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Incidences and Prognostic Impact of *c-KIT*, *WT1*, *CEBPA*, and *CBL* Mutations, and Mutations Associated With Epigenetic Modification in Core Binding Factor Acute Myeloid Leukemia: A Multicenter Study in a Korean Population

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Background: To identify potential molecular prognostic markers in core binding factor (CBF) AML, we analyzed incidences and prognostic impacts of mutations in *c-KIT*, *WT1*, *CEBPA*, *CBL*, and a number of epigenetic genes in CBF AML.

Methods: Seventy one and 21 AML patients with t(8;21) and inv(16) were enrolled in this study, respectively. *NPM1*, *CEBPA*, *c-KIT*, *IDH1/2*, *DNMT3A*, *EZH2*, *WT1*, and *CBL* mutations were analyzed by direct sequencing. Patients were categorized with respect to *c-KIT* and *WT1* mutation status, and both clinical features and prognoses were compared.

Results: The incidences of *FLT3* internal tandem duplication (ITD), *NPM1*, *CEBPA*, *IDH1/2*, *DNMT3A*, *EZH2*, and *CBL* mutations were low (≤5%) in CBF AML patients. However, *c-KIT* and *WT1* mutations occurred frequently (10.9% and 13.8%, respectively). t(8;21) patients with *c-KIT* mutations showed significantly shorter overall survival (OS) and disease free survival (DFS) periods than those without mutations ($P<0.001$, for both); however, although the limited number of t(8;21) patients were analyzed, *WT1* mutation status did not affect prognosis significantly. Relapse or death during follow-up occurred more frequently in t(8;21) patients carrying *c-KIT* mutations than in those without the mutation, although the difference was significant only in a specific patient subgroup with no *WT1* mutations ($P=0.014$).

Conclusions: The incidences of mutations in epigenetic genes are very low in CBF AML; however, *c-KIT* and *WT1* mutations occur more frequently than others. The poor prognostic impact of *c-KIT* mutation in t(8;21) AML patients only applies in a specific patient subgroup without *WT1* mutations. The prognostic impact of *WT1* mutation in CBF AML is not evident and further investigation is required.

Key Words: Acute myeloid leukemia, Core binding factor, *c-KIT*, Epigenetic modification, Incidence, Prognosis, *WT1*

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INTRODUCTION

Mutations related to the development of myeloid malignancy are categorized into five classes, namely those in genes related to signaling pathways (e.g. fms-related tyrosine kinase 3 [*FLT3*], *c-KIT*, and casitas b-lineage lymphoma [*CBL*]; class I), genes encoding transcription factors (e.g. CCAAT/enhancer-binding protein alpha [*CEBPA*] and nucleophosmin1 [*NPM1*]; class II), genes related to epigenetic modification (e.g. enhancer of zeste homolog 2 [*EZH2*], DNA-methyltransferase 3 alpha [*DNMT3A*], and isocitrate dehydrogenase 1/2 [*IDH1/2*]; class III), tumor suppressor genes (e.g. Wilms' tumor 1 [*WT1*]; class IV), and genes associated with RNA maturation (e.g. subunit 1 of splicing factor 3b protein complex [*SF3B1*], serine/arginine-rich splicing factor 2 [*SRSF2*], and U2 small nuclear RNA auxiliary factor 1 [*U2AF1*]; class V) [1, 2].

Of these mutations, *FLT3* internal tandem duplication (ITD) is a commonly observed aberration associated with poor prognosis in AML [3]. *NPM1* and *CEBPA* mutations, associated with a favorable prognosis in normal karyotype (NK) AML, are also used for risk classification [4]. In addition, both *DNMT3A* and *IDH1/2* mutations were recently introduced as potential adverse and favorable prognosis indicators in NK AML, respectively [5-8]. However, both *EZH2* and *CBL* mutations were reported to occur very rarely and offer no prognostic impact for AML [9-11]; in addition, *WT1* mutation was reported to occur only rarely in AML [12]. Despite the low incidence of *WT1* mutation, its prognostic impact in AML cases still needs to be investigated since several studies have shown that *WT1* overexpression, possibly due to *WT1* mutation, may constitute an unfavorable [13-15] or favorable [16] prognostic indicator.

Although a recent study reported that *CBL* mutation occurs in core binding factor (CBF) AML at a frequency of 6% and was associated with a favorable prognosis in CBF AML patients with a *CBL* mutant level of 25% or higher, there have been fewer studies on the identification of prognostic markers for AML when compared with the number of studies concerning NK AML, and a reliable marker other than *c-KIT* mutation has not been identified [17-21]. Given that the five-year survival rate for CBF AML is only 50% and the incidence of *FLT3* ITD mutation in this disease is low, occurring in 5 to 6% of patients [19, 21], an investigation into the identification of potential molecular prognostic markers in CBF AML needs to be performed.

Therefore, we aimed to compare clinical features between patients with t(8;21) and those with inv(16), and to evaluate the incidence and prognostic impact of genetic mutations associated with epigenetic modifications, such as *IDH1/2*, *DNMT3A*, and *EZH2* mutations, as well as tumor suppressor *WT1* mutation, and *CBL* mutation in CBF AML patients, while including *FLT3* ITD, *NPM1*, *CEBPA*, and *c-KIT* mutations in a multicenter study on the Korean population.

METHODS

1. Patient selection and treatment

A total of 92 patients diagnosed with CBF AML at four tertiary hospitals in Korea from January 2002 to December 2010 were retrospectively enrolled in this study, including 71 patients with t(8;21)(q22;q22) and 21 patients with inv(16)(p31.1;q22)/t(16;16)(p13.1;q22). All patients received induction chemotherapy with cytarabine at 100 mg/m^2 per day for seven days plus daunorubicin at 45 mg/m^2 per day for three days (the AD regimen), or cytarabine at 100 mg/m^2 per day for seven days plus idarubicin 12 mg/m^2 per day for three days (the AI regimen). Complete remission (CR) was defined as the presence of $<5\%$ blasts on bone marrow (BM) aspirates and $\geq 20\%$ cellularity in BM biopsy after induction chemotherapy. In total, 67 (72.8%) patients reached CR. Relapse was defined as the presence of $\geq$ 5% leukemic blasts on BM aspirates for patients who had previously achieved CR. In total, 33 (35.9%) patients experienced relapse or died during follow-up periods (median: 18.5 months, range: 0-150 months). Patients underwent stem cell transplantation (SCT) depending on the patient's age and the availability of a suitable donor. In total, 35 (38.0%) patients received SCT during follow-up period. This study was approved by the institutional review board of each institution.

2. Analyses of *FLT3* ITD, *NPM1*, *c-KIT*, and *CEBPA* mutations

FLT3 ITD, *NPM1*, *c-KIT*, and *CEBPA* mutations were analyzed on DNA samples obtained from each patient at initial diagnosis. *FLT3* ITD mutation was analyzed by multiplex PCR using a Seeplex FLT3 Genotyping Kit (Seegene, Seoul, Korea). *NPM1* mutation was analyzed using a primer set designed in-house and PCR conditions as described previously [22]. The size of PCR products was determined by capillary electrophoresis using ABI 3130 genetic analyzer and GeneScan Analysis software (Applied Biosystems Inc., Foster City, CA, USA). For samples with an additional peak in their profile, direct sequencing was performed to confirm the mutation. For analysis of mutations in exons 8 and 17 of *c-KIT*, PCR and direct sequencing were also performed with a primer set designed in-house, using the PCR conditions and analysis strategy described previously [18]. For *CEBPA* mutation analysis, four primer sets were used for PCR and direct

sequencing, using a detection strategy and PCR conditions identical to those applied in a previous study [23]. All information regarding the sequences and melting temperatures of primers used to amplify *c-KIT*, *NPM1*, and *CEBPA* genes, and the size of PCR products, is provided in Supplemental Data Table S1.

3. Analyses of *IDH1*, *IDH2*, *DNMT3A*, *EZH2*, *WT1*, and *CBL* mutations

WT1, *CBL*, and four genes associated with epigenetic modification (*IDH1*, *IDH2*, *DNMT3A*, and *EZH2*) were analyzed by PCR and direct sequencing. Since the quantity of DNA in samples from 11 patients with t(8;21) and one patient with inv(16) was not sufficient for analysis, a total of 80 CBF AML patients were finally included in these analyses. Mutation hotspots in *IDH1* (codon 123 and 132 in exon 4), *IDH2* (codon 140 and 172 in exon 4), *DNMT3A* (codon 882 in exon 23), *EZH2* (exons 17 to 19), *WT1* (exons 7 and 9), and *CBL* (exons 8 and 9) genes were determined as amplification targets to be analyzed. The following PCR conditions were used to amplify a total of 10 exons from six genes: 5 min at 95°C, followed by 35 cycles of 30 sec at 95°C (denaturation), 45 sec at 55°C (annealing) and 30 sec at 72°C (extension), and a final 10 min extension at 72°C. All information regarding the sequences and melting temperatures of primers used to amplify each gene, and the size of PCR products, is provided in Supplemental Data Table S1.

4. Comparison of clinical features and incidences of genetic mutations between AML patients with t(8;21) and those with inv(16)

Both clinical features and incidences of genetic mutations were compared between patients with t(8;21) and those with inv(16). The clinical features compared included gender, age, percentage

Table 1. Comparison of clinical features and incidences of genetic mutations between acute myeloid leukemia patients with t(8;21) and acute myeloid leukemia patients with inv(16)

Variables	AML with t(8;21), 71 patients (% of total patients)	AML with inv(16), 21 patients (% of total patients)	Total, 92 patients	*P*
Sex (M:F)*	38 : 33	13 : 8	51 : 41	0.497
Age, yr, median (range)†	41.0 (5.0-78.0)	47.0 (16.0-82.0)	43.0 (5.0-82.0)	0.126
Additional chromosomal abnormalities*	17/71 (23.9%)	5/21 (23.8%)	22/92 (23.9%)	0.990
SCT during follow-up*	24/71 (33.8%)	11/21 (52.4%)	35/92 (38.0%)	0.123
Relapse or death during follow-up*	22/71 (31.0%)	11/21 (52.4%)	33/92 (35.9%)	0.073
Follow-up period, months, median (range)†	22.0 (0.0-150.0)	17.0 (0.0-119.0)	18.5 (0.0-150.0)	0.955
Laboratory findings at diagnosis†				
WBC ($\times 10^9$/L), median (range)	8.34 (1.2-192.9)	54.4 (2.7-277.6)	10.3 (1.2-277.6)	<0.001
Hemoglobin (g/dL), median (range)	7.8 (1.5-13.2)	8.1 (4.6-12.4)	7.9 (1.5-13.2)	0.488
Platelets ($\times 10^9$/L), median (range)	31.0 (3.0-155.0)	33.0 (6.0-307.0)	31.0 (3.0-307.0)	0.551
PB blasts (%), median (range)	27.0 (0.0-92.0)	63.0 (7.0-87.0)	37.5 (0.0 -92.0)	0.002
BM blasts (%), median (range)	48.0 (21.0-90.0)	65.0 (21.0-91.0)	57.0 (21.0-91.0)	0.003
Mutation analysis results*				
FLT3 ITD	1/71 (1.4%)	2/21 (9.5%)	3/92 (3.3%)	0.129
NPM1	1/71 (1.4%)	0/21 (0.0%)	1/92 (1.1%)	0.584
c-KIT	7/71 (9.9%)	3/21 (14.3%)	10/92 (10.9%)	0.690
CEBPA	0/71 (0.0%)	0/21 (0.0%)	0/92 (0.0%)	NC
IDH1	0/60 (0.0%)	0/20 (0.0%)	0/80 (0.0%)	NC
IDH2	2/60 (3.3%)	0/20 (0.0%)	2/80 (2.5%)	0.408
DNMT3A	1/60 (1.7%)	3/20 (15.0%)	4/80 (5.0%)	0.046
EZH2	2/60 (3.3%)	2/20 (10.0%)	4/80 (5.0%)	0.259
WT1	7/60 (11.7%)	4/20 (20.0%)	11/80 (13.8%)	0.454
CBL	1/60 (1.7%)	0/20 (0.0%)	1/80 (1.3%)	0.561

P values were obtained using Chi-square or Fisher's exact tests (for numbers less than five in each group)* and Mann-Whitney U test†.
Abbreviations: SCT, stem cell transplantation; WBC, white blood cell; PB, peripheral blood; BM, bone marrow; FLT3, fms-related tyrosine kinase 3; *NPM*, nucleophosmin; *CEBPA*, CCAAT/enhancer binding protein alpha; *WT*, Wilms' tumor; *IDH*, isocitrate dehydrogenase; *DNMT3A*, DNA (cytosine-5-)-methyltransferase 3 alpha; *EZH*, enhancer of zeste homolog; *CBL*, casitas b-lineage lymphoma; NC, not calculated.

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of patients with additional chromosomal abnormalities, SCT performance, relapse and death rates, length of follow-up periods, complete blood cell count data at diagnosis, and peripheral blood (PB) and BM blast counts as percentages at diagnosis. In addition, all genetic mutations detected were aligned against reference sequences (NM_004119.2 for *FLT3* ITD, NM_002520.6 for *NPM1*, NM_000222.2 for *c-KIT*, NM_004364.3 for *CEBPA*, NM_005896.2 for *IDH1*, NM_002168.2 for *IDH2*, NM_175629.2 for *DNMT3A*, NM_001203247.1 for *EZH2*, NM_000378.4 for *WT1* and NM_005188.3 for *CBL*) and any resulting protein changes were analyzed. These results are represented in Tables 1 and 2.

5. Comparison of clinical features and prognoses between patients with or without *c-KIT* or *WT1* mutations

Patients with t(8;21) for whom *c-KIT* and *WT1* mutation data were available (71 and 60 patients, respectively) were categorized into two subgroups based on their *c-KIT* and *WT1* mutation status, and their clinical features were compared. *c-KIT* and *WT1* were selected because they showed higher mutation frequencies than other genes. To evaluate the prognostic impact of both *c-KIT* and *WT1* mutations, both overall survival (OS) and disease free survival (DFS) were compared between the two patient subgroups. Since two patients showed *FLT3* ITD or *NPM1* mutations, they were excluded from the survival analysis to exclude the prognostic impact of these mutations. OS was defined as the time from diagnosis to death or last follow-up. DFS was defined as the time from CR to relapse (for patients who experienced relapse), death (for non-relapsed patients who did not survive), or last follow-up (for non-relapsed patients who survived). Patients who underwent SCT were censored at the time of transplantation. In addition, identical comparisons were performed for 21 patients with inv(16). These results are summarized in Table 3 and Fig. 1.

Table 2. Summary of detected mutations in patients with core binding factor acute myeloid leukemia

Genes	AML with t(8;21)		AML with inv(16)		Alignment reference sequence	N of patients with mutation/under mutation analysis
	Mutation results (protein change)	N of patients	Mutation results (protein change)	N of patients		
c-KIT mutation (Exon 8)	c.1250_1255delCTTACG (p.Thr417_Asp419delinsAsn)	1	c. 1256_1257insTTTTCGA	1	NM_000222.2	10/92
c-KIT mutation (Exon 17)	c.2447A>T (p.Asp816Val)	1	c.2447A>T (p.Asp816Val)	1		
	c.2446G>T (p.Asp816Tyr)	2	c.2446G>T (p.Asp816Tyr)	1		
	c.2446G>C (p.Asp816His)	3				
IDH2 mutation	c.419G>A (p.Arg140Gln)	2			NM_002168.2	2/80
DNMT3A mutation	c.2638A>C (p.Met880Val)	1	c.2638A>C (p.Met880Val)	2	NM_175629.2	4/80
			c.2644C>T (p. Arg882Cys)	1		
EZH2 mutation (Exon 17)	c.1978G>A (p.Gly660Arg)	1	c.1996T>C (p.Tyr666Asn)	1	NM_001203247.1	4/80
EZH2 mutation (Exon 18)	c.2068C>T (p.Arg690Cys)	1	c.2068C>T (p.Arg690Cys)	1		
WT1 mutation (Exon 7)	c.1102G>A (p.Val367Met)	1			NM_000378.4	11/80
			c.1105C>G (p.Arg369Gly)	1		
	c.1112C>T (p.Val371Ala)	1				
			c.1131_1132insT	1		
	c.1141T>A (p.Ser381Thr)	2				
	c.1147T>A (p.Ser383Thr)	1	c.1147T>A (p.Ser383Thr)	2		
WT1 mutation (Exon 9)	c.1372C>T (p.Arg458X)	1				
	c.1379T>A (p.Phe460Tyr)	1				
CBL mutation	c.1196T>C (p.Leu399Pro)	1			NM_005188.3	1/80

Abbreviations: *WT*, Wilms' tumor; *IDH*, isocitrate dehydrogenase; *DNMT3A*, DNA (cytosine-5-)-methyltransferase 3 alpha; *EZH*, enhancer of zeste homolog; *CBL*, casitas b-lineage lymphoma; del, deletion; ins, insertion; X, stop codon.

Table 3. Comparison of clinical features between patients with *c-KIT* or *WT1* mutations and those without *c-KIT* or *WT1* mutations

71 patients with t(8;21)	Patient subgroups			
	c-KIT(-), N=64	*c-KIT*(+), N=7	*WT1*(-), N=53	*WT1*(+), N=7
Sex (M:F)*	35:29	3:4	30:23	4:3
Age, yr, median (range)†	41.5 (5.0-78.0)	37 (18.0-51.0)	41 (5.0-78.0)	36 (18.0-64.0)
Additional chromosomal abnormalities*	17/64 (26.6%)	0/7 (0.0%)	12/53 (22.6%)	1/7 (14.3%)
SCT during follow-up*	20/64 (31.3%)	4/7 (57.1%)	21/53 (39.6%)	1/7 (14.3%)
Relapse or death during follow-up*	16/64 (25.0%)	6/7‡ (85.7%)	14/53 (26.4%)	3/7 (42.9%)
Follow-up period, months, median (range)†	29 (0.0-150.0)	10 (4.0-35.0)	29 (0.0-109.0)	15 (0.0-82.0)
Laboratory findings at diagnosis†				
WBC ($\times 10^9$/L), median (range)	8.62 (1.20-192.90)	5.91 (2.90-25.33)	8.34 (1.20-102.37)	10.7 (3.20-192.90)
Hemoglobin (g/dL), median (range)	8 (1.5-13.2)	6.3 (5.4-8.7)	8.3 (2.3-13.2)	8.4 (5.4-12.3)
Platelets ($\times 10^9$/L), median (range)	32 (3.0-155.0)	24 (9.0-59.0)	34 (3.0-155.0)	32 (8.0-59.0)
PB blasts (%), median (range)	24 (0.0-92.0)	45 (13.0-54.0)	26 (0.0-86.0)	47 (15.0-92.0)
BM blasts (%), median (range)	47 (21.0-90.0)	58 (30.0-81.0)	44 (21.0-90.0)	60 (21.0-89.0)
21 patients with inv(16)	**Patient subgroups**			
	c-KIT(-), N=18	*c-KIT*(+), N=3	*WT1*(-), N=16	*WT1*(+), N=4
Sex (M:F)*	11:7	2:1	10:6	3:1
Age, yr, median (range)†	46.5 (16.0-82.0)	58 (47.0-58.0)	46.5 (23.0-69.0)	61.0§ (49.0-82.0)
Additional chromosomal abnormalities*	4/18 (22.2%)	1/3 (33.3%)	4/16 (25.0%)	1/4 (25.0%)
SCT during follow-up*	9/18 (50.0%)	2/3 (66.7%)	9/16 (56.3%)	1/4 (25.0%)
Relapse or death during follow-up*	9/18 (50.0%)	2/3 (66.7%)	7/16 (43.7%)	3/4 (75.0%)
Follow-up period, months, median (range)†	17 (0.0-119.0)	10 (5.0-46.0)	26.5 (1.0-119.0)	6 (0.0-17.0)
Laboratory findings at diagnosis†				
WBC ($\times 10^9$/L), median (range)	51.2 (2.70-277.62)	106.8 (40.06-160.10)	76.5 (11.10-277.62)	28.97 (2.70-181.35)
Hemoglobin (g/dL), median (range)	8.5 (4.6-12.4)	7.1 (6.4-8.1)	7.7 (4.6-11.4)	8.1 (5.8-12.4)
Platelets ($\times 10^9$/L), median (range)	34.5 (13.0-307.0)	15.0‡ (6.0-25.0)	33.5 (13.0-78.0)	21.5 (6.0-307.0)
PB blasts (%), median (range)	60 (7.0-86.0)	74 (71.0-87.0)	63.5 (25.0-87.0)	44 (7.0-71.0)
BM blasts (%), median (range)	62 (21.0-87.0)	85.0‡ (80.0-91.0)	67.5 (43.0-91.0)	57.5 (21.0-87.0)

P values were obtained using Chi-square or Fisher's exact tests (for numbers less than five in each group)* and Mann-Whitney U test†. In t(8;21), the patients with *c-KIT* mutation experienced relapse or died during follow-up more frequently than those without c-KIT mutation (P=0.003). In inv(16), the patients with *c-KIT* mutation showed lower platelet counts (P=0.024) and higher BM blasts (P=0.017) than those without *c-KIT* mutation, and the patients with *WT1* mutation were older than those without *WT1* mutation (P=0.029). Comparison items which showed statistically significant differences with respect to *c-KIT* and *WT1* mutation status were indicated with superscripts (‡) and (§), respectively.
Abbreviations: SCT, stem cell transplantation; WBC, white blood cell; PB, peripheral blood; BM, bone marrow; WT, Wilms' tumor.

6. Comparison of clinical features and prognoses in patients with t(8;21) among 4 patient subgroups categorized by *c-KIT* and *WT1* mutation status

The 60 patients with t(8;21) for whom both *c-KIT* and *WT1* mutation data were available were categorized into four patient subgroups: 1) *c-KIT*(-)/*WT1*(-), N=48; 2) *c-KIT*(+)/*WT1*(-), N=5; 3) *c-KIT*(-)/*WT1*(+), N=5; and 4) *c-KIT*(+)/*WT1*(+), N=2. Both clinical features and survival rates were compared between patient subgroups. As mentioned above, two patients with *FLT3* ITD or *NPM1* mutations were excluded from the survival analysis. These results are summarized in Table 4 and Fig. 2.

7. Statistical analysis

Pearson chi-square or Fisher's exact tests were performed to compare categorical variables. The Mann-Whitney U test was used to compare continuous variables. The log-rank test was applied for the comparison of OS and DFS, and Kaplan-Meier survival curves were generated. All tests were two-tailed, and *P* val-

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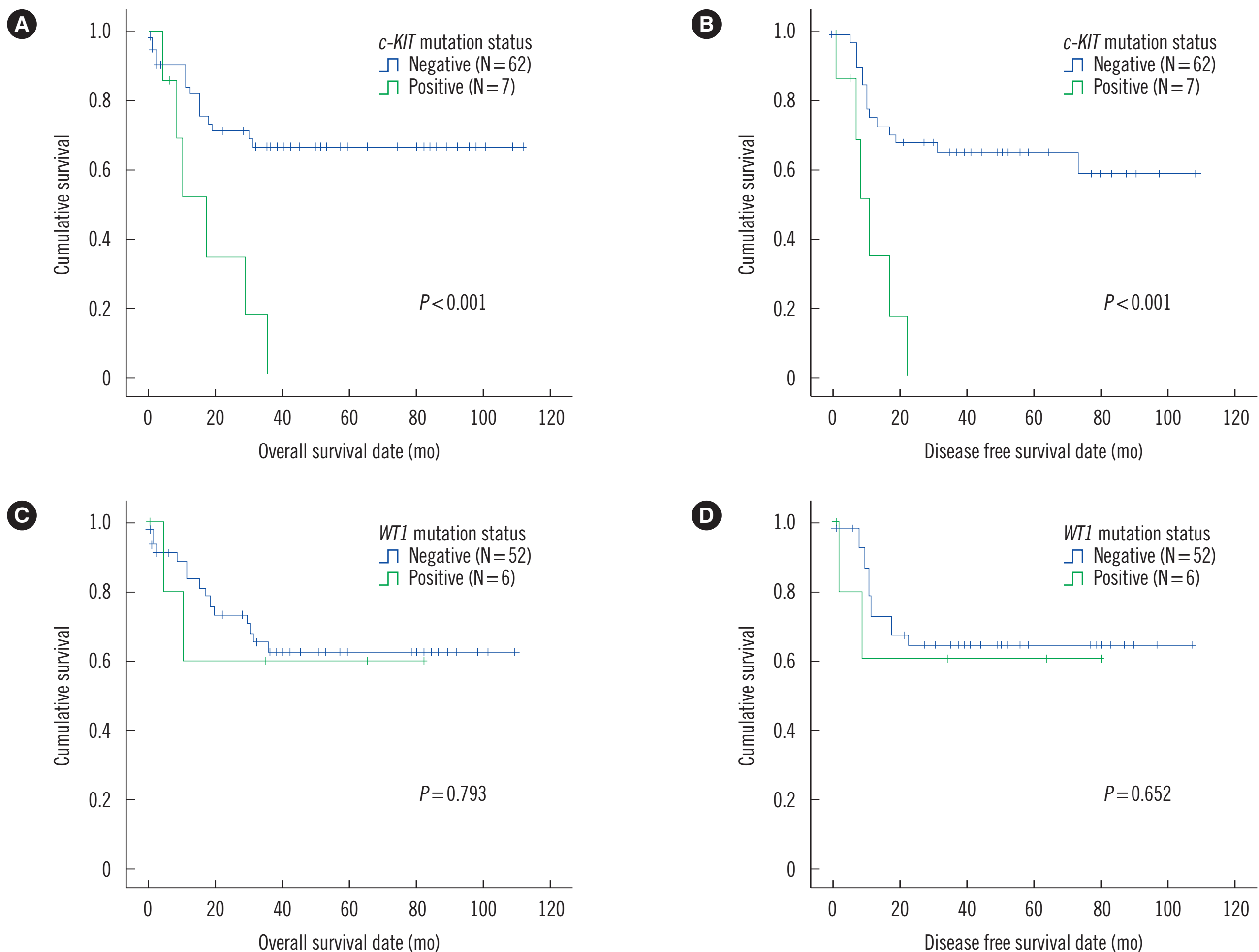

Fig. 1. Comparisons of overall survival and disease free survival lengths in core binding factor acute leukemia patients with t(8;21) and no *FLT3* ITD or *NPM1* mutations, between patients with *c-KIT* mutations and those without *c-KIT* mutations (N=69, A, overall survival; B, disease free survival). Identical comparisons between patients with *WT1* mutations and those without *WT1* mutations (N=58, C, overall survival; D, disease free survival) are also given in this figure.
Abbreviation: WT, Wilms' tumor.

ues <0.05 were considered statistically significant. SPSS 13.0.1 for Windows (SPSS Inc, Chicago, IL, USA) was used for statistical analysis.

RESULTS

1. Comparison of clinical features and incidences of genetic mutations between AML patients with t(8;21) and those with inv(16)

Patients with inv(16) showed significantly higher white blood cell (WBC) counts (median 54.4×10^9/L vs. 8.34×10^9/L, $P<0.001$), and levels of PB blasts (median 63.0% vs. 27.0%, $P=0.002$) and BM blasts (median 65.0% vs. 48.0%, $P=0.003$) than those with t(8;21). Other clinical features were not significantly different between the two patient subgroups.

Among the total of 92 patients, incidences of *FLT3* ITD, *NPM1*, *CEBPA*, *CBL*, *IDH1*, *IDH2*, *EZH2*, and *DNMT3A* mutations were 3.3%, 1.1%, 0%, 1.3%, 0%, 2.5%, 5.0%, and 5.0%, respectively. The incidences of both *c-KIT* mutations (10.9%) and *WT1* mutations (13.8%) were relatively higher than those of the other gene mutations analyzed. Mutation frequencies in patients with t(8;21) and inv(16) were not significantly different, except for that of *DNMT3A*, which showed significantly higher incidence in patients with inv(16) than in those with t(8;21) (15.0% vs. 1.7%, $P=0.046$; Table 1).

2. Summary of detected mutations in 92 CBF AML patients

In total, two and eight patients showed *c-KIT* mutations in exons 8

Table 4. Comparison of clinical features in 60 patients with t(8;21) according to *c-KIT* and *WT1* mutation status

Variables	Patient subgroups			
	c-KIT(-)/*WT1*(-), N=48	*c-KIT*(+)/*WT1*(-), N=5	*c-KIT*(-)/*WT1*(+), N=5	*c-KIT*(+)/*WT1*(+), N=2
Sex (M:F)*	27:21	3:2	4:1	0:2
Age, yr, median (range)†	41.0 (5.0-78.0)	42.0 (37.0-51.0)	53.0 (18.0-64.0)	18.5 (18.0-19.0)
Additional chromosomal abnormalities*	12/48 (25.0%)	0/5 (0.0%)	1/5 (20.0%)	0/2 (0.0%)
SCT during follow-up*	18/48 (37.5%)	3/5 (60.0%)	0/5 (0.0%)	1/2 (50.0%)
Relapse or death during follow-up*	10/48 (20.8%)	4/5‡ (80.0%)	1/5 (20.0%)	2/2 (100.0%)
Follow-up period, months, median (range)†	30.5 (0.0-109.0)	17.0 (6.0-35.0)	35.0 (0.0-82.0)	7.0 (4.0-10.0)
Laboratory findings at diagnosis†				
WBC ($\times 10^9$/L), median (range)	8.62 (1.20-102.37)	5.91 (2.90-8.40)	10.70 (3.20-192.90)	14.27 (3.20-25.33)
Hemoglobin (g/dL), median (range)	8.5 (2.3-13.2)	6.3 (5.6-8.4)	8.4 (6.5-12.3)	7.1 (5.4-8.7)
Platelets ($\times 10^9$/L), median (range)	34.5 (3.0-155.0)	24.0 (9.0-59.0)	35.0 (8.0-59.0)	21.5 (11.0-32.0)
PB blasts (%), median (range)	23.0 (0.0-86.0)	45.0 (13.0-54.0)	52.0 (15.0-92.0)	37.0 (27.0-47.0)
BM blasts (%), median (range)	44.0 (21.0-90.0)	58.0 (30.0-81.0)	60.0 (21.0-89.0)	62.0 (44.0-80.0)

P values were obtained using Chi-square or Fisher's exact tests (for numbers less than five in each group)* and Mann-Whitney U test†. In patients without *WT1* mutation, those with *c-KIT* mutation experienced relapse or died during follow-up more frequently than those without *c-KIT* mutation (P=0.014). Comparison items which showed statistically significant differences between two patient subgroups were indicated with superscript (‡).
Abbreviations: SCT, stem cell transplantation; WBC, white blood cell; PB, peripheral blood; BM, bone marrow; *WT*, Wilms' tumor.

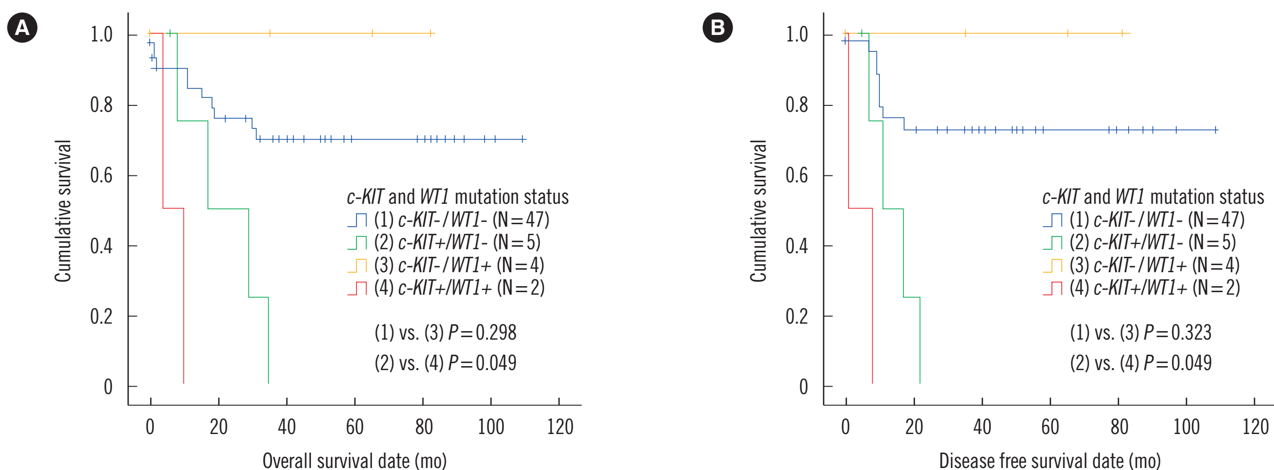

Fig. 2. Comparisons of overall survival and disease free survival in core binding factor acute leukemia patients with t(8;21) and no *FLT3* ITD or *NPM1* mutations, among four patient subgroups categorized by *c-KIT* and *WT1* mutation status (N=58, A, overall survival; B, disease free survival).
Abbreviation: WT, Wilms' tumor.

and 17, respectively. The mutations detected included four previously reported (c.1250_1255delCTTACG, c.2447A>T, c.2446G>T, and c.2446G>C), and one novel mutation (c.1256_1257insTTTTCGA). In the *CEBPA* gene, no mutations were detected but a known polymorphism (c.584_589dup ACCCGC) [23] was observed in 26 (28.3%) patients. In the case of *IDH2*, only two patients harbored a mutation, which was previously reported (c.419G>A), and for *DNMT3A*, four patients showed mutations, both of which were reported previously (c.2638A>C and c.2644C>T). Regarding *EZH2*, three previously reported mutations (c.1978G>A, c.1996T>C, and c.2068C>T) were found in four patients, and one previously reported intronic variant (c.2110+6T>G), predicted as being benign in the ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar/variation/137273/, last reviewed on 14 Mar 2014), was detected in one patient. One previously reported *CBL* mutation (c.1196T>C) was detected in one

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patient. Analysis of *WT1* revealed six previously reported mutations (c.1102G>A, c.1105C>G, c.1141T>A, c.1147T>A, c.1372C>T, and c.1379T>A) and two novel mutations (c.1112C>T and c.1131_1132insT) in 11 patients.

In summary, the present study identified 20 mutations (17 known and three novel mutations) in 32 patients, one intronic variation of the *EZH2* gene from one patient, and one polymorphism of the *CEBPA* gene in 26 patients (Table 2).

3. Comparison of clinical features and prognoses between patients with or without *c-KIT* or *WT1* mutations

With regard to patients with t(8;21), clinical features did not differ significantly between patients with *c-KIT* mutations and those without. However, those with such mutations experienced relapse or died during follow-up more frequently than those without (85.7% vs. 25.0%, $P=0.003$), and both OS and DFS lengths were significantly shorter in patients with *c-KIT* mutations than in those without ($P<0.001$ for both). Neither clinical features nor prognoses differed significantly with respect to *WT1* mutation status.

As for patients with inv(16), neither *c-KIT* nor *WT1* mutation status significantly affected clinical features and prognoses, with the exception of significantly lower platelet counts in patients with *c-KIT* mutations than in those without (median 15.0×10^9/L vs. 34.5×10^9/L, $P=0.024$; Table 3 and Fig. 1).

4. Comparison of clinical features and prognoses in patients with t(8;21) among four patient subgroups categorized by *c-KIT* and *WT1* mutation status

Within the subgroup showing no *WT1* mutation, the patients harboring *c-KIT* mutations were more likely to experience relapse or die during follow-up than those without *c-KIT* mutations (80.8% vs. 20.8%, $P=0.014$). However, this difference was not statistically significant (100.0% vs. 20.0%, $P=0.143$) for the patient subgroup with *WT1* mutations, which suggests that the poor prognostic impact of *c-KIT* mutation in AML patients with t(8;21) may apply only in patients with wild type *WT1*.

Regarding the subgroup showing *c-KIT* mutations, patients with *WT1* mutations did not show significant differences in clinical outcomes, including relapse or death during follow-up (100.0% vs. 80.0%, $P=0.495$), compared with those without *WT1* mutation. In the patient subgroup harboring *c-KIT* mutations, although the survival analysis showed significantly shorter OS and DFS in patients with *WT1* mutations than in those without *WT1* mutations ($P=0.049$ in both), the statistical power of this analysis was seriously limited due to the very low number of patients in each group. In the subgroup without *c-KIT* mutations, the patients with *WT1* mutations also showed no differences in clinical outcomes, including rate of relapse or death during follow-up (20.0% vs. 20.8%, $P=0.965$), and prognosis ($P=0.298$ for OS and $P=0.323$ for DFS), compared with those without *WT1* mutation (Table 4 and Fig. 2).

DISCUSSION

The present study found that the incidences of *FLT3* ITD and *NPM1* mutations were extremely low, representing 3.3% and 1.1% respectively of the CBF AML patients evaluated, and these results are consistent with previous studies, which reported similarly low frequencies of *FLT3* ITD mutation (5 to 6%) and *NPM1* mutation (0%) in cases of CBF AML [19, 21, 24]. Our study also demonstrated that patients with this disease are unlikely to harbor *CEBPA* mutations. These results indicate that *FLT3* ITD, *NPM1*, and *CEBPA* mutations are not involved in leukemogenesis and imply no prognostic impact for CBF AML. Our data also support previous studies that have implicated mutations in both *RAS* and *c-KIT* as major leukemogenic factors in CBF AML [21, 25].

In addition, our study suggests that the frequencies of mutation in *CBL* and in genes associated with epigenetic modification, such as *IDH1*, *IDH2*, *DNMT3A*, and *EZH2*, are low in this disease, present in 1.3%, 0%, 2.5%, 5.0%, and 5.0% of the total number of patients, respectively. These results may support the idea that mutations involved in epigenetic modification do not contribute to leukemogenesis and have no significant prognostic value in CBF AML, since *IDH2*, *DNMT3A*, *EZH2*, and *CBL* mutation status did not significantly affect prognosis in our study (data not shown). In contrast, the present study showed that incidences of *c-KIT* and *WT1* mutation in CBF AML were relatively higher than the mutations in other genes, being found in 10.9% and 13.8% of patients, respectively. Our results confirm the consistency of *c-KIT* mutation frequency in CBF AML between previous publications (6 to 48%) and Korean population [17-21].

The present work demonstrated that the poor prognostic impact of *c-KIT* mutation in patients carrying t(8;21) was evident only in the subgroup lacking *WT1* mutations. Although this trend was also evident in the patient subgroup with *WT1* mutations, the difference was not statistically significant. We also found that *WT1* mutation status does not affect clinical features and prognosis, regardless of *c-KIT* mutation status, in patients with t(8;21). Within the subgroup harboring *c-KIT* mutations, a

worse prognosis was identified for patients with *WT1* mutations compared with those without *WT1* mutations, but the statistical power of this result is seriously limited owing to the fact that only seven patients were included in the survival analysis.

On the basis of these results, we can speculate that both *c-KIT* and *WT1* mutations constitute important genetic aberrations involved in leukemogenesis of CBF AML and show relatively high incidences in these patients. In addition, *c-KIT* mutation is a significant indicator of poor prognosis in CBF AML patients carrying t(8;21), but this prognostic value may exist only for a specific patient subgroup without *WT1* mutations. It may be suggested that the presence of *WT1* mutations does not affect clinical features and prognosis significantly, but further analysis involving a larger number of patients is required to address this point more conclusively.

The present study has two major limitations. First, since only 21 patients with inv(16) were included, comparisons of clinical features and prognoses for inv(16) patients with respect to *c-KIT* and *WT1* mutation status could not be performed with sufficient statistical power. Given that there were also only 71 patients carrying t(8;21) in our study, suggestions and speculations based on this work should be interpreted with much caution. Second, we were unable to evaluate the effect of *WT1* mutation on the expression of the corresponding WT1 protein, which is necessary to interpret our results at the protein expression level. Since previous studies involved evaluation of protein expression, stratification of patients based only on *WT1* mutation status, as performed in the present study, our results need to be interpreted carefully. More focused analysis on this point is required in future studies.

In conclusion, our study demonstrates that the incidences of genetic mutations associated with epigenetic modification are very low and that both *c-KIT* and *WT1* mutations occur more frequently than other mutations in CBF AML. In addition, our results suggest that the poor prognostic impact of *c-KIT* mutation in t(8;21)-positive CBF AML patients may apply only to a specific patient subgroup without *WT1* mutations. The poor prognostic impact of *WT1* mutation was not evident in t(8;21)-positive CBF AML patients owing to the small number of patients in this study; further study will be required to confirm this speculation in a large number of patients.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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