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Prognostic significance of the Musashi-2 (*MSI2*) gene in childhood acute lymphoblastic leukemia

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The prognostic value of the Musashi-2 (*MSI2*) gene has not yet been studied in childhood acute lymphoblastic leukemia (ALL). In our study, *MSI2* mRNA levels of 119 childhood patients with newly-diagnosed ALL were examined and analyzed with regard to clinical characteristics and outcomes. ALL patients demonstrated significantly higher *MSI2* mRNA levels than healthy controls. In addition, *MSI2* mRNA levels were correlated with the disease status and *IK6* mutation status. Survival analyses showed that higher *MSI2* mRNA levels predicted worse outcomes in patients with childhood ALL. Moreover, in multivariate analyses, *MSI2* mRNA overexpression retained its value as an independent risk factor for overall survival (OS), but not for event free survival (EFS). We conclude that high *MSI2* mRNA level predicts adverse prognosis and seems to be useful as a novel prognostic factor for patients with childhood ALL.

Key words: acute lymphoblastic leukemia, childhood, musashi-2, overall survival, prognosis

As the most common malignancy in pediatric population, acute lymphoblastic leukemia (ALL) is a malignant clonal proliferation disease of lymphoid progenitor cells [1]. One important feature of the disease is the acquired chromosomal and genetic abnormalities in the leukemic cells [2]. Together with other disease features such as age, white blood cell (WBC) counts and immunophenotype, some aberrations, like *BCR/ABL1* and *MLL* translocations, have been used as risk factors which can affect patients' treatment protocols and outcomes [3]. With the advances in the frontline protocols design, survival rates for childhood ALL have been improved over the past four decades [4]. However, 6-10% of children with ALL have disease relapse. Thus, understanding the underlying novel genetic aberrations for refractory ALL is essential to discriminate patients with worse outcome and ultimately individualize treatment.

One of the major steps in leukemogenesis is the deregulation of the hematopoietic stem cells (HSCs) [5]. Being a member of an evolutionarily conserved family of RNA binding proteins, Musashi-2 (*MSI2*) serves as a regulator of HSCs and is primarily expressed in HSCs [6,7]. The results of mouse models of chronic myeloid leukemia (CML) show that through Musashi–Numb pathway, high levels of *MSI2* play an important role in CML disease progression [7]. Just as *MSI1* is a risk factor in gallbladder adenocarcinoma, astrocytic, oligodendroglial brain tumors, and breast cancer [8,9], several studies have explored the role of *MSI2* in hematological malignancies. *MSI2* overexpression has been found to be an independent prognostic factor for both acute myeloid leukemia (AML) [5,10] and adult *BCR/ABL*-negative B-ALL [11,12] and is associated with poor outcome. In addition, increased *MSI2* expression is also found in advance disease (AD) CML patients [13].

However, the role of *MSI2* in childhood ALL has not been evaluated. In this study, we analyzed the *MSI2* mRNA levels and its relationship to survival and prognosis in a group of childhood ALL patients, aiming to evaluate its prognostic potential as a risk factor in pediatric ALL patients.

Patients and methods

Patients. We analyzed bone marrow (BM) samples from 119 patients with newly diagnosed ALL and 36 healthy donors treated in the Division of Hematology-oncology, the Children's

Hospital of Zhejiang University School of Medicine from 2007 to 2010. The diagnosis of ALL was established based on morphology (M), immunology (I), cytogenetics (C), according to Recommendations for diagnosis and treatment of acute lymphoblastic leukemia in childhood (3rd revised version) [14]. *BCR/ABL1* fusion transcripts were analyzed at first diagnosis. Additionally, seventeen paired samples at diagnosis and at complete remission (CR) were also collected. Informed consents for laboratory analysis and cryopreservation of BM samples were obtained from parents or guardians. This study abode by the tenets of Declaration of Helsinki and was approved by the Institutional Local Review Board of Ethics.

According to the modified National Cancer Institute (NCI) criteria, low-risk (LR), intermediate risk (IR) and high-risk (HR) groups were defined according to the clinical biologic characteristics, and early response to prednisone [15]. All of these patients were treated by the NPCAC97 protocol. Briefly, the treatment regimen included 7 days of pre-phase treatment with prednisone and 4 weeks of induction therapy consisting of four drugs (vincristine, daunorubicin, L-asparaginase and dexamethasone) which were described elsewhere [16,17].

RNA extraction and cDNA synthesis. BM samples were collected in heparinized glass tubes, and mononuclear cells were isolated by Ficoll gradient centrifugation. Using High Pure RNA Isolation Kit (Roche, Mannheim, Germany), total RNA was extracted from cryopreserved cells stored in liquid nitrogen. Samples were chosen based on the quality and quantity of RNA. Reverse transcription was performed using the ReverTra Ace qPCR RT Kit (Toyobo, Osaka, Japan). Briefly, 1 μg of total RNA was added to a 10-μL volume containing reverse transcriptase and the Primer Mix. The mixture was incubated at 37°C for 15 min, followed by 5 min at 98°C. Then the reacted solution was stored at -20°C until ready to use.

Quantification of *MSI2* mRNA levels and detection of the Ikaros 6 (*IK6*) variant. *MSI2* mRNA levels were quantified using StepOnePlus™ Real-time PCR system (Applied Biosystems) according to the manufacturer's instructions, with co-amplification of the housekeeping gene *GAPDH*. The comparative cycle threshold (CT) method, described in the previous studies [18,19], was used to determine the relative expression levels of *MSI2* to *GAPDH*, using the mean of ΔCT from three replicates and expressed as $2^{\mu(\Delta CT)}$ (ΔCT= *GAPDH* – *MSI2*). The reaction mixture was performed in a final volume of 25μL containing 2μL cDNA, 12.5 μL SYBR Green PCR Master Mix, 1μL of *MSI2* primers (5 nmol/mL) or *GAPDH* primers. PCR conditions used an initial denaturation at 95°C for 1 min followed by 40 cycles of 15 seconds at 95°C, 15 seconds at 55°C and 30 seconds at 72°C. *MSI2* and *GAPDH* primers had been described in the previous study [12].

Moreover, the *IK6* variant of *IKZF1* gene was also examined by nested PCR with the use of primers previously described [20].

The sequences of primers for *MSI2*, *GAPDH* and *IK6* were as follows:

MSI2-1: ACGACTCCCAGCACGACC; *MSI2*-2: GCCAGCTCAGTCCACCGATA

GAPDH-1: ATGGGGAAGGTGAAGGTCG; *GAPDH*-2: GGGTCATTGATGGCAACAATATC

IK6-E1: CTCTTCGCCCCCGAGGATCAGTCTT; *IK6*-E2: GAAGGCGGCAGTCCTTGTGCTTTTC

IK6-I1: CGAGGATCAGTCTTGGCCCCA; *IK6*-I2: GCAGCTGGTACCATCGGGCTGAT

Statistical analysis. To delineate distinct patient subgroups on the basis of *MSI2* mRNA levels, patients with expression values greater than 75th of all measurements were defined as the high *MSI2* mRNA group. To compare the pretreatment clinical features between high *MSI2* mRNA group and low *MSI2* mRNA group, χ^2 test and the nonparametric Mann-Whitney U test were chosen for categorical and continuous variables, respectively. Student t-test for independent samples or paired samples Student t-test was chosen to perform mean comparisons between distinct groups.

Overall survival (OS) was measured from the date of diagnosis to death with any cause or last contact, and event free survival (EFS) was defined as the time from diagnosis to treatment failure, relapse, death, or last follow-up. The last follow-up time was September 30, 2014. Survival analysis was performed for only 108 childhood ALL patients (9 patients who received hematopoietic stem cell transplantation were excluded.). Survival curves were estimated by the Kaplan-Meier method, and *P* values were calculated by the log-rank test. Prognostic factor analysis was performed by univariate analysis and variables with a *P*-value <0.1 in univariate analysis were entered into the Multivariable Proportional Hazards Model. The following covariates were included in the full model: *MSI2* mRNA levels (high *v* low), Age (≤1 year, ≥10 years *v* 1-10 years), WBC counts ($\geq 50\times10^9$/L *v* $<50\times10^9$/L), BM blast (≥median *v* <median), Risk (high + intermediate *v* low), peripheral blood (PB) blast (≥median *v* <median), Day 22 minimal residual disease (MRD) (≥0.01% *v* <0.01%), *BCR/ABL1* (presence *v* absence), and *IK6* (mutated *v* unmutated).

All analyses were performed using SPSS software package, version 17 (SPSS Inc, Chicago, IL), and a *P* value < 0.05 was considered to be statistically significant.

Results

Analysis of *MSI2* mRNA levels in pediatric ALL patients and healthy donors. The characteristics of controls and patients were summarized in supplemental Table I. The median age of healthy controls in our study was 6 years (range, 1 to 14 years), and there was no difference in ages between the healthy controls and ALL patients ($P = 0.501$). We analyzed the *MSI2* mRNA levels in 119 pre-treatment BM specimens with newly-diagnosed ALL and 36 healthy volunteers. Overall, normal controls showed lower *MSI2* mRNA levels compared to ALL patients ($P = 0.030$, Figure 1a).

In order to demonstrate whether the *MSI2* mRNA levels were influenced by disease status, 17 paired BM samples

were also collected and studied at diagnosis and at CR. Our results showed that there was significant reduction in the *MSI2* mRNA levels at CR than those at initial diagnosis ($P < 0.001$, Figure 1b).

Association between *MSI2* mRNA levels and clinical characteristics of childhood ALL patients. Baseline clinical and molecular characteristics of the 119 patients enrolled in this study were summarized in Table I. When we compared the clinical and biological characteristics of the two subgroups classified on the basis of *MSI2* mRNA levels, no significant difference was found for age, gender, WBC count, platelet (PLT) count, BM blasts, PB blasts, extramedullary involvement, immunophenotype, risk, *IK6*, and *BCR/ABL1*.

We then compared the *MSI2* mRNA levels according to patients' molecular features, with the use of *MSI2* mRNA expression values as a consecutive variable. Patients with *IK6* mutation showed higher *MSI2* mRNA levels compared to *IK6* negative group ($P = 0.0037$, Figure 2a). However, no such relationship was found between *BCR/ABL1* presence and absence groups ($P = 0.095$, Figure 2b).

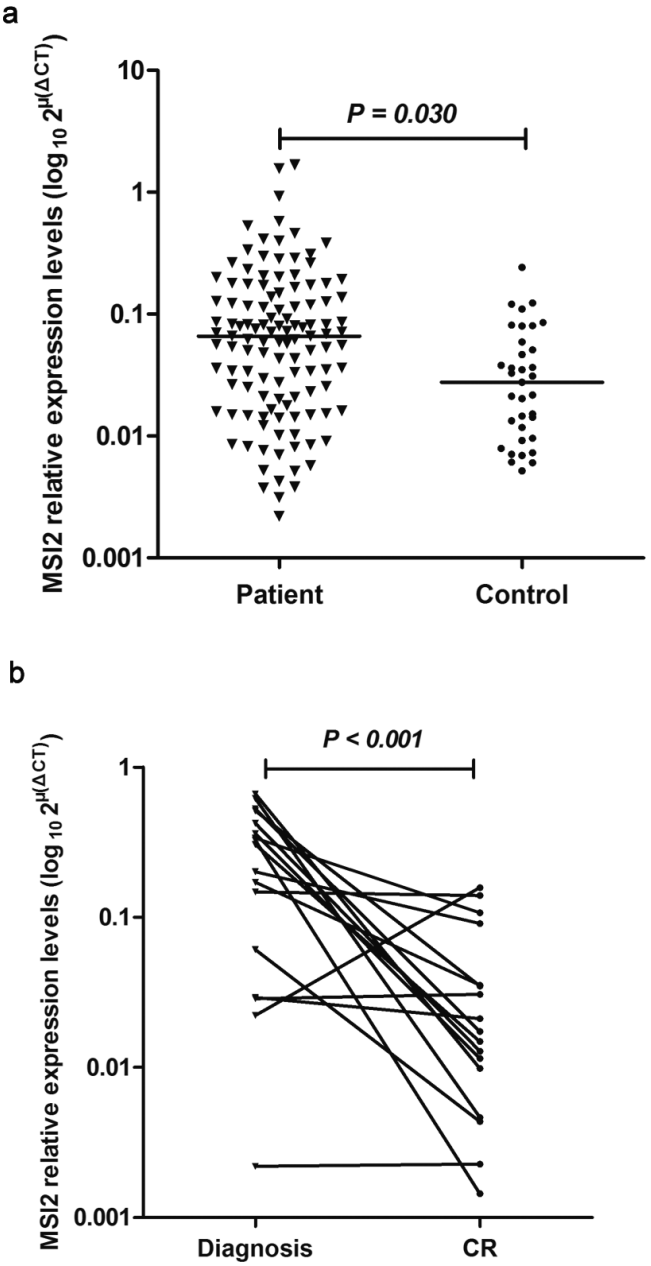

Figure 1. *MSI2* mRNA levels in (a) pediatric ALL patients and healthy controls, and (b) 17 paired samples. (a) Independent-Samples T test and (b) Paired-Samples T test were used for the comparisons of *MSI2* mRNA levels between two groups, using the $2^{\mu(\Delta CT)}$ values as *MSI2* quantitative expression levels. For (a), the line showed the median value. The significant differences were observed in both (a) $P = 0.030$ and (b) $P < 0.001$.

Table I. Clinical characteristics of patients with high and low *MSI2* mRNA levels

Characteristics	Patients (n=119)	Low *MSI2* (n=89)	High *MSI2* (n=30)	*P*
Age, years				.956
Median	6	6	7.5	
Range	1-14	1-14	1-14	
Sex, male, no. (%)	76 (64)	57 (64)	19 (63)	.994
WBC count, x10^9/L				.921
Median	20.3	23.6	22	
Range	0.8-760	0.8-760	2.4-552.9	
PLT count, x10^9/L				.743
Median	55.5	54	58.5	
Range	4-359	4-359	13-339	
Percentage of BM blasts				.099
Median	90%	90%	91%	
Range	61%-98%	61%-98%	70%-98%	
Percentage of PB blasts				.857
Median	25%	25%	26%	
Range	0-85%	0-80%	0-85%	
Extramedullary involvement, no. (%)				.992
Yes	4 (3)	3 (3)	1 (3)	
No	115 (97)	86 (97)	29 (97)	
Immunophenotype, no. (%)				.306
B-ALL	91 (76)	66 (74)	25 (83)	
T-ALL	28 (24)	23 (26)	5 (17)	
Risk, no. (%)				.368
Low	35 (29)	27 (30)	8 (27)	
Intermediate	28 (24)	22 (25)	6 (20)	
High	56 (47)	40 (45)	16 (53)	
Ik6, no. (%)				.133
Mutated	13 (11)	7 (8)	6 (20)	
Unmutated	106 (89)	82 (92)	24 (80)	
BCR/ABL1, no. (%)				.371
Presence	11 (9)	7 (8)	4 (13)	
Absence	108 (91)	82 (92)	26 (87)	

Abbreviations: *MSI2*, Musashi-2; WBC, white blood cell; B-ALL, B-cell acute lymphoblastic leukemia; BM, bone marrow; *Ik6*, Ikaros isoform 6; PB, peripheral blood; PLT, platelet; T-ALL, T-cell acute lymphoblastic leukemia.

Validation of *MSI2* as a risk factor of outcome in pediatric ALL. In the entire cohort of patients, the low *MSI2* mRNA group had significant higher CR rates compared with the high *MSI2* mRNA group (97% *v* 83%, $P = 0.016$, Table II). However, no significant difference was found in the response to prednisone between the low *MSI2* mRNA group and the high *MSI2* mRNA group (88% *v* 79%, $P = 0.325$, Table II). Moreover, regarding to Day 22 MRD, no such association was found between the two groups defined based on *MSI2* mRNA levels ($P = 0.246$, Table II).

With a median follow-up time of 67.5 months (1 – 93 months), there were 108 ALL patients for OS analysis and EFS analysis. When we compared the OS of patients between the low *MSI2* mRNA group and the high *MSI2* mRNA group, the latter group of patients had inferior OS: high *MSI2* mRNA expressers had lower estimated 5-year OS rate (60% *v* 81%; median, 59 *v* 71 months; $P = 0.014$, Table II, Figure 3a). However, no statistical significance ($P = 0.400$, Figure 3b) was found between the estimated 5-year EFS rates for patients in the low *MSI*2 mRNA group and the high *MSI2* mRNA group (67% *v* 60%, respectively, Table II).

Validation of IK6 as a risk factor of outcome in pediatric ALL. We then investigated the association between *IK6* mutation status and the outcomes in childhood ALL patients. The results from both EFS and OS showed that IK6 positive status was a poor prognostic factor in childhood ALL patients: 5-year EFS rate: 68% *v* 40%, $P = 0.010$; 5-year OS rate: 80% *v* 42%, $P = 0.002$ (supplemental Figure 1).

Survival analysis based on *IK6* mutation status and *MSI2* mRNA levels in pediatric ALL patients. Based on the results described above, survival analyses were conducted among the four groups: *MSI2*hi*IK6*+, *MSI2*hi*IK6*-, *MSI2*lo*IK6*+ and *MSI2*lo*IK6*-. The 5-year OS rates were different among the four groups, which were 20%, 68%, 57%, and 83% for *MSI2*hi*IK6*+, *MSI2*hi*IK6*-, *MSI2*lo*IK6*+ and *MSI2*lo*IK6*-, respectively ($P < 0.001$, supplemental Figure 2a). Similar results were also observed for EFS analysis among the four groups ($P = 0.005$, supplemental Figure 2b)

Multivariate analysis. *MSI2* mRNA levels (high *v* low) was still an independent prognostic factor of OS in multivariate

Table II. Clinical outcomes according to *MSI2* mRNA levels

Outcome	Patients	Low *MSI2*	High *MSI2*	*P*
Day22 MRD				0.246
≥0.01% (%)	65 (62%)	53 (65%)	12 (52%)	
Response to prednisone				0.325
Sensitive (%)	98 (86%)	76 (88%)	22 (79%)	
CR				0.016
Yes, no, (%)	110 (94%)	85 (97%)	25 (83%)	
Overall survival				0.014
Median, months	68.5	71	59	
Alive at 5-year, %	76	81	60	
95% CI	0.67-0.84	0.72-0.90	0.41-0.79	
Event-Free survival				0.400
Median, months	61.5	66	56	
Relapse-free at 5-year, %	65	67	60	
95% CI	0.56-0.74	0.57-0.77	0.42-0.78	

Abbreviations: CI, confidence interval; CR, complete remission; MRD: minimal residual disease.

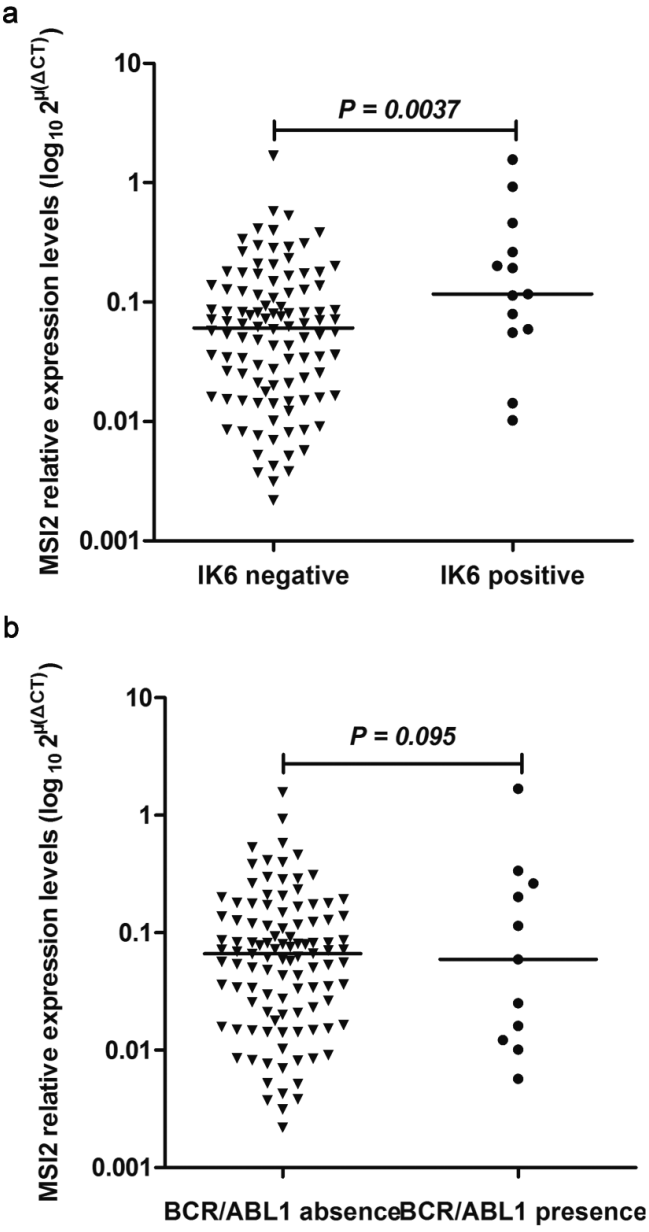

Figure 2. Differences in *MSI2* mRNA levels according to genetic abnormalities status were studied. Independent-Samples T test was chosen to test the differences between groups. *MSI2* mRNA levels were estimated with the $2^{\mu(\Delta CT)}$ method, using *GAPDH* as the control gene. For (a) and (b), the line showed the median value. (a) A significant difference was observed between the *IK6* mutation positive group and the *IK6* mutation negative group ($P = 0.0037$). (b) No statistical difference in *MSI2* mRNA levels was found between the *BCR/ABL1* absence group and the *BCR/ABL1* presence group ($P = 0.095$).

analysis for ALL, when considering age, WBC counts, BM blast, risk, PB blast, Day22 MRD, *BCR/ABL1* and *IK6*, (HR, 2.19; 95 % CI, 1.03-4.67; $P = 0.042$, Table III). Furthermore, BM blast (≥median *v* <median) and *IK6* (mutated *v* unmutated) were also prognostic predictors for OS (Table III). The independent prognostic factors for EFS were WBC counts (≥50×10^9/L *v* <50×10^9/L) and *BCR/ABL1* (presence *v* absence) (Table III).

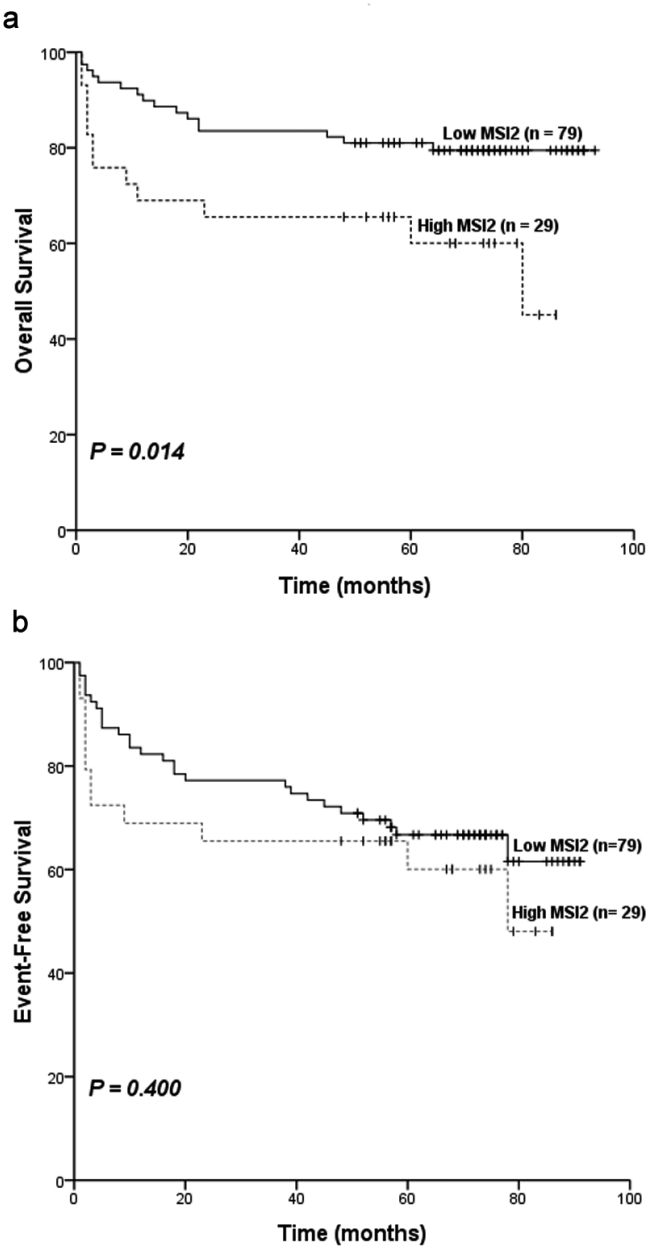

Figure 3. Influence of *MSI2* mRNA levels on outcomes in childhood ALL patients. Patients with high *MSI2* mRNA levels had inferior overall survival (OS) (a) ($P = 0.014$). Kaplan–Meier analysis demonstrated that estimated 5-year OS rate for high *MSI2* mRNA expressers was 60%, whereas the estimated 5-year OS rate for low *MSI2* mRNA expressers was 81%. However, no difference in EFS was found between the high *MSI2* mRNA group and low *MSI2* mRNA group. (b) ($P = 0.400$).

Discussion

The overall long time survival rates of childhood ALL are ranging from 70%-90% [21-23]. In spite of this, the fact that a small number of patients die or relapse during the treatment of diseases cannot be ignored. On the account of the important role which molecular signature plays in ALL risk-stratification refinement and therapy development, we sought to evaluate the relationship between the *MSI2* mRNA levels and ALL in the pediatric population. To our knowledge, this is the first study focusing on *MSI2* mRNA levels in pediatric ALL patients.

Results from Ito T et al [6] show that *MSI2* is important for the establishment and continued propagation of blast crisis for CML in animal models. Similarly, results from 151 CML patients show that *MSI2* levels increase during the advanced phases and decrease in chronic phases (CP) [13]. In this study, we investigated *MSI2* mRNA levels in BM samples from newly-diagnosed ALL patients and compared those to the levels obtained from healthy donors. According to our results, *MSI2* mRNA overexpression was detected in pediatric ALL patients. The result is consistent with the previous studies showing that the expression of *MSI2* in AML patients and adults B-ALL is higher than in healthy volunteers [12,24]. However, the differences in *MSI2* mRNA levels between healthy controls and ALL patients were not as large as expected. One reason may be the relatively small sample size. Further large-scale studies are in preparation and investigation. We then compared the *MSI2* mRNA levels of paired samples collected at diagnosis and at CR, and the patients at CR showed significant lower *MSI2* mRNA levels than at initial diagnosis. As compared to ALL patients at CR, patients at initial diagnosis had higher leukemia burden. Therefore, we can speculate that *MSI2* mRNA overexpression may have an effect on outcomes of ALL patients in childhood population.

Ikaros is a central regulator of hematopoiesis, encoded by the *IKZF1* gene. Due to alternative splicing or genomic deletions, the *IKZF1* gene is transcribed as a number of isoforms [25]. One of the short isoforms is *IK6*, with deletions in exon 4-7 of the *IKZF1* gene [25]. Recent studies have demonstrated that deletions in *IKZF1* are associated with unfavorable outcomes in pediatric B-ALL and adult B-ALL [26,27]. In our group, we have also verified that IK6 positivity was associated with poor outcomes in childhood ALL patients. Consequently, we aimed to evaluate the relevance between *MSI2* mRNA levels and *IK6* mutation status. As demonstrated in Figure 2a, patients with *IK6* mutation showed higher *MSI2* mRNA levels, as compared to *IK6* negative group, suggesting that *MSI2* mRNA overexpression may imply worse clinical features of childhood ALL. Similar to *IK6*, *BCR/ABL1* is also an unfavorable prognostic factor for ALL, and is associated with older age, higher leukocyte count, and more frequent CNS involvement at time

Table III. Multivariate analyses for OS and EFS in pediatric ALL patients

	OS			EFS		
Factor	HR	95%CI	*P*	HR	95%CI	*P*
Age,≤1 year, ≥10 years *v* 1–10 years	1.38	0.65-2.93	0.396	1.44	0.76-2.72	0.259
WBC, ≥50×10^9/L *v* <50×10^9/L	1.50	0.53-4.22	0.094	2.58	1.34-4.96	0.005
Risk, high + intermediate *v* low	1.23	0.4-3.81	0.221	1.56	0.57-4.28	0.385
MSI2 mRNA levels, high *v* low	2.19	1.03-4.67	0.042	1.38	0.70-2.72	0.358
BM blast %, ≥median *v* <median	2.51	1.05-5.97	0.038	1.69	0.84-3.40	0.130
PB blast %, ≥median *v* <median	1.36	0.48-3.86	0.159	1.27	0.49-3.27	0.393
Day 22 MRD, ≥0.01% *v* <0.01%	1.53	0.58-4.03	0.389	1.43	0.67-3.06	0.353
IK6, mutated *v* unmutated	4.60	1.9-11.4	0.001	2.01	0.83-4.87	0.086
BCR/ABL, presence *v* absence	1.89	0.65-5.51	0.191	4.13	1.76-9.66	0.001

Abbreviations: ALL, acute lymphoblastic leukemia; CI, confidence interval; EFS, event-free survival; *Ik6*, Ikaros isoform 6; MRD, minimal residual disease; *MSI2*, Musashi-2; PB, peripheral blood; OS, overall survival; WBC, white blood cell.

of diagnosis [28]. We then compared the *MSI2* mRNA levels between patients with *BCR/ABL1* and those without this fusion gene. However, no such difference was found. Our result is inconsistent with the previous conclusion about the existing correlation between *MSI2* mRNA expression and *BCR/ABL1* [13]. These controversial results about *BCR/ABL1* and *MSI2* mRNA levels may be caused by different sample compositions: Jaspal Kaeda and his colleagues used hematopoietic cell lines to perform their study, whereas we focused on BM samples from pediatric ALL patients. Additionally, as there were only 11 patients with *BCR/ABL1* (9%) in our study, the relationship between *MSI2* mRNA levels and *BCR/ABL1* may be concealed more or less as a result of the small sample size.

Considering the above results, we were interested in demonstrating whether *MSI2* mRNA levels had a prognostic significance for childhood ALL. In our group, patients with *MSI2* mRNA overexpression had poorer overall survival rates than those in the low *MSI2* mRNA group. Compared with the results for OS, no significant difference in EFS was found between the high *MSI2* mRNA group and the low *MSI2* mRNA group, respectively. Drug resistance, lower remission re-induction rates and the persistence of MRD are the features of refractory or recurrent leukemia [29-31]. As expected, patients in the high *MSI2* mRNA group had lower CR rates. Besides, though not reached a significant difference, the rate of sensitive prednisone response in the high *MSI2* mRNA group was lower than in the low *MSI2* mRNA group. The MRD levels at the mid-course of induction (Day22 MRD), which have been proved to be a risk factor for ALL relapse in the previous study [15], were also collected and compared. However, no difference was found in Day22 MRD levels between the two groups classified according to *MSI2* mRNA levels.

We then analyzed the outcomes in patients with childhood ALL according to the *IK6* mutation status and *MSI2* mRNA levels. The results suggested that ALL patients in *MSI2*hi*IK6*+ group had inferior outcomes, either for OS, or for EFS compared to the patients of other three groups. Our finding implies that the combination of *IK6* and *MSI2* mRNA levels could be used to identify a group of patients with poor prognosis in childhood ALL.

In our multivariate analysis, after including most of the known clinical prognostic factors, *MSI2* mRNA overexpression was still an independent predictor for inferior OS, but not for EFS in this cohort. Our finding is in accordance with previous studies conducted in AML patients and adults ALL patients suggesting the negative prognostic influence of high *MSI2* based on mRNA and protein levels [5,10-12]. In addition, the other two genetic abnormalities, i.e. *IK6* and *BCR/ABL1*, had also independently prognostic effects on OS and EFS, respectively, for the whole cohort of childhood ALL patients.

In conclusion, we have shown that *MSI2* mRNA overexpression was signifiantly correlated with poor outcomes in pediatric ALL patients. In addition, we also confirmed that *MSI2* mRNA levels were correlated with *IK6* mutation status. However, there exist several limitations in the present study. Firstly, research design does not allow us to detect whether *MSI2* act through other mechanisms except the NUMB pathway in leukemic cells, which have been implied in the previous studies [5,7]. Thereby, we cannot explain clearly how the high *MSI2* mRNA levels affect outcomes in pediatric ALL patients. Furthermore, our study was conducted in the population with a relatively small sample size of Asian population. As the data on the prognostic impact of the *MSI2* mRNA overexpression in childhood ALL patients are limited, further studies with large-scale and different ethnic backgrounds are needed to confirm the prognostic significance of *MSI2* in children with ALL.

Supplementary information is available in the online version of the paper.

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Supplementary Information

Prognostic significance of the Musashi-2 (*MSI2*) gene in childhood acute lymphoblastic leukemia

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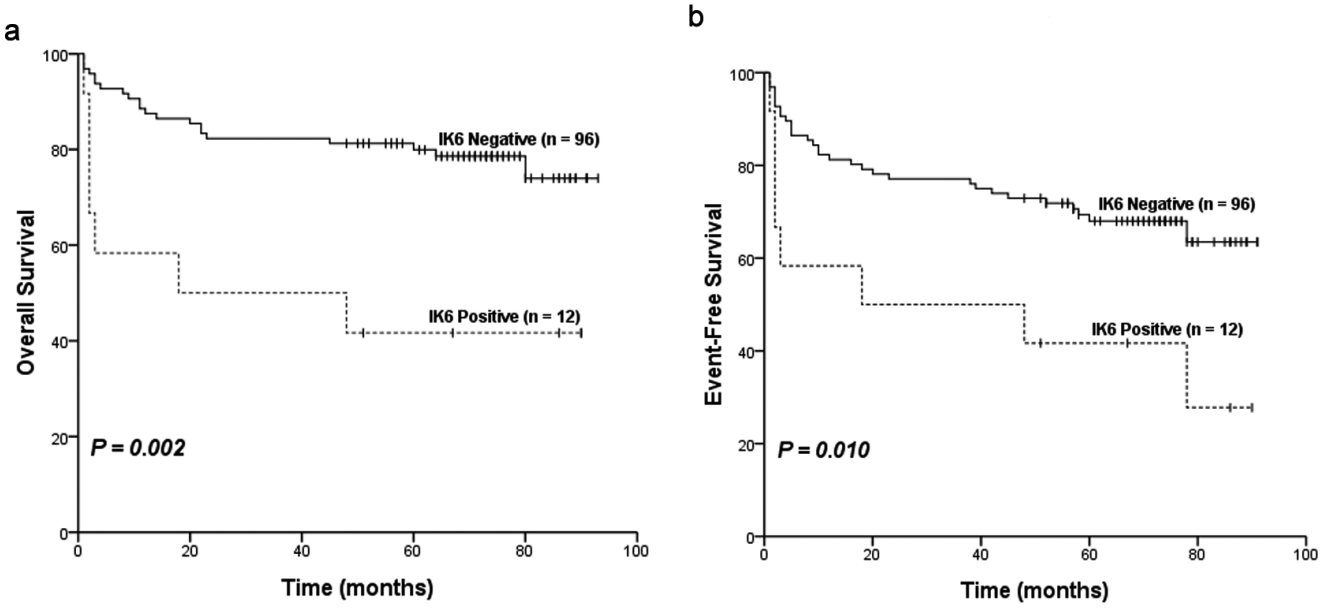
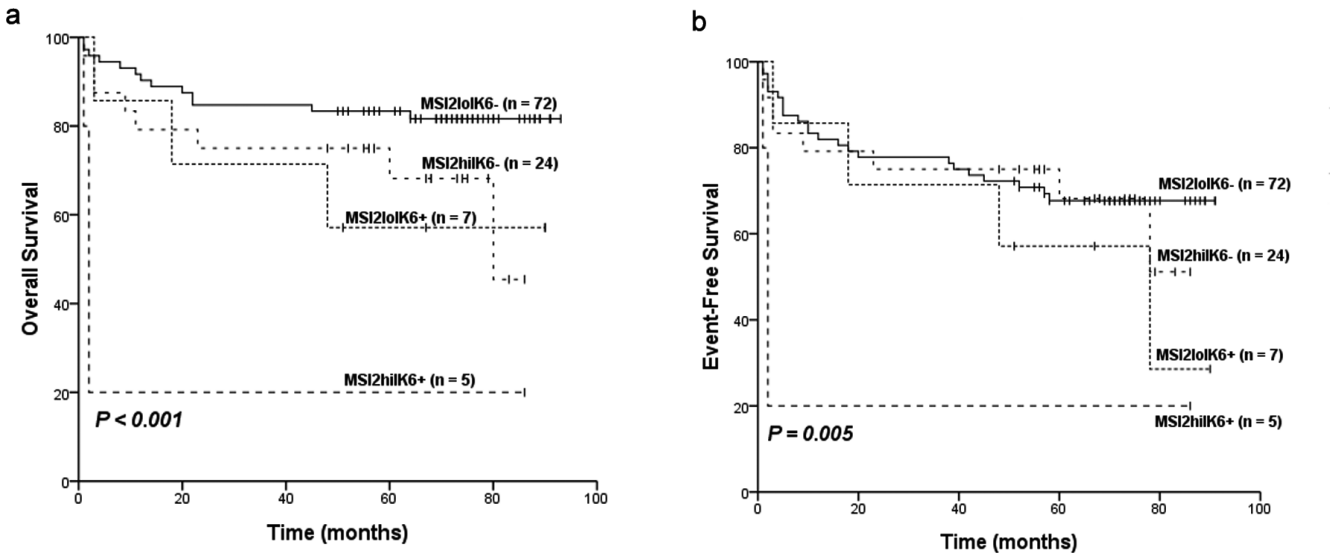


Supplemental Figure 1. Influence of *IK6* on outcomes in childhood ALL patients. Patients with *IK6* mutation had inferior OS (a) (*P* = 0.002) and EFS (b) (*P* = 0.010). Kaplan–Meier analysis demonstrated that estimated 5-year OS rate for patients with *IK6* mutation was 42%, whereas the estimated 5-year OS rate in *IK6* negative group was 80% (a). Similar results were also found in EFS between *IK6* negative group and *IK6* positive group (b) (*P* = 0.010).



Supplemental Figure 2. Survival analysis according to the *IK6* mutation status and *MSI2* mRNA levels in childhood ALL patients. OS (a) ($P < 0.001$) and EFS (b) ($P = 0.005$) were different among patients in the four groups of *MSI2*hi*IK6*+, *MSI2*hi*IK6*-, *MSI2*lo*IK6*+ and *MSI2*lo*IK6*-.

Supplemental Table I. Characteristics of controls and patients

Controls	
Gender, Male/Female	23/13
Age (years), median (range)	6 (1-14)
WBC count (x10^9/L), median (range)	8.43 (4.23-11.7)
Patients	
Gender, Male/Female	76/43
Age (years), median (range)	6 (1-14)
WBC count (x10^9/L), median (range)	20.3 (0.8-760)

Abbreviations: WBC, white blood cell