

## ORIGINAL ARTICLE

# Association of mannose-binding lectin gene polymorphisms with Kawasaki disease in the Japanese

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### Abstract

**Objective:** Kawasaki disease (KD) is a systemic vasculitis in childhood; its etiology is unknown. The possibility that KD is an infectious disease has been discussed and investigated for decades, in light of the implication that infections are involved in the pathogenesis of KD. Young children rely on their innate immune system for protection against virus and micro-organisms. Human mannose binding lectin (MBL) is a C-type serum lectin synthesized by the liver as an acute phase protein and it plays an important role in the innate immune system. Here, we investigate the relationship between the MBL gene polymorphisms and the occurrence of KD in the Japanese population.

**Method:** The frequencies of the genotypes, defined as mutations in codons 52, 54 and 57, and the functional promoter variants of the MBL were determined in 45 patients with KD.

**Results:** The MBL codon-54 polymorphism frequency of heterozygote (GGC/GAC) and promoter variants were significantly higher in the KD group than that in the control group ( $P < 0.05$ ). Neither group showed codon 52 nor 57 polymorphisms.

**Conclusion:** It is possible that mutations of the MBL gene might be related to the trigger of the pathogenesis of Kawasaki disease.

**Key words:** innate immunity, Kawasaki disease, mannose binding lectin, MASP, mutation.

### INTRODUCTION

Kawasaki disease (KD)<sup>1</sup> is an acute systemic vasculitis of children, especially Asian children, and presently the most common acquired heart disease in children. The diagnosis of KD is based entirely on clinical features. The clinical manifestations of KD are high fever plus: (i) bilateral non-exudative conjunctivitis; (ii) polymorphous exanthem; (iii) bilateral non-suppurative cervical lymphadenopathy; (iv) mucous membrane changes; and (v) swelling or erythema of the hands or feet. For classic KD, individuals must have a fever for 5 days and either meet at least 4/5 criteria or have evidence of coronary artery

abnormality.<sup>2</sup> The etiology of KD is unknown. Laboratory findings are nonspecific, and there are no diagnostic tests for KD. Early in the course of the illness parameters of inflammation are increased: C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), white blood cell and neutrophil counts. The disease is most common in children younger than 5 years, with an annual incidence of 90 per 100 000 population in Japan<sup>3</sup> and 5–10 per 100 000 in Europe and the USA.<sup>4</sup> Eighty to ninety percent of cases occur in children younger than 5 years, with an average age of approximately 2 years. KD is relatively uncommon among children younger than 6 months,<sup>5–7</sup> and is rare in infants younger than 3 months old, which suggests the possibility that they are protected from infection by antibodies that are passively acquired from their mothers. Occurrence beyond late-aged children is rare. Widespread immunity to common infectious agents may

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explain the rarity of KD in adults. The variability in the response to infections suggests that innate immune response might be involved in an individual susceptibility to KD during the period before the production of specific antibodies. Mannose binding lectin (MBL) is an important component of host innate immunity, which has a nonspecific role in complement activation and opsonization. MBL is an acute-phase reactant of hepatic origin that can bind through multiple lectin domains, repeating mannose and *N*-acetylglucosamine sugar motifs that are characteristically displayed at high densities on bacterial and fungal cells and on viruses.<sup>8</sup> The gene for MBL is located on the long arm of chromosome 10. There are three single point mutations that have been well characterized in exon 1 of the MBL gene (*MBL2*), at codon 52 (CGT → TGT), codon 54 (GGC → GAC) and codon 57 (GGA → GAA). The normal wild type allele is designated A, whereas the variant alleles are designated as O. Any of these point mutations will lead to substantially reduced concentrations in serum of functional MBL. Polymorphisms of the promoter region of the *MBL2* gene have additional effects on serum MBL concentration. In particular is the one at position -221 (X/Y), with the Y promoter variants being responsible for high and X promoter variants for a low MBL expression activity.<sup>9,10</sup> It was reported that a G to A base change at codon 54 results in a glycine (Gly) to aspartic acid (Asp) substitution in the collagen domain, which can disrupt the interaction between MBL and MBL-associated serine protease (MASP) by causing a disruption of the MBL peptide. Its function is thought to be especially important in the acute stage of primary infections, particularly in young children in the window period when maternal antibodies fall and adaptive immunity starts to develop and become mature.<sup>11</sup>

*MBL2* gene polymorphism at codon 54 has shown to be associated with systemic lupus erythematosus,<sup>12,13</sup> rheumatic disease, and common variable immunodeficiency.<sup>14</sup> In a previous study, Biezeveld *et al.* examined the frequency of *MBL2* genotypes in 90 Dutch patients with KD. They described an increased risk of coronary artery lesions in patients younger than 1 year who expressed either low or medium levels of an *MBL2* genotype.<sup>9</sup>

The frequencies of KD and *MBL2* genotypes are different in different populations. Therefore, we think it is valuable to assess whether the *MBL2* gene polymorphisms, which cause the defect in the activation of the innate immune system, are associated with susceptibility to KD in the Japanese population.

## MATERIALS AND METHODS

### Study population

The subjects enrolled in this study included 45 Japanese patients (29 boys and 16 girls) with KD and 34 healthy controls. The diagnosis of KD was made according to the previous criteria and echocardiographic scoring guidelines for KD.<sup>15</sup> DNA samples were obtained from 45 patients aged 4–139 months (mean, 30.02 ± 26.4 months). Six children were diagnosed as KD with coronary artery lesions. All patients were hospitalized at Tokyo Medical University Hospital. Thirty-four healthy blood donors were recruited as controls. All controls were adults who had not been diagnosed as KD. The gender distributions in the group of patients with KD and the control group were not significantly different. All participants gave written informed consent. The local ethics committee of the Tokyo Medical University Hospital approved the study protocol.

### MBL assay and genotyping

Venous blood was drawn from each individual, and genomic DNA was extracted from peripheral blood mononuclear cells using a QIA amp blood kit (Qiagen, Hilden, Germany). We amplified the region of the MBL gene including codon 54 using polymerase chain reaction (PCR) with a set of specific primers: 5'-GAG-GCCAGGGATGGGTCATC-3' (sense) and 5'-CCA ACA CGT ACC TGG TTC CC-3' (antisense). To amplify the Y/X promoter fragment, the primers BaR: 5'-GAT-GAGCAGTGGGGATCCTAAGGA-3' and MBL kurz: 5'-GGCTAGGCTGCTGAGGTTTC-3' were used. A direct sequence was carried out using Taq Dye Primer Cycle Sequencing kit (Applied Biosystems, Tokyo, Japan). The nucleotide sequence was analyzed with an automated nucleotide analyzer, the 373A DNA Sequencer (Applied

**Table 1** *MBL2* exon 1 genotype frequencies in Kawasaki disease (KD) patients and controls

Genotype	KD number (%)	Controls no. (%)	<i>P</i> value	Odds ratio (95% CI)
Wild type A/A	23 (51.1)	25 (73.5)	0.036*	2.657 (1.017–6.941)
Heterozygous type A/O	20 (44.4)	8 (23.5)		
Homozygous type O/O	2 (4.4)	1 (2.9)		

\*Listed when *P* was significant.

Biosystems, Foster City, CA, USA). The combination of structural mutations and promoter variants in these gene results was well described in three expression groups (high, medium and low).

### Data analysis

Genotype frequencies were calculated by direct counting. Differences in MBL genotype frequencies among KD patients and healthy controls were determined using Fisher's exact test. The odds ratio (OR) and 95% confidence interval (CI) were also calculated. A probability value of  $< 0.05$  was considered statistically significant in all analyses.

## RESULTS

### Findings in healthy controls

The distribution of codon 54 gene polymorphisms in healthy Japanese is shown in Table 1. The heterozygous type of codon 54 was seen in 23.5%, and the homozygous type was 2.9%. Neither group showed codon 52 or 57 polymorphisms.

### Findings in patients with KD

We defined structural mutations in the *MBL2* gene (A = exon 1 wild-type; O = exon 1 mutation.) and functional promoter variants of this gene (X/Y at -221 bp [X → C and Y → G]) by DNA sequencing. Table 1 shows genotypic and allelic frequencies of codon 54 in the patients with KD. A mutation in codon 54 (genotypes A/O or O/O) was reported in 22 (48.9%) of 45 patients with KD compared with nine (26.5%) of 34 healthy Japanese controls ( $P = 0.036$ , OR 2.657, 95% CI 1.017–6.941). Table 2 shows the combination of structural mutations and promoter variants (X/Y at -221 bp) in the gene (high, medium and low). The distributions of *MBL* genotypes in patients and controls are summarized in Table 2. The *MBL* genotype distributions between KD patients and controls indicated high frequencies of XA/O and XA/XA types ( $P = 0.028$ , OR 2.743, 95% CI 1.069–7.035). Six children were diag-

nosed as having KD with coronary artery lesions. Two of six patients had *MBL2* gene mutations.

## DISCUSSION

Human mannose binding lectin is one of the most important constituents of the innate immune system. Many studies have reported a deficiency of MBL in different populations. The different distributions of genotypic and allelic frequencies in different populations might exist as a result of ethnic differences, in addition to migration.<sup>16</sup> The genotype and allele A frequencies of codon 54 did not shown significant differences compared with those of our healthy controls from other Japanese, Chinese and Europeans. In Japan, codon 52 and 57 mutations are probably absent.<sup>17</sup> MBL is a liver-derived serum protein that binds to sugars on the surface of pathogenic micro-organisms and triggers complement fixation. Low serum levels of MBL are found in association with single nucleotide polymorphisms in the promoter region and the structural gene coding region of the *MBL2* gene. Deficiency of MBL due to point mutations of the *MBL2* gene has been shown to predispose to infections in children.

However, the roles of *MBL2* genotypes in KD are still under debate. Previous studies in the Netherlands suggested a possible association between MBL dysfunctional alleles and KD. Biezeveld *et al.*, described that in 31 randomly selected patients with KD, median serum concentration of MBL in patients in the medium and low expression groups was significantly lower than in those in the high expression group.<sup>9</sup> An infectious trigger is generally believed to cause KD. Several pathogens, such as HCoV-NH, retroviruses, Epstein-Barr virus, parvovirus B19, coronavirus, and chlamydia, have been suggested by different studies as possibly important in the pathogenesis of KD.<sup>18,19</sup> We recognize that the enhanced risk of the development of infection indicates the importance of the complement system in protection against diseases. Many studies have shown that the MBL pathway is an independent pathway of a

**Table 2** *MBL2* genotype in Kawasaki disease patients and control.

Genotype	KD no. (%)		Controls no. (%)		P-value	Odds ratio (95% CI)
High YA/YA YA/XA	21 (46.7)		24 (70.6)		0.028*	2.743 (1.069–7.035)
Medium YA/O XA/XA	22 (48.9)	24 (53.3)	9 (26.5)	10 (29.4)		
Low XA/O O/O	2 (4.4)		1 (2.9)			

A  $2 \times 2$  contingency table was created to compare the high MBL expression genotypes *vs.* the combined medium and low MBL expression genotypes. The *P*-values were calculated by Fisher's exact test. A, exon 1 wild type; O, exon 1 mutation. X/Y are functional promoter polymorphism.

\*Listed when *P* was significant.

complement activation. Mutation and deficiency of the MBL2 codon could impair the ability of the pathogen or immune complex clearance, facilitating the development of autoimmunity. Therefore, we hypothesize that opsonization dysfunction of the complement system caused by MBL2 gene mutation is involved in the immune response to infection. Recently, Cheung described an association between premature atherosclerosis among KD patients and MBL.<sup>10</sup> In this study, the combination of codon-54 polymorphism and promoter variants in the MBL2 gene were significantly higher in the KD group than that in the control group ( $P < 0.05$ ). However, there was no significant difference in the allele frequency of GAC and serum MBL levels between patients with and without coronary artery lesions in KD cases. It is thought that MBL deficiency is a trigger for infection and KD. This study has shown that, in a population of individuals born in Japan, having codon 54 variants in the MBL2 gene is significantly associated with susceptibility to KD but not with coronary arterial lesions. The disease is common among children younger than 5 years, but rare in those younger than 6 months. It is suggesting that MBL plays a role in the defense against infection at a young age, when innate immunity becomes involved in the presence of waning levels of maternal antibodies. In contrast, MBL becomes less important to counter infectious triggers at an older age, when the primary host defense against invading pathogens and subsequent clearance reactions are taken up by more mature and specific arms of the immune system, such as lymphocytes and immunoglobulins. Our study suggests that MBL polymorphisms do not influence clinical manifestations of KD but influence susceptibility to KD in Japanese children. This indicates that the MBL2 gene may play a role in the pathogenesis of this disease. Because this study included a small number of subjects, further research in large cohorts of patients should be carried out to reach a stronger conclusion.

## CONFLICT OF INTEREST

None.

## REFERENCES

- 1 Kawasaki T, Kosaki F, Okawa S, *et al.* (1974) A new infantile acute febrile mucocutaneous lymph node syndrome (MLNS) prevailing in Japan. *Pediatrics* **54**, 271–6.
- 2 American Academy of Pediatrics (2000) *Red Book: Report of the Committee on Infectious Diseases*, 25th edn. American Academy of Pediatrics, Elk Grove Village, IL.
- 3 Yanagawa H, Nakamura Y, Yashiro M, *et al.* (1998) Results of the nationwide epidemiologic survey of Kawasaki disease in 1995 and 1996 in Japan. *Pediatrics* **102**, E65.
- 4 Holman RC, Curns AT, Belay ED, *et al.* (2003) Kawasaki syndrome hospitalizations in the United States, 1997 and 2000. *Pediatrics* **112**, 495.
- 5 Chang LY, Chang IS, Lu CY, *et al.* (2004) Epidemiologic features of kawasaki disease in Taiwan, 1996–2002. *Pediatrics* **114**, e678.
- 6 Yanagawa H, Yashiro M, Nakamura Y, *et al.* (1995) Epidemiologic pictures of Kawasaki disease in Japan: from the nationwide incidence survey in 1991 and 1992. *Pediatrics* **95**, 475.
- 7 Rosenfeld EA, Corydon KE, Shulman ST (1995) Kawasaki disease in infants less than one year of age. *J Pediatr* **126**, 524.
- 8 Neth O, Jack DL, Dodds AW, *et al.* (2000) Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition. *Infect Immun* **2000**, 688–93.
- 9 Biezeveld MH, Kuipers IM, Geissler J, *et al.* (2003) Association of mannose-binding lectin genotype with cardiovascular abnormalities in Kawasaki disease. *Lancet* **12**, 1268–70.
- 10 Cheung YF, Ho MH, Ip WK, Fok SF, Yung TC, Lau YL (2004) Modulating effects of mannose binding lectin genotype on arterial stiffness in children after Kawasaki disease. *Pediatr Res* **56**, 591–6.
- 11 Koch A, Melbye M, Sorensen P, *et al.* (2001) Acute respiratory tract infections and mannose-binding lectin insufficiency during early childhood. *JAMA* **14**, 1316–21.
- 12 Mok MY, Ip WK, Lau CS, Lo Y, Wong WH, Lau YL (2007) Mannose-binding lectin and susceptibility to infection in Chinese patients with systemic lupus erythematosus. *J Rheumatol* **34**, 1270–6.
- 13 Garred P, Voss A, Madsen HO, Junker P (2001) Association of mannose-binding lectin gene variation with disease severity and infections in a population-based cohort of systemic lupus erythematosus patients. *Genes Immun* **2**, 442–50.
- 14 Mullighan CG, Marshall SE, Welsh KI (2000) Mannose binding lectin polymorphisms are associated with early age of disease onset and autoimmunity in common variable immunodeficiency. *Scand J Immunol* **51**, 111–22.
- 15 Dajani AS, Taubert KA, Gerber MA, *et al.* (1993) Diagnosis and therapy of Kawasaki disease in children. *Circulation* **87**, 1776–80.
- 16 Turner MW, Dinan L, Heatley S, *et al.* (2000) Restricted polymorphism of the mannose-binding lectin gene of indigenous Australians. *Hum Mol Genet* **9**, 1481–6.
- 17 Sasaki K, Tsutsumi A, Wakamiya N, *et al.* (2000) Mannose-binding lectin polymorphisms in patients with hepatitis C virus infection. *Scand J Gastroenterol* **35**, 960–5.
- 18 Esper F, Shapiro ED, Weibel C, *et al.* (2005) Association between a novel human coronavirus and Kawasaki disease. *J Infect Dis* **15**, 499–502.
- 19 Barron KS (2002) Kawasaki disease: etiology, pathogenesis, and treatment. *Cleve Clin J Med* **69**(Suppl. 2), SII69–78.