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小児急性骨髄性白血病の新たな予後因子の同定

概要

上武大学医学生理学研究所の林泰秀副所長(上武大学副学長)，群馬県立小児医療センター血液腫瘍科の鏑木多映子部長，横浜市立大学附属病院小児科の柴徳生准教授らは，日本小児がん研究グループ(JCCG)が実施する急性骨髄性白血病（Acute myeloid leukemia; AML）の臨床試験に登録され治療を受けた小児患者に対し、次世代シーケンサーを用いた遺伝子解析を行い，小児 AML において Upstream binding transcription factor（*UBTF*)遺伝子の遺伝子内縦列重複変異(Internal tandem duplication, ITD): *UBTF*-ITD が新たな予後不良因子である可能性を報告しました.

AML は血液中に存在する白血球ががん化する血液がんの一種です．小児 AML ではこれまでの研究で *RUNX1::RUNX1T1* 融合遺伝子や、*FLT3*-ITD 遺伝子変異などの予後予測因子*1 が同定されており、疾患リスクに合わせた治療を行う層別化治療*2 が進められてきました。しかしながら、既存の予後予測因子を同定できない症例も多く存在し、治療成績の向上のためには新規の予後予測因子の同定を行うことが重要とされています。

今回の遺伝子解析の結果、小児初発 AML の 503 例中 6 例に *UBTF*-ITD を同定しました。*UBTF*-ITD を有する症例は小児 AML でしばしば認められる 8 番染色体が 1 本多い 8 トリソミーとの合併が多く、全生存率*3、無イベント生存率*4 ともに有意に不良であることがわかりました。これまで、8 トリソミー陽性例には予後良好例、不良例が混在していましたが、今回の *UBTF*-ITD の同定により 8 トリソミー陽性例の予後を正確に予測することが可能となりました。これらの結果は昨年米国の小児再発 AML を中心とした解析からも同様の報告がなされており、小児 AML における *UBTF*-ITD が重要な予後因子であることを示唆しています。今後の症例数の蓄積により予後因子として確立することで正確な層別化治療や新規治療薬の開発につながり小児 AML の治療成績の向上に結び付くことが期待されます。

本研究成果は、科学誌『Genes chromosomes and Cancer』に掲載されます。
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【ポイント】

- 小児 AML503 例中 6 例(1.2%)に *UBTF*-ITD を同定し、成人 AML には認められず小児 AML に特徴的な遺伝子異常と考えられました。
- *UBTF*-ITD を有する AML の特徴として、*FLT3*-ITD、*PRDM16* 遺伝子高発現※5、*WT1* 遺伝子変異、8 トリソミーとの合併が多くみられることがわかりました。
- 本研究の結果は 2022 年度に米国から報告された小児再発 AML で同定された *UBTF*-ITD 症例と同様の分子生物学的特徴や予後の傾向を示しており、*UBTF*-ITD が小児 AML において重要な予後不良因子である可能性が示されました。
- *UBTF*-ITD が予後因子として確立することで適切な層別化や新規治療薬の開発を目指すことが期待されます。

【研究の背景】

AML は血液のがんである白血病の一種で、小児白血病の中では急性リンパ性白血病の次に多い疾患です。AML においてはこれまで数多くの遺伝子解析が行われ、予後良好因子である *RUNX1::RUNX1T1*、*CBFB::MYH11* といった融合遺伝子や予後不良因子である *FLT3*-ITD などの遺伝子変異が複数同定されています。小児 AML ではそれらの予後因子や治療反応性を組み合わせた層別化治療が行われており、長期生存率は 60-70％まで上昇していますが、30-40%の患者さんは再発や死亡に至り、急性リンパ性白血病の生存率が 80-90%であることと比べると未だに十分な治療成績とは言えない疾患です。近年の遺伝子解析研究により多数の遺伝子変異や融合遺伝子が予後因子として同定され、以前と比較しリスクの層別化も進んでいますが、いまだに明らかな予後因子を持たずに適切な層別化治療が受けられない症例が存在します。新規の予後因子を同定して、より適切な層別化治療を行うことが小児 AML の治療成績向上のために重要とされています。

【研究の方法と結果】

本研究グループは、小児 AML131 例に対して次世代シーケンサーを用いて遺伝子解析を行ったところ、*UBTF*-ITD という新しい遺伝子異常を同定しました。多くの症例で解析するため、小児 AML503 例、成人 AML175 例、小児悪性腫瘍(AML、急性リンパ性白血病、神経芽腫)の細胞株 65 株を対象にサンガーシーケンス法という方法で解析したところ、小児 AML503 例中 6 例(1.2%)に *UBTF*-ITD を認めましたが、成人 AML には一例も認めませんでした。*UBTF*-ITD を有する AML の症例を詳細に解析したところ、*FLT3*-ITD、*WT1* 遺伝子変異、*PRDM16* 遺伝子高発現、8 トリソミーといった遺伝子異常、染色体異常との合併が多いことがわかりました(図 1)。次に小児 AML139 例を対象に RNA シークエンスによる遺伝子発現解析を行ったところ、*UBTF*-ITD を有する症例は、近年予後不良との関連が注目されている *PRDM16* 遺伝子の発現が高く、他の *PRDM16* 高発現の症例と比較しても *UBTF*-ITD を有する症例は *PRDM16* 遺伝子の発現程度がより高いことがわかりました。

UBTF-ITD を有する症例とそれ以外の症例の予後を比較すると、*UBTF*-ITD を有する症例は全生存率、無イベント生存率ともに不良であることがわかりました(図 2)。また、8 トリソミーの症例の中で比較すると、通常予後因子として扱われない 8 トリソミーですが、*UBTF*-ITD を有する 8 トリソミーの症例は有意に予後不良であることがわかりました。8 トリソミーには他の染色体異常を合併することも知られていますが、*UBTF*-ITD を有する症例には他の染色体異常がなく、特徴的な集団であると示唆されました。*UBTF*-ITD は昨年米国の再発 AML を中心とした解析で初めて詳細に報告され、併存する分子生物学的異常や遺伝子発現パターン、予後への影響などが我々の結果と同様であり、今回の解析で再現性を確認することができました。*UBTF* は ITD 以外に急性リンパ性白血病では他の遺伝子と融合するキメラ

遺伝子を作ることが最近報告されて注目されており、血液腫瘍において重要な遺伝子である可能性が示唆されています。*UBTF*-ITD は小児 AML おいて重要な予後因子となる可能性があります。

図 1 A　*UBTF*-ITD を有する症例の分子生物学的特徴と臨床的特徴

UBTF-ITD を有する 6 症例を 6 列に示している。8 トリソミー、*FLT3*-ITD、*WT1* 遺伝子変異、*PRDM16* 遺伝子高発現との合併を多く認めた。

図 1 B 各症例の *UBTF*-ITD の遺伝子配列

各症例で同定された *UBTF*-ITD の遺伝子配列を示している。各症例の上段は塩基配列、下段はアミノ酸配列を示している。いずれも Exon 13 内に重複を認め(黄色部分)、全症例に共通した重複配列(点線四角)を有していた。

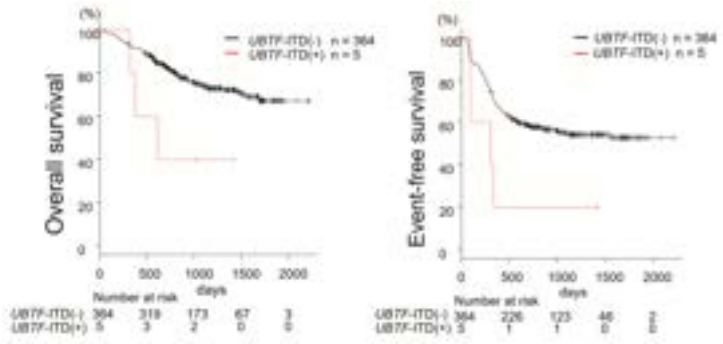

図 2．*UBTF*-ITD の有無による生存率の比較

UBTF-ITD を有する症例は全生存率(Overall survival)、無イベント生存率(Event-free survival)とも有意に不良であった。

【研究成果の意義と今後の展望】

本研究の成果として、*UBTF*-ITD が小児 AML における予後因子として確立した場合、より適切な層別化治療に結び付く可能性や、治療標的としての薬剤開発につながる可能性があり、今後の小児 AML の治療成績の向上が期待されます。

【論文情報】

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論文タイトル：*UBTF*-ITD は小児急性骨髄性白血病における予後不良因子である

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【用語解説】

※１　予後因子：治療後、その病気の状態がどうなるかを判断するための因子で、遺伝子異常の有無などが含まれる。

※２　層別化治療：予後因子に基づいて患者を分類し、それぞれのリスクに応じた最適と思われる治療を行うこと。

※３　全生存率：診断されてから一定の期間が経過した後に生存している人の割合。

※４　無イベント生存率：診断されてから一定の期間が経過した後、現病の進行や再発なく生存している人の割合。

※５　遺伝子高発現：細胞の中で DNA の遺伝情報をもとにたんぱく質が合成される際、通常よりもその合成が増強していること。

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RESEARCH ARTICLE

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UBTF-internal tandem duplication as a novel poor prognostic factor in pediatric acute myeloid leukemia

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Abstract

The prognosis of pediatric acute myeloid leukemia (AML) has improved via stratification therapy. However, relapse or death occurs in 30%–40% of cases. Novel genetic factors for pediatric AML need to be elucidated to improve prognosis. We detected recurrent internal tandem duplication in upstream binding transcription factor (*UBTF*-ITD) in 1.2% (6/503) of Japanese pediatric patients with de novo AML. No *UBTF*-ITD was detected in 175 adult patients with AML or in 65 cell lines that included 15 AML, 39 acute lymphoblastic leukemia, five chronic myeloid leukemia, and six neuroblastoma cell lines. All *UBTF*-ITDs were found in exon 13 and shared a duplicated region. *UBTF*-ITD was more frequently detected in patients with trisomy 8, *FLT3*-ITD, *WT1* mutation, and/or high *PRDM16* expression (trisomy 8, 3/6; *FLT3*-ITD, 5/6; *WT1* mutation, 2/6; and high *PRDM16* expression, 6/6). Gene expression patterns of patients with *UBTF*-ITD were similar to those of patients with *NUP98::NSD1* or *FUS::ERG*. Survival analysis

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of the AML-05 cohort revealed that patients with *UBTF*-ITD had worse outcomes than those without *UBTF*-ITD (3-year event-free survival, 20% vs. 55%; 3-year overall survival, 40% vs. 74%). Moreover, among the 27 patients with trisomy 8, all three patients with *UBTF* -ITD had a poor prognosis resulting in early events (relapse or non-complete remission) within 1 year. Our findings suggest that *UBTF*-ITD may be a novel and significant prognostic factor for pediatric patients with AML.

KEYWORDS
AML, *FLT3*-ITD, pediatric, *PRDM16*, trisomy 8, *UBTF*-ITD

1 | INTRODUCTION

The prognosis of pediatric acute myeloid leukemia (AML) has improved owing to stratification therapy using cytogenetic aberrations and therapeutic response.[1] However, there are still poor prognosis cases for which the detailed molecular background remains unknown. Thus, further investigation is needed to identify novel prognostic factors in pediatric AML.

While *FLT3*-ITD has been identified as an important prognostic factor in pediatric and adult AML, evidence has also shown that *FLT3*-ITD alone does not determine the prognosis, which has been found to depend on other coexisting genetic factors.[2–4] Our previous RNA sequencing analysis of 139 pediatric AML found that 37 of 47 patients with *FLT3*-ITD had some type of fusion gene, but no other factors were identified in the remaining 10 patients.[5] Focusing on these 10 patients with *FLT3*-ITD without any fusion genes, we performed a custom panel sequencing on 131 pediatric AML and identified recurrent internal tandem duplication in upstream binding transcription factor (*UBTF*-ITD) in 3 of 10 patients with *FLT3*-ITD without any fusion genes. *UBTF* encodes a member of the HMG-box DNA-binding protein family and plays a critical role in ribosomal RNA transcription. We further analyzed *UBTF*-ITD in an expanded cohort comprising pediatric AML, adult AML, and leukemia and neuroblastoma cell lines by Sanger sequencing and investigated the correlation between *UBTF*-ITD and other cytogenetic aberrations, gene expressions, clinical features, and prognosis.

2 | MATERIALS AND METHODS

2.1 | Patients

This study enrolled patients with de novo AML who participated in the Japanese AML-99 trial conducted by the Japanese Childhood AML Cooperative Study from January 2000 to December 2002, as well as the AML-05 trial conducted by the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) from November 2006 to December 2010.[6,7] The AML-05 trial is registered with the UMIN Clinical Trials Registry (UMIN-CTR, http://www.umin.ac.jp/ctr/index.htm, number UMIN000000511). A total of 503 patients with leukemic samples were included in this study. There were 134 patients from a total of 280 in the AML-99 trial and 369 from a total of 443 patients in the AML-05 trial. The clinical characteristics of the patients and a comparison between available and unavailable samples in each study are described in Tables S1–S3. Patients diagnosed with acute promyelocytic leukemia, Down syndrome-associated AML, and secondary AML were excluded from these studies. Details pertaining to the diagnosis, treatment, and risk stratification have been previously reported.[6,7] The treatment protocols and procedures for data and sample collection were approved by the institutional review boards of each participating institution; written informed consent was obtained from all patients or their parents/guardians. These studies were conducted in accordance with the Declaration of Helsinki and approved by the institutional review board of the Gunma Children's Medical Center and the ethical review board of the JPLSG. Furthermore, 175 adult patients with AML were enrolled as a control cohort.

2.2 | Cell lines

The following 65 cell lines were analyzed: 15 AML, 39 acute lymphoblastic leukemia, five chronic myeloid leukemia, and six neuroblastoma cell lines. Detailed information regarding the cell lines is presented in Table S4.

2.3 | Custom panel sequencing

Custom panel sequencing was performed in 131 of 369 patients in the AML-05 study. This panel contained 343 genes that have been previously reported as driver or targetable genes in hematological malignancies or solid tumors, including *UBTF*, which was reported in integrated analysis in erythroleukemia.[8] All coding exons were captured using the SureSelect custom kit (Agilent Technologies, Santa Clara, CA) and sequenced using the Hiseq 2500.

2.4 | Sanger sequencing for *UBTF*-ITD

Analysis of *UBTF*-ITD was performed via polymerase chain reaction (PCR) followed by Sanger sequencing. Sanger sequencing was performed to detect *UBTF*-ITD in 369 patients from the AML-05 study,

157 patients from the AML-99 study, 175 adult patients with AML, and 67 cell lines. The following primers were used: forward primer, 5′-ACCCTCTGCACCTGGACA-3′ and reverse primer, 5′-TGACAG CTTCCCGCCTTC-3′.

2.5 | Molecular characterization other than *UBTF*-ITD

Various gene mutations, gene rearrangements, and gene expressions were analyzed using Sanger sequencing and quantitative RT-PCR. Detailed methods have been reported previously.[9]

2.6 | RNA sequencing

RNA sequencing was performed in 139 of the 369 patients from the AML-05 study. The RNA-seq data are available at the European Genome-Phenome Archive (EGAS00001003701). Detailed patient characteristics and methods are presented in Table S6 and in our previous report.[5]

2.7 | Statistical methods

The EZR software (version 1.35; Saitama Medical Center, Jichi Medical University, Saitama, Japan) was used for all analyze.[10] Detailed methods are described in Supplementary Methods. Inter-group differences in clinical characteristics were analyzed using Fisher's exact and Mann–Whitney U tests. The Kaplan–Meier method and log-rank test were used to estimate the survival rate. Overall survival (OS) was defined as the time from diagnosis to death or the last follow-up. Event-free survival (EFS) was defined as the time from diagnosis to the date of failure (induction failure, relapse, second malignancy, or death) for patients who experienced treatment failure or the date of last contact for all other patients. Hazard ratio and 95% confidence interval were estimated using Cox regression analysis. The Kruskal–Wallis test was used to compare gene expression levels among the various genetic abnormalities. For all analyses, two tailed p values of <0.05 were considered indicative of statistical significance.

3 | RESULTS

3.1 | Detection of *UBTF*-ITD in pediatric AML

Three patients with *UBTF*-ITD were detected from 131 patients via custom panel sequencing. Thereafter, we detected additional *UBTF*-ITDs in two patients from the AML-05 study and one patient from the AML-99 study via Sanger sequencing, respectively. Thus, six of 503 (1.2%) pediatric AML cases were found to have *UBTF*-ITD (Table 1 and Figure 1A). In contrast, no *UBTF*-ITDs were detected in the 175 adult AMLs and 65 cell lines with leukemia and neuroblastoma. All

TABLE 1 Clinical characteristics of patients with *UBTF*-ITD

UPN	Duplicated length	Insertion	Sex	Age, year	WBC, $\times 10^3/\mu l$	FAB	Karyotype	*FLT3*-ITD	High *PRDM16* expression	CR	SCT	Relapse	Dead
AML-05-21	40 bp	11 bp	F	14.9	1.1	M6a	46,XX[20]	–	+	+	+	–	–
AML-05-47	38 bp	10 bp	M	12.8	7.0	M4	47,XY,+8[1]/46,XY[19]	+	+	+	+	+	+
AML-05-67	90 bp	3 bp	M	7.4	168.1	M1	47,XY,+8[20]	+	+	–	–	+	+
AML-05-275	81 bp	–	F	8.3	87.5	M1	47,XX,+8[20]	+	+	–	+	–	–
AML-05-365	27 bp	36 bp	F	8.5	95.9	M5a	47,XX,+8[20]	+	+	+	+	+	+
AML-99-154	39 bp	15 bp	F	8	25.7	M0	46,XX	+	+	+	+	+	+

Abbreviations: CR, complete remission; FAB, French–American–British classification; N/A, not available; SCT, stem cell transplantation; WBC, white blood cell counts; UPN, unique patient number.

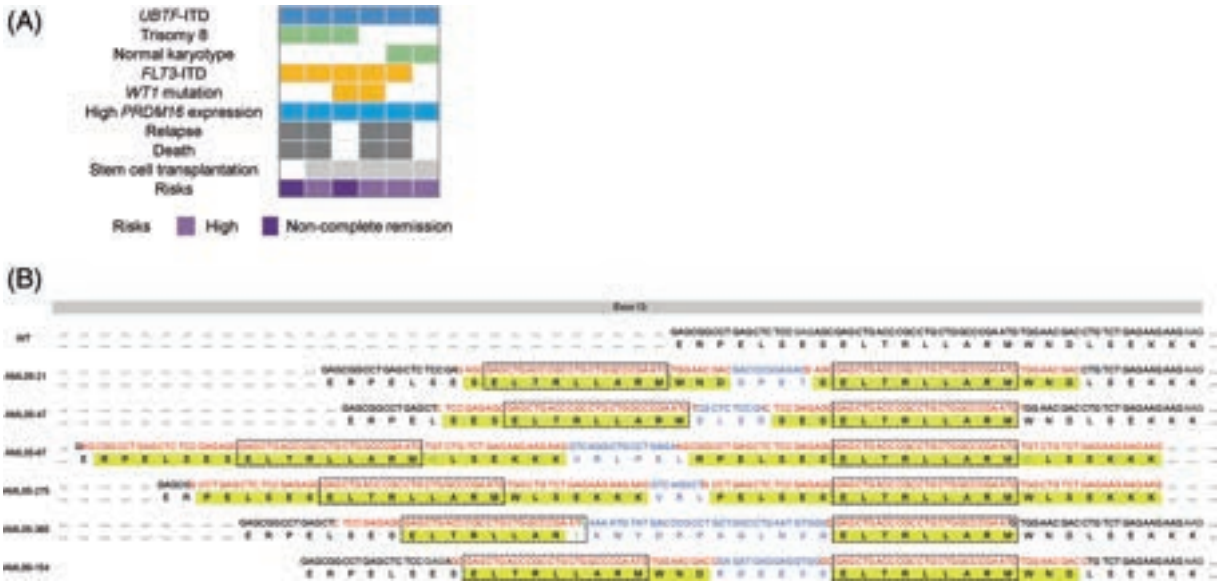

FIGURE 1 (A) Molecular and cytogenetic aberrations in six pediatric acute myeloid leukemia (AML) with *UBTF*-ITD in the AML-05 and AML-99 studies. Among the six patients with *UBTF*-ITD, four had trisomy 8, and the other two had a normal karyotype. Moreover, five and two of the six patients with *UBTF*-ITD had concomitant *FLT3*-ITD or *WT1* mutations, respectively. High *PRDM16* expression was also detected in all patients with *UBTF*-ITD. Each column displays the cytogenetic aberration pattern and clinical status of an individual sample. Light blue indicates *UBTF*-ITD. Green indicates chromosomal aberrations. Yellow indicates genetic aberrations. Blue indicates gene expression. Gray indicates a clinical outcome. Blanks indicate the absence of a chromosomal aberration, genetic alteration, or prognostic event. Light and dark purple indicate risk classification. (B) *UBTF*-ITD sequences in each patient. The image shows the sequences of *UBTF*-ITD in exon 13. The top row of each case shows the nucleotide sequences, whereas the bottom row shows the amino acid sequence. Duplicated regions are depicted in red characters and a yellow marker. Commonly duplicated regions are indicated by the dotted line.

UBTF-ITDs were detected in exon 13 and had common duplicated region (Figure 1B).

3.2 | Clinical and cytogenetic characteristics of patients with *UBTF*-ITD

The landscape of patients with *UBTF*-ITD is described in Figure 1A. Trisomy 8, *FLT3*-ITD, *WT1* mutation, and high *PRDM16* expression were more frequently detected in patients with *UBTF*-ITD from the AML-05 study, respectively (trisomy 8, $n = 3$, $p = 0.003$; *FLT3*-ITD, $n = 4$, $p = 0.001$; *WT1* mutation, $n = 2$, $p = 0.039$; high *PRDM16* expression, $n = 5$, $p = 0.001$) (Table 2).

3.3 | Gene expression analysis of cases with *UBTF*-ITD

Unsupervised hierarchical clustering analysis of the 139 pediatric AML is shown in Figure 2A,B. Five clusters were generated, with each cluster having unique characteristics related to gene alterations. Cases with *UBTF*-ITD were categorized into clusters 1 and 3 (Figure 2A). Region B consisted of *HISH1Hs* and *HOXAs*, the gene expression of which were higher in clusters 1A and 3 (Figure 2B). In contrast, genes belonging to region A were lower in clusters 1A and 3. Genes categorized to region C differed significantly between clusters 1A and 3, including *CTNNB1* and *BCL3*. Thereafter, we compared gene expressions between AML with and without *UBTF*-ITD. The expression of *UBTF*, *PRDM16*, *HOXB5*, *HIST1H1C, and CLU* differed significantly (Figure S1). Finally, gene expressions were compared among representative genetic abnormalities, including *NUP98::NSD1*, *FUS::ERG*, *NPM1*, biallelic *CEBPA* mutations, *KMT2A* rearrangements, or others (Figure S2). *UBTF* expression tended to differ among these seven groups ($p = 0.057$, Figure S2), and the expression of *PRDM16*, *HOXA9*, *HOXB5*, *FLT3*, *CEBPA*, *HIST1H1C, and CLU* differed significantly among these groups. The expression levels of these genes in cases with *UBTF*-ITD were similar to those in AML with *NUP98::NSD1* or *NPM1* mutation (Figure S2). Among them, the expression level of *CLU* was higher in cases with *UBTF*-ITD or *NUP98::NSD1* but lower in patients with *NPM1* mutation. Intriguingly, patients with *UBTF*-ITD had a significantly higher *PRDM16* expression level than those without *UBTF*-ITD among the 64 patients with high *PRDM16* expression ($p = 0.010$, Figure S3).

3.4 | Survival analysis of patients with *UBTF*-ITD-positive AML in the AML-05 study

Survival analysis was performed in the AML-05 study using the Kaplan–Meier method to reveal the prognostic impact of *UBTF*-ITD. Notably, patients with *UBTF*-ITD had significantly poorer outcomes in terms of both EFS and OS than those without *UBTF*-ITD (3-year EFS,

TABLE 2 Clinical characteristics of patients with and without *UBTF*-ITD

	***UBTF*-ITD ($n = 5$)**	**w/o *UBTF*-ITD ($n = 364$)**
Age median at diagnosis, year (range)	8.5 (7.4–14.9)	7.8 (0.0–17.9)
Sex, Male, *n* (%)	2 (40)	192 (53)
WBC median, $\times 10^9$/L (range)	87.5 (1.1–168.1)	20.1 (0.62–985.0)
Stem cell transplantation, *n*	4	171
Relapse, *n*	3	129
FAB classification, *n*		
M0	0	7
M1	2	46
M2	0	105
M4	1	55
M5	1	72
M6	1	7
M7	0	33
Risk classification, *n*		
Low	0	126
Intermediate	0	144
High	3	46
Non-complete remission	2	48
Cytogenetic features, *n*		
Normal karyotype	1	69
Trisomy 8	3	24
RUNX1-RUNX1T1	0	106
CBFB-MYH11	0	31
KMT2A-rearrangement	0	55
WT1 mutation	2	23
FLT3-ITD	4	43
High *PRDM16* expression	5	79

Abbreviations: FAB, French–American–British; WBC, white blood cell counts.

20% vs. 55%; 3-year OS, 40% vs. 74%) (Figure 3). Further analyses were performed, focusing on patients with trisomy 8 ($n = 27$). The clinical characteristics of patients with trisomy 8 are described in Figure 4A and Table S7. All three patients with trisomy 8 had no other cytogenetic aberrations, such as *KMT2A* rearrangement or *CBFB::MYH11* (Figure 4A). Furthermore, all three patients with trisomy 8 with *UBTF*-ITD had significantly worse outcomes, resulting in early events (relapse or non-complete remission) within 1 year (Figure 4B). Patients with trisomy 8 with *NUP98::NSD1*, *FUS::ERG*, or *UBTF*-ITD had an extremely poor prognosis, whereas patients with trisomy 8 without those had a favorable prognosis (3-year EFS, 11% vs. 64%; 3-year OS, 33% vs. 82%) (Figure 4C).

4 | DISCUSSION

UBTF-ITD was detected in 6 (1.2%) out of 503 pediatric AML cases in this study. *UBTF*-ITD has recently been reported mainly in relapsed pediatric patients with AML.[11,12] As indicated in previous studies, all these ITDs coexisted with various insertions and deletions in exon 13, resulting in in-frame changes.[11,12] Consistent with previous reports,[11,12] *UBTF*-ITD was detected specifically in pediatric AML in this study. Also similar to the previous report,[11] *UBTF*-ITD was strongly associated with trisomy 8, *FLT*-ITD, *WT1* mutation, and high *PRDM16* expression (Table 2) in this study. Patients with *UBTF*-ITD also had a significantly worse prognosis, which is consistent with the previous report.[11] Patients with *FLT3*-ITD were classified as high-risk and were all indicated for stem cell transplantation in this study.[6] High *PRDM16* expression was reported to be associated with poor prognosis in our previous study.[13] Furthermore, we also showed, that among patients with pediatric AML, the prognosis of those with *FLT3*-ITD can be divided according to the presence or absence of high *PRDM16* expression.[14] Thus, high *PRDM16* expression has recently been recognized as a strong poor prognostic factor. Considering that patients with *UBTF*-ITD concomitantly had these poor prognostic factors, it was difficult to determine the significance of *UBTF*-ITD alone. However, our findings suggested that *UBTF*-ITD enhanced the expression of *PRDM16* and contributed to the poor prognosis given that patients with *UBTF*-ITD had more higher expression of *PRDM16* (Figure S3). Further analysis of *UBTF*-ITD should better elucidate the mechanism by which *PRDM16* expression is enhanced in leukemic cells. Furthermore, gene expression analysis revealed that patients with *UBTF*-ITD expression patterns were similar to those with *NUP98::NSD1* or *FUS::ERG* (Figure 2A). *UBTF*-ITD can be considered a novel abnormality characteristic to a similar group with a poor prognosis, such as those with *NUP98::NSD1* or *FUS::ERG*. In particular, the prognosis of patients with trisomy 8 can be clearly determined according to the presence or absence of *UBTF*-ITD, *NUP98::NSD1*, *or FUS::ERG* (Figure 4C). Trisomy 8 is sometimes detected with other cytogenetic aberrations such as *KMT2A* rearrangement or *CBFB::MYH11*. In fact, 17 of 27 patients with trisomy 8 harbored additional cytogenetic abnormalities. Moreover, the patients with *UBTF*-ITD had no other cytogenetic aberrations other than trisomy 8. In a previous report, despite the high frequency of *FLT3*-ITD in patients with trisomy 8 alone, no significant difference was detected between the prognosis of patients with trisomy 8 alone and those with trisomy 8 harboring other cytogenetic alterations.[15] On the contrary, the results of this study showed, that among the patients with trisomy 8 alone, those with *UBTF*-ITD may have a worse prognosis.

Given the small number of patients, it is difficult to determine the clinical significance of *UBTF* -ITD based on the results of this study alone. However, the results of this study are consistent with previous studies.[11] Although further cases are needed to establish the clinical significance of *UBTF*-ITD, it may be useful for more accurate stratification and may contribute toward improving the prognosis of pediatric patients with AML.

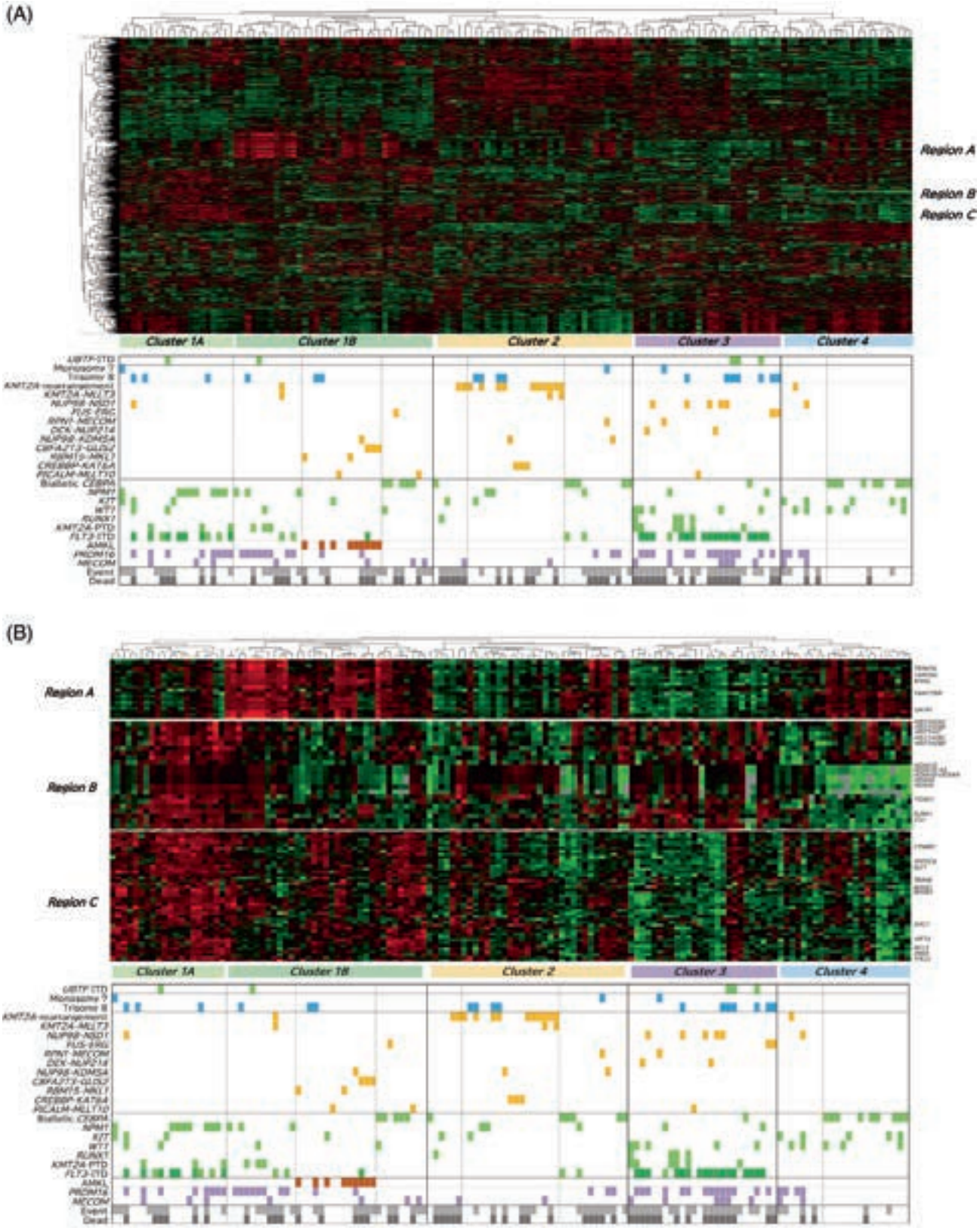

FIGURE 2 (A) Unsupervised hierarchical clustering analysis of the 139 pediatric AML. The 139 cases were classified into five clusters. Each cluster had the following characteristics: cluster 1A, AML with *NPM1* mutation; cluster 1B, acute megakaryoblastic leukemia, and some cases with biallelic *CEBPA* mutation, cluster 2, *KMT2A* rearrangement; cluster 3, *NUP98*::*NSD1*, *UBTF*-ITD, or *RUNX1* mutation; cluster 4, some cases with *CEBPA* biallelic mutation. (B) Distinct three regions from unsupervised hierarchical clustering analysis of the 139 pediatric AML. Region A consisted of low-expression genes in clusters 1A and 3. Region B consisted of high-expression genes in clusters 1A and 3. Region C consisted of genes with significantly different expression patterns between clusters 1A and C.

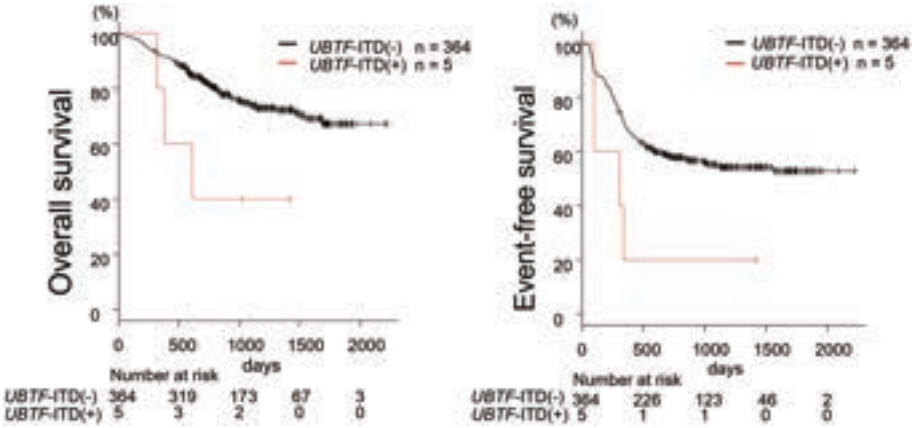

FIGURE 3 Prognostic significance of *UBTF*-ITD in pediatric AML from the AML-05 study. The Kaplan–Meier curves indicate the overall and event-free survival of patients with or without *UBTF*-ITD.

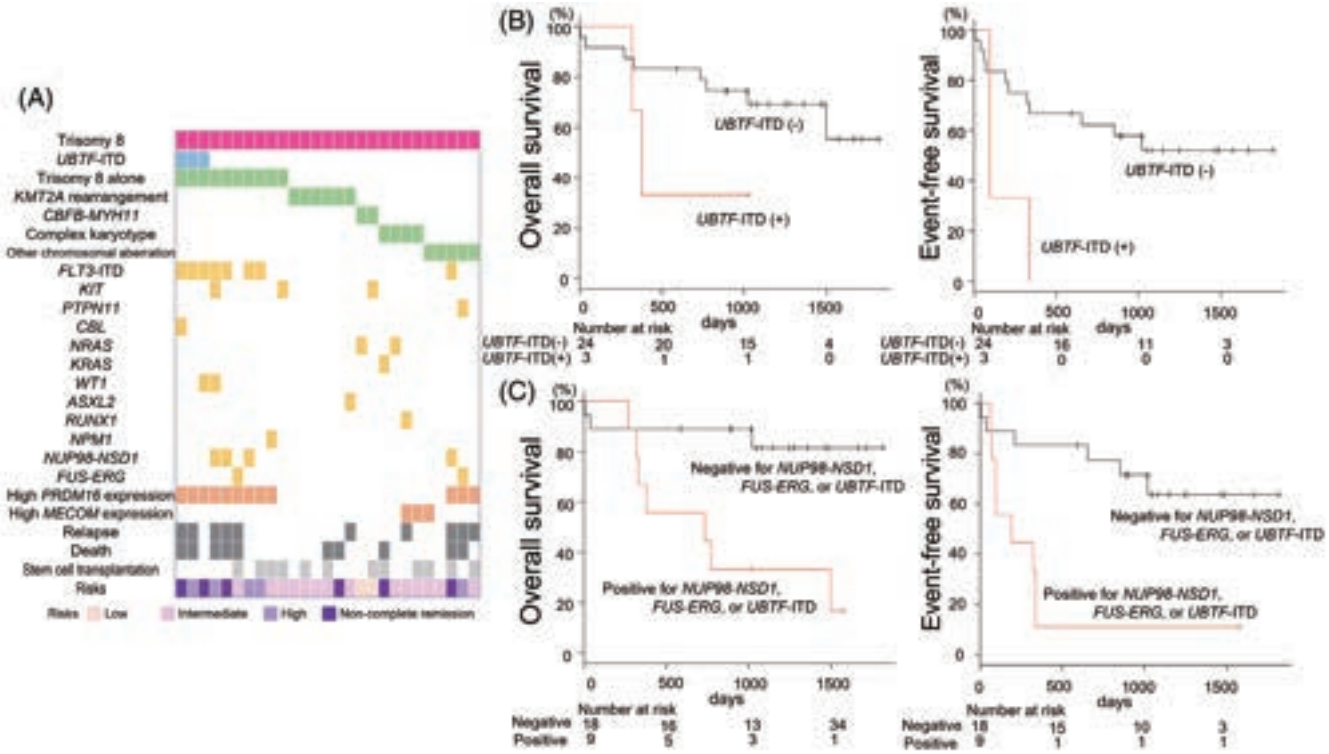

FIGURE 4 (A) Molecular and cytogenetic aberrations in 27 pediatric AMLs with trisomy 8 from the AML-05 study. Each column displays the cytogenetic aberration pattern and clinical status of an individual sample. Pink indicates trisomy 8. Light blue indicates *UBTF*-ITD. Green indicates chromosomal aberrations. Yellow indicates genetic aberrations. Orange indicates gene expression. Gray indicates a clinical outcome. Blanks indicate the absence of a chromosomal aberration, genetic alteration, or prognostic event. Light orange, pink, light purple, and dark purple indicate risk classification. (B) Prognostic significance of *UBTF*-ITD in pediatric AML with trisomy 8 participated in the AML-05 study. Kaplan–Meier curves indicate the overall and event-free survival of patients with or without *UBTF*-ITD in AML with trisomy 8. (C) Prognostic significance of *NUP98::NSD1*, *FUS::ERG*, *or UBTF*-ITD among patients with AML with trisomy 8. Kaplan–Meier curves indicate the overall and event-free survival of cases that are positive or negative for *NUP98::NSD1*, *FUS::ERG*, *or UBTF*-ITD.

AUTHOR CONTRIBUTIONS

Taeko Kaburagi, Norio Shiba, Genki Yamato, Etsuko Ishikita, and Yusuke Hara designed and performed the research, analyzed the data, and wrote the article. Yasuhide Hayashi designed the research, led the project, and wrote the article. Ken Tabuchi performed statistical analysis. Kenichi Yoshida, Yuichi Shiraishi, Satoru Miyano, and Seishi Ogawa performed the research. Kentaro Ohki, Manabu Sotomatsu, Hirohide Kawasaki, Takumi Takizawa, Tomohiko Taki, Nobutaka Kiyokawa, Daisuke Tomizawa, Keizo Horibe, Takashi Taga, and Souichi Adachi provided patient samples and data. All authors critically reviewed and revised the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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