

# Structural Polymorphisms in the Mannose-Binding Lectin Gene Are Associated with Juvenile Idiopathic Arthritis

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**ABSTRACT.** *Objective.* To investigate the possible association between polymorphisms of the mannose-binding lectin gene (*MBL2*) and susceptibility to juvenile idiopathic arthritis (JIA).

*Methods.* We performed a case-control association study including 118 Hungarian patients with JIA and 118 sex-matched healthy controls. *MBL* genotyping for the 3 mutant structural alleles at codons 54 (B), 57 (C), and 52 (D) in exon 1 and the promoter polymorphisms at position –550 (HL) and –221 (YX) were carried out by real-time PCR allelic discrimination. Serum level of MBL was determined by ELISA.

*Results.* Variant allele frequencies of both codon 52 and 57 polymorphisms in the *MBL2* gene were significantly overrepresented in JIA ( $p = 0.001$  and  $p = 0.004$ , respectively). The frequency of low *MBL* genotypes (XA/XA, YA/YO, XA/YO, and YO/YO) in JIA was higher than that in healthy controls ( $p = 0.001$ ). Serum MBL concentrations were found to be significantly lower in JIA patients versus control subjects ( $p = 0.001$ ). The 2 promoter polymorphisms and codon 54 SNP of the *MBL2* gene were not associated with JIA.

*Conclusion.* Our findings suggest that genetically determined low MBL levels may predispose children to JIA in a Hungarian population. These data warrant further research to investigate the role of the lectin-dependent complement system in the pathogenesis of JIA. (First Release March 15 2009; *J Rheumatol* 2009;36:843–7; doi:10.3899/jrheum.080681)

## Key Indexing Terms:

MANNOSE-BINDING LECTIN  
JUVENILE IDIOPATHIC ARTHRITIS

LECTIN PATHWAY

COMPLEMENT  
POLYMORPHISM

Juvenile idiopathic arthritis (JIA) belongs to a clinically heterogeneous group of arthritides of unknown cause that begin before 16 years of age. Although the immune-mediated pathogenesis is not completely understood, several studies point to a genetic component in the susceptibility for this disease, with environmental factors also contributing to the

etiology<sup>1</sup>. Recent findings confirm the association or linkage between JIA and the HLA region and provide evidence for additional loci in other non-HLA candidate genes involved in susceptibility to JIA<sup>2,3</sup>. Genetically determined deficiencies of the complement system have been demonstrated in JIA<sup>4,5</sup>; however, the relationship between the disease and the lectin-dependent pathway, a distinct complement activating mechanism through the mannose-binding lectin (MBL), is still poorly understood.

MBL, a serum protein that is structurally related to certain surface sugar residue patterns, is secreted by the liver as part of the acute-phase response of the innate immune defense. The ligands for MBL are expressed by a wide variety of microorganisms, and binding of the protein leads to opsonization as well as activation of the complement system. The serum level of MBL is influenced by the presence of 2 promoter polymorphisms and 3 mutations in the first exon of the *MBL2* gene<sup>6</sup>. Genetically determined low serum MBL levels have been shown to be associated with an increased rate of infection and with autoimmune diseases such as rheumatoid arthritis (RA)<sup>7,8</sup> or systemic lupus erythematosus (SLE)<sup>9,10</sup>. Recently, the downregulating G-221C (Y/X) promoter polymorphism was described as a risk factor for juvenile-onset SLE in a Hungarian population<sup>11</sup>.

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Supported by the Hungarian National Science Fund (OTKA) T042637 and the National Research and Development Programme GVOP-2004-3.1.1-2004-05-0183/3.0.

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Accepted for publication October 6, 2008.

Several studies have shown MBL deficiency as a potential prognostic characteristic in RA<sup>7,8</sup>. In our study, however, we examined MBL deficiency as a predisposing factor for JIA.

In order to elucidate the role of a major genetic determinant of the lectin-dependent complement-activating pathway in JIA, we investigated, in a case-control study, whether functional polymorphisms of the *MBL2* gene were associated with JIA in a Hungarian population.

### MATERIALS AND METHODS

**Patients and controls.** DNA samples were obtained from 118 patients with JIA (84 female, 34 male) aged 1–15 years (mean age 6.5 ± 4.2). All patients were hospitalized in the National Institute of Rheumatology and Physiotherapy, Budapest. In the JIA cohort, 10 patients had systemic onset disease, 48 had oligoarticular type (35 persistent, 13 extended), and 45 had polyarticular type [27 rheumatoid factor (RF)-negative and 18 RF-positive]. The diagnosis was enthesitis-related JIA in 11 cases and juvenile psoriatic arthritis in 4 cases. Subgroup definition was according to the International League of Associations for Rheumatology criteria<sup>12</sup>. One hundred eighteen healthy blood donors were recruited from the National Medical Centre, Institute of Haematology and Immunology, Budapest, to serve as controls. Seventy-eight of them were adults (49 female, 29 male) aged 19–56 years (mean age 34.9 ± 10.7) and 40 were children (17 female, 23 male) aged 1–15 years (mean age 8.5 ± 4.4). Pediatric controls were hematopoietic stem cell donors acquired from the National Medical Center, Institute of Haematology and Immunology. All individuals and parents of children participating in the study provided written informed consent in accordance with regulations of the institutional review boards.

**MBL2 genotyping.** DNA was isolated from peripheral blood samples with the Genomic DNA Purification Tray II (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions using a 6100 Nucleic Acids PreStation instrument (Applied Biosystems). DNA samples were stored at 4°C until use. MBL2 genotyping was performed with reverse transcriptase-polymerase chain reaction (RT-PCR) allelic discrimination

TaqMan assays (Applied Biosystems) using the following forward primers for the wild and variant alleles, respectively: +52 (C→T, rs5030737): VIC-CAC ACC CAT CTT TGC C, FAM-ACG CCC ATC TTT GCC; +54 (G→A, rs1800450): VIC-TCC TTG GTG TCA TCA CG, FAM-CTT GGT GCC ATC ACG; +57 (G→A, rs1800451): VIC-CAC CAA GGA AGA AAA, FAM-ACC AAG GGA GAA AA; –221 (G→C, rs7096206): VIC-CAT GCT TTC GGT GGC AG, FAM-CAT GCT TTC CGT GGC AG; 550 (G→C, rs11003125): VIC-AAG CCT GTG TAA AAC, FAM-AAG CCT GTC TAA AAC. RT-PCR analysis was carried out in a total volume of 10 µl with 10 ng of genomic DNA, 1 pmol gene-specific forward and reverse primers in 1× TaqMan 2× Universal PCR Master Mix No AmpErase UNG (Applied Biosystems). RT-PCR was performed in an ABI 7300 Real-Time PCR System (Applied Biosystems) according to the manufacturer's instructions. Designation of common *MBL2* haplotypes (HYA, LYA, LXA, HYD, LYB, LYC, and LYD) was used according to Schmiegelow, *et al*<sup>13</sup> (Table 1). *MBL2* genotypes were determined on the basis of short haplotypes known to modulate MBL levels such as YA/YA, YA/XA for normal serum MBL concentrations, YA/YO, XA/XA for intermediate serum MBL concentrations — an allele was designated O when allelic variant B (codon 54), C (codon 57), or D (codon 52) was present — and XA/YO, YO/YO for low serum MBL concentrations as described by Schmiegelow, *et al*<sup>13</sup>.

**MBL serum concentration.** Out of the original patient and control groups, 30 serum samples per group were available for serum MBL measurements. Mean age and boy/girl ratio were similar in these subgroups. *MBL2* genotype distribution of each subgroup was representative of that seen in the original patient or control group, respectively. Sera were collected and stored at –70°C. MBL was determined using the Human MBL ELISA test kit (HyCult Biotechnology, Uden, The Netherlands) according to the manufacturer's instruction. The optical density was measured using a Multiskan RC reader (Thermo Electron Corp., Waltham, MA, USA). The lower detection limit of the assay was 20 µg/l.

**Statistical analysis.** All allele and genotype frequencies were tested for the Hardy-Weinberg equilibrium in both case and control groups. Associations between the investigated polymorphisms and JIA were analyzed using the chi-square test or the Fisher test if appropriate. The magnitude of association was expressed as the odds ratio with a 95% confidence interval.

Table 1. Frequencies of 5 single-nucleotide polymorphisms in the *MBL2* gene in JIA patients and controls. All data are number (%).

	Controls, n = 118	JIA, n = 118	p; OR (95% CI)*	Oligoarticular JIA, n = 48	p; OR (95% CI)*	Polyarticular JIA, n = 45	p; OR (95% CI)*
Promoter –221 G→C (Y/X variant alleles)							
Y	180 (76.27)	189 (80.10)	NS	75 (72.0)	NS	66 (66.67)	NS
X	56 (23.73)	47 (19.90)		21 (28.0)		14 (33.33)	
Promoter –550 G→C (H/L variant alleles)							
L	157 (66.52)	169 (71.61)	NS	65 (67.71)	NS	65 (72.22)	NS
H	79 (33.48)	67 (28.39)		31 (32.29)		25 (27.78)	
Codon 52 C→T (allele D)							
C	224 (94.92)	201 (85.17)	< 0.001; 3.25	80 (83.33)	0.001; 3.73	81 (90.0)	NS
T	12 (5.09)	35 (14.83)	(1.64–6.43)	16 (16.67)	(1.72–8.11)	9 (10.0)	
Codon 54 G→A (allele B)							
G	213 (90.25)	199 (84.32)	NS	81 (84.38)	NS	74 (82.22)	NS
A	23 (9.75)	37 (15.68)		15 (15.62)		16 (17.78)	
Codon 57 G→A (allele C)							
G	229 (97.08)	214 (90.68)	0.004; 3.36	90 (93.75)	NS	78 (86.67)	0.001; 5.03
A	7 (2.97)	22 (9.32)	(1.41–8.03)	6 (6.25)		12 (13.33)	(1.97–12.85)

\* Listed when p was significant.

Correlation between genotype and serum level was estimated with Spearman rank correlation coefficient. Serum MBL levels were compared by the Mann-Whitney U test, and values were given as mean  $\pm$  standard error of the mean. A correction for multiple comparisons (Bonferroni) was introduced and the significance level in accordance with this correction was considered 0.0125. Statistical calculations were performed with the Prism Version 3.0 software for Windows (GraphPad, San Diego, CA, USA).

## RESULTS

Hardy-Weinberg equilibrium for each single-nucleotide polymorphism (SNP) studied was confirmed in all cohorts. Codon 52 and 57 polymorphisms of the *MBL2* gene were significantly associated with JIA, whereas allele frequencies of any of the promoter polymorphisms and those of codon 54 SNP were similar in the JIA and control groups. After stratification for the extent of disease in the JIA group, an association of codon 52 SNP was observed with oligoarticular disease, whereas codon 57 SNP was associated with the polyarticular type (Table 1). In comparison to the control group, distribution of *MBL2* haplotypes (HYA, LYA, LXA, LYB, LYC, HYD, LYD) was significantly different in the JIA cohort ( $p = 0.001$ ) due to the overrepresentation of the LYB ( $p = 0.039$ , OR 1.86, 95% CI 1.07–3.24), LYC ( $p = 0.004$ , OR 3.36, 95% CI 1.41–8.03), and the rare LYD ( $p = 0.001$ , OR 7.94, 95% CI 1.79–35.12) haplotypes. Genotypes that determine intermediate and low plasma concentrations (YA/YO, XA/XA and XA/YO, YO/YO, respectively) were overrepresented among JIA patients. Stratification analysis showed overrepresentation of the XA/YO and YO/YO genotypes in oligoarticular JIA and the YA/YO and XA/XA genotypes in polyarticular JIA as compared to the control group (Table 2).

Although it has been well established that serum MBL level is strongly determined genetically, we investigated whether overrepresentation of the low *MBL* genotypes would result in significantly lower serum MBL levels in our study population. As expected, serum MBL concentrations of JIA patients ( $707 \pm 429$   $\mu\text{g/l}$ ) were significantly lower than those of healthy children ( $1411 \pm 576$   $\mu\text{g/l}$ ;  $p = 0.001$ ). We observed lower serum MBL concentrations in oligoarticular JIA ( $549 \pm 298$   $\mu\text{g/l}$ ) than in the polyarticular form

( $834 \pm 520$   $\mu\text{g/l}$ ), although the difference was not significant between these 2 groups (Figure 1). MBL serum levels in both JIA patients and controls correlated with the *MBL2* genotypes ( $R = 0.89$ ,  $p = 0.001$ ), suggesting that decreased serum MBL concentrations are most likely to occur due to the higher frequency of the low *MBL* genotypes in JIA.

## DISCUSSION

The complement system is essential for the operation of the innate and adaptive immune defense, but its activation can significantly contribute to inflammation-mediated tissue damage; moreover, inherited or acquired complement deficiencies highly favor the development of autoimmunity<sup>14</sup>. Our study provides evidence that polymorphisms of the *MBL2* gene, a major determinant of the lectin-dependent complement pathway, are associated with susceptibility to JIA and result in decreased serum concentrations of MBL in a Hungarian population.

JIA is a very complex and heterogeneous disease in which laboratory and clinical characteristics in each subgroup can vary significantly. We observed an increased frequency of low *MBL* genotypes along with decreased serum MBL levels in both oligoarticular and polyarticular JIA; however, presence of rheumatoid factor, antinuclear antibody, age at onset, or sex seemed to be unrelated to MBL deficiency (data not shown). Further investigations including larger numbers of cases in each subgroup are necessary to make firm statements about the associations of different *MBL2* alleles across the different JIA subgroups.

Several studies have provided evidence of the role for MBL deficiency in immune-mediated rheumatic diseases<sup>7,8</sup>; however, few data are available suggesting a possible role for the *MBL2* gene or MBL protein in JIA. Similarly to our findings, a Finnish study reported that patients with JIA are overrepresented among children with MBL deficiency<sup>15</sup>. Recently, a Chinese investigation failed to show association between codon 54 polymorphism and JIA<sup>16</sup>. This is in accordance with our results, since we found associations only with codon 52 and 57. MBL insufficiency was shown to be associated with elevated IgM RF, increased joint ero-

Table 2. *MBL2* genotypes in JIA patients and controls. Data are number (%).

	Controls, n = 118	JIA, n = 118	p; OR (95% CI)	Oligoarticular JIA, n = 48	p; OR (95% CI)	Polyarticular JIA, n = 45	p; OR (95% CI)
Normal MBL plasma concentrations* (YA/YA, YA/XA)	74 (62.71)	38 (32.20)	< 0.001; 0.28 (0.17–0.48)	18 (37.50)	0.003; 2.80 (1.41–5.58)	12 (26.67)	< 0.001; 4.63 (2.18–9.78)
Intermediate MBL plasma concentrations* (YA/YO, XA/XA)	31 (26.27)	49 (41.53)	0.013; 0.99 (1.15–3.45)	16 (33.3)	NS	23 (51.11)	0.005; 2.93 (1.45–5.96)
Low MBL plasma concentrations* (XA/YO, YO/YO)	13 (11.02)	31 (26.27)	0.003; 2.88 (1.43–5.78)	14 (29.17)	0.004; 3.33 (1.44–7.67)	10 (22.22)	NS

\* As determined by Schmiegelow, et al<sup>13</sup>.

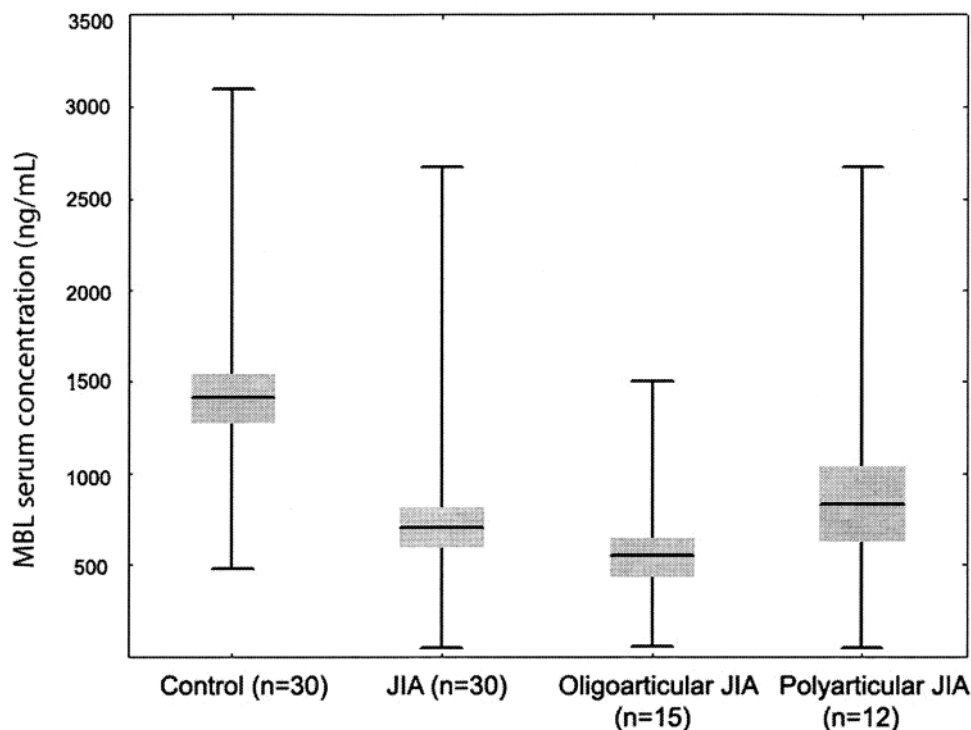


Figure 1. Mannose-binding lectin (MBL) serum concentrations in healthy controls and patients with different clinical forms of JIA. Serum levels were compared using the Mann-Whitney U test. Boxes represent mean  $\pm$  SE with a line at the mean; whiskers show the highest and lowest values. Bonferroni corrected p value of 0.0125 was considered significant. Serum MBL levels ( $707 \pm 429 \mu\text{g/l}$ ) of JIA patients ( $n = 30$ ) were significantly lower ( $1411 \pm 576 \mu\text{g/l}$ ;  $p = 0.001$ ) than those of healthy controls ( $n = 30$ ). The difference between MBL levels of patients with oligoarticular JIA ( $549 \pm 298 \mu\text{g/l}$ ) and those with the polyarticular form ( $834 \pm 520 \mu\text{g/l}$ ) did not attain statistical significance.

sion, inflammation, and early onset of RA. This may be explained in part by the ability of MBL to bind RF complexes, thus assisting RF clearance by the reticuloendothelial system<sup>7</sup>. In SLE, a reduced functional activity of the MBL pathway of complement, in relation to expression of MBL variant alleles, was shown to be associated with increased levels of autoantibodies against cardiolipin and C1q, but not against MBL<sup>17</sup>. Thus, an enhanced production of autoantibodies in RA or SLE may be related to disturbed clearance of apoptotic material due to impaired MBL function. In JIA, we could not confirm the association of MBL insufficiency with elevated autoantibody levels (data not shown), which suggests that MBL deficiency may predispose to JIA through a different mechanism. MBL also facilitates the phagocytosis of cells undergoing programmed cell death by binding carbohydrates expressed on the surface of dying cells; thus, an impaired clearance of apoptotic cells due to low serum MBL levels can lead to an aggressive immune response, which in turn generates autoimmunity<sup>18,19</sup>.

Besides predisposing to autoimmunity, low MBL serum levels have also been shown to enhance the risk of infections, which strengthens the hypothesis that infective agents might play a role in triggering JIA in children. Indeed, several studies reported that infections by various microbial

agents occur more frequently in JIA and often precede the disease onset<sup>20</sup>. As of today, however, only Aittoniemi, *et al*<sup>15</sup> have suggested that MBL insufficiency might contribute to these observations. It remains elusive whether JIA patients with MBL deficiency are indeed at an increased risk for recurrent or severe childhood infections, and how this can contribute to disease pathogenesis. Our data warrant further research to investigate the role of the lectin-dependent complement system in the pathogenesis of JIA.

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