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Genetic susceptibility to Kawasaki disease: Analysis of pattern recognition receptor genes

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ABSTRACT

Kawasaki disease (KD) is a systemic vasculitis of unknown etiology occurring in infants and children. Several lines of evidence suggested the importance of genetic factors and infectious triggers for the pathogenesis of KD. We have reported that oral administration of a pure NOD1 ligand induces coronary arteritis in mice without fail. Since NOD1 is one of the pattern recognition receptors (PRRs) which play important roles in the innate immunity for the detection of microbial substances and induce inflammatory responses, we have investigated the association of PRR genes with the development of KD. Forty-six tagging-SNPs in 19 PRR genes were genotyped in Japanese KD patients ($n = 356$, consisting of two groups) and controls ($n = 215$). The genotypes and allele frequencies of each SNP or haplotype were compared between KD patients and controls. As a result, we did not find any genes with strongly contributed to the development of KD. A haplotype, G-T-C-C, in the *NOD1* gene, was associated with lower risk for KD development (KD 1st group versus controls: 23.2% versus 35.3%, $Pc = 0.0385$). The second-round case-control study in KD group 2 demonstrated that a haplotype, T-T-C-G-A-C, in the *NLRP1* gene was associated with a higher risk for KD development (4.9% versus 1.2%, $Pc = 0.035$). From the association analysis of SNPs and haplotypes of 19 PRR genes, *NOD1* and *NLRP1* seemed to partly contribute to the development of KD. Further analysis with larger samples of another independent set would be needed to find confirmative results.

1. Introduction

Kawasaki disease (KD) is an acute febrile illness of childhood characterized by prolonged fever, polymorphous skin rashes, injected conjunctiva, diffuse mucosal inflammation, indurative edema in the palms and soles, and non-suppurative cervical lymphadenopathy [1]. The histopathological findings in KD comprise vasculitis with infiltration of lymphocytes, macrophages and neutrophils in the vascular walls [2–4]. Coronary artery lesions (CALs) are the most important complication of KD. The patients with coronary artery aneurysms are at risk for rupture and development of stenosis, which cause ischemic heart disease. Even though treatment with intravenous immunoglobulin (IVIG) reduces the development of aneurysms, about 5% of KD patients still suffer from this critical complication [5].

The etiology of KD remains unknown, however, a combination of microbial infection and the immune response, or genetic susceptibility, is believed to contribute to the development of KD. Several lines of evidence have suggested the importance of genetic factors for the disease susceptibility and CAL formation. The incidence of KD is significantly elevated in the Asian population, and siblings or children of affected patients are at higher risk for the occurrence of KD [6]. In previous reports, much attention has focused on the relationship between KD and the polymorphisms of a variety of genes related to inflammation [7,8].

The clinical and epidemiological features of KD also suggested that infectious agents might trigger the development of this disease [9], although no specific pathogens have been identified. A significant contribution of the innate immune system to the

Abbreviations: CAL, coronary artery lesion; CLR, C-type lectin receptor; IVIG, intravenous immunoglobulin; KD, Kawasaki disease; LD, linkage disequilibrium; MAF, minor allele frequency; NLR, nod-like receptor; PAMP, pathogen-associated molecular pattern; PGLYRP, peptidoglycan recognition protein; PRR, pattern recognition receptors.

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pathophysiology of acute phase of KD has been demonstrated in recent studies. We reported that the expression levels of genes related to innate immunity (*NAIP, IPAF*) were up-regulated during acute phase KD [10]. Popper et al. also showed that the expression levels of innate immunity-associated genes were upregulated during the acute phase of KD [11]. Furthermore, we recently reported that the administration of FK565, a pure ligand of *NOD1*, caused coronary arteritis in mice [12].

Based on these findings, we speculated that the inflammatory or immunologic process of the innate immune system has a pivotal role in the development of KD. We focused on the PRRs which recognize microbial components, known as pathogen associated molecular patterns (PAMPs), and play a role in the initiation of innate immunity. We herein report the results of an association study that was performed for the 19 selected genes from four families of PRRs to evaluate whether any of the genes may have genetic contribution to the development of KD.

2. Materials and methods

2.1. Subjects

The KD patients, consisting of two groups, who were admitted to Kyushu University Hospital or its affiliated hospitals, were enrolled in the present study. All patients fulfilled the diagnostic criteria for KD (http://www.kawasaki-disease.org/diagnostic/index.html). All patients were treated with aspirin and high-dose IVIG. The characteristics of the two groups are shown in Table 1. The KD patients in Group 1 consisted of 129 KD patients whose samples were collected from 1991 to 2003. These samples were mainly collected when patients were hospitalized at Kyushu University Hospital to receive cardiac catheterization during the convalescent period of KD, including 42 with CALs (32.5%). The second KD group consisted of 227 patients whose samples were collected during the acute phase of KD at Fukuoka Children's Hospital from 2005 to 2008, including eight patients (3.5%) with CALs. The prevalence of CALs in Group 2 was similar to that observed in the Nationwide Survey on KD in Japan.

A CAL was defined as when the lumen diameter was at least 3 mm (4 mm in the cases when the patients were older than 5 years of age) or the internal diameter of one or more segments at least 1.5 times larger than that of the adjacent normal-size segments. Two hundred fifteen healthy children, with no history of Kawasaki disease, living in the northern area of Kyushu Island in Japan, served as normal controls. Informed consent was obtained from all of the patients of their parents. This study was approved by the Ethics Committee of Kyushu University.

2.2. Selection of SNPs

Haplotype-tagging SNPs for *NOD1*, *NOD2*, *TLRs* (*TLR2*, *TLR4*, *TLR5*, *TLR6*) and *PGLYRPs* (*PGLYRP1*, *PGLYRP2*, *PGLYRP4*) were selected using the genotypic data by the JPT plus CHB population, available from the HapMap project, HapMap Genome Browser release#24 (Phase 1 & 2 – full dataset) (released on 21 July 2006; http://www.hapmap.org/cgi-perl/gbrowse/hapmap24_B36/). The haplotype-tagging SNPs for *DECTIN2* and *MINCLE* were selected using the genotypic data by JPT population data, available from the HapMap Genome Browser release #27 (Phase 1, 2 & 3 – merged genotypes & frequencies) and those for *DC-SIGN* and *DECTIN1* were from JPT population data using SNPbrowser™ Software v4.0 (Applied Biosystems LLC, Foster city, CA, USA). The selection setting for the above SNPs was as follows: a minor allele frequency (MAF) greater than 0.2 at a threshold of $R^2 > 0.75$ in the linkage disequilibrium (LD) blocks of each gene. For *PGLYRP3*, *NLRP1*, *NLRP3*, *NLRC4* and *MRC1*, representing SNPs were chosen from the haplotype-tagging SNPs picked-up by HapMap Genome Browser and/or SNPbrowser, based on sizes of estimated haplotype blocks, or availability and consistency of SNP assay supplied from Applied Biosystems. Because the MAFs of all of the SNPs in the *NAIP* gene were <0.2, the SNP with the highest MAF in the gene was selected (rs4976210).

Table 1
Baseline characteristics of the 2 groups of KD patients.

	KD Group 1	KD Group 2	*P*-value
Total	129	227	
Sex (M/F)	85/44	120/107	0.019
Age at diagnosis (month)			
Median	20	23	n.s
Range	1–151	2–125	
Patient with CAL	42 (32.6%)	8 (3.5%)	<0.001

2.3. DNA extraction and genotype analysis of SNPs

Genomic DNA was extracted from peripheral whole blood using a DNA blood mini kit (QIAGEN, Tokyo, Japan). Each SNP was analyzed by the TaqMan SNP genotyping assay (Applied Biosystems) using an ABI StepOnePlus Real-Time PCR System or an ABI PRISM 7700 sequence Detection System (Applied Biosystems).

2.4. Haplotype analysis

The calculation of the haplotype frequencies and the chi-square test for linkage disequilibrium among pairs of alleles were performed with use of the THESIAS software program (http://genecanvas.ecgene.net/news.php). As parameters, we used the number of alleles of haplotypes with frequencies >0.01 for each model. The haplotype analysis of *PGLYRP3* and *PGLYRP4* was jointly performed, because they are positioned in a head-to-tail orientation on Chromosome 1.

2.5. Statistical analysis

The results of the genotyping were analyzed by the chi-square test with a 2×3 contingency table for the trend or Fisher's exact test (if the cell number was less than 5). The allele frequencies were then analyzed by a chi-square analysis with a 2×2 contingency table. A *P*-value of <0.05 was considered to be significant except for multiple comparisons. In order to account for multiple comparisons, we used a strictly conservative approach by applying the Bonferroni correction to the numbers, and the corrected *P*-values (*Pc*) were calculated as follows: *Pc* = *P*-value × (all haplotype or SNP numbers at the analysis).

3. Results

3.1. Selected SNPs for PRR genes

We analyzed 46 SNPs of 19 PRRs genes, as shown in Table 2. Based on the method of SNP selection described in the Materials and methods section, 18 SNPs in six selected *NLR* genes (*NOD1*, *NOD2*, *NLRP1*, *NLRP3*, *NLRC4*, *NAIP*), nine SNPs in four selected *TLR* genes (*TLR2*, *TLR4*, *TLR5*, *TLR6*), 11 SNPs in four *PGLYRP* genes (*PGLYRP-1*, *-2*, *-3* and *-4*) and eight SNPs in five *CLR* genes (*DC-SIGN*, *DECTIN1*, *DECTIN2*, *MINCLE*, *MRC1*) were chosen.

Table 2
The information of 46 SNPs selected from 19 PRR genes.

Gene	Chromosome	SNP ID	Location in the gene	Amino acid alteration
NOD1	Chr 7	rs932272	intron 1	
		rs3823773	intron 1	
		rs2075820	exon 6	
		rs2970498	intron 9	Glu ⇒ Lys
NOD2	Chr 16	rs6500328	intron 2	
		rs2111235	intron 2	
NLRP1	Chr 17	rs3744717	intron4	
		rs11078571	intron 5	
		rs11651270	exon 13	
		rs16954813	intron 17	Met ⇒ Val
		rs8079727	intron 17	
		rs8079034	intron 17	
NLRP3	Chr 1	rs4925650	intron 2	
		rs4612666	intron 6	
		rs10754558	UTR 3	
NLRC4	Chr 2	rs385076	intron 1	
		rs212717	intron 6	
NAIP	Chr 5	rs4976210	intron 4	
TLR2	Chr 4	rs1898830	intron 1	
		rs3804099	exon 3	Asn ⇒ Asn
		rs3804100	exon 3	Ser ⇒ Ser
TLR4	Chr 9	rs1927907	intron 3	
		rs2149356	intron 3	
		rs11536889	UTR 3	
TLR5	Chr 1	rs2241096	intron 3	
		rs2241097	intron 5	
TLR6	Chr 4	rs3775073	exon 1	Lys ⇒ Lys
PGLYRP1	Chr 19	rs2041992	intron 1	
		rs2072561	intron 1	
PGLYLP2	Chr 19	rs3813135	exon 2	Thr ⇒ Ala
		rs733731	exon 2	Arg ⇒ Gln
		rs892145	exon 2	Met ⇒ Lys
		rs4264508	intron 2	
PGLYLP3	Chr 1	rs2771112	intron 2	
		rs843971	exon 3	Gly ⇒ Ser
PGLYLP4	Chr 1	rs1754134	intron 7	
		rs821434	intron 7	
		rs2570440	UTR 3	
DC-SIGN	Chr 19	rs2287886	promoter	
		rs1544767	UTR 3	
		rs8112310	UTR 3	
DECTIN 1	Chr 12	rs7959451	UTR 3	
DECTIN 2	Chr 12	rs4459385	intron1	
MINCLE	Chr 12	rs4620776	intron 1	
		rs10841845	UTR 3	
MRC1	Chr 10	rs1926736	exon 7	Ser ⇒ Gly

3.2. Analysis of the PRR genes in Group 1

As a case-control study, 129 Japanese KD patients (Group1) and 215 healthy controls were analyzed for the 46 SNPs in the PRR genes. The allele, genotype, and haplotype frequencies are listed in Tables 3 and 4. The gene views and LD plots of *NOD1* and *NLRP1* gene are shown in Fig. 1a and b, respectively. As shown in Table 4, we found that a major haplotype, G-T-C-C, in the *NOD1* gene, was associated with lower risk for KD development (KD 1st group versus controls: 23.2% versus 35.3%, $Pc = 0.0385$) among the haplotypes. In the *NLRP1* gene, the frequency of a rare haplotype, T-T-C-G-A-C, was slightly higher in KD patients than in controls (KD versus controls, 4.3% versus 1.2%), however, the significance disappeared after Bonferroni's correction. No difference was found in the haplotype distribution of the other genes.

For the single SNP analysis, the allele or genotype frequencies of 12 SNPs among the 46 were found to be different between all KD patients or between KD patients with CALs, and controls (Table 3), however, the differences in the polymorphisms did not reach statistical significance after Bonferroni correction.

3.3. Analysis of the PRR genes in Group 2

Next, we selected two genes for further study (*NOD1* and *NLRP1*), since these genes showed slight differences in the single SNP or haplotype analysis in KD Group 1. The second-round case-control study was performed in 227 Japanese KD patients (Group 2) for the 10 SNPs of the *NOD1* and *NLRP1* genes.

In the single SNP analysis, no statistically significant differences were found in the frequencies of any genotype or allele distribu-

Table 3
Results of association analysis for Japanese KD patients (Group1) and controls.

Gene	SNP ID	Genotype	Allele	Frequency			Uncorrected *P* value		Corrected *P* value	
				Control	Patients KD	Patients (CAL+)	Patients versus controls	CAL+ versus controls	Patients versus controls	CAL+ versus controls
NOD1	rs932272	AA		0.39	0.52	0.48	0.054	0.537	NS	NS
		AG		0.49	0.38	0.40				
		GG		0.13	0.10	0.12				
			A	0.63	0.71	0.68	**0.034**	0.399	NS	NS
			G	0.37	0.29	0.32				
DC-SIGN	rs1544767	AA		0.62	0.68	0.71	**0.025**	**0.021**	NS	NS
		AT		0.36	0.26	0.21				
		TT		0.01	0.05	0.07				
			A	0.80	0.81	0.82	0.764	0.721	NS	NS
			T	0.20	0.19	0.18				
DECTIN-2	rs4459385	CC		0.44	0.38	0.43	0.112	**0.033**	NS	NS
		CT		0.48	0.47	0.36				
		TT		0.08	0.16	0.21				
			C	0.68	0.61	0.61	0.086	0.216	NS	NS
			T	0.32	0.39	0.39				
TLR5	rs2241096	CC		0.59	0.67	0.76	0.205	**0.046**	NS	NS
		CT		0.36	0.27	0.18				
		TT		0.05	0.06	0.07				
			C	0.77	0.81	0.84	0.235	0.129	NS	NS
			T	0.23	0.19	0.16				
TLR6	rs3775073	AA		0.50	0.56	0.67	0.417	0.121	NS	NS
		AG		0.40	0.37	0.29				
		GG		0.10	0.07	0.04				
			A	0.70	0.75	0.81	0.210	**0.041**	NS	NS
			G	0.30	0.25	0.19				
PGLYRP1	rs2072561	TT		0.63	0.51	0.52	0.089	0.143	NS	NS
		TG		0.33	0.41	0.36				
		GG		0.05	0.08	0.12				
			T	0.79	0.72	0.70	**0.028**	0.076	NS	NS
			G	0.21	0.28	0.30				
NLRP1	rs3744717	TT		0.23	0.33	0.36	0.076	**0.043**	NS	NS
		CT		0.58	0.54	0.60				
		CC		0.19	0.12	0.05				
			T	0.52	0.61	0.65	**0.038**	**0.024**	NS	NS
			C	0.48	0.40	0.35				
	rs11078571	TT		0.33	0.26	0.19	0.216	0.078	NS	NS
		AT		0.52	0.53	0.55				
		AA		0.15	0.21	0.26				
			T	0.59	0.53	0.46	0.091	**0.029**	NS	NS
			A	0.41	0.47	0.54				
	rs11651270	TT		0.49	0.41	0.29	0.333	**0.039**	NS	NS
		CT		0.46	0.53	0.67				
		CC		0.05	0.06	0.05				
			T	0.72	0.67	0.62	0.196	0.061	NS	NS
			C	0.28	0.33	0.38				
	rs16954813	GG		0.44	0.36	0.31	0.739	**0.023**	NS	NS
		AG		0.40	0.55	0.62				
		AA		0.16	0.09	0.07				
			G	0.64	0.63	0.62	**0.014**	0.721	NS	NS
			A	0.36	0.37	0.39				
	rs8079727	AA		0.48	0.39	0.36	**0.006**	**0.041**	NS	NS
		AC		0.37	0.53	0.57				
		CC		0.15	0.08	0.07				
			A	0.67	0.66	0.64	0.739	0.663	NS	NS
			C	0.33	0.34	0.36				
	rs8079034	CC		0.60	0.51	0.50	0.057	0.134	NS	NS
		CT		0.33	0.45	0.48				
		TT		0.07	0.04	0.02				
			C	0.76	0.74	0.74	0.480	0.662	NS	NS
			T	0.24	0.26	0.26				

Among the analyzed 46 SNPs, the 12 SNPs with at least one genotype or allele frequency having uncorrected *P*-values less than 0.05 are shown in the table.
The *P* value has been subjected to the Bonferroni correction: the *P* value was multiplied by the number of comparisons made (46 SNPs) and shown as corrected *P* values ($Pc = P \times 46$). The number in bold indicated the uncorrected value <0.05. NS, not significant ($Pc > 0.05$).

tion between the KD patients and healthy controls (Supplementary Table). The results of the haplotype analysis are shown in Table 5. We again found that a rare haplotype, T-T-C-G-A-C, in the *NLRP1* gene showed an association with a higher risk of KD (4.9% versus

Table 4
Results of haplotype analysis of PRR genes (Group1).

Gene	Haplotypes	Frequency			Uncorrected *P* value		Corrected *P* value	
		Control	Patients KD	Patients (CAL+)	Patients versus controls	CAL+ versus controls	Patients versus controls	CAL+ versus controls
NOD1	A-T-C-C	0.019	0.065	0.058	**0.0020**	0.039	NS	NS
	G-T-C-C	0.353	0.232	0.240	**0.0009**	0.040	**0.0385**	NS
	A-C-T-C	0.184	0.117	0.122	0.020	0.161	NS	NS
	A-T-T-C	0.218	0.288	0.237	0.039	0.736	NS	NS
	A-T-C-T	0.175	0.171	0.186	0.905	0.828	NS	NS
	A-T-T-T	0.027	0.018	0.029	0.467	0.915	NS	NS
	G-T-T-C	0.005	0.028	0.023	0.015	0.098	NS	NS
	G-T-C-T	0.011	0.000	0.000	0.094	0.624	NS	NS
NLRP1	C-T-T-G-A-C	0.412	0.347	0.333	0.111	0.075	NS	NS
	C-T-T-A-C-T	0.008	0.015	0.000	0.396	–	NS	NS
	T-A-C-A-C-C	0.013	0.008	0.012	0.554	0.846	NS	NS
	T-A-C-A-C-T	0.021	0.021	0.041	0.944	0.317	NS	NS
	T-A-C-G-A-C	0.096	0.139	0.185	0.070	0.031	NS	NS
	T-A-T-A-C-T	0.184	0.196	0.209	0.640	0.791	NS	NS
	T-A-T-G-A-C	0.076	0.069	0.064	0.771	0.593	NS	NS
	T-T-C-A-A-C	0.033	0.034	0.024	0.915	0.612	NS	NS
	T-T-C-A-C-C	0.067	0.058	0.070	0.675	0.984	NS	NS
	T-T-C-G-A-C	0.012	0.043	0.037	**0.009**	0.121	NS	NS
NLRP3	A-C-C	0.114	0.124	0.092	0.695	0.559	NS	NS
	A-C-G	0.302	0.359	0.315	0.121	0.801	NS	NS
	A-T-C	0.039	0.031	0.042	0.578	0.902	NS	NS
	A-T-G	0.031	0.014	0.015	0.146	0.403	NS	NS
	G-C-C	0.114	0.089	0.076	0.296	0.299	NS	NS
	G-C-G	0.049	0.041	0.100	0.608	0.067	NS	NS
	G-T-C	0.285	0.295	0.302	0.808	0.769	NS	NS
	G-T-G	0.065	0.049	0.058	0.385	0.828	NS	NS
NLRC4	C-A	0.402	0.446	0.429	0.257	0.647	NS	NS
	C-G	0.117	0.085	0.048	0.191	0.059	NS	NS
	T-G	0.455	0.469	0.524	0.726	0.249	NS	NS
PGLYRP2	T-C-A-C	0.171	0.213	0.214	0.243	0.413	NS	NS
	C-T-T-C	0.331	0.353	0.333	0.753	0.893	NS	NS
	T-C-A-T	0.438	0.419	0.429	0.417	0.706	NS	NS
PGLYRP3-4	A-C-G-A-T	0.076	0.088	0.120	0.597	0.215	NS	NS
	A-C-G-C-G	0.173	0.177	0.143	0.931	0.429	NS	NS
	A-C-G-C-T	0.021	0.026	0.022	0.682	0.990	NS	NS
	T-C-G-A-T	0.092	0.126	0.094	0.162	0.985	NS	NS
	T-T-A-C-G	0.595	0.530	0.570	0.074	0.451	NS	NS
	T-T-G-A-T	0.009	0.015	0.024	0.487	0.251	NS	NS
DC-SIGN	A-A-A	0.047	0.038	0.040	0.592	0.782	NS	NS
	A-A-T	0.500	0.496	0.448	0.920	0.337	NS	NS
	A-T-A	0.158	0.168	0.179	0.747	0.671	NS	NS
	A-T-T	0.027	0.008	0.000	0.074	0.124	NS	NS
	G-A-A	0.018	0.036	0.019	0.140	0.944	NS	NS
	G-A-T	0.240	0.244	0.314	0.987	0.212	NS	NS
	G-T-A	0.010	0.010	0.000	0.932		NS	

The *P* value has been subjected to the Bonferroni correction: the *P* value was multiplied by the number of comparisons made (45 alleles) and shown as corrected *P* values ($Pc = P \times 45$). The number in bold indicated the uncorrected value <0.01. NS, not significant ($Pc > 0.05$).

1.2%, $Pc = 0.035$). We compared each haplotype frequency between all KD patients and controls, and found again that the frequency of the T-T-C-G-A-C haplotype in the *NLRP1* was different between all KD patients and controls ($Pc = 0.033$; data not shown).

4. Discussion

In the present study, we evaluated whether any of the PRR genes might be involved in the pathogenesis of KD in the Japanese population but we did not find any genes with significantly strong contribution. The haplotype analysis demonstrated that rare haplotypes in the *NOD1* and *NLRP1* genes seemed to have associations with the development of KD, even after the adjustment by the conservative Bonferroni method.

Several lines of epidemiologic evidence have indicated the importance of genetic factors in the susceptibility and outcome of KD. In the previous reports, the relationships between KD and inflammatory or acquired immunity-related genes have been primarily analyzed. Onouchi et al. demonstrated that the *ITPKC* and *CASP3* genes were implicated in the onset of KD and development of CAL by a linkage analysis [7,13]. The genes encoding inflammatory cytokines such as IL-1, IL-1b, IL-4, IL-8, IL-10, TNF alpha and TGF beta, were also evaluated in many association studies. The latest GWAS study identified *FCGR2A* and *ITPKC* as susceptibility loci for KD from 62 SNPs exceeded $P < 1.0 \times 10^{-4}$ by the first-line selection [14].

Previous studies about the clinical presentation, seasonal and geographic variations of KD suggested that infectious agents might trigger the development of KD [9], although no specific pathogen has been isolated. Innate immunity has an important role in the protection against a wide range of infectious pathogens, especially in children, whose acquired immune system is still developing. The PRRs can activate specific signaling pathways, including the NF-κB

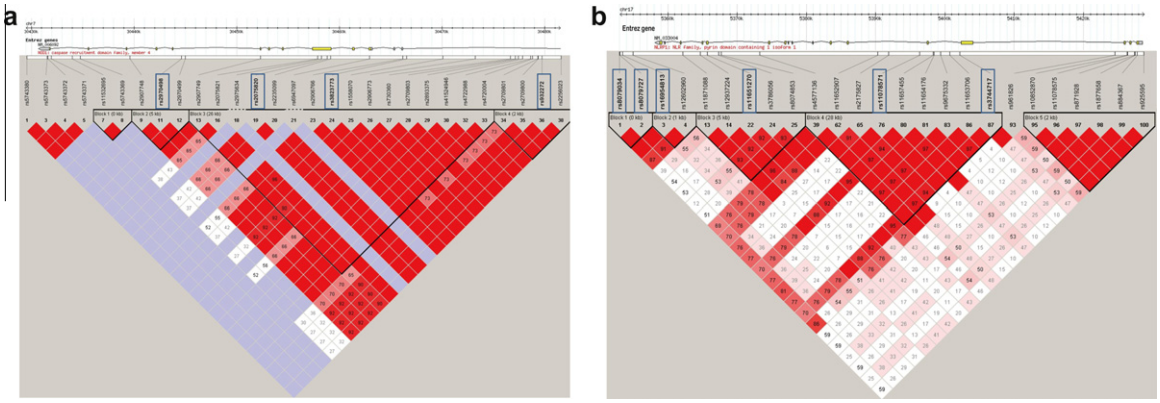

Fig. 1. The positions of genotyped SNPs and linkage disequilibrium (LD) plots in (a) *NOD1* and (b) *NLRP1* genes. The top bar in each figure shows the intron/exon structure of *NOD1* or *NLRP1* gene, with exons being represented by the boxes. The chromosomal range covered by the LD plot is shown by the horizontal bars bordering the gene. The triangle plot below the gene shows LD (red shading = high LD) and the number in each box represents the R^2 value generated by Haploview (v4.2) using data from Japanese patient cohort. The SNP numbers for the haplotype analysis are highlighted in boxes.

Table 5
Results of haplotype analysis of *NOD1* and *NLRP1* genes for KD patients Group 2 and control samples.

Gene	Haplotypes	Frequency			Uncorrected *P* value		Uncorrected *P* value	
		Control	Patients KD	Patients (CAL+)	Patients versus controls	CAL+ versus controls	Patients versus controls	CAL+ versus controls
NOD1	A-T-C-C	0.019	0.011	0.000	0.337	0.575	NS	NS
	G-T-C-C	0.353	0.320	0.375	0.240	0.887	NS	NS
	A-C-T-C	0.184	0.172	0.250	0.570	0.524	NS	NS
	A-T-T-C	0.218	0.259	0.188	0.192	0.750	NS	NS
	A-T-C-T	0.175	0.204	0.187	0.304	0.915	NS	NS
	A-T-T-T	0.027	0.014	0.000	0.176	0.503	NS	NS
	G-T-C-T	0.011	0.019	0.000	0.323	0.674	NS	NS
NLRP1	C-T-T-G-A-C	0.412	0.455	0.438	0.424	0.990	NS	NS
	C-T-T-A-C-T	0.008	0.011	0.062	0.749	0.035	NS	NS
	T-A-C-A-C-C	0.013	0.016	0.000	0.783	0.640	NS	NS
	T-A-C-A-C-T	0.021	0.035	0.000	0.242	0.550	NS	NS
	T-A-C-G-A-C	0.096	0.069	0.062	0.106	0.610	NS	NS
	T-A-T-A-C-T	0.184	0.183	0.188	0.748	0.940	NS	NS
	T-A-T-G-A-C	0.076	0.071	0.125	0.667	0.523	NS	NS
	T-T-C-A-A-C	0.033	0.035	0.000	0.933	0.451	NS	NS
	T-T-C-A-C-C	0.067	0.059	0.125	0.542	0.409	NS	NS
	T-T-C-G-A-C	0.012	0.049	0.000	**0.002**	0.650	**0.035**	NS

The *P* value has been subjected to the Bonferroni correction: the *P* value was multiplied by the number of comparisons made 17 haplotypes) and shown as corrected *P* values ($Pc = P \times 17$). The number in bold indicated the uncorrected value < 0.01. NS, not significant ($Pc > 0.05$).

pathway, in response to microbial pathogens [15]. Several families, such as the Toll-like receptors (TLRs), Nod-like receptors (NLRs), and C-type lectin receptors (CLRs), have been identified as PRRs that can detect microbial pathogens [16–20]. Peptidoglycan recognition protein (PGLYRP) is also an innate immune molecule with bactericidal function [21]. Recently, several studies have demonstrated the importance of the innate immune system in the pathophysiology of the acute phase of KD [11,12].

In this study, we evaluated the association of PRR genes and KD susceptibility, and found that the *NOD1* and *NLRP1* genes might have contribution to the development of KD. *NOD1* is one of the NLR family members located in the cytosol, and is activated by constituents of most Gram-negative and some Gram-positive bacteria [22]. The activation of *NOD1* induces NF-κB activation, leading to increased inflammatory cytokine transcription [23], and the activation of Nod1 by ligands recruits neutrophil to the administration site [24]. We have actually detected significantly high NOD1 ligand activity in specimens from a portion of KD patients (manuscript in preparation). *NLRP1* is also a member of the NLR family, and is activated by *Bacillus anthracis* lethal toxin (LT) [25]. *NLRP1* interacts with the adapter protein, ASC, as well as caspase-1 and caspase-5, to form an inflammasome, resulting in the maturation of pro-inflammatory cytokines (interleukin-18 and interleukin-1b) [26]. On the basis of these findings, we speculated that individuals with risk haplotypes of the *NOD1* and *NLRP1* genes might be susceptible to the development of KD by the activation of innate immunity by environmental factors.

Several studies have demonstrated the association of genetic variations in *NOD1* or *NLRP1* with inflammatory diseases. The *NOD1* insertion-deletion polymorphism (ND(1)+32656) was associated with the risk of asthma and inflammatory bowel disease [27,28]. A previous report demonstrated that carrying the *NOD1* *c.156C > G* and *g.27606C > T* variants showed an association with the risk of Crohn' disease. The *c.156C > G* variant was under strong LD with rs2075820 of our study. In addition, this polymorphism in the *NOD1* gene would modify the expression of the splice variant of the LRR domain in the *NOD1* gene and alter its activation by PAMPs [28,29]. It is possible that specific *NOD1* haplotypes may influence the structure and function of *NOD1*, thus leading to unusual immune responses and systematic vasculitis.

The polymorphism of interest in the *NLRP1* gene in this study was previously reported to be associated with vitiligo-associated

autoimmune disease [30], celiac disease [31] and congenital toxoplasmosis [32]. It is also possible that variants of the *NLRP1* gene may modify the function of this gene, and contribute to immune reactions. Further studies are, therefore, needed to elucidate these possible effects.

Taken together, our present data and the previous studies suggest that the individuals carrying rare haplotypes of PRRs might experience an abnormal innate immune reaction triggered by common infectious agents, resulting in the development of KD. However, there are some limitations to this study that should be kept in mind in the interpretation of the results. First, the significant difference in the rare *NOD1* haplotype found in KD Group 1 was not seen in KD Group 2. Since the clinical phenotype and severity are outbreak-dependent ([33], and our observation), a distinct etiologic agent that stimulates a different innate immune receptor might have been involved in the development of KD in each outbreak. Second, sample sizes seemed small as compared with low frequencies of a risk haplotype of the *NLRP1* gene. Further study with larger sample size would be needed to find confirmative results.

In conclusion, we herein investigated the relationship between various PRR genes and KD susceptibility. We did not found specific genes with strong contribution to the development of KD, but *NOD1* and *NLRP1* genes appeared to be partly involved in the pathogenesis of KD. Further studies in other ethnic groups of larger samples are needed to confirm the importance of these genes in the development of KD.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.humimm.2012.03.011.

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