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ORIGINAL ARTICLE

# Prognostic implications of *CEBPA* mutations in pediatric acute myeloid leukemia: a report from the Japanese Pediatric Leukemia/Lymphoma Study Group

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CCAAT/enhancer-binding protein alpha (*CEBPA*) mutations are a favorable prognostic factor in adult acute myeloid leukemia (AML) patients; however, few studies have examined their significance in pediatric AML patients. Here we examined the *CEBPA* mutation status and clinical outcomes of pediatric AML patients treated in the AML-05 study. We found that 47 (14.9%) of the 315 evaluable patients harbored mutations in *CEBPA*; 26 cases (8.3%) harbored a single mutation (*CEBPA*-single) and 21 (6.7%) harbored double or triple mutations (*CEBPA*-double). After excluding core-binding factor-AML cases, patients harboring *CEBPA* mutations showed better overall survival (OS; $P = 0.048$), but not event-free survival (EFS; $P = 0.051$), than wild-type patients. Multivariate analysis identified *CEBPA*-single and *CEBPA*-double as independent favorable prognostic factors for EFS in the total cohort (hazard ratio (HR): 0.47 and 0.33; $P = 0.02$ and 0.01, respectively). *CEBPA*-double was also an independent favorable prognostic factor for OS (HR: 0.30; $P = 0.04$). *CEBPA*-double remained an independent favorable factor for EFS (HR: 0.28; $P = 0.04$) in the normal karyotype cohort. These results suggest that *CEBPA* mutations, particularly *CEBPA*-double, are an independent favorable prognostic factor in pediatric AML patients, which will have important implications for risk-stratified therapy.

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## INTRODUCTION

CCAAT/enhancer-binding protein alpha (CEBPA) is a transcription factor that co-ordinates cellular differentiation. CEBPA is expressed in myeloid precursors during hematopoiesis, where it regulates the expression of several granulocyte-specific genes.[1] CEBPA inhibits E2F pathways, thereby downregulating c-Myc and allowing myeloid precursors to enter the granulocytic differentiation pathway.[2,3] The *CEBPA* gene is located on chromosome 19 band q13.1. Approximately 10% of acute myeloid leukemia (AML) patients harbor mutations in *CEBPA* genes, and these mutations can occur across the whole gene, but there are two main hotspots.[4,5] N-terminal out-of-frame mutations are located between the major translational start site and a second ATG further downstream. They abolish translation of the full-length p42 isoform of CEBPA, leading to overexpression of a shorter dominant-negative p30 isoform.[6] C-terminal mutations are generally in-frame insertions/deletions located in the basic leucine zipper (bZIP) domain; these mutations disrupt binding to DNA or dimerization.[7] Most AML patients with double *CEBPA* mutations harbor both N- and C-terminal mutations, which are typically present on different alleles; however, homozygous mutations have also been described.[8]

*CEBPA* mutations are a favorable prognostic factor for AML, particularly in patients harboring double *CEBPA* mutations and a normal karyotype.[8–13] However, the prognostic value of *CEBPA* mutations has been studied mostly in adult AML patients, with few studies examining mutations in pediatric AML patients. The first set of pediatric data was presented by the Taiwan Pediatric Oncology Group, but the report lacked data regarding clinical outcome.[14] The prognostic impact of *CEBPA* in pediatric AML was reported by two other groups, namely, the Children's Oncology Group and the Dutch Childhood Oncology Group/the Berlin–Frankfurt–Münster Study Group,[15,16] which both reported that, after excluding core-binding factor (CBF)-AML cases, patients harboring *CEBPA* mutations had a significantly better clinical outcome than those harboring the wild-type (WT) gene; however, the clinical implications of single vs double mutations were unclear. A more recent study conducted by the Nordic Society of Pediatric Hematology and Oncology suggests that *CEBPA* mutations in pediatric AML patients are not associated with improved survival;[17] thus the clinical significance of *CEBPA* mutations in pediatric AML patients is unclear. Although we previously reported the characteristics of *CEBPA* mutations in Japanese children with AML, the small sample size meant that further study was required.[18]

Here we examined the *CEBPA* mutation status and clinical outcomes of pediatric AML patients treated in the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) AML-05 study.

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## SUBJECTS AND METHODS

### Patients and study protocol

The AML-05 study is a Japanese nationwide multi-institutional study of children (age <18 years) with *de novo* AML, all of whom were enrolled between 1 November 2006 and 31 December 2010. The trial is registered with the UMIN Clinical Trials Registry (UMIN–CTR; http://www.umin.ac.jp/ctr/index.htm; number UMIN000000511).

In all, 485 patients with suspected AML (diagnosed at 118 centers and hospitals in Japan) were registered in the AML-05 study. Patients with acute promyelocytic leukemia, Down's syndrome, secondary AML, myeloid/natural killer cell leukemia and myeloid sarcoma, were not eligible. Overall, 38 patients were excluded, mainly because of misdiagnosis, while four additional patients were excluded for the following reasons: the patient's guardian refused permission to participate ($n=1$); there was a significant protocol violation during the initial induction course ($n=1$); the hospital withdrew from the JPLSG ($n=1$); and the patient was transferred to a non-JPLSG member hospital ($n=1$). Patients were stratified into three risk groups according to specific cytogenetic characteristics and morphological responses to treatment. CBF-AML patients were assigned to the low-risk group; those with unfavorable cytogenetics ($-7$, 5q-, t(16;21)(p11;q22), Ph1, Fms-like tyrosine kinase 3 internal tandem duplications (*FLT3*-ITD)) and poor induction responders were assigned to the high-risk group; and the rest were assigned to the intermediate-risk group. Details of the patient disposition, treatment schedules and risk stratification have been described previously.[19,20] In the present study, morphology was diagnosed prospectively using a central review system. Cytogenetic tests were performed in regional laboratories, but the reports were reviewed centrally. The study was conducted in accordance with the principles set down in the Declaration of Helsinki and was approved by the Ethics Committees of all participating institutions. All patients, or the patients' parents/guardians, provided written informed consent.

### Mutation analysis

cDNA was synthesized from RNA obtained from diagnostic bone marrow samples using the Omniscript Reverse Transcription Kit (Qiagen, Chatsworth, CA, USA), according to the manufacturer's recommendations. The entire coding region of the *CEBPA* gene was amplified using the overlapping PCR primer pairs followed by direct sequencing, as previously described.[6,18]

### Statistical analysis

Patient characteristics were analyzed using Fisher's exact test (categorical variables) and the Kruskal–Wallis test (continuous variables). Event-free survival (EFS) was defined as the time from the diagnosis of AML to the last follow-up or the first event (failure to achieve remission, relapse, secondary malignancy or any-cause death). Overall survival (OS) was defined as the time from the diagnosis of AML to any-cause death. The Kaplan–Meier method was used to estimate EFS and OS, and data were compared using the log-rank test. To determine the prognostic value of *CEBPA* mutation, Cox regression analysis was performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). All tests were two-tailed, and a *P*-value $<0.05$ was considered statistically significant.

## RESULTS

### Mutation analysis

Diagnostic samples from 315/443 (71.1%) eligible AML patients were analyzed for *CEBPA* mutations; *CEBPA* data were unavailable for 128 patients. There were no significant differences in the major characteristics or clinical outcomes of the 315 patients and the 128 patients for whom no data were available (EFS $P=0.78$, OS $P=0.30$). We found that 47/315 patients (14.9%) harbored a mutation in *CEBPA*, 26 (8.3%) harbored a single mutation and 21 (6.7%) harbored double or triple mutations. The location and combination pattern of all the detected mutations are shown in Figure 1 and Table 1. Single mutations were distributed across the entire gene, but most in-frame insertions/deletions were located in the bZIP domain. By contrast, double or triple mutations were clustered in the N- and C-terminal hotspots. Thirteen out of the 21 cases (61.9%) harbored both an N-terminal out-of-frame mutation and an in-frame mutation in the bZIP domain, which were predicted to result in a lack of WT CEBPA p42 expression. We identified five patients with triple mutations but could not exclude the possibility that these mutations occurred in different cells. Moreover, the method we used cannot identify whether mutations are located on different alleles. Further study is required to overcome these limitations.

### Polymorphisms in the *CEBPA* mutations

Overall, 131 patients (41.6%) harbored an in-frame 6-bp insertion (ACCCGC) in the transactivation domain 2 (TAD2), resulting in a His-Pro duplication (HP196–197 insertion). This mutation is observed in approximately 10% of healthy controls and AML patients and is reported as a germline polymorphism.[21,22] We did not identify any differences in characteristics between the HP196–197 insertion-positive and -negative groups, and the clinical outcomes of both groups were similar (data not shown). Therefore, we ignored this mutation during our analysis of clinical outcome, along with other mutations that did not result in amino-acid changes.

### Patient characteristics

Patient characteristics according to *CEBPA* mutation status are shown in Table 2. Patients harboring a single *CEBPA* mutation were described as '*CEBPA*-single' and those harboring double or triple *CEBPA* mutations were described as '*CEBPA*-double'. *CEBPA*-double patients showed a significantly higher percentage of M1 or M2 French–American–British subtypes ($P<0.001$). Compared with WT patients, patients with *CEBPA* mutations were older ($P=0.03$) at the time of diagnosis. *CEBPA* mutations were predominant in those with an intermediate risk ($P=0.002$) and a normal karyotype ($P<0.001$). There was a well-balanced gender distribution ($P=0.84$), and there were no significant differences in the number of patients with *FLT3*-ITD and *NPM1* mutations among the three *CEBPA* subgroups.

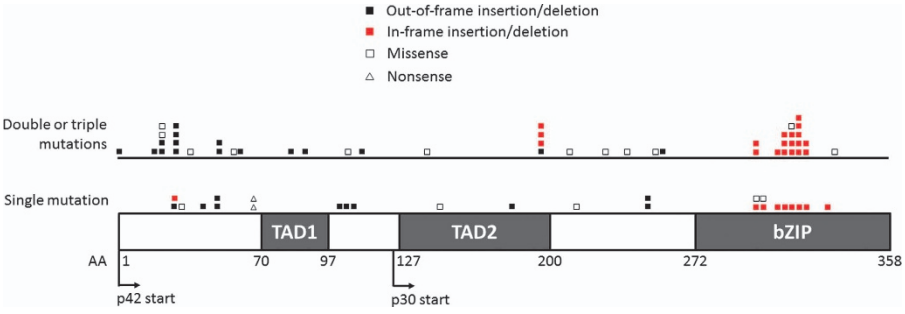


**Figure 1.** Location and type of the mutations detected in pediatric AML patients enrolled in the AML-05 study. AA, amino acid; BZIP, basic leucine zipper; TAD, transactivation domain.

**Table 1.** Summary of *CEBPA* mutations detected in 315 acute myeloid leukemia patients

| *Mutation status* | *Mutation 1* | | | *Mutation 2 (Mutation 3)* | | | |
|---|---|---|---|---|---|---|---|
| | *N-terminal* AA 1-120 | *Middle* AA 121-277 | *C-terminal* AA 278-358 | *N-terminal* AA 1-120 | *Middle* AA 121-277 | *C-terminal* AA 278-358 | *No. of patients* |
| *CEBPA*-single | Out-of-frame ins/del | | | | | | 7 |
| | In-frame ins/del | | | | | | 1 |
| | Missense | | | | | | 1 |
| | Nonsense | | | | | | 2 |
| | | Out-of-frame ins/del | | | | | 3 |
| | | Missense | | | | | 2 |
| | | | In-frame ins/del | | | | 8 |
| | | | Missense | | | | 2 |
| Total | | | | | | | 26 |
| *CEBPA*-double | Out-of-frame ins/del | | | | | In-frame ins/del | 11 |
| | Missense | | | Missense | | | 1 |
| | Missense | | | | Missense | | 1 |
| | Missense | | | | | Missense | 1 |
| | | Out-of-frame ins/del | | | | In-frame ins/del | 2 |
| | Out-of-frame ins/del | | | Missense | (In-frame ins/del) | | 1 |
| | Out-of-frame ins/del | | | | In-frame ins/del | (In-frame ins/del) | 2 |
| | | Missense | | | Missense (Missense) | | 1 |
| | | Missense | | | | Missense (In-frame ins/del) | 1 |
| Total | | | | | | | 21 |

Abbreviations: AA, amino acid; *CEBPA*, CCAAT/enhancer-binding protein alpha; del, deletion; ins, insertion. Note: Patients harboring a single *CEBPA* mutation were described as *CEBPA*-single and those harboring double or triple *CEBPA* mutations were described as *CEBPA*-double.

### Prognostic impact of *CEBPA* in the total cohort

We first analyzed the clinical outcomes of patients harboring *CEBPA* mutations and then compared them with the outcomes of CBF-AML patients and patients without CBF or *CEBPA* mutations (denoted 'WT non-CBF') (Figure 2). The CBF-AML group included AML patients harboring t(8;21)(q22;q22) along with inv(16)(p13.1q22) or its variant t(16;16)(p13.1;q22). Seven CBF-AML patients harboring *CEBPA* mutations were categorized as '*CEBPA*-mutant'. Patients harboring *CEBPA* mutations showed better OS ($P = 0.048$), but not EFS ($P = 0.051$), than WT non-CBF patients (Figures 2a and b). However, patients with *CEBPA* mutations showed poorer OS ($P = 0.0006$) than patients with CBF-AML. Furthermore, we examined whether the number of *CEBPA* mutations had an impact on prognosis (Figures 2c and d). *CEBPA*-double patients did not show significantly better EFS and OS than *CEBPA*-single patients ($P = 0.33$ each). There was also no significant difference in EFS and OS between *CEBPA*-double patients and WT non-CBF patients ($P = 0.055$ and $P = 0.057$, respectively).

### Prognostic impact of *CEBPA* in the normal karyotype cohort

We next examined prognosis in the normal karyotype cohort, because *CEBPA* mutations have been described as a favorable prognostic factor, particularly in cytogenetically normal AML (Figure 3). There was no significant difference in EFS and OS between *CEBPA*-double patients and WT or *CEBPA*-single patients (EFS: *CEBPA*-double vs WT, $P = 0.15$; *CEBPA*-double vs *CEBPA*-single, $P = 0.21$; OS: *CEBPA*-double vs WT, $P = 0.28$; *CEBPA*-double vs *CEBPA*-single; $P = 0.44$). Patients with *CEBPA*-single showed almost identical EFS ($P = 0.97$) and OS ($P = 0.77$) to those of WT patients.

### Prognostic impact of *CEBPA* mutation type

We also examined the prognostic impact of the location of the *CEBPA* mutations, which has never been examined in pediatric AML patients. Only patients with hotspot mutations predicted to cause translation of the p30 isoform and/or disruption or loss of the C-terminal bZIP domain were included in the analysis. In the total cohort, patients with an N-terminal out-of-frame mutation and a C-terminal in-frame mutation ($n = 13$, denoted as *CEBPA*-double N + C-term) had significantly better EFS ($P = 0.01$), but not OS ($P = 0.06$), than WT non-CBF patients (Figures 4a and b). This patient group also had significantly better EFS, but not OS, than other *CEBPA*-double patients ($n = 8$), suggesting that a combination of N-terminal and C-terminal mutations results in a better prognosis for *CEBPA*-double patients (data not shown). We also investigated differences in outcome between *CEBPA*-single patients with an N-terminal mutation and those with a C-terminal mutation and found that the clinical outcomes were nearly identical. In the normal karyotype cohort, we found no significant difference in the outcome of four groups: patients with an N-terminal out-of-frame mutation, patients with a C-terminal in-frame mutation, patients with an N-terminal out-of-frame mutation and a C-terminal in-frame mutation, and WT patients, which may be due to the small sample size (Figures 4c and d).

### Multivariate analysis

Multivariate Cox regression analysis, including age and white blood cell count at the time of diagnosis, was performed to examine whether *CEBPA* mutations were a favorable prognostic factor (Table 3). *FLT3*-ITD and *NPM1* mutations were not included as variables owing to the small number of positive cases and

**Table 2.** Characteristics of patients categorized according to *CEBPA* mutation status

| | *Total* | *WT* | *CEBPA-single* | *CEBPA-double* | P-*value* |
|---|---|---|---|---|---|
| Number | 315 | 268 | 26 | 21 | |
| *Age, years* | | | | | 0.03[b] |
| Median | 7.9 | 7.6 | 9.9 | 9.6 | |
| Range | 0.0–17.5 | 0.0–17.5 | 0.3–16.2 | 1.3–15.9 | |
| *Sex,* n *(%)* | | | | | 0.84[a] |
| Male | 167 (53) | 144 (54) | 13 (50) | 10 (48) | |
| Female | 148 (47) | 124 (46) | 13 (50) | 11 (52) | |
| *WBC ($\times 10^9$/l)* | | | | | 0.052[b] |
| Median | 57.8 | 52.6 | 58.1 | 124 | |
| Range | 0.8–985 | 0.8–552 | 1.9–381 | 3.9–985 | |
| *Risk groups,* n *(%)* | | | | | 0.002[a] |
| Low | 87 (28) | 81 (30) | 4 (15) | 2 (10) | |
| Intermediate | 132 (42) | 101 (38) | 15 (58) | 16 (76) | |
| High | 43 (14) | 37 (14) | 6 (23) | 0 (0) | |
| Unclassified | 53 (17) | 49 (18) | 1 (4) | 3 (14) | |
| *FAB,* n *(%)* | | | | | $<0.001$[a] |
| M0 | 7 (2) | 7 (3) | 0 (0) | 0 (0) | |
| M1 | 43 (14) | 28 (10) | 5 (19) | 10 (48) | |
| M2 | 79 (25) | 63 (24) | 9 (35) | 7 (33) | |
| M4 | 52 (17) | 46 (17) | 6 (23) | 0 (0) | |
| M5 | 70 (22) | 67 (25) | 1 (4) | 2 (10) | |
| M6 | 8 (3) | 5 (2) | 2 (8) | 1 (5) | |
| M7 | 31 (10) | 29 (11) | 2 (8) | 0 (0) | |
| RAEB-T | 25 (8) | 23 (9) | 1 (4) | 1 (5) | |
| *Karyotype,* n *(%)* | | | | | $<0.001$[a] |
| Normal | 62 (20) | 35 (13) | 14 (54) | 13 (62) | |
| t(8;21) | 75 (24) | 69 (26) | 3 (12) | 3 (14) | |
| inv(16) | 25 (8) | 24 (9) | 1 (4) | 0 (0) | |
| 11q23 | 48 (15) | 47 (18) | 0 (0) | 1 (5) | |
| other | 105 (33) | 93 (35) | 8 (31) | 4 (19) | |
| *Molecular abnormalities,* n *(%)* | | | | | |
| *FLT3*-ITD | 42 (13) | 35 (13) | 6 (23) | 1 (5) | 0.21[a] |
| *NPM1* | 12/167 (7) | 10/128 (8) | 2/22 (9) | 0/17 (0) | 0.59[a] |

Abbreviations: *CEBPA*, CCAAT/enhancer-binding protein alpha; FAB, French–American–British; *FLT3*-ITD, Fms-like tyrosine kinase 3 internal tandem duplications; *NPM1*, nucleophosmin; WBC, white blood cell count; WT, wild type. [a]Fisher's exact test. [b]Kruskal–Wallis test.

because no statistically significant differences were detected by univariate analysis. For the total cohort ($n=315$), multivariate analysis identified both *CEBPA*-single and *CEBPA*-double as independent favorable prognostic factors for EFS (hazard ratio (HR): 0.47 and 0.33; $P=0.02$ and 0.01, respectively; upper column, Table 3). C*EBPA*-double was also identified as an independent favorable prognostic factor for OS (HR: 0.30; $P=0.04$). For the normal karyotype cohort ($n=62$), *CEBPA*-double was also identified as an independent prognostic factor for favorable EFS (HR: 0.28; $P=0.04$; lower column, Table 3). This may indicate that other factors, such as age and white blood cell count, had masked the benefit of *CEBPA* mutations in the univariate analysis.

## DISCUSSION

Here we examined *CEBPA* mutations in 315 pediatric AML patients enrolled in the AML-05 study. We detected *CEBPA* mutations in 47 patients (14.9%), which is comparable to the reported frequency in adult and pediatric AML patients (approximately 10%).[8–17] In all, 26 out of the 47 cases (55.3%) harbored a single *CEBPA* mutation; this percentage is higher than that reported in previous studies of pediatric AML patients.[15,16] We detected the HP196–197 insertion in 131/315 cases (41.6%). This well-known polymorphism was previously observed in approximately 10% of AML cases; thus the percentage identified in the present study was rather high.[21,22] Whether this polymorphism is also common in healthy Japanese populations remains to be seen. A recent study by a Korean group reported the incidence of this polymorphism as 30%; thus the frequency of this polymorphism may vary considerably according to geographical region.[23] The majority of *CEBPA*-double patients comprised M1 or M2 French–American–British subtypes, which is in agreement with the findings of previous studies.[15,16] *CEBPA* mutations were predominant in the intermediate risk and normal karyotype group, which is also consistent with previous findings.[15–17]

With regard to prognosis, the results presented herein suggest that *CEBPA* mutations, especially *CEBPA*-double, are an independent favorable prognostic factor in pediatric AML patients. Multivariate analysis of the normal karyotype cohort identified *CEBPA*-double as an independent favorable prognostic factor for EFS, but not OS; this finding may be due to the small sample size. As the majority of pediatric AML patients lack markers that indicate a favorable or poor prognosis, it is important to identify prognostic markers in intermediate-risk patients. *CEBPA* mutations show promise as markers of a favorable prognosis in pediatric AML patients, because they are strongly associated with intermediate risk.

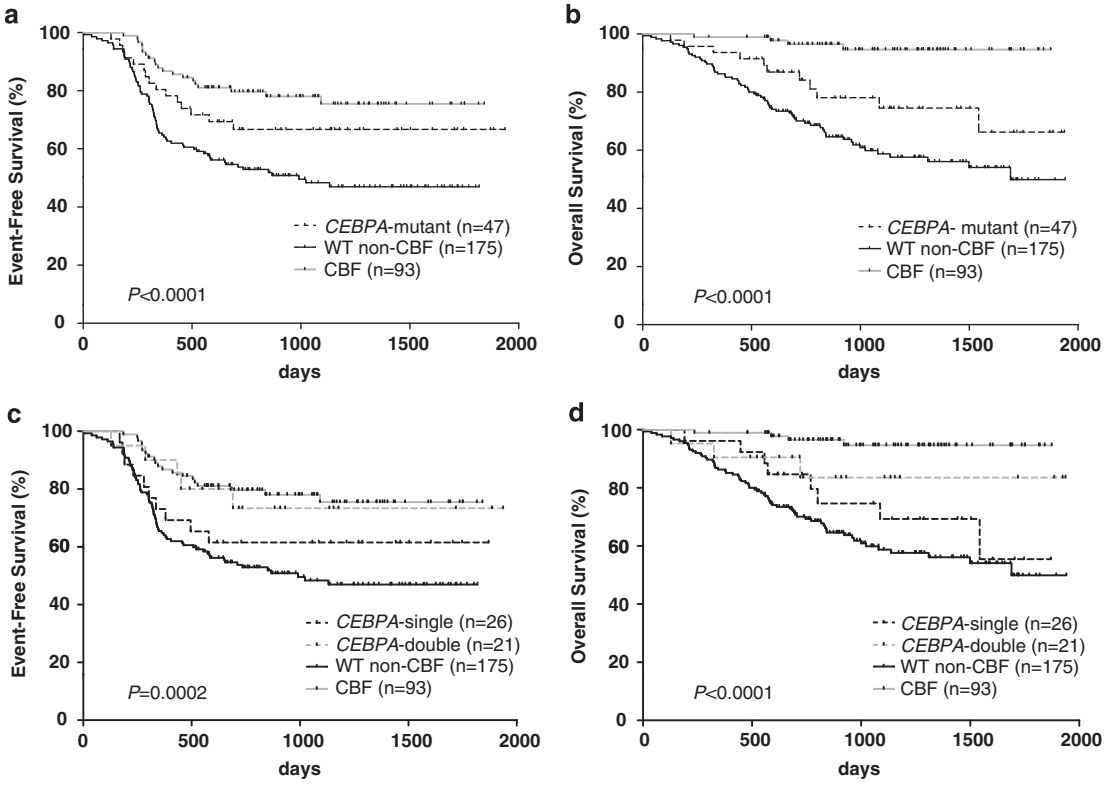


**Figure 2.** Kaplan–Meier survival curves showing EFS and OS from the time of diagnosis according to *CEBPA* mutation status. (**a**) EFS and (**b**) OS of patients harboring *CEBPA* mutations, patients harboring WT *CEBPA* (excluding core-binding factor-acute myeloid leukemia (CBF-AML) cases (WT non-CBF)) and patients with CBF-AML. (**c**) EFS and (**d**) OS of patients harboring a single *CEBPA* mutation (*CEBPA*-single), patients harboring double or triple *CEBPA* mutations (*CEBPA*-double), WT patients (excluding CBF-AML cases (WT non-CBF)) and patients with CBF-AML. *P*-values were determined using the log-rank test.

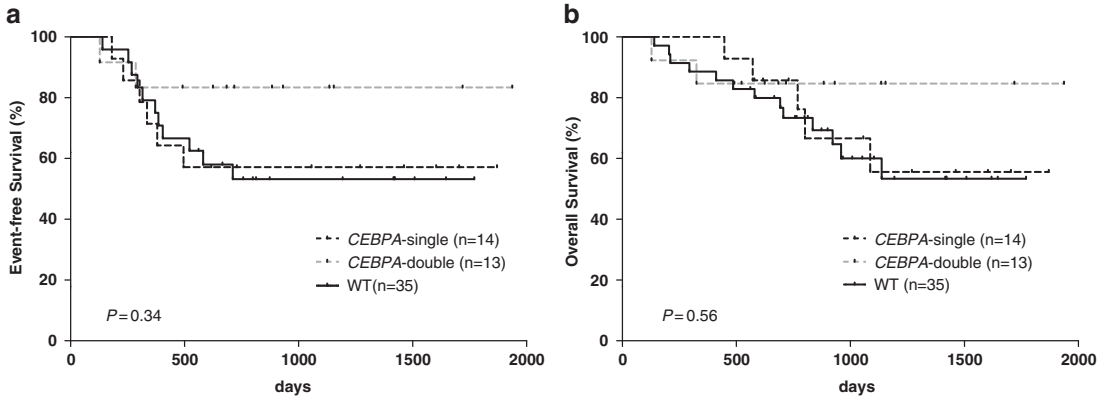


**Figure 3.** Kaplan–Meier survival curves showing EFS and OS in acute myeloid leukemia patients with a normal karyotype. (**a**) EFS and (**b**) OS of patients harboring a single *CEBPA* mutation (*CEBPA*-single), patients harboring double or triple *CEBPA* mutations (*CEBPA*-double) and WT patients. *P*-values were determined using the log-rank test.

Consistent with our results, several studies (including one pediatric study) postulated that AML patients harboring double *CEBPA* mutations have a favorable prognosis.[9–12] Two different *CEBPA* mutations have a synergistic effect on AML development, and the mechanism underlying leukemogenesis is likely to be different from that in AML patients harboring a single *CEBPA* mutation.[24,25] We found that a combination of N-terminal and C-terminal mutations is essential for a better prognosis in *CEBPA*-double patients (data not shown), indicating that a favorable prognosis is restricted in patients who lack WT CEBPA p42 expression among *CEBPA*-double patients. Moreover, a recent study of a large cohort of adult AML patients suggests that patients harboring double *CEBPA* mutations belong to a genetically distinct subtype and should be clearly distinguished from patients harboring a single mutation.[13] In this study, we could not examine the prognostic impact of concomitant molecular mutations because of their low incidence; therefore further analyses of pediatric AML patients is required.

In contrast to double *CEBPA* mutations, the prognostic value of single *CEBPA* mutation is currently under debate because of its small number. We detected a relatively large number of cases harboring a single *CEBPA* mutation in the total cohort, and

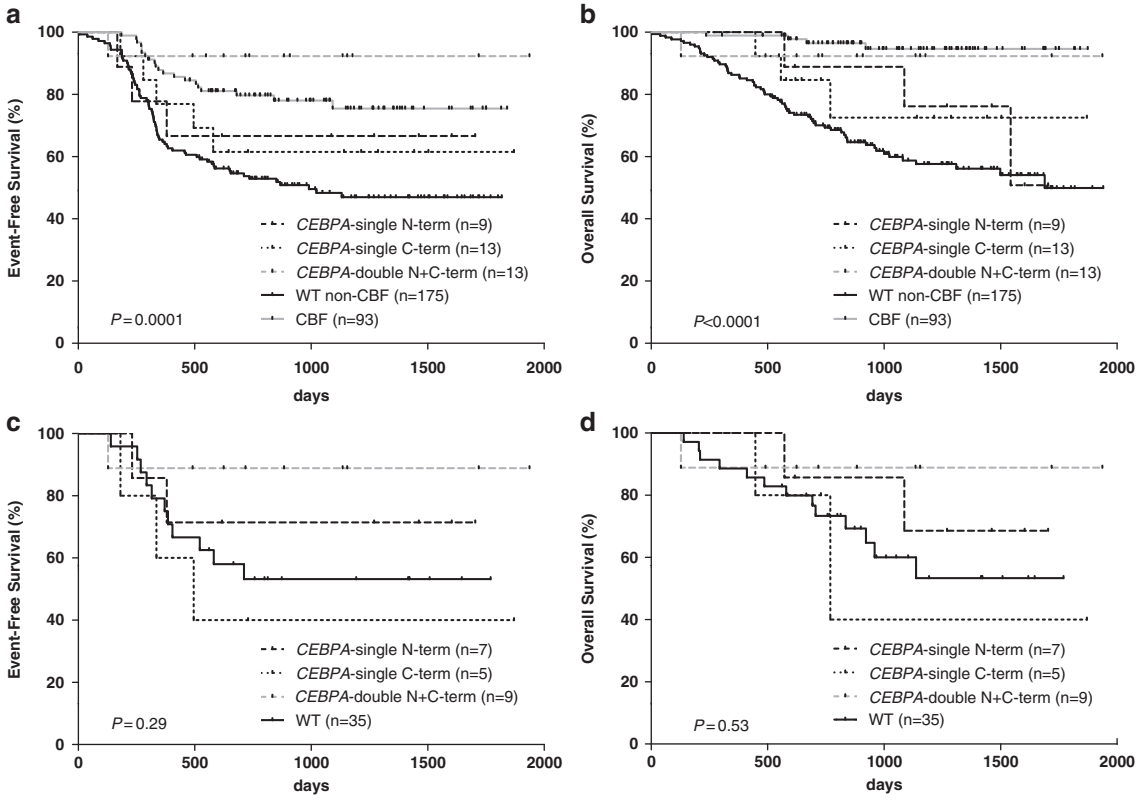


**Figure 4.** Kaplan–Meier survival curves showing EFS and OS according to the location and number of *CEBPA* mutations. Only patients with hotspot mutations predicted to cause p30 isoform translation and/or disruption or loss of the C-terminal bZIP domain were included in the analysis. (**a**) EFS and (**b**) OS in patients harboring a single N-terminal mutation (*CEBPA*-single N-term), patients harboring a single C-terminal mutation (*CEBPA*-single C-term), patients harboring both N and C-terminal mutations (*CEBPA*-double N + C-term), WT patients (excluding core-binding factor-acute myeloid leukemia (CBF-AML) cases (WT non-CBF)) and patients with CBF-AML. (**c**) EFS and (**d**) OS of AML patients with a normal karyotype. *P*-values were determined using the log-rank test.

**Table 3.** Multivariate Cox regression analysis for EFS and OS

| | *EFS* | | | *OS* | | |
|---|---|---|---|---|---|---|
| | *HR* | *95% CI* | P-*value* | *HR* | *95% CI* | P-*value* |
| *Total cohort* (n = *315)* | | | | | | |
| Mutation status, vs WT non-CBF | | | | | | |
| CBF | 0.31 | 0.19–0.49 | <0.01 | 0.09 | 0.03–0.25 | <0.01 |
| *CEBPA*-single | 0.47 | 0.24–0.91 | 0.02 | 0.60 | 0.29–1.26 | 0.18 |
| *CEBPA*-double | 0.33 | 0.14–0.76 | 0.01 | 0.30 | 0.09–0.94 | 0.04 |
| Age (+1 year) | 1.00 | 0.97–1.03 | 0.86 | 1.03 | 0.99–1.07 | 0.17 |
| WBC (⩾50 000) | 1.81 | 1.29–2.55 | <0.01 | 1.50 | 0.96–2.33 | 0.07 |
| *Normal karyotype cohort* (n = *62)* | | | | | | |
| Mutation status, vs WT | | | | | | |
| *CEBPA*-single | 0.54 | 0.22–1.33 | 0.18 | 0.88 | 0.31–2.47 | 0.81 |
| *CEBPA*-double | 0.28 | 0.08–0.95 | 0.04 | 0.49 | 0.11–2.17 | 0.35 |
| Age (+1 year) | 0.95 | 0.89–1.02 | 0.14 | 0.94 | 0.86–1.02 | 0.13 |
| WBC (⩾50 000) | 2.05 | 1.00–4.22 | 0.05 | 1.34 | 0.55–3.29 | 0.52 |

Abbreviations: CBF, core-binding factor; *CEBPA*, CCAAT/enhancer-binding protein alpha; CI, confidence interval; EFS, event-free survival; HR, hazard ratio; OS, overall survival; WBC, white blood cell count; WT, wild type.

multivariate analysis identified single mutation as an independent prognostic factor for favorable EFS (Table 3). Two adult AML studies (but no pediatric studies) showed that a single *CEBPA* mutation can be an independent favorable prognostic factor in patients harboring *NPM1* mutations.[26,27] Indeed, the two patients in the present study that harbored both a single *CEBPA* mutation and an *NPM1* mutation showed good long-term survival without any events. We also tried to examine the clinical significance of the location of the mutation in *CEBPA*-single patients but found no significant difference in outcomes for patients harboring N-terminal or C-terminal mutations. However, the *CEBPA*-single patients in the normal karyotype cohort who harbored a

C-terminal mutation may have slightly poorer EFS and OS than those who harbored an N-terminal mutation (Figures 4c and d), which is not consistent with previous adult AML studies.[12,13] Gene expression profiling suggests that *CEBPA*-single patients harboring a C-terminal mutation are more similar to *CEBPA*-double patients than to *CEBPA*-single patients harboring N-terminal mutations.[10] This latter study was performed in adult AML patients and needs to be validated in pediatric AML patients.

So far, the biological mechanisms underlying a favorable clinical outcome for AML patients harboring *CEBPA* mutations (including relative drug sensitivity) are not clear. Further studies of single and double *CEBPA* mutations and the underlying biology are required to enable better risk assessment and therapeutic approaches in pediatric AML.

## CONCLUSION

This is the first nationwide study to examine the clinical significance of *CEBPA* mutations in Japanese pediatric AML patients. The results suggest that *CEBPA* mutations, especially double or triple *CEBPA* mutations, are an independent favorable prognostic factor for pediatric AML patients. *CEBPA*-double patients should be stratified into the favorable risk group, and the prognostic significance of these mutations should be validated prospectively.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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