

## 소아 급성백혈병에서 *MLL* 유전자의 부분일렬반복의 유병률과 임상적 의의

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### Prevalence and Clinical Implication of Partial Tandem Duplication of the Mixed Lineage Leukemia Gene in Pediatric Acute Leukemia

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**Background:** The mixed lineage leukemia (*MLL*) gene may induce hematopoiesis and leukemia. Partial tandem duplication of *MLL* (*MLL*-PTD) is associated with poor prognosis in acute myeloid leukemia (AML); however, the significance of *MLL*-PTD in acute lymphoblastic leukemia (ALL) has not been thoroughly studied. We evaluated the incidence, relationship with other cytogenetic abnormalities, and the prognostic role of *MLL*-PTD in ALL.

**Methods:** We reviewed medical records from pediatric patients diagnosed with ALL in Severance Hospital, Yonsei University Health System, South Korea from 2002 to 2008. *MLL*-PTD was detected by nested reverse transcriptase polymerase chain reaction.

**Results:** In ALL patients, 50.0% (42/84) were positive for *MLL*-PTD. There was no significant difference in the 10-year overall survival (10Y OS) and event-free survival (EFS) between *MLL*-PTD-positive (+) and *MLL*-PTD-negative (-) groups (69.4% vs. 76.2%,  $P=0.609$ , and 62.6% vs. 66.7%,  $P=0.818$ , respectively). The combination of high level of lactate dehydrogenase ( $>1,100$  IU/L) and *MLL*-PTD(+) [*MLL*-PTD(+)/High LDH] was a statistically significant negative prognostic factor for 10Y OS and EFS ( $P=0.0226$  and  $P=0.0230$ , respectively). In multivariate analysis, National Cancer Institute risk stratification and very high risk features were independent significant prognostic factors but *MLL*-PTD (+)/High LDH was not.

**Conclusion:** *MLL*-PTD was observed frequently in pediatric ALL patients. *MLL*-PTD was not an independent prognostic factor. *MLL*-PTD (+)/High LDH should be evaluated further for its prognostic potential in ALL.

**Key Words:** Acute leukemia, Cytogenetics, Partial tandem duplication, *MLL*, Prognostic factors, Pediatric leukemia

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## Introduction

Chromosomal rearrangements in the hematopoietic system are regarded as key events in development and leukemogenesis [1,2]. The mixed lineage leukemia (*MLL*) gene is located on chromosome 11q23 and has been extensively investigated. *MLL* is a human homolog of *Drosophila melanogaster* trithorax. In *Drosophila*, the trithorax group and the polycomb group appear to play opposing roles in the regulation of gene expression [1]. The 90-kb *MLL* gene encodes a 430-kDa protein comprising 3969 amino acid residues [2] that has methylase and acetylase activities. The *MLL* gene forms various rearrangements with over 50 partner genes, and the most common *MLL* fusion partners are *AF4*, *AF9*, *ENL*, *AF6*, *ELL*, and *AF10* [3]. *MLL* is associated with leukemia transformation, and the *MLL* gene rearrangement is associated with poor prognosis of both acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML).

*MLL* partial tandem duplication (*MLL*-PTD) is an *MLL* gene rearrangement that is observed in 5-10% of adults with AML [3]; however, the incidence is quite varied depending on karyotype. Although the incidence of *MLL*-PTD is about 11% in unselected AML, it is 5.7-21% for karyotypically normal AML and even higher, 13.6-54%, in AML with trisomy 11 [3]. Most of these studies were conducted in the adult population, and it is possible that the incidence of *MLL*-PTD is higher in pediatric AML. For example, Ross et al. [4] reported that 27.7% of karyotypically normal pediatric AML subjects had *MLL*-PTD.

There are fewer studies on the incidence of *MLL*-PTD in ALL patients. *MLL* is an essential factor in the development of hematopoietic cells [5]. Since *MLL* translocations are thought to be involved in leukemogenesis, certain translocations might result in ALL. In one study, Pallisgaard et al. found *MLL*-PTD in 12.9% (8/62) of ALL patients [6], but more data on *MLL*-PTD in ALL are needed.

In this study, we investigated the frequency, relationship with other cytogenetic abnormalities, and prognostic implications of *MLL*-PTD in ALL. We also studied *MLL*-PTD in the pediatric population, the field where there is the least

amount of data. This study was conducted in an Asian population and could be used to elucidate ethnic differences in the incidence of *MLL*-PTD.

## Methods

### 1) Patients

Between March 2002 and May 2008, 149 pediatric patients were diagnosed with ALL at Severance Hospital in Yonsei University Health System. During this period, 106 patients with ALL were treated in the institution, but 22 patients with ALL were excluded from the study because they were treated for relapse during the study period, and their *MLL*-PTD status, including their initial diagnosis and treatment before March 2002, was unclear. As a result, 84 patients diagnosed with ALL were used in this study. All cases were morphologically diagnosed according to French-American-British (FAB) classification. Bone marrow samples from patients were drawn and analyzed in the laboratory department of the institution. The samples were subject to immunophenotyping and molecular subtyping. The *MLL*-PTD test (described below) was performed at the time of diagnosis for patients diagnosed with ALL from 2002 to 2008. The positive *MLL*-PTD status was verified by correlating with patient medical records.

### 2) Treatment Strategy

ALL patients were classified as standard risk (ALL-SR) or high risk (ALL-HR) according to age and white blood cell (WBC) count using the National Cancer Institute (NCI) risk stratification. Patients who were initially assigned to the ALL-SR group and were later found to have adverse translocations, including t(9;22), various 11q23 translocations, and *MLL*-PTD, were treated with the ALL-HR protocol. ALL-SR and -HR protocols were based on the Children's Cancer Group (CCG) 1991 and CCG 1961 protocols, respectively. Hematopoietic stem cell transplant was considered for patients with the following characteristics: WBC count >100,000/ $\mu$ L, presence of t(9;22) or t(4;11) translocation, infantile leukemia (age younger than 1 year at diagnosis), induction failure, or patients in second or more remission after relapse or with secondary leukemia.

### 3) Detection of *MLL*-PTD using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Total cellular RNA was isolated from bone marrow samples at diagnosis using Tri Reagent (BD, Molecular Research Center, Inc., 5645 Montgomery Road, Cincinnati, OH, USA). RNA was reverse transcribed to cDNA using a cDNA Synthesis Kit (Roche, USA). Nested RT-PCR was performed using Taq polymerase (Poschem, Korea) for 30 cycles with the following primers: 3.1c (5'-AGGAGAGAGTTTACCTGCTC-3') from exon 3, 5.3 (5'-GGAAGTCAAGCAAGCAGGTC-3') from exon 5, 6.1 (5'-GTCCAGAGCAGAGCAAACAG-3') from exon 6, and 3.2c (5'-ACACAGATG-GATCTGAGAGG-3') from exon 3 based on the literature [7]. PCR products were analyzed by electrophoresis on 1.5% agarose gels. We included a sample known to contain *MLL*-PTD as a positive control and distilled water as a negative control.

### 4) Comparison of prognosis

In ALL, patients were grouped into ALL-SR and ALL-HR groups according to NCI risk stratification. Parameters known to affect prognosis for ALL were recorded and analyzed. Early death was defined as death within 6 months from diagnosis. *MLL*-PTD (+) and *MLL*-PTD (-) groups were defined according to the status of *MLL*-PTD detection. The patient population was divided into two groups based on lactate dehydrogenase (LDH) level. The high-LDH group was defined by an LDH level higher than 1,100 IU/dL. The low-LDH group was defined by an LDH level of 1,100 IU/dL or lower. The cut-off point of LDH classification was determined using receiver operating characteristic analysis for death event. We combined *MLL*-PTD status and LDH level in the survival analysis as follows: *MLL*-PTD (+)/High LDH compared to all others [*MLL*-PTD (+)/Low LDH, *MLL*-PTD (-)/High LDH, and *MLL*-PTD (-)/Low LDH]. Very high risk factor (VHR factor), which was considered as acceptable transplant indication at first complete remission, was defined as the presence of t(9;22) or t(4;11) translocation, infantile leukemia (age younger than 1 year at diagnosis), and induction failure.

### 5) Statistical Analysis

The chi-square test was used to evaluate the difference between categorical variables in patient characteristics. For survival analysis, the start point was diagnosis date and the end point was the occurrence of an adverse event (relapse or death). Event-free survival (EFS) was defined as time from diagnosis to the occurrence of the first adverse event, including relapse, progression, or death. Overall survival (OS) was defined as time from diagnosis to the death incurred by any cause. The Kaplan-Meier method was used for survival analysis, and the difference in OS between groups was evaluated by the log-rank test. Multivariate analysis was performed using a Cox proportional hazards regression model. Statistical significance was considered when the *P*-value was less than 0.05.

## Results

### 1) *MLL*-PTD in ALL

*MLL*-PTD was detected in 50.0% (42/84) of ALL patients (Table 1). The mean age at diagnosis of the *MLL*-PTD (+) group was  $5.63 \pm 3.79$  years, and was not statistically different from that of the *MLL*-PTD (-) group ( $6.26 \pm 4.28$  years,  $P=0.480$ ). There was no difference in initial WBC count between the *MLL*-PTD (+) group and the *MLL*-PTD (-) group ( $56,100 \pm 20,200$  vs.  $47,500 \pm 12,500$ , respectively,  $P=0.717$ ). There was no statistical difference between the two groups in the proportion of high-risk patients, hemoglobin levels, platelet counts, or FAB subtypes (Table 1).

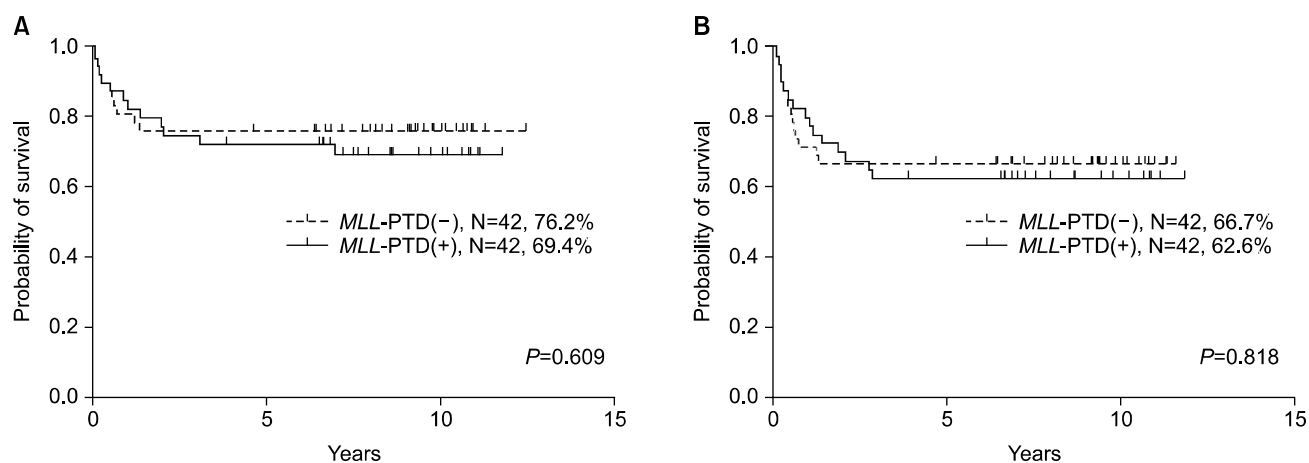
The LDH level was significantly higher in the *MLL*-PTD (-) group than in the *MLL*-PTD (+) group ( $3,380 \pm 650$  vs.  $1,230 \pm 210$ ,  $P=0.003$ ). *MLL*-PTD (+) was significantly correlated with low LDH defined by an LDH level less than 1,100 IU/dL ( $P=0.011$ ).

There was no difference between the two groups in the frequency of minor *BCR-ABL* and *TEL-AML1* rearrangements, whereas the frequency of *E2A-PBX* rearrangement was greater in the *MLL*-PTD (-) group than in the *MLL*-PTD (+) group ( $P=0.054$ ). In the *MLL*-PTD (+) group, 31/40 (77.5%) patients were cytogenetically normal. In cytogenetically normal patients, 31/62 (50%) had *MLL*-PTD.

**Table 1.** Clinical characteristics of acute lymphoblastic leukemia patients

	<i>MLL</i> -PTD(+)	<i>MLL</i> -PTD(-)	<i>P</i> -value
No. of cases	42	42	
Age at diagnosis (mean, years)	5.63±3.79	6.26±4.28	0.480
Sex (male : female)	22:20	20:22	0.663
WBC (mean, 10 <sup>9</sup> /L)	56,100±20,200	47,500±12,500	0.717
Hemoglobin (mean, g/dL)	7.42±2.71	8.05±2.93	0.319
Platelets (mean, 10 <sup>9</sup> /L)	100,800±16,200	104,600±18,400	0.879
LDH (mean, IU/L)	1230±210	3,380±650	0.003
NCI risk group			
Standard	27	27	1.000
High	15	15	
FAB subtype			
L1	33	36	0.365
L2	8	7	
L3	0	2	
Gene rearrangement			
<i>BCR-ABL</i> (n=78)	1	1	1.000
<i>TEL-AML1</i> (n=80)	10	8	0.591
<i>E2A-PBX</i> (n=71)	0	4	0.054
<i>MLL-AF4</i> (n=40)	0	0	
CALLA-positive	31	30	0.949
CR rate	2 (95.3%)	2 (95.3%)	1.000

*MLL*, mixed lineage leukemia gene; CALLA, common ALL antigen; CI, confidence interval; CR, complete remission; FAB, French-American-British; LDH, lactate dehydrogenase; NCI, National Cancer Institute; WBC, white blood cell.

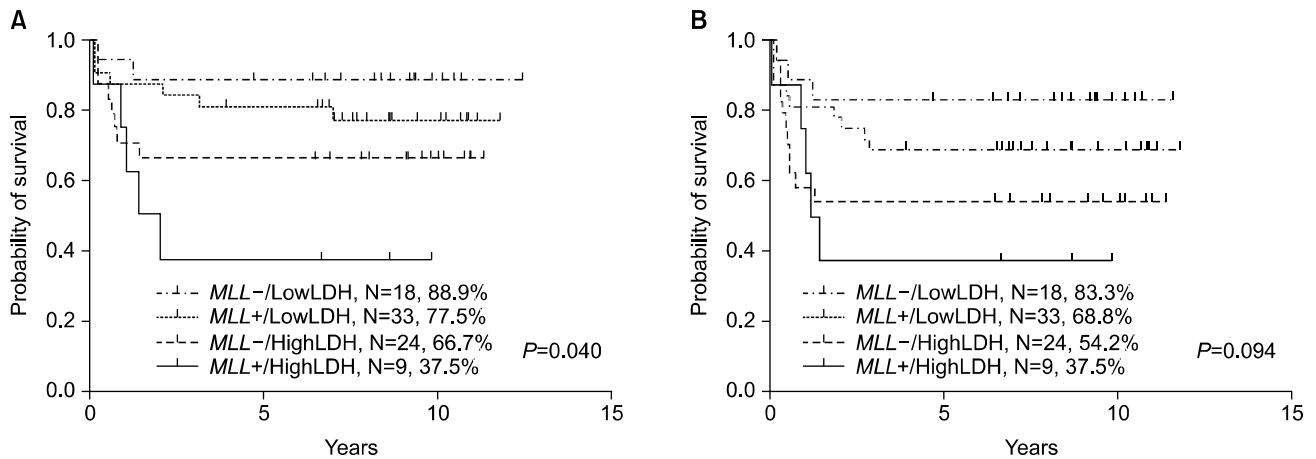


**Fig. 1.** Probability of Survival in Acute Lymphoblastic Leukemia (ALL) Patients according to the Mixed Lineage Leukemia Gene - Partial Tandem Duplication (*MLL*-PTD) status. (A) Overall Survival. (B) Event-Free Survival.

In this study population, there were 4 infantile leukemia patients, and 3/4 (75%) of them had *MLL*-PTD. However, the *MLL-AF4* translocation was not observed in these patients. The complete remission rate was not significantly different between the groups ( $P=0.345$ ).

## 2) Outcome in ALL

The median follow-up duration for leukemia patients who survived was 8.8 years (range, 0.9-12.4 years). The 10-year overall survival (10Y OS) rate was  $72.4\pm 4.9\%$ . The 10-year event-free survival (10Y EFS) rate was  $64.7\pm 5.3\%$ . The 10Y OS of the ALL-SR group was significantly higher



**Fig. 2.** Probability of Survival in Acute Lymphoblastic Leukemia (ALL) Patients according to Mixed Lineage Leukemia Gene - Partial Tandem Duplication (*MLL*-PTD) and Lactate Dehydrogenase (LDH) Status. (A) Overall Survival. (B) Event-Free Survival.

than that of the ALL-HR group (84.4% vs. 51.9%  $P < 0.0001$ ). The 10Y EFS of the ALL-SR group was significantly higher than that of the ALL-HR group (79.6% vs. 38.0%  $P < 0.0001$ ).

### 3) OS and *MLL*-PTD (Fig. 1A)

The 10Y OS rate was not significantly different between the *MLL*-PTD (+) and (-) groups (69.4% vs. 76.2% respectively,  $P=0.609$ ). The 10Y EFS rate was  $64.7\% \pm 5.3\%$ . In both the ALL-HR and ALL-SR groups, the *MLL*-PTD (+) group had a similar but slightly lower survival rate than the *MLL*-PTD (-) group (ALL-SR,  $79.3 \pm 8.4\%$  vs.  $88.9 \pm 6.0\%$ ,  $P=0.441$ ; ALL-HR,  $50.3 \pm 13.4\%$  vs.  $53.3 \pm 12.9\%$ ,  $P=0.904$ ).

### 4) EFS and *MLL*-PTD (Fig. 1B)

The 10Y EFS rate was not significantly different between the *MLL*-PTD (+) and (-) groups (62.6% vs. 66.7% respectively,  $P=0.818$ ).

In both the ALL-HR and ALL-SR groups, the *MLL*-PTD (+) group had a similar but slightly lower survival rate than the *MLL*-PTD (-) group (ALL-SR,  $76.9 \pm 8.3\%$  vs.  $81.5 \pm 7.5\%$ ,  $P=0.763$ ; ALL-HR,  $35.9 \pm 12.8\%$  vs.  $40.0 \pm 12.6\%$ ,  $P=0.900$ ) (Fig. 1D, 1E).

### 5) Treatment Strategy in the *MLL*-PTD (+) Group

Most of the patients (39/42, 92.9%) in the *MLL*-PTD (+) group were treated with the HR risk protocol regardless of

NCI risk stratification; the other 3 patients in the *MLL*-PTD (+) group were treated with the SR risk protocol. There was no survival difference between the 3 patients treated with the SR protocol and those treated with the HR protocol (66.7% vs. 71.8%,  $P=0.972$ ). In the *MLL*-PTD (+) group, hematopoietic stem cell transplantation was performed in 11 patients: 3 of them received the transplantation because of relapse, 3 for an initial WBC count  $>100,000/\mu\text{L}$ , 2 for infantile leukemia, 1 for uncontrolled initial central nervous leukemia, and 2 for induction failure. There was no difference in survival among initial SR protocol approach vs. HR protocol approach vs. transplantation approach ( $P=0.445$ ).

### 6) Outcome Analysis According to LDH and *MLL*-PTD Status

The patient population was divided into two groups based on LDH level. The 10Y OS and 10Y EFS of the high-LDH group (LDH level  $>1,100$  IU/L) was significantly lower than that of the low-LDH group (59.4% vs. 81.6%,  $P=0.0226$ ; 50.0% vs. 74.1%,  $P=0.0230$ , respectively) (Fig. 2A). In the ALL-SR group, the 10Y OS rate of the high-LDH group was significantly lower than that of the low-LDH group (70.6% vs. 90.9%,  $P=0.0369$ ). The 10Y EFS rate according to LDH group showed a trend of higher survival in the low-LDH group (86.1% vs. 64.7%,  $P=0.064$ ). In the HR group, the 10Y OS of the high-LDH group was not significantly different from that of the low-LDH group (46.7% vs. 57.1%,  $P=0.467$ ; 33.3% vs. 42.9%,  $P=0.634$ , respectively).

**Table 2.** Multivariate analysis of prognostic factors for survival in acute lymphoblastic leukemia patients

Factor	Overall survival			Event-free survival		
	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value
NCI high risk	2.45	0.94-6.42	0.680	3.06	1.33-7.03	0.008 <sup>a)</sup>
VHR	4.90	1.78-13.50	0.002 <sup>a)</sup>	2.65	1.05-6.69	0.040 <sup>a)</sup>
High LDH	2.18	0.81-5.86	0.122	1.79	0.78-4.12	0.170
<i>MLL</i> -PTD	1.22	0.45-3.32	0.690	1.13	0.49-2.62	0.768

CI, confidence interval; LDH, lactate dehydrogenase; *MLL*-PTD, mixed lineage leukemia-partial tandem duplication; NCI, National Cancer Institute; VHR, very high risk; WBC, white blood cell.

<sup>a)</sup>Statistically significant ( $P < 0.05$ ).

Combining *MLL*-PTD status and LDH level in the survival analysis, the group with both *MLL*-PTD (+) and high LDH [*MLL*-PTD(+)/High LDH group] showed the lowest 10Y OS and EFS rates (37.5%,  $P=0.040$  and 37.5%,  $P=0.094$ , respectively) (Fig. 2).

### 7) Multivariate Analysis (Table 2)

In the multivariate analysis for OS, VHR factor was an independent risk factor (Hazard Ratio [HR]=4.90,  $P=0.002$ ). NCI high risk showed an inferior trend (HR 2.45,  $P=0.068$ ), but high LDH and *MLL*-PTD status were not independent risk factors (HR 2.18,  $P=0.122$  and HR 1.22  $P=0.690$ , respectively). For EFS, VHR factor and NCI high-risk stratification were independent risk factors (HR 2.65,  $P=0.040$ , and HR 3.06,  $P=0.008$ , respectively) but high LDH and *MLL*-PTD status were not (HR=1.79,  $P=0.170$  and HR=1.13,  $P=0.768$ ).

We examined the combined factor as *MLL*-PTD (+)/High LDH in the analysis. VHR factor and NCI high risk were independent predictors for OS and EFS, but *MLL*-PTD (+)/High LDH was not an independent risk factor (HR 1.77,  $P=0.296$  for OS, HR 1.28,  $P=0.633$  for EFS) (Table 2).

## Discussion

In this study, *MLL*-PTD was frequently observed in ALL and AML, which are well known leukemias associated with *MLL*-PTD. We evaluated the prognostic significance of *MLL*-PTD, but it was not an independent risk factor in ALL for survival in univariate analysis. However, *MLL*-PTD was significantly associated with LDH level in ALL and influ-

enced the prognosis of the ALL-SR group. Overall, *MLL*-PTD (+)/High LDH group was an independent risk factor for survival in ALL.

In this study, the prevalence of *MLL*-PTD was high compared with other reports [3]. This difference may reflect the detection method used for *MLL*-PTD. When using a nested RT-PCR, *MLL*-PTD can be detected in 17-100% of whole blood and bone marrow samples from normal healthy donors [8,9]. According to the authors of these studies, a mutation such as *MLL*-PTD that is not lethal might be observed in healthy individuals throughout life. *MLL*-PTD is even detected in cord blood samples of newborns, suggesting it is present from the early stages of development [10]. Therefore, the *MLL* gene is believed to play a role in normal hematopoiesis and leukemogenesis, although it is not clear whether the *MLL* gene acts as an oncogene, a suppressor gene, or a dominant negative gene [2]. The high positivity rate of *MLL*-PTD in healthy donors is not much different from that of other gene rearrangements found in leukemias: t(9;22), *BCR-ABL*, t(8;21), and *AML1-ETO*. Many other translocations are detectable in healthy donors and are involved in non-neoplastic hematopoiesis and leukemogenesis [11].

However, there is a major difference in the frequency of *MLL*-PTD transcripts between leukemia patients and healthy donors. When conventional nested PCR was used for detection, *MLL*-PTD could be detected in healthy individuals in the nested cycle only, whereas in AML patients, the amplification was evident during the primary PCR [3]. To reduce false positives during detection of *MLL*-PTD, many researchers use simple, single-round RT-PCR instead of nest-

ed RT-PCR [12-14]. The quantitation of *MLL*-PTD and its clinical application to treat leukemia are currently under investigation [15].

Although nested RT-PCR has certain limitations, it is a standard method for the detection of *MLL*-PTD, and many researchers have reported valuable findings using this approach [16-18]. When results from other studies using nested PCR were compared with our findings, the detection rate of *MLL*-PTD in our study was higher. In previous studies, *MLL*-PTD was detected in 5-11% of the subjects, whereas we detected *MLL*-PTD in over 40% of our patients with ALL. One possible reason for this discrepancy is the difference in age of the study population. In the adult population, 5-10% of *MLL*-PTD positivity is considered a normal range [3], but the positivity in pediatric populations tends to be somewhat higher and has been reported to be 13.3% (21/158), 27.7% (13/47), and 40% (2/5) in different studies [4,13,18]. Geographic or ethnic differences could also affect the positivity rate. Recent studies of Asian populations, including those in Japan and Taiwan, reported a higher frequency of *MLL*-PTD than in other studies elsewhere [13,18]. To validate these hypotheses, further studies on the pediatric population with varying ethnicities are needed.

To our knowledge, this is the first large study of *MLL*-PTD in ALL. Using nested RT-PCR, we detected *MLL*-PTD in a very high percentage (47.6%) of pediatric ALL patients. Theoretically, since the *MLL* gene acts in hematopoiesis and is thought to be associated with leukemogenesis, the mutation of this gene could lead to various types of leukemia. Quentmeier et al. screened a panel of cell lines (66 AML and 73 ALL cell lines) for the *MLL*-PTD mutation [19]. They proposed that the *MLL* breakpoint cluster region (BCR) of the *MLL* gene might be involved in *MLL*-PTD in addition to translocations, and therefore *MLL*-PTD might be common in ALL and AML. However, of the 139 cell lines tested, only four carried the *MLL*-PTD mutation, and all of these were AML-derived. In contrast, Pallisgaard et al. reported that 12.9% (8/62) of pediatric ALL patients had *MLL*-PTD [6], and this is one of the few studies to report *MLL*-PTD in ALL other than our study.

*MLL*-PTD in AML is accepted as a poor prognostic factor, but this remains controversial [3,20]. Schnittger et al. re-

ported that the median survival and relapse free interval of the *MLL*-PTD (+) group were significantly worse than those of an age-matched karyotypically normal control group [14]. Yu et al. studied *MLL*-PTD in normal karyotype adult AML subjects and showed that 7 out of 34 patients had a median survival of 2.7 months, compared to a 6.8-month median survival for all other patients [21]. However, several other reports do not support the notion that *MLL*-PTD is an independent risk factor for AML. Dohner et al. studied adult AML patients with normal cytogenetics and showed that the OS rate between *MLL*-PTD-positive and -negative groups was not statistically different ( $P=0.427$ ), but the duration of remission of the *MLL*-PTD-positive group was significantly lower than that of the *MLL*-PTD-negative group ( $P=0.02$ ) [12]. However, Shih et al. reported no difference in OS rate or remission duration between *MLL*-PTD-positive AML patients and patients with other 11q23 rearrangements [22]. Moreover, Whitman et al., in a Cancer and Leukemia Group B (CALGB) study, and Steudel et al. concluded that *MLL*-PTD was not an independent prognostic factor for OS or EFS [16,17]. Considering these controversial results, the prognostic impact of *MLL*-PTD might be weaker than previously thought.

Prognosis of *MLL*-PTD (+) ALL patients has not previously been reported. In our study, *MLL*-PTD was not an independent prognostic factor for ALL in the SR or the HR group. LDH is a well-known prognostic factor in leukemia [23]. The LDH level was associated with the prognosis of ALL, mainly due to its effect on ALL-SR. The cut-off point was 1,100 IU/L in this study, and this was consistent with other ALL studies [24,25]. Pui et al. studied the prognostic implication of LDH in 293 standard risk ALL patients, and the cut-off value was 1,000 IU/dL [23]. ALL-SR is defined by age and WBC count. ALL-SR is homogenous, and LDH might have a clear impact on the survival rate in this group of patients. In contrast, the ALL-HR group is quite heterogeneous and includes several patient characteristics with strong adverse prognostic values such as BCR/ABL and *MLL/AF4*. Therefore, the prognosis of ALL-HR is not influenced by weak individual prognostic factors such as LDH.

Initially, *MLL*-PTD was associated with a lower LDH level. However, few patients showed high LDH levels com-

bined with positive *MLL*-PTD (N=9), and it was significantly associated with adverse outcomes (Fig. 2). *MLL*-PTD and high LDH were not independent risk factors for OS or EFS in multivariate analysis. NCI high risk and VHR factor were strong risk factors for survival. One of the reasons is the association between *MLL*-PTD (+)/high LDH and VHR factor. In the *MLL*-PTD (+)/High LDH group, 33.3% (3/9) of patients had VHR factor, which was significantly higher than the VHR factor frequency of other patients (9.3%, 7/75,  $P=0.036$ ). In the ALL-SR patients without VHR factors, *MLL*-PTD (+)/High LDH showed inferior OS and EFS (50.0% vs. 91.0%,  $P=0.010$  and 50.0% vs. 85.1%,  $P=0.083$ , respectively). Therefore, *MLL*-PTD (+)/High LDH would be another prognostic factor next to the NCI risk stratification and VHR features, especially in the cytogenetically normal ALL group. The implication of this combined factor should be evaluated later with much more cytogenetically normal ALL patients.

Our study has some unique features. To our knowledge, this is the first large study of *MLL*-PTD in ALL patients. The patients were followed up for a long period of time (over 9 years). This study provides information about *MLL*-PTD in the pediatric population, which has not been extensively studied. Moreover, the study involved Asian patients and may reveal characteristics specific to ethnicity. This study also has some limitations: *MLL*-PTD was detected by nested RT-PCR, not by single-round RT-PCR. *MLL*-PTD-positive patients were treated with high-risk ALL chemotherapy protocols, and so this high intensity might have influenced the survival results in the *MLL*-PTD-positive group. High-intensity chemotherapy might overcome the potential adverse prognostic implication of *MLL*-PTD in ALL.

In conclusion, this is the first report on the prevalence of *MLL*-PTD in ALL patients. As an independent factor, *MLL*-PTD did not show prognostic power for survival. The *MLL*-PTD-positive and high LDH group should be evaluated further for prognostic potential.

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