Pediatric RESEARCH

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CLINICAL RESEARCH ARTICLE

Identification of increased expression of activating Fc receptors and novel findings regarding distinct IgE and IgM receptors in Kawasaki disease

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BACKGROUND: Kawasaki disease (KD) is associated with expression and methylation of Fc gamma receptor genes. We characterized immunoglobulin A (IgA), IgE, IgG, and IgM receptor expression levels in KD.

METHODS: Fc receptor expression levels were characterized using GeneChip Human Transcriptome Array 2.0 (HTA 2.0) with 18 KD patients, 18 non-febrile controls, and 18 febrile controls. Another 48 control individuals and 46 patients with KD were measured using pyrosequencing for the methylation levels.

RESULTS: The mRNA expression levels of *FCER1A* and *FCER2* were significantly lower in KD patients than in non-febrile controls and then rose following treatments with intravenous immunoglobulin (IVIG). Expression levels of *FCER1G* increased compared to the non-febrile subjects and then subsided after IVIG. *FCER1A* methylation was significantly lower among KD patients and even lower in KD patients with IVIG resistance. HTA analysis revealed higher mRNA levels of *FCAR*, *FCGR1C*, and *FCGR2A* in KD patients. *FCMR* mRNA expression levels were significantly lower in KD patients. *FCMR* expression levels rose after IVIG treatment. After IVIG, *FCGR1A*, *B*, and *C* decreased even lower than the febrile controls.

CONCLUSION: This is the first study indicating that IgA, IgE, IgG, and IgM receptors are associated with KD. We highlighted potential biomarkers related to Fc receptors and their regulation.

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INTRODUCTION

Kawasaki disease (KD) is an acute febrile mucocutaneous lymph node illness of early childhood mostly under the age of 5 years.[1] Various mechanisms including abnormal inflammatory response contribute to the pathogenesis of KD. In Taiwan, the incidence of KD rose rapidly from 29.3 to 62.0 per 100,000 children from 1997 to 2011.[1] Meta-analysis confirmed significant effect of 2 g/kg intravenous immunoglobulin (IVIG) on preventing coronary artery lesions (CAL).[2] Because of the binding to Fc receptors on antigen-presenting cells, IVIG suppresses harmful inflammation. Scholars studying KD have been interested in Fc receptors. Current literature supports a correlation between KD and genetic polymorphisms, gene copy number, expression, and methylation with ethnic variation in Fc gamma receptor genes.[3–6] However, no research has yet focused on immunoglobulin E (IgE) receptors encoded by *FCER1*, *LGALS3*, IgA, and IgM receptors in KD.

The different antibody classes exert multiple activities that are important for the response to pathogens and allergies. The expression levels of antibodies vary with different antibody classes and stages of KD. Biological role of IgM antibodies is critical for a primary immune response to an immunogen or pathogen. Ko and colleagues first reported the disappearance of clonally expanded IgM clonotypes, which have primarily been observed in acute-phase patients.[7] Individuals with KD have significantly elevated values of IgA, G, M than pretreatment levels over 2 weeks after diagnosis.[8] IgG antibodies contribute directly to an immune response, including neutralization of toxins and viruses. Recent reports suggest that post-IVIG higher IgG levels are biomarkers indicating better clinical outcomes.[9] In addition, IgA levels at mucosal surfaces and in secretions tend to be much higher than in serum to protect mucosal surfaces from toxins, virus, and bacteria. On the other hand, IgE plays a vital role in the response to hypersensitivity and allergic reactions as well as parasitic infections.

Our previous study suggested that the following plasma eosinophils and eosinophil-related mediators: IgE, eosinophil-related T helper 2 cytokines interleukin (IL)-4, IL-5, eotaxin, and eosinophil cationic protein, were distinctively higher in patients with KD than in control individuals.[10,11] In addition, previous studies have identified the role of post-IVIG treatment eosinophilia in IVIG-responsive KD patients.[12] Of particular note, children with KD had a higher susceptibility to allergic diseases, including atopic dermatitis, urticaria, and allergic rhinitis, recognized from before the age of 1 year.[10] It has not yet been clear whether multiple IgE receptors exert different effects on KD. Therefore, effects of multiple IgE receptors on KD require elucidation.

Immunoglobulin receptors (Fc receptors) play an important role in immunoregulatory processes and mediate such diverse functions as phagocytosis, the triggering of the degranulation of basophils and mast cells, promotion of Ig class switching, and

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prevention of excessive activation, which may also contribute to disease pathogenesis.[13] Fc receptors are associated with transmembrane proteins with cytoplasmic domains that contain immune-receptor tyrosine-based activation motifs (ITAM) and comprise a conserved sequence present in the cytoplasmic domain of Fc gamma receptors and shared by different signaling subunits associated with such receptors as *FCAR* encoding FcαR, *FCER1G* encoding FcεR1γ, *FCGR1* encoding CD64, *FCGR2A* encoding CD32, and *FCGR3A* encoding CD16A.[14] Binding to phosphorylated ITAMs relieves spleen tyrosine kinase (SYK), which is expressed considerably by all hematopoietic lineage cells. These cells conduct cellular responses like cytokine release.

Receptors have already been characterized as being able to recognize and bind to IgG (Fc gamma receptor family), IgE (*FCER1* encoding FcεRI, *FCER2* encoding CD23, and *LGALS3* encoding galectin-3), IgA (*FCAR* encoding CD89; *FCAMR*), and IgM (*FCAMR* and *FCMR* encoding FcμR). The cell surface's Fc receptor for the IgM antibody encoded by *FCMR* has most recently been identified among Fc receptors on both T and B cells.[15] Fc gamma receptors, which recognize the Fc portion of IgG, can be categorized by function (FcγRIIB receptor is the only inhibitory receptor, while the others are activating receptors) or affinity to IgG (FcγRI is the only high-affinity receptor).

Evolving technologies enable the broad analysis of transcriptional profiles in small cell quantities. In this article, we investigated the expression levels of Fc receptors in the leukocytes of KD patients using transcriptome array technologies. Our comprehensive analysis of the Fc receptors will greatly contribute to a better understanding of the relevance of the different Fc receptors in KD.

METHODS

Subjects

In this study, we recruited 54 subjects from Kaohsiung Chang Gung Memorial Children's Hospital in Taiwan during the period of 2012–2014. Of those, 18 were KD patients, 18 non-febrile subjects, and 18 fever control (FC) patients who had a fever but were not diagnosed with KD.

Eighteen KD subjects had been diagnosed according to KD diagnosis criteria and had been hospitalized.[16] All recruited KD patients were treated with IVIG. The subjects in the FC set consisted of patients admitted to the hospital, 11 of which for lower airway infections, including 1 patient with adenovirus and mycoplasma infection, 2 respiratory syncytial-related viruses, and 6 upper airway infections, including 1 herpangina and 1 gastroenteritis. Among the 11 FC with lower airway infections, there were 3 patients complicated with acute otitis media and 1 complicated with sinusitis. We obtained peripheral blood samples from the KD subjects both before IVIG treatment (KD1) and 3 weeks after IVIG treatment (KD3), as described in our previous study.[17] This study was approved by Chang Gung Memorial Hospital's Institutional Review Board (IRB number: 201700270A3C501). Upon providing a thorough explanation of the study, we obtained written informed consent from the parents or guardians of all the participants.

Experiment design and transcriptome analysis of whole-blood RNA

For robust, unbiased results, we created pooled RNA libraries by evenly pooling six RNA samples. We pooled samples in order to minimize the difference between subjects, as in our previous reports.[18,19] We subjected the pooled RNA samples to microarray assay to determine their gene expression profiles using GeneChip® Human Transcriptome Array 2.0 (HTA 2.0, Affymetrix, Santa Clara). The venous blood of both patients and controls was collected in heparin blood collection tubes without EDTA. We obtained RNA samples extracted from the total leukocytes for three pairs of six pooling samples. Since the blood volume could not exceed 5 ml from the pediatric patients in our study, we were unable to arrange a density gradient enrichment of leucocytes, which is one of this study's limitations. We extracted RNA from the leukocytes and reverse transcribed mRNA to synthesize cDNA, which was fragmented and biotinylated. We washed and stained the microarrays and scanned their fluorescence signals with GeneChip Scanner 3000 7G (Affymetrix, CA, USA). Then we calculated the transcriptome level ratio and significance of those changes using one-way analysis of variance conducted by Partek. Fold changes were defined as 1/ratio.

Methylation status of *FCER1A* genes in KD patients

Methylation microarray assays (M450K) identified two CpG sites of *FCER1A* from the sample group with a significant p value, cg09581644 and cg24642483.[18] We selected cg09581644 because of its hypomethylation following IVIG, which was compatible with HTA. White blood cells were taken at two points from a separate cohort with a total of 48 controls (24 non-febrile controls, 24 patients with fever) and 46 KD patients who were enrolled in this study, without pooling: prior to IVIG administration and at least 3 weeks after IVIG treatment. *FCER1A* DNA methylation levels were measured using pyrosequencing.

To extract DNA, we first treated the collected white blood cells with a 0.5% sodium dodecyl sulfate lysis buffer and then protease K (1 mg/ml) for 4 h at 60 °C to digest the nuclear protein. We isolated DNA from the whole-blood samples using Non-Organic Solvent Reagents Purification. Like the protocol of a previous study in our laboratory, we processed 0.5 μg genomic DNA bisulfited using an EZ DNA Methylation Kit (Zymo Research) and then eluted it in 20 μl of Tris buffer (10 mM).[20] We detected *FCER1A* loci cg09581644 across the gene region of the major transcription regulators. Then we applied polymerase chain reaction (PCR) to a 25-μl reaction mixture containing 25 ng of bisulfite-converted DNA, 1× PyroMark PCR Master Mix (Qiagen), and 0.2 μM *FCER1A* biotinylated forward primer 5′-AAGAGGGAGAGTGATTAATAGTG as well as the biotinylated reverse primer 5′-AAACCCTAAATCATAAAAACTACACCA. After amplification, the PCR products were purified and treated with the sequencing primer 5′-AAGTAGGGAAAGAATTAAGA, which was designed to bind adjacent to the CpG sites of interest. We then applied a PyroMarkQ24 instrument to calculate the percentage of methylation levels using the Qiagen software 1.0.10.

RESULTS

Fc receptor subtypes in KD

Table 1 summarizes previous studies focused on the association between Fc receptors and the increased risk of KD development.

Activated Fc gamma receptors, particularly FcγRI and FcγRIIA, have been detected in children with KD and FcγRIIIA in monocytes. In our previous study, we demonstrated that *FCGR2A*, which encoded CD32 transcriptional expression, was significantly increased in KD patients and then decreased after they received IVIG treatment.[5] Furthermore, children with hypomethylated *FCGR2A* were associated with IVIG resistance. This finding indicates that increased *FCGR2A* expression may be relevant to KD. CD14++CD16+ cells are also of clinical interest because they expand in acute KD.[20,21] While CD14++CD16+ monocytes are reduced through IVIG treatment, CD16 encoded by *FCGR3A* expression on neutrophils in KD was significantly lower following reverse kinetics during a clinical course.[21]

Several studies have addressed the relationship between the genetic polymorphisms of Fc receptors and the occurrence of KD. In a study of 443 KD subjects, the polymorphism *FCGR3B NA1* variant was related to IVIG resistance, and individuals with the *FCGR3B NA1* variant had an increased risk of KD (odds ratio (OR) 3.67, confidence interval (CI) 1.75–7.66; $p = 0.0006$). From a functional perspective, *FCGR3B NA1* demonstrates a greater attraction to IgG than the NA2 variant. Shrestha et al. also

Table 1. Fc receptors in Kawasaki disease.

Fc receptor genes		Study	Origin	Method	Result
IgE receptors	*FCER2* (CD23)	Furukawa et al.[27]	Japan	Fluorescence-activated cell sorter	Increased Fc epsilon R2/CD23 antigen present
		Furukawa et al.[28]	Japan	Fluorescence-activated cell sorter	Increased number of CD23-positive B lymphocytes
		Matsubara et al.[29]	Japan		Patients with KD had increased soluble CD23 levels
	FCER1	—			
IgG receptors	*FCGR*				
	FCGR1 (CD64)	Nakatani et al.[21]	Japan	Flow cytometry	Higher FcγRI in KD
		Hokibara et al.[36]	Japan	Flow cytometry	Higher FcγRI in KD
	FCGR2A (CD32A)	Nakatani et al.[21]	Japan	Flow cytometry	Higher FcγRII in KD
		Shrestha et al.[22]	USA	SNP	*FCGR2A-131H* in KD
		Kuo et al.[5,31]	Taiwan	RTPCR, pyrosequencing Luciferase	The KD group had the highest *FCGR2A* mRNA level by hypomethylation
		Kwon et al.[23]	Korea	Whole-exome sequencing	Male-specific *FCGR2A* His167Arg SNP in KD
	FCGR2B (CD32B)	Shrestha et al.[24]	USA	SNP	Functional *FCGR2B* SNP influence IVIG response
		Chang et al.[17]	Taiwan	RTPCR	Higher *FCGR2A/2B* in KD patients with CAL
		Xia et al.[25]	China	Flow cytometry	Lower FcγRIIB in KD patients with CAL
	FCGR2C (CD32C)	Makowsky et al.[3]	USA	Gene copy number (GCN)	*FCGR2C* GCN associated with KD
	FCGR3A (CD16A)	Nakatani et al.[21]	Japan	Flow cytometry	Lower FcγRIII on neutrophils in KD Higher FcγRIII on monocytes in KD
		Katayama et al.[20]	Japan	Flow cytometry	Increased CD14+CD16+ monocytes with acute KD
	FCGR3B (CD16B)	Shrestha et al.[22]	USA	SNP	*FCGR3B* NA1 variant among IVIG non-responders
		Makowsky et al.[3]	USA	Gene copy number	*FCGR3B* GCN associated with KD
IgA and IgM receptors		—			

CAL coronary artery lesions, *IVIG* intravenous immunoglobulin, *R* receptor, *RTPCR* reverse transcription polymerase chain reaction, *SNP* single-nucleotide polymorphism

observed that the *FCGR2A-131H* variant granted a higher affinity for IgG2 and IgG3 than *FCGR2A-131R/R* and contributed to an increased risk of KD (OR 1.51, CI 1.16–1.96; $p = 0.001$).[22] Meanwhile, Kwon et al. demonstrated a male-specific association among Korean and Japanese populations between the *FCGR2A* His167Arg polymorphism and KD.[23] The minor allele A at *FCGR2B* 120T/a was observed to be three times more frequent (15%) in IVIG responders than in non-responders (5%) in Caucasian subjects.[24] Makowsky et al. previously found that the gene copy numbers of *FCGR2C* and *FCGR3B* were associated with KD and IVIG treatment response. Xia et al. also found KD patients who developed CAL to have a lower FcγRIIB than those without CAL.[25]

Gene expression changes in IgE receptors affected by KD

We first analyzed IgE receptors' expression between KD children and control subjects, as shown in Fig. 1. We observed no significant difference in gender and age distribution between the KD patients and controls (Table 2). Transcriptional levels of *FCER1A* and *FCER2* were significantly lower in KD patients than in non-febrile controls ($p = 0.0003$ and 0.001, respectively). The mRNA levels of *FCER2* were considerably lower in acute-stage KD patients than in febrile controls ($p = 0.002$). Both gene expression levels significantly increased in KD patients after they underwent IVIG treatment. In patients with KD, the mRNA of *FCER1G* increased ($p = 0.004$) but then decreased following IVIG treatment. In *LGALS3*, we observed no significant difference between KD patients and controls. The *LGALS3* levels in patients receiving IVIG were very similar to those before IVIG.

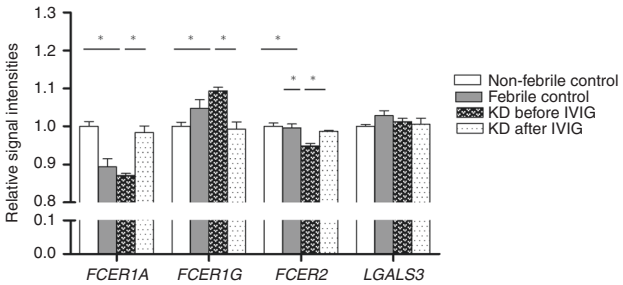

Fig. 1 For each Fc receptor, the distributions of signal intensities for three pairs of six pooling samples are displayed with the average of Fc receptors' values in the non-febrile control samples set to 1. Asterisk (*) indicates a p value of <0.05 between the two groups. The expression levels of mRNA levels of *FCER2* were significantly downregulated in KD patients compared to the non-fever and febrile controls. After KD patients received IVIG treatment, *FCER2* and *FCER1A* values increased considerably. We also observed the mRNA level of *FCER1G* to be higher in KD patients. *LGALS3* is a galectin-3 protein coding gene. Data are expressed as mean ± standard error.

FCER1A methylation in KD: clinical significance

We examined whether DNA methylation of the *FCER1A* promoter region impacted KD using a total of 94 participants (46 KD patients and 48 controls). Of the 46 KD patients, 24 patients had CAL complications. We observed no significant differences between the control group and the KD group regarding gender and age

Table 2. Demographic characteristics of KD patients and matched individuals.

	Kawasaki disease patients	Controls	*p* value
HTA array			
Number of subjects	18	18	
Male/female	12/6	10/8	0.508
Age at study (years)	1.49 ± 0.20	2.19 ± 0.37	0.108
FCER1A methylation			
Number of subjects	46	48	
Male/female	23/23	23/25	0.842
Age at study (years)	1.44 ± 0.13	2.09 ± 0.32	0.068

The data were presented as the mean ± standard error

($p = 0.842$ and 0.068, respectively) in the matched cohort (Table 2). As shown in Supplementary Table S1, KD individuals who responded poorly to IVIG had a higher C-reactive protein value ($p = 0.001$). In contrast, KD patients who responded well to IVIG treatment had lower Kobayashi scores, but the difference did not reach statistical significance (Supplementary Table S1). Two patients with IVIG-resistant KD received corticosteroids. *FCER1A* is often deactivated by promoter methylation in leukocytes. The methylation levels of the cg09581644 CpG site of *FCER1A* were significantly lower in KD patients compared to controls (97.98 ± 0.29 vs. 99.21 ± 0.23, $p = 0.001$) (Fig. 2a). Nonparametric analysis with Mann–Whitney *U* test indicated that DNA methylation of the cg09581644 CpG sites of the *FCER1A* gene promoter was lower in KD patients with IVIG resistance ($n = 16$) when compared to those who responded to IVIG treatment with normal methylation patterns ($n = 30$, $p = 0.038$) (Fig. 2b). No statistically significant associations were found between *FCER1A* promoter methylation and CAL ($p = 0.78$). We also found no significant difference regarding KD3 *FCER1A* methylation between KD patients with/without IVIG responsiveness ($p = 0.639$). Furthermore, the *FCER1A* methylation in patients 3 weeks after undergoing IVIG treatment was very similar to that before IVIG among the matched cohort using paired *t* test (98.54 ± 0.269 and 97.98 ± 0.29, $p = 0.103$).

Comparisons of Fc receptors' expression levels between controls and KD patients

The gene expression profile indicated that the expression levels of ten gene transcripts were deregulated in KD patients compared to the non-febrile controls. Of these, the expression levels of seven genes, *FCGR1A*, *B*, *C*, *FCGR2A*, *TRIM21*, *FCAR*, and *FCER1G*, increased. Meanwhile, the mRNA expression levels of *FCMR* were down-regulated in KD patients ($p = 0.001$; Figs. 1, 3, and 4). We observed no differences in *FCGR2B*, *C*, *FCGR3A*, *B*, *FCGRT*, or *FcRL5* between KD patients and non-febrile controls (Fig. 4b).

FCAR, *FCGR1C*, and *FCGR2A* expression levels were all significantly elevated in the KD group compared with those in the other infectious disease groups ($p = 0.020$, $p = 0.047$, and $p = 0.017$, respectively). In contrast, both *FCMR* and *FCER2* values in KD patients' white blood cells were markedly lower than those of the febrile individuals ($p = 0.001$ and $p = 0.002$, respectively). Despite the *FCER1G*, *FCGR1A*, *B*, and *TRIM21* expression levels being significantly higher in KD than in non-febrile controls, we observed no significant difference in their expression levels between KD and FC.

Expression patterns of selected candidate differentially expressed genes in KD1 and KD3 patients

To examine the effects of IVIG on the transcriptome profile of Fc receptors, we compared the Fc receptor gene expression patterns

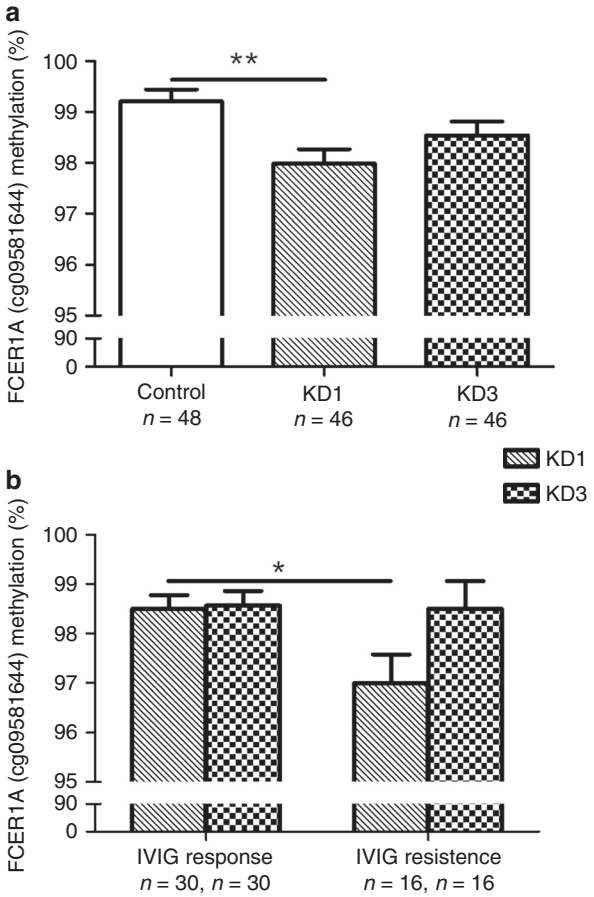

Fig. 2 **a** *FCER1A* methylation may decrease in patients with KD. At 3-week follow-up (KD3), *FCER1A* methylation change showed no significant difference compared with before treatment (KD1). **b** *FCER1A* methylation is significantly associated with IVIG resistance. Data are expressed as mean ± standard error (*$p < 0.05$, **$p < 0.001$).

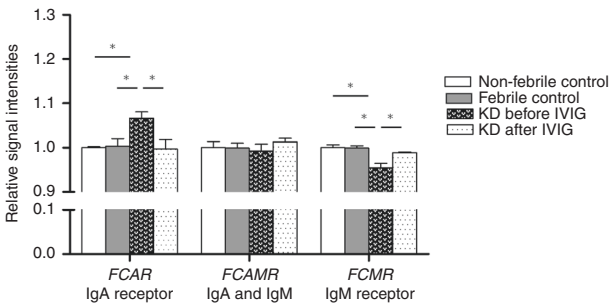

Fig. 3 The microarray expression data of IgA and IgM receptors between controls and KD patients. Data are expressed as mean ± standard error (*$p < 0.05$).

of the patients at KD3 with the gene expression patterns at KD1 using a similar protocol to the one described above. The expression levels of only 14 gene transcripts were differentially expressed with significance between KD3 and KD1 ($p < 0.05$), 11 of which were downregulated (*FCAR*, *FCGR1G*, *FCGR1A*, *B*, *C*, *FCGR2A*, *B*, *C*, *FCGR3A*, *B*, and *TRIM21*). After patients received IVIG treatment, *FCGR1A*, *B*, *C* dropped (fold change = −6.19, fold change = −5.42, and fold change = −6.84, respectively) even lower than the febrile controls ($p = 0.013$, 0.014, and 0.010, respectively) (Fig. 4a). We also observed a 1.81-fold decline in the IgG receptor *FCGR2A* (Fig. 4a), and *TRIM21* decreased slightly

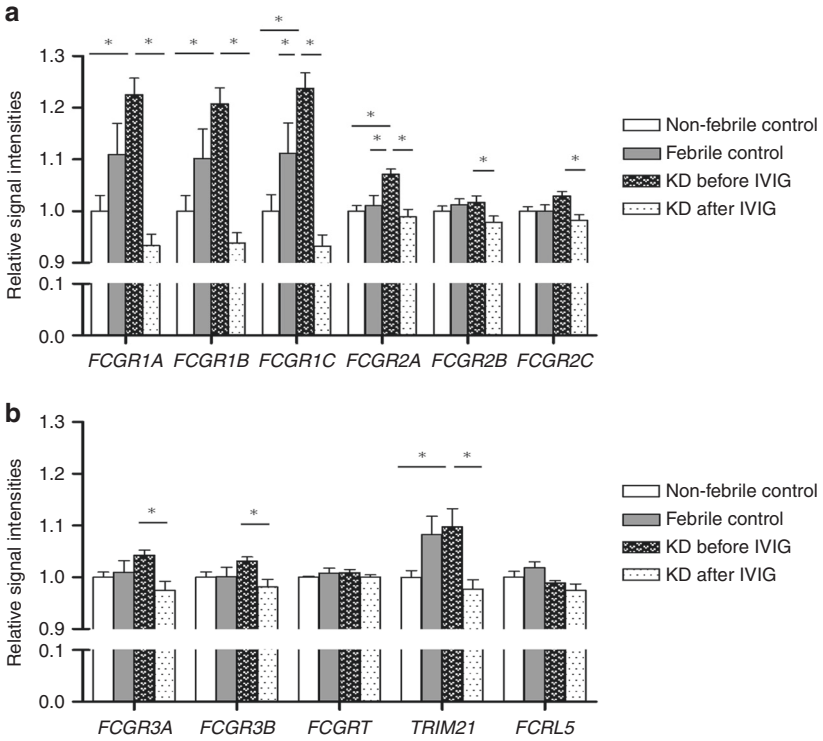

Fig. 4 The microarray expression data of IgG receptors between controls and KD patients. a *FCGR1* and *FCGR2*; **b** other IgG receptors. The mRNA levels of *FCGR1C* and *FCGR2A* were significantly higher in KD patients than in the non-febrile control and febrile control groups. These values significantly decreased in KD patients following IVIG treatment. Data are expressed as mean ± standard error (*$p < 0.05$).

following IVIG administration (fold change = −2.10) (Fig. 4b). Only *FCMR*, *FCER1A*, and *FCER2* values increased following IVIG administration ($p = 0.006$, $p = 0.026$, and $p = 0.024$, respectively; Figs. 1 and 3). Interestingly, *FCMR* levels were lower in KD1 children. We observed no significant changes in *FCGRT* and *FcRL5* expression levels during the clinical course of KD.

These results indicate that the gene expression patterns in KD1 and KD3 demonstrated similar trends when compared to the non-febrile groups; therefore, the genes that were downregulated or upregulated in the KD1 samples were found to be conversely upregulated or downregulated, respectively, in the KD3 samples.

DISCUSSION

To the best of our knowledge, this is the first study to comprehensively report that Fc receptors, including *FCAR*, *FCER1G*, *FCGR1*, and *FCGR2A*, are significantly elevated in acute-stage KD patients and drop greatly following IVIG treatment. This finding also suggests that overall immune activation is increased in KD. Furthermore, the current study is the first to demonstrate that the *FCMR* and *FCER2* mRNA levels in peripheral blood are lower in patients with KD than in healthy and febrile individuals. The human transcriptome array identified both known and novel Fc receptor biomarkers.

In our previous studies, we found IL-5 and IgE levels to be significantly higher in KD patients.[11] Our survey demonstrated that the peripheral mRNA expression levels of *FCER1A* may be lower in patients with KD than in healthy individuals. This finding supports the hypothesis that *FCER1A*, which encodes the high-affinity IgE receptor FcεRIα, actually promotes immune homeostasis and regulation. The high-affinity IgE FcεRIα receptors constitutively expressed by mast cells and basophils, which are encoded by *FCER1A*, are the receptor subunit responsible for IgE binding. FcεRIγ encoded by *FCER1G* is an intracellular component of FcεRI with a transmembrane domain. Cells obtained from KD patients demonstrate elevated levels of CD23 (low-affinity IgE receptors), as well as an increase in CD23-positive B lymphocytes. Furukawa et al. compared the decreased absolute counts of CD23 +CD14+ macrophage/monocyte expression levels in five patients with CAL with those of 35 patients without CAL.[26–29] However, the current study by HTA demonstrated an association between KD and the lower mRNA of *FCER2* in total leukocytes. The discordant findings may have been due to sampling serum or different white blood cell populations and varied experimental methods, including transcriptional array and the fluorescence-activated cell sorter.

The DNA methylation changes of inflammatory, inflammation-associated anemia and coronary vasculitis genes are vital to the pathogenesis of KD. Our previous study found an association between genomic hypomethylation of *FCGR2A*, susceptibility to KD and IVIG resistance.[5] We found that children with KD receiving IVIG had higher methylation of both *FCGR2A* and *FCGR2B* than before IVIG treatment.[17] Recent results have indicated that changes in toll-like receptors (TLRs), NOD-like receptors, matrix metalloproteinases, and *HAMP* transcriptional expression may depend on the extent of DNA methylation in gene promoter regions in children with KD.[30–32] This study is the first to indicate that *FCER1A* methylation can function as a valuable biomarker for predicting unfavorable clinical features. Such findings are consistent with the hypomethylation of *FCGR2A* in KD children and IVIG-resistant patients with KD, reflecting the hypomethylation patterns of CpG markers in KD1 samples. These results thus indicate that DNA hypomethylation may be a prognostic biomarker in KD children who receive IVIG treatment. Methylation appears to be an important component of disease pathogenesis in IVIG resistance of KD. These results may further extend the possible use of methylation therapy for preventing poor response to IVIG. Furthermore, additional global genetic methylation studies between IVIG resistance and response are warranted to verify our

results. These similar methylation patterns of Fc receptors also suggest the role of genetic de/methylation enzymes in global methylation.

This current study is the first to indicate that the peripheral mRNA expression levels of *FCER2* and *FCMR* may be lower in patients with KD than in both febrile and non-febrile individuals. TOSO/FAIM3 has recently been identified as the long-sought-after FcR for IgM encoded by *FCMR*. A previous study found that the activation of TLRs strongly downregulated *FCMR* in chronic lymphocytic leukemia cells.[33] Another recent study found that TLR2 may also downregulate the expression of IgE receptors and their transcription factor PU.1 in human Langerhans cells.[34] Our previous survey showed that KD patients had significantly higher *TLR* mRNA levels compared to both healthy and febrile controls.[35] The transcriptomic findings of lower *FCER2* and *FCMR* levels in KD patients correspond to higher *TLR* mRNA levels.

An increase in neutrophil-CD64 encoded by an *FCGR1* surface expression was observed in KD.[36] CD64-expressing neutrophils have also been used to distinguish KD patients from those with other viral or bacterial infections.[21] Some febrile diseases may mimic the immunological response of KD. The CD64 receptor has been described as an interesting biomarker for bacterial infection or sepsis biomarker.[37]

High-dose IVIG is commonly used to treat KD. Recent research has uncovered that high-dose IVIG downregulates *FCGR2A* and IFN-γR2 in humans.[38] We have demonstrated that the altered transcriptional values of high-affinity *FCGR1A*, *B*, *C* after treatment were lower than those of the febrile controls. High-dose IVIG is part of a homeostatic mechanism to limit excessive inflammation and tissue damage. The lower post-IVIG *FCGR1A*, *B*, *C* in the KD than in the febrile controls suggested anti-inflammatory activities of high-dose IVIG.

Given the role of IgA antibodies in mucosal immunity and the Fc receptor for IgA encoded by *FCAR* in triggering IgA-mediated immune responses to pathogens, growing evidence has indicated that a *FCAR*-mediated immune system is associated with inflammatory and infectious diseases.[39] Its potential significance in KD has not yet been reported. This report is the first to describe a transcriptional *FCAR*' change in KD, which suggests that *FCAR* is involved in KD.

Recently, the use of corticosteroids has garnered considerable clinical and academic interest. Kobayashi et al. conducted a retrospective cohort study of patients who underwent IVIG+prednisolone as first-line rescue therapy to treat IVIG non-responders and reported that IVIG+prednisolone groups had a significantly higher rate of treatment success and lower coronary abnormalities.[40] In accordance with these promising findings, RAISE and post-RAISE studies demonstrated that additional prednisolone in initial treatment improved coronary artery outcomes in patients predicted to be non-responders according to their Kobayashi score.[41]

This study is the first to demonstrate that the peripheral mRNA expression levels of IgE receptors' genes may be altered in KD patients. The mRNA expression levels of IgE receptors differed significantly among the pre-IVIG and post-IVIG groups. In this study, we also reported upregulation of *FCAR*, *FCER1G*, *FCGR1*, and *FCGR2A*, which suggests that children with KD are susceptible to triggering overactivated inflammatory reactions. Fc-receptor-mediated myeloid cell functions and macrophage dectin-1/Syk-mediated pathway have all been implicated in the pathogenesis of KD and they all have been shown to require SYK. Therefore, SYK may become a major therapeutic target in KD in the future. As the first transcriptome array study of Fc receptors for KD, this study provided novel findings regarding IgA, IgE, and IgM receptors, and these findings will contribute to a better understanding of KD.

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AUTHOR CONTRIBUTIONS

L.-S.C. drafted the article, carried out conception, design, and initial analyses, and approved the final manuscript as submitted. M.M.-H.G. and M.-H.L. acquired data, reviewed the manuscript, and approved the final manuscript as submitted. H.-C.K. carried out conception and design, acquired data, reviewed the manuscript, revised it for intellectual content, and approved the final manuscript as submitted.

ADDITIONAL INFORMATION

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