# Increased frequency of mannose-binding lectin insufficiency among children with acute lymphoblastic leukemia

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**Epidemiological data indicate that acute lymphoblastic leukemia (ALL) could be induced by interactions between the immune system and early childhood infections. Mannose-binding lectin (MBL) plays a critical role in the immune response in early childhood before specific immune protection develops. We investigated whether there may be an association between childhood ALL and low-producing *MBL* genotypes. Serum MBL levels depend on normal *(A)* or defective (*O*) alleles, and on normal (*Y*) or reduced (*X*) promoter activities. For this study, 137 noninfants with ALL and 250 controls were classified into 3 *MBL* genotype groups according to their influence on the serum level of functional MBL: group I, *YA/YA* and *YA/XA* (higher levels); group II, *XA/XA* and *YA/O* (intermediate levels); and group III, *MBL* insufficiency with *XA/O* or *O/O* (*MBL*-deficient) genotypes. Compared with controls, cases more often had low-level genotypes (I/II/III: 63 [46%]/44 [32%]/30 [22%] vs 145 [58%]/65 [26%]/40 [16%]; *P* = .02) and MBL deficiency (8.8% vs 2.8%; *P* = .009). Thus, the ALL odds ratio for *MBL*-deficient versus non-deficient individuals was 3.3 (95% CI, 1.3-8.7), whereas the ALL odds ratio for group I versus group II/III genotypes was 0.62 (95% CI, 0.41-0.94). MBL group III patients were significantly younger at diagnosis than patients in group I/II (median, 3.9 vs 5.2 years; *P* = .04). The study shows that the presence of low-level *MBL* genotypes is associated with an increased risk of childhood ALL, particularly with early age at onset. (Blood. 2002;100:3757-3760)**

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## Introduction

Acute lymphoblastic leukemia (ALL) is a group of closely related yet cytogenetically distinct hematological malignancies. Approximately 50% of all cases of ALL have either a high-hyperdiploid genotype (chromosome modal number higher than 51) or the chromosomal translocation t(12;21)(p13;q22) (*TEL/AML1* gene fusion).[1,2] Epidemiological data have indicated that childhood ALL may occur as a consequence of an abnormal interaction between the immune system and certain unidentified infections.[3-6] Given that the characteristic incidence peak of ALL emerges by the age of 2 to 3 years, it has been proposed that this abnormal interaction should take place during early childhood when the immune system is immature.[6,7] Mannose-binding lectin (MBL) is a serum protein that plays a critical role in the innate immune response, with a particular role in the vulnerable period of infancy before adequate specific immune protection is established.[8,9] A single functional gene (*mbl2*) at chromosome 10 codes for human *MBL*.[10,11] The normal wild-type *MBL* allele is designated *A*, whereas the 3 common variant alleles that cause low serum MBL levels are designated *O*.[12] The variant alleles are relatively common in many ethnic groups including Eskimos (12%) and whites (20%).[13] Serum MBL concentrations are further dependent on polymorphisms in the promoter region of the *mbl2* gene.[14,15] In particular, a polymorphism at position −221 (*X* or *Y* genotypes) has a significant effect on the MBL serum concentration, with the *Y* promoter variant being responsible for high and the *X* variant for low *MBL*-expressing activity, respectively.[14]

Presently, knowledge is lacking with respect to those factors that influence the development of critical leukemogenic cytogenetic aberrations in lymphoblasts. Because of the importance of MBL for the infectious pattern during early childhood, we investigated whether the presence or absence of variant MBL alleles may influence the occurrence of ALL.

## Patients, materials, and methods

### Patients

The study included all patients who were (1) diagnosed with non–B ALL between January 1, 1992, and December 31, 2000; (2) 1.0 to 14.9 years of age at diagnosis; and (3) diagnosed and treated at the University Hospital, H:S Rigshospitalet, Copenhagen, Denmark, which takes care of all cases of childhood ALL from the eastern part of Denmark, Greenland, and the Faeroe Islands (approximately 2.5 million inhabitants). A total of 137 patients fulfilled these criteria. Approval for the study was obtained from the Ethical Committee of Copenhagen. Informed consent was provided according to the Declaration of Helsinki. Both the parents of 10 of the patients and the fathers of 2 additional patients were from Mediterranean countries. Furthermore, 2 patients had immigrant parents or an immigrant father from Africa. Finally, 4 patients were Eskimos, and the mother of one additional patient was an Eskimo. All other parents were white. All patients but the 4 Eskimos were born in and grew up in Denmark. The median age of the patients was 4.8 years (50% range, 2.9-8.5 years), and their median white blood cell (WBC) count at diagnosis was $13 \times 10^9$/L (50% range, $4 \times 10^9$/L-$41 \times 10^9$/L). The patients included 50 girls and 87 boys, with

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38 cases of standard-risk (SR), 57 cases of intermediate-risk (IR), and 42 cases of higher-risk (HR) ALL.[16] Risk classification was determined by age and white blood cell counts at diagnosis (SR, 2-10 years and WBC $< 10 \times 10^9$/L; IR, $< 2$ years or $\geq 10$ years and/or WBC $= 10 \times 10^9$/L-$49 \times 10^9$/L; HR, WBC $\geq 50 \times 10^9$/L), T-cell disease, a mediastinal mass, t(9;22), t(4;11), and the presence of central nervous system or testicular leukemia, lymphomatous leukemia, or an M3 bone marrow day 15 or an M2 bone marrow day 29 (all HR criteria).[16] Of the patients, 24 had T-cell leukemia. All patients were treated according to the Nordic Society of Paediatric Haematology and Oncology (NOPHO) ALL-92 protocol.[16]

### Cytogenetics

Of the patients, 31 had high-hyperdiploid ALL with modal numbers higher than 51 by G-band karyotyping and/or comparative genomic hybridization (HR-CGH), and 28 of the 137 patients were shown to have t(12;21)(p13; q22) by reverse transcriptase–polymerase chain reaction (n = 131) or fluorescence in situ hybridization (n = 6).[17]

### Treatment outcome

Using a clone-specific competitive PCR method, the minimal residual disease (MRD) was determined after 4 weeks of induction therapy in 68 patients who had clonal immune gene rearrangements and for whom DNA was available.[18] All patients were followed until December 31, 2001.

### Controls

A total of 250 healthy adult, Danish, white volunteers served as background controls (190 unselected blood donors and 60 from unselected hospital staff).[15]

### Mannose-binding lectin

The *MBL* genotypes in patients and controls were determined by PCR as previously described.[13,14] We defined 3 *MBL* genotype subgroups: group I included *YA/YA* patients (normal structural genes and promoter activities) and *YA/XA* patients (one high- and one low-expression promoter). Group II included *XA/XA* patients (2 low-expression promoters) and patients with only one normal structural allele with a high-expression promoter (*YA/O*). Group II patients will have serum MBL levels that are reduced 2- to 4-fold.[13] Group III included the patients with 2 defective alleles (*O/O* = *MBL* deficient) and the *XA/O* patients (those who had one variant gene and a normal gene with a low-expression promoter), of which both subsets have virtually undetectable MBL in the blood.[19]

### Statistics

Subgroups were compared by the Mann-Whitney *U* test, Fisher exact test, and the Chi-squared test, including analysis for trend for the distribution of the 3 MBL genotype subgroups. In the event-free survival (EFS) analyses, we included as events death during induction or death in remission, relapse, or the diagnosis of a second malignant neoplasm, whichever occurred first. The Kaplan-Meier method was applied for estimation of probability of EFS. Subgroups were compared with the log-rank test. In all analyses, 2-sided *P* values less than .05 were regarded as being significant. All statistical analyses were done with SPSS 11.0 software (SPSS, Chicago, IL).

## Results

The ALL cases and controls differed in their *MBL* genotypes, with a significantly higher frequency of *MBL* low-level genotypes among ALL cases (*MBL* group I/II/III: 63 (46%)/44 (32%)/30 (22%) vs 145 (58%)/65 (26%)/40 (16%); $P = .02$, analysis for trend; Table 1). Of the 30 ALL patients (22%) in *MBL* group III, 12 (9%) were *MBL* deficient (*O/O*), a significantly higher frequency than that among controls ($P = .009$). The odds ratio for ALL for *MBL*-deficient versus nondeficient individuals was 3.3 (95% confidence interval, 1.3-8.7). In contrast, the odds ratio for ALL for individuals with high-level *MBL* genotypes compared with the intermediate or low-level individuals was 0.62 (95% confidence interval, 0.41-0.94).

Nonwhite and white patients did not differ in their *MBL* group I-III distribution (*MBL* group I/II/III: 6/7/6 vs 57/37/24; $P = .35$).

The increased frequency of low-level genotypes was found in both pre–B-lineage and T-lineage ALL and seemed to be most pronounced in the 56 patients for whom successful cytogenetic analyses revealed neither t(12;21) positivity nor high-hyperdiploidy (Table 1). The 30 patients classified as *MBL* group III were diagnosed with ALL at a significantly earlier age than the remaining 107 *MBL* group I/II patients (median, 3.9 vs 5.2 years; $P = .04$). The lower age at diagnosis was found in both pre–B-lineage and T-lineage ALL (median age, pre–B-lineage [n = 113]: 3.4 vs 4.6 years; $P = .02$; T-lineage [n = 24]: 6.5 vs 9.1 years; $P = .26$).

The day-29 MRD levels did not differ between the 3 MBL groups (median MRD, group I: 0.02%, group II: 0.01%, group III: 0.2%; $P = .6$). After a median follow-up of 5.5 years for patients who stayed in remission, 5 patients died during induction therapy, 18 patients developed a relapse 0.2 to 6.7 years from diagnosis (median, 1.9 years), 2 patients developed a second myeloid malignancy, and one patient died in remission. At 7 years the pEFS is 0.76, which is identical to the overall outcome of the NOPHO ALL-92 protocol in the 5 Nordic countries.[16] The 7-year pEFS did not differ significantly between the 3 *MBL* genotype subgroups

**Table 1. Mannose-binding lectin genotypes in leukemia patients and controls**

| Mannose-binding lectin genotypes | *YA/YA* and *YA/XA* | *YA/O* and *XA/XA* | *XA/O* and *O/O* | *O/O* |
|---|---|---|---|---|
| Controls | 145 (58) | 65 (26) | 40 (16) | 7 (2.8) |
| Leukemias* | 63 (46)† | 44 (32) | 30 (22) | 12 (8.8)† |
| Pre-B | 53 (47)† | 38 (34) | 22 (20) | 9 (8.0)† |
| T | 10 (42) | 6 (25) | 8 (33)† | 3 (12.5)† |
| t(12;21) positive | 14 (50) | 11 (39) | 3 (11) | 0 |
| t(12;21) negative* | 49 (45) | 33 (30) | 27 (25) | 12 (11)† |
| High hyperdiploid | 17 (55) | 9 (29) | 5 (16) | 2 (6.5) |
| High hyperdiploid or t(12;21) positive | 31 (53) | 20 (34) | 8 (14) | 2 (3.4) |
| Non-high hyperdiploid, t(12;21) negative* | 23 (41) | 18 (32) | 15 (27) | 5 (8.9)† |
| Standard or intermediate risk leukemia | 46 (48) | 30 (32) | 19 (20) | 9 (9.5)† |
| Higher risk leukemia* | 17 (41) | 14 (33) | 11 (26) | 3 (7.1) |

Frequencies in percentage are given in parentheses. Pre-B/T = B-precursor or T-lineage acute lymphoblastic leukemia; t(12;21) = translocation t(12;21)(p13;q22).

*$P < .05$ for the distribution of the mannose-binding genotype subgroups in cases and controls (trend analysis).

†$P < .05$ for the frequency of the specific mannose-binding genotype subgroup in cases versus controls.

(group I: 0.73 ± 0.07, group II: 0.79 ± 0.07, group III: 0.84 ± 0.08; $P = .54$). Similarly, the 3 MBL groups did not differ significantly in their 7-year risk of relapse (group I: 0.21 ± 0.06, group II: 0.16 ± 0.06, group III: 0.10 ± 0.07; $P = .43$).

## Discussion

Circumstantial evidence has been provided for a relationship between childhood ALL in general and the pattern of infections during early childhood, but the mechanisms by which these infections may influence the natural history of ALL have been unclear.[3-6,20,21] More specifically, the hypotheses put forward suggest that childhood ALL may be caused by a combination of a lack of common infections in infancy (hygiene hypothesis), leading to a failure of normal immune system modulation that could leave the individual susceptible to leukemogenic bacterial or viral infections later in early childhood (delayed infection hypothesis).[6] Even if these patterns of childhood infections could influence the risk of leukemia, the biologic mechanisms involved in these 2 settings could be completely unrelated. In addition, each of them may influence only certain subsets of childhood ALL.

Although there has been some evidence that HLA class II alleles could influence the risk of childhood ALL,[22] there has so far been little to support a link between common polymorphisms in the immune system and the risk of childhood ALL. The present study suggests an *MBL* gene dose-dependent association to the risk of ALL with a significantly increased leukemia odds ratio for individuals with low-level *MBL* genotypes. This finding lends support to the proposed theory of an increased risk of ALL subsequent to an abnormal response to infections during early childhood (delayed infection hypothesis).[1] Due to more frequent and/or more severe infections, the proliferative stress on the developing immune system, possibly leading to critical leukemogenic DNA damages, would be more pronounced in children with low-level compared with those with high-level *MBL* genotypes. The mechanisms by which the low-level *MBL* genotypes lead to an increased risk of deleterious cytogenetic aberrations is unclear and could simply involve random events second to an increased turnover of B- and T-lineage lymphoblasts. It remains to be explored whether the association is specific for *MBL* or whether other polymorphisms in the immune system may have a similar impact on the risk of childhood ALL.

The study indicates that the *MBL* genotype distribution could be normal, specifically among the ALL subsets that are especially frequent in the high-incidence 2- to 7-years age group (ie, the translocation t(12;21)-positive and high-hyperdiploid leukemias). This is especially intriguing, because t(12;21)-positive and high-hyperdiploid ALL so far are the only noninfant groups of childhood ALL for which a high prevalence of cells with leukemia-associated genetic markers have been demonstrated at birth.[23-27] However, future studies are needed to confirm this finding and to explore whether other pathogenic mechanisms are involved in these patient subsets.

The differences between cases and controls in *MBL* genotype distributions found in the present study are unlikely to be due to ascertainment bias of our adult control population. Thus, a previous study of young Danish white cystic fibrosis patients showed the same *MBL* genotype frequencies as our controls.[19] In addition, a study on cord blood from healthy English children was in accordance with the genotype distribution both in healthy adult British controls and in the present control population.[28,29] Finally, the younger age at diagnosis for the *MBL* group III compared with group I/II individuals also supports the theory that the distribution of *MBL* genotypes in children with ALL is truly skewed compared with the background population.

This is the first study that indicates a critical interaction between the developing immune system and the risk of childhood ALL. Hence, further case-control studies are needed both to confirm this finding and to explore in detail whether more frequent and/or severe infections in early childhood may lead to ALL. Such epidemiological studies should be stratified by the *MBL* genotypes of the individuals as well as by the cytogenetically defined ALL subtypes.

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