Brief report

KIT mutations, and not *FLT3* internal tandem duplication, are strongly associated with a poor prognosis in pediatric acute myeloid leukemia with t(8;21): a study of the Japanese Childhood AML Cooperative Study Group

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Patients with t(8;21) acute myeloid leukemia (AML) are considered to have a good prognosis; however, approximately 50% of them relapse. The genetic alterations associated with a poor outcome in t(8;21) AML remain unknown. Recently, aberrations of receptor tyrosine kinases (RTKs) were frequently found in patients with AML. However, the prevalence and prognostic impact of RTK aberrations in pediatric t(8;21) AML remains undetermined. Here, we found the kinase domain mutations of the *KIT* gene in 8 (17.4%) of 46 patients with t(8;21) AML among newly diagnosed pediatric patients with AML treated on the AML99 protocol in Japan. Significant differences between patients with or without *KIT* mutations were observed in the 4-year overall survival (50.0% versus 97.4%, $P = .001$), disease-free survival (37.5% versus 94.7%, $P < .001$) and relapse rate (47.0% versus 2.7%, $P < .001$). Furthermore, *FLT3* internal tandem duplication was found in only 2 (4.3%) patients. These results suggested that *KIT* mutations are strongly associated with a poor prognosis in pediatric t(8;21) AML. (Blood. 2006; 107:1806-1809)

Introduction

Patients with t(8;21) acute myeloid leukemia (AML) have been reported to have a good prognosis; however, approximately 50% of them relapse.[1,2] A high presenting leukocyte count, CD56 expression, or extramedullary disease has been reported to be associated with a poor prognosis in t(8;21) AML.[1,3,4] However, the genetic alterations associated with a poor outcome in patients with t(8;21) AML remain unknown. Recent studies revealed that internal tandem duplication (ITD) of *FLT3* is considered to be one factor predicting poor prognosis in adult and pediatric patients with AML.[5-9] More recently, *KIT* mutations were found in 12.7% to 48.1% of adult patients with AML with t(8;21)[10-12] and were reported to be associated with a poor prognosis.[13,14] The prevalence and prognostic impact of *KIT* mutations in pediatric t(8;21) AML remain unknown. We performed the mutational analysis of *KIT* and *FLT3* in pediatric patients with t(8;21) AML who were treated on the Japanese Childhood AML Cooperative Study Group Protocol, AML99. We report here that *KIT* mutations are strongly associated with a poor prognosis in pediatric patients with t(8;21) AML.

Study design

Patients and samples

The diagnosis of AML was based on the French-American-British (FAB) classification, and cytogenetic analysis was performed using a routine G-banding method. From January 2000 to December 2002, 318 patients were newly diagnosed as having de novo AML. Of 240 patients, 77 (32.1%), except for 29 AML-M3 and 49 Down syndrome, had t(8;21)(q22; q22) according to cytogenetics or *AML1-MTG8* fusion transcript with the reverse-transcriptase–polymerase chain reaction (RT-PCR) (Figure S1; see the Supplemental Materials link at the top of the online article, at the *Blood* website). Samples were available from 135 (56.3%) of 240 patients with AML, including 46 (59.7%) of 77 patients with t(8;21) AML. Of 46 patients with t(8;21) AML, 3 patients were classified into M1, 39 into M2, and 4 into

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A list of the participating members of the Japanese Childhood AML Cooperative Study Group appears in "Appendix."

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A.S. performed genetic analysis and wrote the paper; T.T. assisted with the genetic analysis; K.T. performed the statistical analysis; A.T., K.H., M.T., and R.H. arranged the clinical data; I.T. designed the AML cooperative study in Japan; and Y.H. designed the study and wrote the paper.

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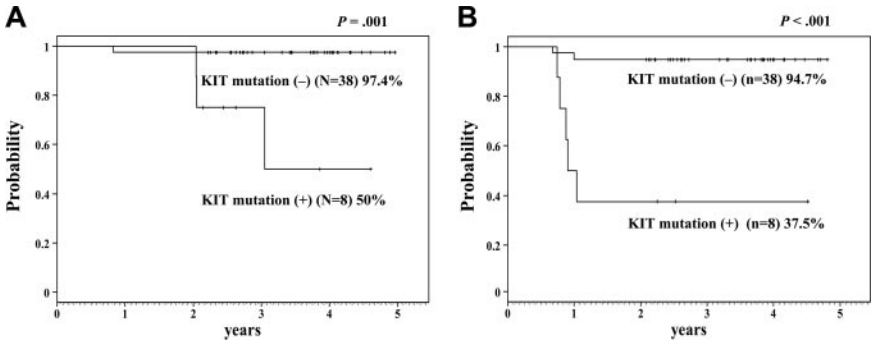

Figure 1. Kaplan-Meier analysis. This analysis shows 4-year overall survival (A) and disease-free survival (B) of the patients with or without *KIT* mutation. The difference is statistically significant (A: $P = .001$; B: $P < .001$).

M4. There were no statistical differences between 46 analyzed patients with t(8;21) AML and the 31 nonanalyzed patients in age (median 7.5 years [range: 2-15 years] versus 9 years [range: 1-15 years]), initial white blood cell (WBC) count (median: 14.4 × 10^9/L; range: 1.65 × 10^9/L-107.7 × 10^9/L; versus 9.1 × 10^9/L; range: 1.4 × 10^9/L-136 × 10^9/L), induction rate (100% versus 93.5%), relapse rate (15.2% versus 19.4%), and 4-year overall survival rate (4y-OS; 87% versus 91%). In the AML99 protocol, patients with t(8;21) with initial WBC count lower than 50 × 10^9/L were categorized into a low-risk group. Thus, after patients with t(8;21) AML obtained complete remission (CR) with induction chemotherapy (cytarabine, etoposide, and mitoxantrone), they were treated with 5 additional courses of intensive chemotherapy (high-dose cytarabine [HDCA], etoposide, idarubicine, and mitoxantron; Figure S2 and Tsukimoto et al[15]). If the initial WBC count was greater than 50 × 10^9/L, patients were categorized into an intermediate-risk group and received allogeneic stem cell transplantation (allo-SCT) in the case of the presence of a donor. Informed consent was obtained from the patients or patients' parents, according to guidelines based on the tenets of the revised Helsinki protocol. The institutional review board of Gunma Children's Medical Center approved this project.

KIT mutation analysis

Mutational analysis of the extracellular (EC) domain (exons 8, 9), transmembrane (TM) domain (exon 10), juxtamembrane (JM) domain (exon 11), and the second intracellular kinase (TK) 2 domain (exons 17 and 18) of *KIT* gene was performed with RT-PCR followed by direct sequencing. Primers used are shown in Table S1.

FLT3 mutation analysis

Mutational analysis of ITD within the JM domain and D835 mutation (D835Mt) within the TK2 domain of the *FLT3* gene was performed as previously reported.[16-18]

Statistical analysis

Estimation of survival distributions was performed using the Kaplan-Meier method and the differences were compared using the log-rank test. Disease-free survival (DFS), event-free survival (EFS), and overall survival (OS) were defined as the times from diagnosis to relapse, from diagnosis to event (relapse or death of any cause), and from diagnosis to death of any cause or the last follow-up. Statistical difference analysis was performed using the χ^2 test.

Results and discussion

KIT and *FLT3* expressions were found in all of the 46 t(8;21) AML samples. Although *KIT* mutations have been reported in a small number of pediatric patients with t(8;21) AML,[8,19] TK2 domain mutations of the *KIT* gene were found in 8 (17.4%) of 46 patients in this study (Table 1). However, we could not find any mutation other than the TK2 domain. The N822K mutation, which has been frequently reported so far,[12] was found in 3 of 8 patients in this study.

The statistical differences between patients with or without *KIT* mutations were not significant in age (median 8 years [range: 1-15 years] versus 7 years [range: 2-15 years]), and the initial WBC count (median: 20.65 × 10^9/L; range: 4.6 × 10^9/L-66.2 × 10^9/L; versus 14.3 × 10^9/L; range: 1.65 × 10^9/L-107.7 × 10^9/L). Interestingly, *KIT* mutations were observed only in M2 patients according to FAB classification. Another report also suggested that *KIT* mutations were frequently found in M2 patients with t(8;21).[19] Significant differences between patients with or without *KIT* mutations were observed in 4-year OS (50.0% versus 97.4%, $P = .001$, Figure 1), DFS (37.5% versus 94.7%, $P < .001$), and relapse rate (47.0% versus 2.7%, $P < .001$). Short CR duration and high relapse rate were more significant than those of the previous report in adults.[14] *KIT* mutations have recently been reported not to influence the clinical outcome in pediatric core-binding factor (CBF) leukemia patients.[20] Although they found *KIT* mutations in 5 of 16 cases of t(8;21) AML, they did not describe the clinical outcome of patients with t(8;21) AML with or without *KIT* mutations. Furthermore, the clinical outcome of the patients

Table 1. Clinical characteristics of patients with t(8;21) AML with *KIT* mutations

Patient no.	Age, y	Sex	WBC count, × 10^9 cells/L	Additional chromosome abnormalities	Time of relapse, mo	Status of allo-SCT	Survival, mo	*KIT* mutation
1	8	F	14.10	None	12	Second CR	37	A814S
2	8	M	27.60	−Y	14	Second CR	47*	N822K
3	8	F	10.77	−X	10	Second CR	25	D816H
4	6	M	34.50	−Y, +4	12	Second CR	26*	N822K
5	3	F	20.50	None	11	—	25	N822K
6	1	F	4.60	−X, t(7;9)	—	—	32*	N822T
7	15	M	20.80	−Y	—	First CR	56*	D816V
8	13	M	66.20	None	—	First CR	30*	V825A

— indicates not applicable.
*Patient still alive.

without *KIT* mutations in their study was poorer than the outcome of those in our study (EFS 63% versus 92.1%). Our result may depend on our good clinical outcome of patients with t(8;21) AML without *KIT* mutations.

Except for 2 patients who received allo-SCT in first CR (patients no. 7 and no. 8 in Table 1), 5 of 6 (83.3%) patients with the mutation relapsed within 14 months after diagnosis. Allo-SCT was performed in 6 of 8 patients with t(8;21) AML with *KIT* mutations (2 in first CR, 4 in second CR) and 4 patients are still alive. In contrast, allo-SCT was also performed in only 1 of 38 patients with t(8;21) AML without *KIT* mutation in second CR, and this patient is still alive.

A high presenting leukocyte count and extramedullary disease were not associated with the poor prognosis in this study. Notably, *KIT* was mapped to chromosome 4 at band q11 and trisomy 4 was reported to be associated with *KIT* mutation.[21] One patient with trisomy 4 in addition to t(8;21) had N822K mutation (patient no. 4). As for additional chromosome abnormality, loss of sex chromosome was observed in 5 (62.5%) of 8 patients with *KIT* mutation and 14 (37%) of 38 patients without mutations, although the difference between them was not statistically significant. Recently, it has been reported that AML blasts with N822K mutation are sensitive to the tyrosine kinase inhibitor Gleevec/STI571/imatinib mesylate.[12] The effectiveness of imatinib mesylate for the patient with AML with *KIT* mutation was also reported.[22] Thus, tyrosine kinase inhibitors may be applicable for these patients in the future.

Two samples examined at relapse showed the same mutations as those at diagnosis (patients no. 3 and no. 5), and these *KIT* mutations disappeared in samples in remission, suggesting that *KIT* mutation was not a constitutional abnormality.

Recently, clonal leukemic cells with *AML1-MTG8* fusion transcript have been reported to arise in utero.[23] Moreover, it was reported that this fusion transcript was not sufficient for full leukemogenesis, and that additional genetic events were required.[24,25] *KIT* mutations may be one of the secondary genetic events of the stepwise leukemogenesis of t(8;21) AML.

FLT3-ITD was found in only 2 (4.6%) of 46 patients with t(8;21). One patient died during chemotherapy, and the other patient was disease free for 42 months from diagnosis. *FLT3*-ITD is considered to be strongly associated with a poor prognosis in AML.[6,7] However, *FLT3*-ITD was rarely reported in patients with t(8;21) AML.[8,9,13,14,20] Our data also confirmed the low incidence of *FLT3*-ITD in patients with t(8;21) AML. As for D835Mt of the *FLT3* gene, we found the mutation in 1 of 46 patients, who was alive for 31 months after diagnosis.

In total, 11 (23.9%) of 46 patients with t(8;21) AML in this study had *KIT* or *FLT3* mutations, suggesting that the pediatric patients with t(8;21) AML had genetic heterogeneity. In conclusion, *KIT* mutations are considered to be strongly associated with poor prognosis in pediatric t(8;21) AML.

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Appendix

Members of the Japanese Childhood AML Cooperative Study Group who contributed data to the study include Akira Morimoto, Department of Pediatrics, Kyoto Prefectural University of Medicine; Ryoji Kobayashi, Department of Pediatrics, Hokkaido University School of Medicine; Hiromasa Yabe, Department of Pediatrics, Tokai University School of Medicine; Kazuko Hamamoto, Department of Pediatrics, Hiroshima Red Cross Hospital; Shigeru Tsuchiya, Department of Pediatric Oncology, Institute of Development, Aging, and Cancer, Tohoku University; Yuichi Akiyama, Department of Pediatrics, National Hospital Organization Kyoto Medical Center; Hisato Kigasawa, Department of Hematology, Kanagawa Children's Medical Center; Akira Ohara, Department of First Pediatrics, Toho University School of Medicine; Hideki Nakayama, Department of Pediatrics, Hamanomachi Hospital; Kazuko Kudo, Department of Pediatrics, Nagoya University Graduate School of Medicine; and Masue Imaizumi, Department of Hematology/Oncology, Miyagi Prefectural Children's Hospital.

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