521.
9. Lee YC, Kuo HC, Chang JS, Chang LY, Huang LM, Chen MR, *et al*. Two new susceptibility loci for Kawasaki disease identified through genome-wide association analysis. *Nat Genet* 2012;44:522-525.
10. Khor CC, Davila S, Breunis WB, Lee YC, Shimizu C, Wright VJ, *et al*. Genome-wide association study identifies FCGR2A as a susceptibility locus for Kawasaki disease. *Nat Genet* 2011;43:1241-1246.
11. Chang CJ, Kuo HC, Chang JS, Lee JK, Tsai FJ, Khor CC, *et al*. Replication and meta-analysis of GWAS identified susceptibility loci in Kawasaki disease confirm the importance of B lymphoid tyrosine kinase (*BLK*) in disease susceptibility. *PLoS One* 2013;8:e72037.
12. Newburger JW, Takahashi M, Gerber MA, Gewitz MH, Tani LY, Burns JC, *et al*. Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. *Circulation* 2004;110:2747-2771.
13. McCrindle BW, Rowley AH, Newburger JW, Burns JC, Bolger AF, Gewitz M, *et al*. Diagnosis, treatment, and long-term management of Kawasaki disease: a scientific statement for health

professionals from the American Heart Association. *Circulation* 2017;135:e927-e999.
14. Kim JJ, Yun SW, Yu JJ, Yoon KL, Lee KY, Kil HR, *et al*. A genome-wide association analysis identifies NMNAT2 and HCP5 as susceptibility loci for Kawasaki disease. *J Hum Genet* 2017;62:1023-1029.
15. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, *et al*. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;81:559-575.
16. No SJ, Kim DO, Choi KM, Eun LY. Do predictors of incomplete Kawasaki disease exist for infants? *Pediatr Cardiol* 2013;34:286-290.
17. Park YW, Han JW, Park IS, Kim CH, Cha SH, Ma JS, *et al*. Epidemiologic study of Kawasaki disease in 6 months old and younger infants. *Korean J Pediatr* 2008;51:1320-1323.
18. Burns JC, Wiggins JW Jr, Toews WH, Newburger JW, Leung DY, Wilson H, *et al*. Clinical spectrum of Kawasaki disease in infants younger than 6 months of age. *J Pediatr* 1986;109:759-763.
19. Oosterwegel M, van de Wetering M, Dooijes D, Klomp L, Winoto A, Georgopoulos K, *et al*. Cloning of murine TCF-1, a T cell-specific transcription factor interacting with functional motifs in the CD3-epsilon and T cell receptor alpha enhancers. *J Exp Med* 1991;173:1133-1142.
20. Travis A, Amsterdam A, Belanger C, Grosschedl R. LEF-1, a gene encoding a lymphoid-specific protein with an HMG domain, regulates T-cell receptor alpha enhancer function [corrected]. *Genes Dev* 1991;5:880-894.
21. van Genderen C, Okamura RM, Fariñas I, Quo RG, Parslow TG, Bruhn L, *et al*. Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1-deficient mice. *Genes Dev* 1994;8:2691-2703.
22. Reya T, Duncan AW, Ailles L, Domen J, Scherer DC, Willert K, *et al*. A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature* 2003;423:409-414.
23. Arce L, Yokoyama NN, Waterman ML. Diversity of LEF/TCF action in development and disease. *Oncogene* 2006;25:7492-7504.
24. Gutierrez A, Jr., Tschumper RC, Wu X, Shanafelt TD, Eckel-Passow J, Huddleston PM 3rd, *et al*. LEF-1 is a prosurvival factor in chronic lymphocytic leukemia and is expressed in the preleukemic state of monoclonal B-cell lymphocytosis. *Blood* 2010;116:2975-2983.
25. Petropoulos K, Arseni N, Schessl C, Stadler CR, Rawat VP, Deshpande AJ, *et al*. A novel role for Lef-1, a central transcription mediator of Wnt signaling, in leukemogenesis. *J Exp Med* 2008;205:515-522.
26. Reya T, O'Riordan M, Okamura R, Devaney E, Willert K, Nusse R, *et al*. Wnt signaling regulates B lymphocyte proliferation through a LEF-1 dependent mechanism. *Immunity* 2000;13:15-24.
27. Okada Y, Shimane K, Kochi Y, Tahira T, Suzuki A, Higasa K, *et al*. A genome-wide association study identified AFF1 as a susceptibility locus for systemic lupus eyrthematosus in Japanese. *PLoS Genet* 2012;8:e1002455.
28. Sabatti C, Service SK, Hartikainen AL, Pouta A, Ripatti S, Brodsky J, *et al*. Genome-wide association analysis of metabolic traits in a birth cohort from a founder population. *Nat Genet* 2009;41:35-46.
29. Berndt SI, Skibola CF, Joseph V, Camp NJ, Nieters A, Wang Z, *et al*. Genome-wide association study identifies multiple risk loci for chronic lymphocytic leukemia. *Nat Genet* 2013;45:868-876.
30. Yeom JS, Woo HO, Park JS, Park ES, Seo JH, Youn HS. Kawasaki disease in infants. *Korean J Pediatr* 2013;56:377-382.

SUPPLEMENTARY INFORMATION

Identification of *LEF1* as a Susceptibility Locus for Kawasaki Disease in Patients Younger than 6 Months of Age

Hea-Ji Kim[1], Sin Weon Yun[2], Jeong Jin Yu[3], Kyung Lim Yoon[4], Kyung-Yil Lee[5], Hong-Ryang Kil[6], Gi Beom Kim[7], Myung-Ki Han[8], Min Seob Song[9], Hyoung Doo Lee[10], Kee Soo Ha[11], Sejung Sohn[12], Ryota Ebata[13], Hiromichi Hamada[14], Hiroyuki Suzuki[15], Yoichiro Kamatani[16], Michiaki Kubo[17], Kaoru Ito[18], Yoshihiro Onouchi[18§], Young Mi Hong[12§], Gi Young Jang[11§], Jong-Keuk Lee[1§*], The Korean Kawasaki Disease Genetics Consortium

[1]Asan Institute for Life Sciences, University of Ulsan College of Medicine, Seoul 05505, Korea, [2]Department of Pediatrics, Chung-Ang University Hospital, Seoul 06973, Korea, [3]Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul 05505, Korea, [4]Department of Pediatrics, Kyung Hee University Hospital at Gangdong, Seoul 05278, Korea, [5]Department of Pediatrics, Daejeon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Daejeon 34943, Korea, [6]Department of Pediatrics, Chungnam National University Hospital, Daejeon 35015, Korea, [7]Department of Pediatrics, Seoul National University Children's Hospital, Seoul 03080, Korea, [8]Department of Pediatrics, Gangneung Asan Hospital, University of Ulsan College of Medicine, Gangneung 25440, Korea, [9]Department of Pediatrics, Inje University Busan Paik Hospital, Busan 47392, Korea, [10]Department of Pediatrics, Pusan National University Hospital, Busan 49241, Korea, [11]Department of Pediatrics, Korea University Ansan Hospital, Ansan 15355, Korea, [12]Department of Pediatrics, Ewha Womans University Hospital, Seoul 07985, Korea, [13]Department of Pediatrics, Chiba-University Graduate School of Medicine, Chiba 260-8670, Japan, [14]Department of Pediatrics, Tokyo Women's Medical University Yachivo Medical Center, Yachivo 276-8524, Japan, [15]Department of Pediatrics, Wakayama Medical University, Wakayama 641-8509, Japan, [16]Laboratory for Statistical Analysis, RIKEN Center for Integrative Medical Sciences, Yokohama 230-0045, Japan, [17]Laboratory for Genotyping Development, RIKEN Center for Integrative Medical Sciences, Yokohama 230-0045, Japan, [18]Laboratory for Cardiovascular Diseases, RIKEN Center for Integrative Medical Sciences, Yokohama 230-0045, Japan

https://doi.org/10.5808/GI.2018.16.2.36

Supplementary Table 1. Association results of 12 candidate SNPs selected from KD patients younger than 6 months of age

Locus	Chr	SNP	Risk allele	Collection	KD (age ≤0.5 y) vs. Controls				KD (age 3< to ≤5 y) vs. Controls			
					No. (case/control)	RAF (case/control)	OR (95% CI)	p-value	No. (case/control)	RAF (case/control)	OR (95% CI)	p-value
CSF1	1	rs333949	G	GWAS	19/1,000	0.079/0.012	7.36 (2.11-25.66)	2.45×10^{-4}	80/1,000	0.013/0.012	1.09 (0.25-4.65)	0.911
				Replication	46/3,553	0.012/0.010	1.15 (0.16-8.36)	0.891				
				Combined	65/4,553	0.032/0.010	3.16 (1.14-8.73)	0.019				
CD84	1	rs1570707	T	GWAS	19/1,000	0.211/0.050	5.12 (2.29-11.46)	1.04×10^{-5}	80/1,000	0.071/0.050	1.46 (0.76-2.78)	0.251
				Replication	46/3,553	0.058/0.065	0.88 (0.36-2.19)	0.789				
				Combined	65/4,553	0.105/0.062	1.78 (0.99-3.18)	0.049				
IL1RN	2	rs2902452	A	GWAS	19/1,000	0.237/0.074	3.91 (1.82-8.42)	1.76×10^{-4}	80/1,000	0.0500.074	0.66 (0.32-1.38)	0.268
				Replication	46/3,553	0.089/0.074	1.22 (0.58-2.53)	0.601				
				Combined	65/4,553	0.133/0.074	1.91 (1.14-3.21)	0.012				
CXCR4	2	rs6716987	C	GWAS	19/1,000	0.421/0.183	3.25 (1.69-6.24)	1.95×10^{-4}	80/1,000	0.194/0.183	1.07 (0.71-1.61)	0.736
				Replication	46/3,553	0.152/0.175	0.85 (0.48-1.50)	0.568				
				Combined	65/4,553	0.231/0.177	1.40 (0.93-2.11)	0.109				
LEF1	4	rs4365796	A	GWAS	19/1,000	0.105/0.020	5.92 (2.00-17.48)	2.68×10^{-4}	80/1,000	0.025/0.020	1.29 (0.45-3.65)	0.632
				Replication	46/3,553	0.076/0.024	3.32 (1.51-7.28)	0.001				
				Combined	65/4,553	0.085/0.023	3.90 (2.07-7.34)	5.58×10^{-6}				
DEFB1	8	rs2738165	C	GWAS	19/1,000	0.763/0.492	3.33 (1.57-7.08)	9.07×10^{-5}	80/1,000	0.494/0.492	1.01 (0.73-1.39)	0.956
				Replication	46/3,553	0.522/0.498	1.10 (0.73-1.66)	0.644				
				Combined	65/4,553	0.592/0.496	1.48 (1.04-2.10)	0.029				
ST3GAL1	8	rs9643302	T	GWAS	19/1,000	0.237/0.076	3.77 (1.75-8.12)	2.71×10^{-4}	80/1,000	0.081/0.076	1.08 (0.60-1.94)	0.810
				Replication	46/3,553	0.043/0.074	0.57 (0.21-1.55)	0.263				
				Combined	65/4,553	0.100/0.075	1.38 (0.77-2.46)	0.275				
PAX5	9	rs1536876	A	GWAS	19/1,000	0.395/0.163	3.35 (1.73-6.49)	1.50×10^{-4}	80/1,000	0.206/0.163	1.33 (0.89-1.99)	0.157
				Replication	46/3,553	0.198/0.175	1.16 (0.68-1.98)	0.587				
				Combined	65/4,553	0.258/0.173	1.67 (1.11-2.50)	0.013				
CCND2	12	rs11063069	G	GWAS	19/1,000	0.211/0.042	6.08 (2.71-13.67)	7.16×10^{-7}	80/1,000	0.063/0.042	1.52 (0.77-2.99)	0.221
				Replication	46/3,553	0.078/0.050	1.60 (0.73-3.48)	0.234				
				Combined	65/4,553	0.117/0.048	2.61 (1.51-4.52)	3.53×10^{-4}				
BCL11B	14	rs2693689	A	GWAS	19/1,000	0.105/0.018	6.61 (2.22-19.62)	9.16×10^{-5}	80/1,000	0.006/0.018	0.35 (0.05-2.59)	0.285
				Replication	46/3,553	0.000/0.015	0	0.245				
				Combined	65/4,553	0.031/0.015	2.07 (0.75-5.67)	0.150				
IL29	19	rs7247086	T	GWAS	19/1,000	0.105/0.022	5.23 (1.78-15.37)	8.00×10^{-4}	80/1,000	0.031/0.022	1.43 (0.56-3.67)	0.450
				Replication	46/3,553	0.033/0.024	1.36 (0.43-4.34)	0.603				
				Combined	65/4,553	0.054/0.024	2.34 (1.08-5.08)	0.026				
FPR1	19	rs11667868	A	GWAS	19/1,000	0.684/0.389	3.40 (1.71-6.78)	2.26×10^{-4}	80/1,000	0.381/0.389	0.97 (0.69-1.35)	0.847
				Replication	46/3,553	0.369/0.392	0.91 (0.58-1.42)	0.674				
				Combined	65/4,553	0.467/0.391	1.37 (0.95-1.95)	0.087				

SNP, single nucleotide polymorphism; KD, Kawasaki disease; Chr, chromosome; RAF, risk allele frequency; OR, odds ratio; 95% CI, 95% confidence interval; GWAS, genome-wide association study.

Supplementary Table 2. Association results of 14 candidate SNPs selected from KD patients older than 5 years of age

Locus	Chr	SNP	Risk allele	Collection	KD (age > 5 y) vs. Controls				KD (age ≤ 5 y) vs. Controls			
					No. (case/control)	RAF (case/control)	OR (95% CI)	p-value	No. (case/control)	RAF (case/control)	OR (95% CI)	p-value
SLAMF1	1	rs2295613	T	GWAS	45/1,000	0.422/0.259	2.09 (1.36–3.21)	6.08×10^{-4}	251/1,000	0.285/0.259	1.14 (0.92–1.42)	0.240
				Replication	48/3,553	0.177/0.267	0.59 (0.35–1.00)	0.048				
				Combined	93/4,553	0.296/0.265	1.16 (0.85–1.60)	0.351				
IL28RA	1	rs7552086	A	GWAS	45/1,000	0.600/0.370	2.55 (1.66–3.93)	1.09×10^{-5}	251/1,000	0.376/0.370	1.03 (0.84–1.26)	0.788
				Replication	48/3,553	0.344/0.361	0.93 (0.61–1.42)	0.732				
				Combined	93/4,553	0.468/0.363	1.54 (1.15–2.07)	0.003				
INPP5D	2	rs4663337	G	GWAS	45/1,000	0.089/0.016	6.00 (2.68–13.43)	7.93×10^{-7}	251/1,000	0.022/0.016	1.38 (0.69–2.75)	0.362
				Replication	48/3,553	0.031/0.020	1.57 (0.49–5.02)	0.442				
				Combined	93/4,553	0.059/0.019	3.21 (1.71–6.01)	1.19×10^{-4}				
IRAK2	3	rs2302862	G	GWAS	45/1,000	0.289/0.143	2.44 (1.52–3.91)	1.45×10^{-4}	251/1,000	0.151/0.143	1.07 (0.81–1.41)	0.633
				Replication	48/3,553	0.135/0.145	0.92 (0.51–1.66)	0.789				
				Combined	93/4,553	0.210/0.145	1.57 (1.10–2.24)	0.013				
IL33	9	rs12349559	C	GWAS	45/1,000	0.244/0.121	2.36 (1.43–3.89)	5.24×10^{-4}	251/1,000	0.112/0.121	0.92 (0.67–1.25)	0.580
				Replication	48/3,553	0.056/0.112	0.47 (0.19–1.16)	0.093				
				Combined	93/4,553	0.150/0.113	1.38 (0.91–2.09)	0.127				
NFIL3	9	rs13297268	A	GWAS	45/1,000	0.133/0.042	3.51 (1.84–6.69)	5.23×10^{-5}	251/1,000	0.036/0.042	0.85 (0.50–1.42)	0.531
				Replication	48/3,553	0.083/0.059	1.46 (0.70–3.02)	0.312				
				Combined	93/4,553	0.108/0.055	2.07 (1.29–3.31)	0.002				
CXCL12	10	rs800314	G	GWAS	45/1,000	0.189/0.077	2.89 (1.66–5.03)	9.12×10^{-5}	251/1,000	0.094/0.077	1.26 (0.89–1.77)	0.191
				Replication	186/3,553	0.104/0.091	1.16 (0.82–1.65)	0.407				
				Combined	231/4,553	0.122/0.088	1.44 (1.07–1.93)	0.014				
C1RL	12	rs11613834	A	GWAS	45/1,000	0.122/0.029	4.75 (2.40–9.40)	9.47×10^{-7}	251/1,000	0.038/0.029	1.34 (0.79–2.28)	0.275
				Replication	48/3,553	0.042/0.031	1.36 (0.49–3.72)	0.554				
				Combined	93/4,553	0.081/0.031	2.79 (1.62–4.79)	1.08×10^{-4}				
KLRD1	12	rs2270238	T	GWAS	45/1,000	0.589/0.371	2.42 (1.58–3.72)	3.26×10^{-5}	251/1,000	0.378/0.371	1.03 (0.84–1.26)	0.772
				Replication	48/3,553	0.351/0.368	0.93 (0.61–1.42)	0.737				
				Combined	93/4,553	0.467/0.369	1.50 (1.12–2.01)	0.006				
FAM174B	15	rs8033443	T	GWAS	45/1,000	0.367/0.173	2.78 (1.78–4.33)	2.85×10^{-6}	251/1,000	0.185/0.173	1.09 (0.85–1.41)	0.501
				Replication	48/3,553	0.167/0.193	0.84 (0.49–1.43)	0.513				
				Combined	93/4,553	0.263/0.189	1.54 (1.11–2.14)	0.010				
DYNLRB2	16	rs1401197	T	GWAS	45/1,000	0.089/0.010	9.66 (4.13–22.58)	1.92×10^{-10}	251/1,000	0.016/0.010	1.60 (0.70–3.66)	0.258
				Replication	48/3,553	0.135/0.117	1.18 (0.66–2.14)	0.573				
				Combined	93/4,553	0.199/0.114	1.92 (1.34–2.77)	3.61×10^{-4}				
HS3ST4	16	rs3112543	G	GWAS	45/1,000	0.267/0.110	3.08 (1.89–5.03)	2.20×10^{-6}	251/1,000	0.108/0.110	1.02 (0.74–1.40)	0.893

				Replication	48/3,553	0.021/0.013	1.64 (0.40–6.76)	0.489				
				Combined	93/4,553	0.054/0.012	4.60 (2.37–8.95)	7.38×10^{-7}				
MAFB	20	rs6029245	C	GWAS	45/1,000	0.133/0.032	4.65 (2.41–8.97)	5.18×10^{-7}	251/1,000	0.042/0.032	1.32 (0.80–2.18)	0.279
				Replication	48/3,553	0.022/0.035	0.62 (0.15–2.55)	0.506				
				Combined	93/4,553	0.078/0.035	2.36 (1.35–4.12)	0.002				
ZBED4	22	rs2295407	A	GWAS	45/1,000	0.144/0.038	4.33 (2.31–8.15)	7.74×10^{-7}	251/1,000	0.040/0.038	1.07 (0.64–1.76)	0.806
				Replication	48/3,553	0.052/0.053	0.98 (0.40–2.42)	0.962				
				Combined	93/4,553	0.097/0.050	2.05 (1.25–3.36)	0.004				

SNP, single nucleotide polymorphism; KD, Kawasaki disease; Chr, chromosome; RAF, risk allele frequency; OR, odds ratio; 95% CI, 95% confidence interval; GWAS, genome-wide association study.

Supplementary Table 3. Age-stratified association results of *LEF1* (rs4365796; risk allele: T) in Korean KD

Locus	Chr	SNP	Risk allele	Collection	Age group	No. (case/control)	RAF (case/control)	OR (95% CI)	p-value
LEF1	4	rs4365796	A	Combined	All KD	1,669/4,553	0.030/0.023	1.29 (1.01–1.64)	0.042
					KD ≤ 0.5 y	118/4,553	0.068/0.023	3.07 (1.81–5.19)	1.10×10^{-5}
					KD 0.5 to ≤1 y	225/4,553	0.020/0.023	0.87 (0.44–1.70)	0.681
					KD 0 to ≤1 y	343/4,553	0.037/0.023	1.60 (1.05–2.45)	0.027
					KD 1 to ≤2 y	335/4,553	0.020/0.023	0.84 (0.48–1.49)	0.558
					KD 2 to ≤3 y	305/4,553	0.035/0.023	1.52 (0.96–2.40)	0.071
					KD 3 to ≤4 y	266/4,553	0.032/0.023	1.40 (0.85–2.31)	0.190
					KD 4 to ≤5 y	178/4,553	0.014/0.023	0.60 (0.25–1.48)	0.264
					KD >5 y	231/4,553	0.037/0.023	1.63 (0.98–2.69)	0.056

KD, Kawasaki disease; Chr, chromosome; SNP, single nucleotide polymorphism; RAF, risk allele frequency; OR, odds ratio; 95% CI, 95% confidence interval.

Supplementary Table 4. The effect of *LEF1* (rs4365796; risk allele: T) genotypes on clinical parameters of KD patients in KD age subgroups (either KD ≤0.5 y or KD >0.5 y)

Clinical variable	Clinical subgroup	KD ≤0.5 y (n = 118)			KD >0.5 y (n = 1,538)		
		CC (n=102)	CT/TT (n=16/0)	p-value	CC (n=1,457)	CT/TT (n=80/1)	p-value
Age (y)		0.4 (0.3–0.5)	0.3 (0.3–0.5)	0.311	2.7 (1.5–4.2)	2.8 (1.9–4.4)	0.108
Sex, n (%):							
	Male	64 (62.7)	10 (62.6)	0.985	863 (59.2)	52 (62.2)	0.376
	Female	38 (37.2)	6 (37.5)		594 (40.8)	29 (35.8)	
Type of KD, n (%):							
	iKD	29 (28.4)	5 (31.3)	0.835	372 (25.5)	15 (18.5)	0.184
	cKD	72 (70.6)	11 (68.8)		1,078 (74.0)	64 (79)	
IVIG response, n (%):							
	Responder	95 (93.1)	14 (87.5)	0.430	1,207 (82.8)	63 (77.8)	0.310
	Non-responder	7 (6.9)	2 (12.5)		197 (13.5)	14 (17.3)	
Coronary artery lesion							
	Normal	86 (84.3)	14 (87.5)	0.742	1,130 (77.6)	69 (85.2)	0.050
	CAL	16 (15.7)	2 (12.5)		318 (21.8)	10 (12.4)	
Baseline laboratory finding							
	CRP (mg/L)	6.7 (4.0–9.7)	6.3 (2.6–12.6)	0.786	6.5 (3.4–10.9)	8.5 (3.8–14.7)	**0.032**
	ESR (mm/hr)	41.0 (25.5–61.0)	35.0 (17.0–81.3)	0.890	54.0 (36.0–75.0)	51.0 (36.5–84.0)	0.743
	WBC (10^9/L)	14.6 (12.1–18.4)	15.0 (11.6–17.4)	0.847	13.3 (10.5–16.5)	12.7 (9.5–15.9)	0.247
	Neutrophil (%)	54.6 (46.9–63.9)	49.0 (40.4–67.3)	0.571	65.8 (53.1–76.3)	69.2 (54.0–78.8)	0.281
	Neutrophil (10^9/L)	7.8 (5.9–10.8)	8.7 (5.4–10.0)	0.751	8.4 (6.1–11.4)	8.3 (4.9–11.6)	0.476
	Non-neutrophil (10^9/L)	6.5 (4.8–8.4)	6.3 (4.6–7.9)	0.864	4.3 (3.0–5.8)	3.9 (2.8–5.4)	0.054
	Platelet (10^9/L)	374.5 (315.8–458.3)	406.0 (332.5–508.0)	0.389	325.0 (271.0–393.0)	331.0 (274.0–401.0)	0.652
	Hb (g/L)	10.9 (10.2–11.5)	10.9 (10.3–11.5)	0.691	11.5 (10.9–12.1)	11.6 (11.1–12.2)	0.183
	Albumin (mg/dL)	4.0 (3.6–4.2)	4.0 (3.9–4.2)	0.420	3.9 (3.6–4.1)	3.9 (3.5–4.1)	0.493
	AST (IU/L)	33.0 (25.0–48.0)	34.0 ([28.3–60.3)	0.385	34.0 (25.0–70.0)	37.0 (27.0–67.0)	0.467
	ALT (IU/L)	26.0 (19.0–48.0)	24.5 (17.3–33.3)	0.448	27.0 (14.0–108.8)	42.0 (17.0–103.0)	0.089
	Total protein (mg/dL)	6.2 (5.9–6.4)	6.1 (5.9–6.6)	0.719	6.6 (6.3–7.0)	6.6 (6.3–7.0)	0.791

The results for the categorical variables are presented as numbers and percentages in parentheses. For the normality test of the continuous variables, the Kolmogorov-Smirnov test was used. The continuous variables were found to be non-normally distributed and described by median and interquartile range in brackets. The difference between the groups was tested by Chi-square test for the categorical variables and Mann-Whitney test for the continuous variables.

A p-value of < 0.05 was considered as statistically significant. Significant p-values ($p < 0.05$) are shown in bold.

KD, Kawasaki disease; iKD, incomplete KD; cKD, complete KD; IVIG, intravenous immunoglobulin; CAL, coronary artery lesion; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; WBC, white blood cell; Hb, hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase.