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ORIGINAL ARTICLE

WT1 mutation in pediatric patients with acute myeloid leukemia: a report from the Japanese Childhood AML Cooperative Study Group

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Abstract Mutations in *Wilms tumor 1* (*WT1*) have been reported in 10–22 % of patients with cytogenetically normal acute myeloid leukemia (CN-AML), but the prognostic implications of these abnormalities have not been clarified in either adults or children. One hundred and fifty-seven pediatric AML patients were analyzed for *WT1* mutations around hotspots at exons 7 and 9; however, amplification of the *WT1* gene by the reverse transcriptase-polymerase chain reaction was not completed in four cases (2.5 %). Of the 153 evaluable patients, 10 patients (6.5 %) had a mutation in *WT1*. The incidence of *WT1* mutations was significantly higher in CN-AML than in others (15.2 vs. 4.5 %, respectively, $P = 0.03$). Of the 10 *WT1*-mutated cases, eight (80 %) had mutations in other genes, including *FLT3*-ITD in two cases, *FLT3*-D835 mutation in two, *KIT* mutation in three, *MLL*-PTD in three, *NRAS* mutation in one, and *KRAS* mutation in two (in some cases, more than one additional gene was mutated). The incidences of *KIT* and *FLT3*-D835 mutations were significantly higher in patients with than in those without *WT1* mutation. No significant differences were observed in the 3-year overall survival and disease-free survival; however, the presence

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of *WT1* mutation was related to a poor prognosis in patients with CN-AML, excluding those with *FLT3*-ITD and those younger than 3 years.

Keywords Acute myeloid leukemia · *WT1* mutation · Cytogenetically normal acute myeloid leukemia · Prognosis

Introduction

The prognosis of pediatric acute myeloid leukemia (AML) patients has improved markedly over the past decade, with an overall survival rate of about 60–70 % according to the results of various clinical trials; however, relapse remains a major cause of treatment failure, occurring in 30–40 % of patients in their first complete remission (CR) [1–6]. Several study groups have shown that chromosome abnormalities are independent and strong predictors of the outcome in both childhood and adult AML [1, 4, 7]. On the other hand, cytogenetically normal acute myeloid leukemia (CN-AML) is the largest cytogenetic subgroup of AML, representing approximately 40 % of pediatric AML patients [7]. Recently, CN-AML has been recognized as highly heterogeneous molecularly, since several abnormalities were discovered, including mutations in *FLT3*, *NPM1*, *CEBPA*, and *MLL* genes and aberrant expression of *BAALC*, *ERG*, and *MN1* genes [8]. These alterations have been associated with the treatment outcome and serve as a basis for risk assessment in CN-AML [8, 9]. Discovering novel genetic markers may lead to an improvement in molecular risk stratification and allow a more accurate prediction of the response to therapy.

Wilms tumor 1 (*WT1*) is located at chromosome 11p13 [10] and encodes a transcription factor capable of activating or repressing gene transcription, depending on the cell type, WT1 protein isoform, and target gene [11]. Although initially considered a tumor suppressor gene [12], *WT1* has also been demonstrated to act as an oncogene [11, 13–15]. Mutations of the *WT1* gene have been reported in 10–22 % of cases of CN-AML in both adults [16–18] and children [19]. *WT1* gene mutations cluster to exons 7 and 9, and are associated with induction failure and/or relapse in adults and children [16–22]. However, there have been few reports on *WT1* gene mutation in pediatric AML patients. Thus, we performed mutational analysis of *WT1* in pediatric AML patients who were treated on the Japanese Childhood Cooperative Study Group Protocol, AML99 [5], and demonstrated that *WT1* mutations were related to a poor prognosis in patients older than 2 years with CN-AML excluding those with *FLT3*-ITD. Furthermore, we analyzed the association between *WT1* mutations and other gene aberrations including *RAS* and *KIT* mutations, *FLT3*-ITD, *FLT3*-D835, and *MLL*-PTD.

Materials and methods

Patients

The diagnosis of AML was based on the FAB classification, and cytogenetic analysis was performed using a routine G-banding method. From January 2000 to December 2002, 318 patients were newly diagnosed with de novo AML. Of these, samples from 157 patients were available for molecular analysis, including 13 with FAB-M3 and 10 with Down syndrome (DS), who were treated on different treatment protocols [5, 23–25]. There were no significant differences between the 134 patients without FAB-M3 or DS and the 106 non-analyzed patients in terms of the age [median 6 years (range 0–15 years) vs. 6 years (range 0–15 years), respectively] and initial WBC count [median 24.8×10^9/L (range 1.65-621.0 $\times 10^9$/L) vs. 13.8×10^9/L (range 1.0-489.0 $\times 10^9$/L, $P = 0.08$), respectively]. Patients who were younger than 2 years or had an initial WBC count <100,000/μL were treated using the induction A regimen [etoposide (VP-16), cytarabine, and mitoxantrone (MIT), (ECM)]. Patients who were older than 2 years and had an initial WBC count > 100,000/μL were treated using the induction B regimen [VP-16, cytarabine, and idarubicin (IDA), (ECI)]. If patients achieved a complete remission (CR), they were classified into three risk groups (62 low, 57 intermediate, and 10 high) according to the results of cytogenetic analyses or the achievement of CR after the 2 initial courses of chemotherapy [5, 23–25]. AML patients with t(8;21)(q22;q22) (except for those with WBC counts >50,000/μL) or inv(16)(p13q22) were classified into the low-risk (LR) group. Patients with monosomy 7, 5q-, t(16;21), or Philadelphia (Ph) chromosome were classified into the high-risk (HR) group. Patients were treated with additional chemotherapy or allogeneic hematopoietic stem cell transplantation (allo-HSCT) in each risk group.

Informed consent was obtained from the patients or their 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.

Detection of *WT1* mutations

Total RNA (4 μg) extracted from the bone marrow or peripheral blood samples at diagnosis was reverse transcribed to cDNA with a cDNA Synthesis Kit (GE Healthcare Japan Corporation, Tokyo, Japan). Mutations of exons 7 and 9 of the *WT1* gene were directly sequenced using the following primers: exon 7 WT1-1s 5′-TACG AGAGCGATAACCACAC-3′; exon 7 WT1-4as 5′-GTC CTTGAAGTCACACTGGT-3′; exon 9 WT1-3s 5′-ACCA GTGTGACTTCAAGGAC-3′; exon 9 WT1-2as 5′-TCAA AGCGCCAGCTGGAGTT-3′.

Table 1 Ten cases with *WT1* mutation in this study

No.	Age (year)	Sex	Karyotype	*WT1* mutation	*FLT3*-ITD or Mt	*KIT* Mt	*NRAS* Mt	*KRAS* Mt	*MLL*-PTD	*WT1* mRNA expression (copies/μgRNA)	Outcome [observation period (year)]
1	14	M	Normal	(1) 7 bp ITD in exon 7[a]	D835Y	Wt	Wt	G12D	Wt	65,000	5.6+[b]
				(2) H357R							
2	6	F	Normal	(1) R462W	Wt	Wt	Wt	Wt	PTD	21,000	1.4
3	8	F	t(8;21)	(1) W395X	Wt	A814S	Wt	Wt	Wt	1,400	3.0
				(2) 101 bp deletion in exons 9 to 10[a]							
4	13	F	t(8;21)	(1) 16 bp insertion in exon 9[a]	Wt	Wt	Wt	Wt	Wt	32,000	4.7+
5	14	M	Other[c]	(1) 115 bp deletion in exons 7 to 8[a]	D835Y	Wt	Wt	Wt	Wt	16,000	4.2+
				(2) 137 bp insertion and 110 bp deletion in exon 7[a]							
				(3) S381X							
6	12	M	Other[d]	(1) 11 bp ITD in exon 7[a]	Wt	Wt	Wt	Wt	PTD	7,000	4.5+
7	13	M	Normal	(1) P376L and 3 bp insertion and 8 bp ITD in exon 7[a]	ITD	V474L	Wt	Wt	PTD	18,000	4.4+
8	10	M	Normal	(1) 3 bp deletion and 4 bp insertion in exon 7[a]	ITD	Wt	Wt	Wt	Wt	14,000	1.7
9	3	F	Inv(16)	(1) 7 bp deletion and 2 bp insertion in exon 7[a]	Wt	Wt	G12D	Wt	Wt	12,000	3.6+
10	6	M	Normal	(1) 157 bp deletion in exons 8 to 10[a]	Wt	V540L	Wt	G13D	Wt	23,000	0.6

M male, *F* female, *Mt* mutation, *Wt* wild-type

[a] Resulting in the frameshift and premature stop codon

[b] Non-CR- > off study, but surviving

[c] 46,XY[2/8]/ 46,XY,del(6)(q15q21),-7,-9,-10,+3mar[1/8]/ 46,XY,?del(3)(p25)[1/8]/ 47,XY,-5,-8,-10,add(12)(q24.1),-16,-18,+6mar[1/8]/ 46,XY,-2,-6,-8,+3mar[1/8]/ 46,XY,-8,+mar[1/8]/ 46,Y,?add(X)(p11.2)[1/8]

[d] 46,XY,t(1;7;11)(q32;p15;p15)[14/20]/ 46,XY[6/20]

Detection of *FLT3*-ITD, *FLT3*-D835, *MLL*-PTD, *KIT*, and *RAS* mutations

Mutation analysis of internal tandem duplication (ITD) within the JM domain and D835 mutation (D835Mt) within the TK2 domain of *FLT3* was performed as previously reported [23, 25–27]. Mutation analysis of partial tandem duplication (PTD) of *MLL* was performed using the primer pair 6.1 (located in exon 9) and E3AS (located in exon 4), as previously reported [25]. Mutation analysis of the kinase domain, extracellular domain, and transmembrane domain of the *KIT* gene was performed with the reverse transcriptase-polymerase chain reaction (RT-PCR) followed by direct sequencing, as previously reported [23]. Mutation analysis of the *RAS* gene around hot spots at codons 12, 13, and 61 was performed as previously reported [28].

Detection of *WT1* mRNA expression by quantitative RT-PCR (qRT-PCR)

WT1 expression at diagnosis was measured using the qRT-PCR system, as previously reported [29]. We determined the cut-off value of *WT1* expression to be 2,500 copies/μg RNA, because the value for the 90th percentile of *WT1* expression in normal bone marrow samples was 2,519 copies/μg RNA [29].

Statistical analysis

The χ^2 test was used to compare the frequencies of mutations. Fischer's exact test was used when data were sparse. The survival distribution was assessed using the Kaplan–Meier method, and differences were compared using the log-rank test [30]. Overall survival (OS) was defined as the time from diagnosis until death owing to any cause or the last follow-up. Disease-free survival (DFS) was defined as the time from the date of complete remission until relapse or death; patients alive and relapse-free at last follow-up were censored. Multivariate analyses were performed to investigate whether *WT1* mutation might serve as a prognostic factor in 130 AML patients, excluding those with FAB-M3 and Down syndrome. *FLT3*-ITD, *FLT3*-D835, *MLL*-PTD, *KIT*, *RAS*, and *WT1* mutation were examined whether these alterations influenced about the 3-year OS and DFS. Karyotypic abnormalities were not included in analytic variables since they were apparently confounded with aforementioned genomic alterations. These statistical analyses were based on Dr. SPSS II for Windows (release 11.0.1J, SPSS; Japan, Inc.).

Results

WT1 mutations

Of the 157 analyzed pediatric AML patients, amplification of the *WT1* gene by RT-PCR was not completed in 4 (2.5 %) cases. Therefore, the following analyses were conducted with the 153 evaluable cases excluding these four.

Ten (6.5 %) of the 153 cases had an activating mutation (Table 1). In 7 cases (70 %) with *WT1* mutation, two or more mutations were detected in the *WT1* gene (Table 1). There was no significant difference in the age, sex, WBC count at diagnosis, or frequency of extramedullary infiltration of leukemic cells between patients with and without *WT1* mutations; however, the frequency of allo-HSCT was significantly higher in patients with than in those without *WT1* mutation (70.0 vs 35.7 %, respectively, $P = 0.03$) (Table 2).

Table 2 Clinical characteristics of 153 evaluable patients with and without *WT1* mutations

	All patients	*WT1*-Mt	*WT1*-Wt
Age, median (years)	6 (0–15)	11 (3–14)	6 (0–15)
≥3	113	10	103
<3	40	0	40
WBC count, median ($\times 10^9$/L)	20.7 (1.0–620.0)	35.1 (3.6–440.0)	20.5(1.0–620.0)
Male/female	87/66	6/4	81/62
Patients with Down syndrome	10 (6.5 %)	0	10 (7.0 %)
Patients who underwent allo-HSCT	58 (37.9 %)	7 (70.0 %)	51 (35.7 %)
Total	153	10	143
Risk group (excluding FAB-M3 and Down syndrome)			
Low	58 (44.6 %)	2 (20.0 %)	56 (46.7 %)
Intermediate	57 (42.5 %)	6 (60.0 %)	51 (41.1 %)
High	10 (7.5 %)	1 (10.0 %)	9 (7.3 %)
Non-CR	5 (3.7 %)	1 (10.0 %)	4 (3.2 %)
Total	130	10	120

WT1-Mt patients with *WT1* mutation, *WT1-Wt* patients lacking *WT1* mutation, *HSCT* hematopoietic stem cell transplantation

Table 3 FAB classification, other genomic alterations, and karyotypic abnormalities of 153 evaluable patients with *WT1* mutations

	All patients	*WT1*-Mt
FAB classification		
M0	5	0
M1	24	3 (12.5 %)
M2	44[a]	4 (9.1 %)
M3	13	0
M4	21	1 (4.8 %)
M5	24	1 (4.2 %)
M6	1	0
M7	19[a]	0
Unclassified	2	1 (50.0 %)
Other genomic alterations		
FLT3-ITD	17	2 (11.8 %)
FLT3 D835 mutation	8	2 (25.0 %)
KIT mutation	11	3 (27.3 %)
MLL-PTD	21	3 (14.3 %)
NRAS	11	1 (9.1 %)
KRAS	18	2 (11.1 %)
Total	153	10 (6.5 %)
Karyotypic abnormalities (excluding Down syndrome)		
Normal	33	5 (15.2 %)
t(8;21)	44	2 (4.5 %)
11q23 abnormality	19	0
t(15;17)	13	0
inv(16)	7	1 (14.3 %)
Others	25	2 (8.0 %)
Unknown	2	0
Total	143	10 (7.0 %)

WT1-Mt patients with *WT1* mutation

[a] Of 10 cases with Down syndrome, 9 were classified into FAB-M7 and 1 into FAB-M2. None of them had *WT1* mutations

The incidence of mutations in *WT1* was significantly higher in pediatric CN-AML (15.2 vs. 4.5 %, respectively, $P = 0.04$) (Table 3, in which DS patients were not included in karyotypic abnormalities).

Correlations between *WT1* mutations and other gene aberrations

The incidence of mutations in *KIT* was significantly higher in patients with than in those without the *WT1* mutation (30 vs. 5.6 %, respectively, $P < 0.01$). Moreover, the incidence of *FLT3*-D835 mutation was also significantly higher in patients with than in those without the *WT1* mutation (20.0 vs. 4.2 %, respectively, $P = 0.03$). The distribution of *FLT3*-ITD, *MLL*-PTD, and mutations in *NRAS* and *KRAS* was not different from those without *WT1* mutation (Table 3).

Correlation between *WT1* mutation and *WT1* mRNA expression

A higher *WT1* expression (≥2,500 copies/μg RNA) was detected in 9 (90 %) of 10 cases with *WT1* mutation (Table 1). On the other hand, a higher *WT1* expression was detected in 113 (77 %) of 147 cases without *WT1* mutation. The difference was not significant ($P = 0.33$).

Clinical outcome and prognostic significance of *WT1* mutations

There were no differences in the 3-year OS and DFS between those with and without *WT1* mutation in 130 evaluable AML patients, excluding those with FAB-M3 and DS (Fig. 1). The frequency of *WT1* mutation was not different between patients with and without CR after induction therapy (6.7 vs. 20.0 %, respectively, $P = 0.13$). Among patients with a normal karyotype, *WT1* mutation tended to be related to a poorer 3-year OS and DFS than those without *WT1* mutation, although the differences were not significant ($P = 0.38$ and $P = 0.45$, respectively) (Fig. 2).

WT1 mutations were not randomly distributed over the different cytogenetic subgroups. The frequency of *WT1* mutation in CN-AML was higher than in other cytogenetic subgroups ($P = 0.04$). This trend was similar to previous pediatric reports [19, 21, 22]. Moreover, the frequency of *WT1* mutation in patients <3 years was lower than in patients aged 3 years or older; however, this difference was not significant (0 vs. 8.6 %, respectively, $P = 0.06$). In other pediatric reports, the frequency of *WT1* mutation was significantly lower in patients <3 years old than in patients aged 3 years or older [19, 21, 22]. Furthermore, *FLT3*-ITD in AML was too strong a prognostic factor to assess whether or not *WT1* mutation has a prognostic impact [25]. Thus, we analyzed the clinical impact of *WT1* mutation in patients with CN-AML excluding those with *FLT3*-ITD and <3 years. In patients with a normal karyotype, aged 3 years or older, and showing no evidence of *FLT3*-ITD, *WT1* mutation was related to a poorer prognosis based on the 3-year OS and DFS ($P = 0.17$ and $P < 0.01$, respectively) (Fig. 3). *WT1* mutation was not a significant risk factor on 3-year OS and DFS by multivariate analyses (Tables 4, 5).

Discussion

Although several papers reported the differences in clinical outcome between patients with and without *WT1* mutation, we could not identify any differences between them in this study. However, other studies demonstrated that these

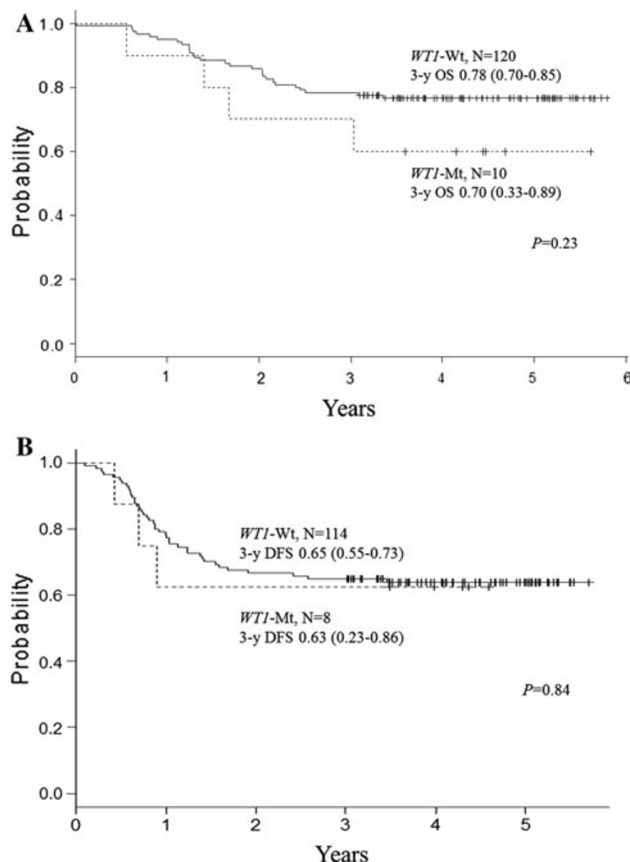

Fig. 1 Probability of 3-year OS (**a**) and DFS (**b**) in 130 AML patients, excluding those with FAB-M3 and Down syndrome. Kaplan–Meier estimates for patients with and without *WT1* mutation are shown

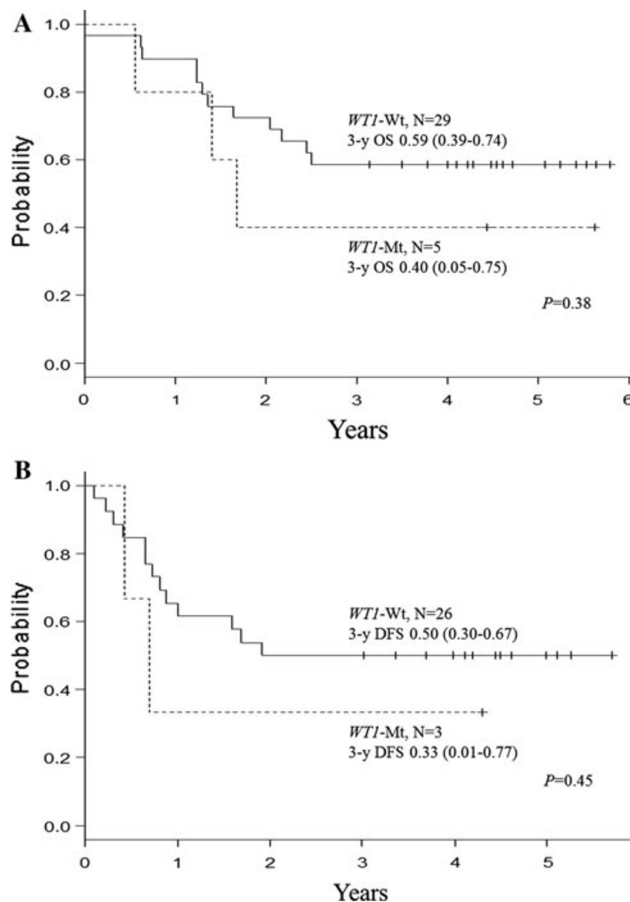

Fig. 2 Probability of 3-year OS in 34 patients (**a**) and DFS in 29 patients (**b**) with CN-AML, excluding those with FAB-M3 and Down syndrome. Kaplan–Meier estimates for patients with and without *WT1* mutation are shown

mutations had no independent effects on the outcome when the *FLT3*-ITD status was taken into account [18, 21, 22]. These results suggest an effect on the clinical outcome due to the different treatment regimens used in each study. For example, when comparing the treatment protocols, the cumulative doses of high-dose cytarabine given for consolidation treatment were markedly different. The current patients were treated on the Japanese Childhood AML Cooperative Study Group Protocol, AML99, in which the intensive use of cytarabine, including high-dose cytarabine, was considered to improve the outcome. Improvement of the clinical outcome of patients with *WT1* mutation in this study might have decreased the differences in the 3-year OS and DFS between patients with and without *WT1* mutation, although *WT1* mutation was not a significant risk factor on 3-year OS and DFS by multivariate analyses. *WT1* mutations have been reported to be an adverse prognostic factor in some studies because of the high frequency of coexisting *FLT3*-ITD [18, 21, 22]; however, only 2 cases had both *WT1* mutation and *FLT3*-ITD in our study. Because of the small number of cases, prognostic analysis for each status of *WT1* mutation and *FLT3*-ITD was not performed. On the other hand, *WT1* mutations were associated with a poor prognosis in patients with CN-AML excluding those with *FLT3*-ITD and less than 3 years old. There has been no similar report of this result. Although further validation of the present results is required, *WT1* mutation might be a prognostic factor in patients with CN-AML excluding those with *FLT3*-ITD and <3 years old.

The frequencies of *WT1* mutations (6.5 % of total AML and 15.2 % of CN-AML) tended to be low compared with previous pediatric reports (8.2–11.7 and 14.3–22.3 %, respectively) [19, 21, 22], although the differences were not significant. This might be due to racial differences or the samples used. Previous reports used genomic DNA for analyzing *WT1* mutation; however, we could use only cDNA. We could not amplify *WT1* genes by RT-PCR in 4 cases (2.5 %). In a previous study, Hollink et al. [19] described 2 cases (1 %) with homozygous deletion out of 298 patients. If homozygous deletions occur in tumor suppressor genes, expressions will be lost and analyses

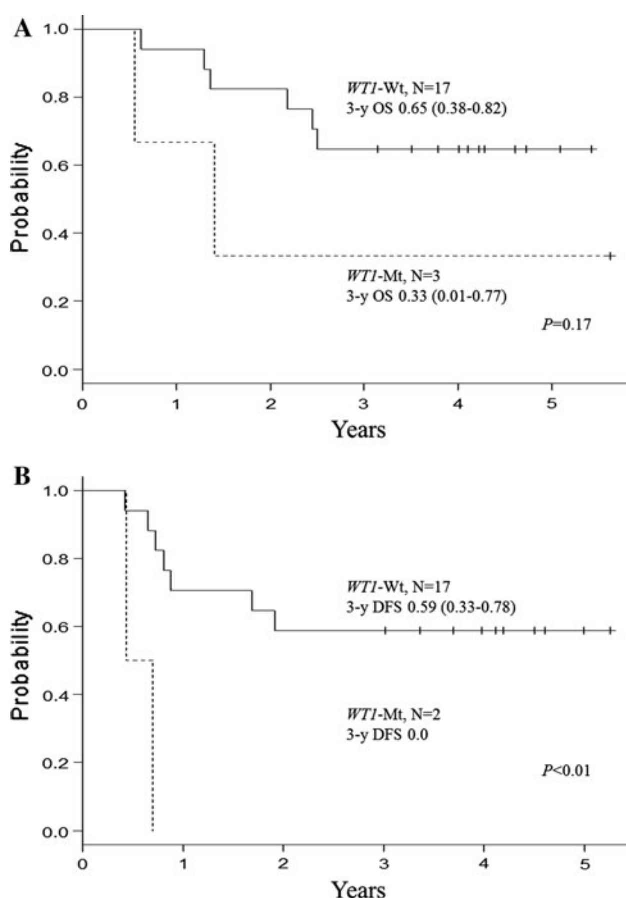

Fig. 3 Probability of 3-year OS in 20 patients (**a**) and DFS in 19 patients (**b**) aged 3 years or older with CN-AML, excluding those with *FLT3*-ITD, FAB-M3, and Down syndrome. Kaplan–Meier estimates for patients with and without *WT1* mutation are shown

Table 4 Multivariate model of prognostic risk factors (overall survival, $N = 130$)

Variables	Hazard ratio	*P* value	95 % CI
FLT3-ITD	5.62	<0.01	2.41–13.12
MLL-PTD	1.96	0.11	0.87–4.46
RAS mutation	1.89	0.15	0.80–4.47
WT1 mutation	1.36	0.62	0.40–4.57
KIT mutation	0.75	0.64	0.22–2.50
FLT3 D835 mutation	<0.01	1.00	0.00–∞

CI confidence intervals

using cDNA will be impossible. As a result, the rate of detecting mutations in these genes is generally higher in genomic DNA than in cDNA [31–33]. Therefore, genomic DNA is usually used for the analysis of these genes. Loss of *WT1* amplification in our study might be partially explained by homozygous deletions of *WT1* genes; however, the frequency of homozygous deletion based on a previous report [19] seemed to be low. Thus, its influence on our research might be limited.

Table 5 Multivariate model of prognostic risk factors (disease-free survival, $N = 122$)

Variables	Hazard ratio	*P* value	95 % CI
MLL-PTD	2.37	0.21	1.14–4.96
FLT3-ITD	2.29	0.10	0.86–6.09
KIT mutation	1.70	0.26	0.68–4.22
RAS mutation	1.27	0.50	0.63–2.57
WT1 mutation	0.57	0.41	0.15–2.20
FLT3 D835 mutation	0.47	0.45	0.06–3.45

CI confidence intervals

When combined with three previous pediatric reports and our data, *WT1* mutations were identified in 50 out of 286 (17.5 %) patients with CN-AML [19, 21, 22]. On the other hand, they were identified in 146 out of 1,283 (11.4 %) adult patients when combined with three large-scale reports [16–18]. The frequency of *WT1* mutation in patients with CN-AML was significantly higher in pediatric compared with adult patients ($P < 0.01$). It was impossible to compare the frequencies of *WT1* mutations in AML patients other than CN-AML because the analyses were usually performed focusing on patients with CN-AML in adult reports.

In 70 % of cases with *WT1* mutations, two or more mutations were detected in the *WT1* gene (Table 1). This frequency was higher than those in previous reports by Hollink et al. [19] (16/35, 46 %, $P = 0.28$), and Ho et al. [21] (15/70, 21 %, $P < 0.01$). It became clear that the existence of multiple mutations of *WT1* genes was not rare. Meanwhile, 90 % of cases with *WT1* mutations were accompanied by other mutations, including *FLT3*-ITD, *MLL*-PTD, and mutations of *FLT3* D835, *KIT*, or *RAS* (Table 1). This frequency was higher than in patients without *WT1* mutation (67/143, 47 %, $P < 0.01$). From these results, there is a possibility that mutations of *WT1* and other genes collaborate and participate in the development of AML. The traditional model of molecular-genetic cooperativity in myeloid leukemogenesis states that "class II" events, which impair differentiation, must be coupled with "class I" events, which confer a proliferative advantage [34]. In our study, *WT1* mutations showed significant overlap with class I mutation, such as *FLT3*-ITD, *MLL*-PTD, and mutations of *FLT3* D835 and *KIT*, so the role of *WT1* mutation in the stepwise evolution might be associated with the arrest of differentiation.

WT1 mRNA expression at diagnosis tended to be higher in patients with compared with those without *WT1* mutation, although the difference was not significant, probably due to the low number of patients with *WT1* mutation. Overexpression of wild-type *WT1* is a common finding in AML [35–37], although *WT1* mutations in AML appear to

result in a loss of *WT1* function. This contradiction, in which a single gene might function as both an oncogene as well as a tumor suppressor, may stem from the ability of the WT1 protein to function either as a transcriptional activator or repressor, depending on a multitude of factors [11]. There is still much to be learned about the biology of *WT1* in AML.

In conclusion, *WT1* mutations were the most common in patients with normal karyotype AML, and showed no correlation with the 3-year OS and DFS. However, these mutations were associated with a poor prognosis in patients with CN-AML excluding those with *FLT3*-ITD and <3 years old.

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Conflict of interest There is no conflict of interest.

Appendix

Committee members of the Japanese Childhood AML Cooperative Study Group who contributed data to this study include Akira Morimoto, Department of Pediatrics, Kyoto Prefectural University 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, First Department of 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 and Oncology, Miyagi Children's Hospital.

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