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ORIGINAL ARTICLE

Evaluation of *ETV6/RUNX1* Fusion and Additional Abnormalities Involving *ETV6* and/or *RUNX1* Genes Using FISH Technique in Patients with Childhood Acute Lymphoblastic Leukemia

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Abstract Childhood acute lymphoblastic leukemia (ALL) is the most common type of childhood leukemia. Specifically, ALL is a malignant disorder of the lymphoid progenitor cells, with a peak incidence among children aged 2–5 years. The t(12;21)(p13;q22) translocation occurs in 25 % of childhood B cell precursor ALL. In this study, bone marrow samples were obtained from 165 patients with childhood ALL. We analyzed the t(12;21) translocation and other related abnormalities using the fluorescent in situ hybridization (FISH) technique with the *ETV6*(*TEL*)/*RUNX1*(*AML1*) ES dual color translocation probe. Conventional cytogenetic analyses were also performed. *ETV6* and *RUNX1* related chromosomal abnormalities were found in 42 (25.5 %) of the 165 patients with childhood ALL. Among these 42 patients, structural changes were detected in 33 (78.6 %) and numerical abnormalities in 9 (21.4 %). The frequency of FISH abnormalities in pediatric ALL cases were as follows: 8.5 % for t(12;21)(p13;q22) *ETV6/RUNX1* fusion, 6.0 % for *RUNX1* amplification, 3.0 % for tetrasomy/trisomy 21, 1.8 % for *ETV6* deletion, 1.21 % for *ETV6* deletion with *RUNX1* amplification, 1.21 % for *ETV6* amplification with *RUNX1* amplification, 0.6 % for polyploidy, 0.6 % for *RUNX1* deletion, and 0.6 % for diminished *ETV6* signal. The most common structural abnormality was the t(12;21) translocation, followed by *RUNX1* amplification and *ETV6* deletion, while the most commonly observed numerical abnormality was trisomy 21.

Keywords Acute lymphoblastic leukemia · ETV6 · RUNX1 · t(12;21) translocation · FISH

Introduction

Acute lymphoblastic leukemia (ALL) is characterized by clonal proliferation, accumulation and tissue infiltration of neoplastic hematopoietic cells in the bone marrow. These abnormal cells are arrested in the lymphoblast stage of the normal maturation pathway. Aberrations in proliferation and differentiation of these cells are common and hematopoiesis is suppressed. Symptoms may be due to the presence of anemia, neutropenia, or thrombocytopenia [1]. ALL is the most common form of leukemia that occurs in children, accounting for about 75–80 % of childhood leukemias and with a sharp peak incidence at 2–5 years of age [2, 3]. Recurrent chromosomal abnormalities including t(12;21), t(9;22), rearrangement of 11q23, hyperdiploidy, hypodiploidy, trisomy/polysomy 21 and duplication of the der(21)t(21;21), have been observed in pediatric ALL cases. Detection of these cytogenetic abnormalities is very important for the prediction of prognosis. Among these abnormalities, t(12;21) translocation which is the most frequently observed abnormality among childhood ALL cases, is a good prognostic marker for disease course [4, 5].

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The translocation t(12;21)(p13;q22), resulting in the *ETV6–RUNX1* fusion gene, is a chromosomal abnormality with an approximate frequency of 25 % in childhood ALL patients [6–8]. The *ETV6–RUNX1* fusion is associated with early onset of disease, a B-lineage immunophenotype, and favourable prognosis following conventional therapeutic strategies [8, 9]. The t(12;21) translocation may be an initiating mutation in the leukemic process. However, secondary chromosomal abnormalities are believed to be necessary for the development of leukemia. For instance, the non-translocated *ETV6* allele is deleted in approximately 70 % of ALL patients with t(12;21) and this subtype has also a favorable prognosis [10–12]. Whether the loss of normal *ETV6* function as a putative tumor suppressor gene alone or the presence of other chromosome anomalies have any influence on the occurence of relapse is still not known [13–15].

Since the t(12;21) translocation is virtually undetectable with conventional cytogenetic procedures, the two preferred screening methods are reverse transcriptase polymerase chain reaction (RT-PCR) and fluorescence in situ hybridization (FISH). In this study, to detect the t(12;21) translocation; we used these two methods and we present t(12;21) translocation and atypical FISH signal patterns detected by FISH techniques using *ETV6*(*TEL*)/*RUNX1*(*AML1*) extra signal (ES) dual-color translocation probe on bone marrow in 165 pediatric patients with ALL.

Materials and Methods

Patients

Between 2010 and 2013, 165 pediatric patients with newly diagnosed ALL were included in this study. The patients were classified according to the French–American–British criteria and treated according to ALL-BFM protocols. Immunophenotyping was performed at the European Group for the Immunological Classification of Leukemia criteria by flow cytometry with a large panel of commercial monoclonal antibodies directed against the following surface and intracellular antigens. Of these patients, 110 were males and 55 were females (sex ratio 2.04:1). The mean age was 7 years ranging between 3 months and 18 years of age.

Conventional Cytogenetics

The Chromosome Kit M (EuroClone, Milan, Italy) was used for the preparation of bone marrow cell cultures in accordance with the manufacturer's protocols. Synchroset (EuroClone, Milan, Italy) was used in order to obtain chromosome preparations at high band level and a high number of metaphases. Cultures were incubated for 24 h at 37 °C. Cultures were treated with Colcemid (0.1 μg/ml) (Gibco, USA) for 30 min before harvesting and then harvested. Standard cytogenetic preparations were made. Metaphase chromosomes were analyzed using GTG banding. At least 20 metaphases were evaluated from each case by a motorised microscope (Axio Imager. Z2. Carl Zeiss, Germany) with the software Metafer 4 (version 3.8 MetaSystems GmbH, Altlussheim, Germany) for metaphase scanning and capturing. The karyotypes were analyzed using Ikaros software (version 5.4 MetaSystems GmbH, Altlussheim,Germany) and reported according to the International System for Human Cytogenetic Nomenclature recommendations (ISCN 2013).

Fluorescence In Situ Hybridization (FISH)

FISH was performed according to the manufacturer's instructions using a LSI *ETV6*(*TEL*)/*RUNX1*(*AML1*) *ES* dual-color translocation probe (Vysis-Abbott Molecular Inc. Des Plaines, IL 60018, USA) on bone marrow slides. At least 200 interphase nuclei were analyzed from each case under a motorised fluroscence microscope (Axio Imager. M1. Carl Zeiss, Germany) with the software Metafer 4 (version 3.9.0 MetaSystems GmbH, Altlussheim, Germany). This probe set includes a SpectrumGreen *ETV6* probe and a SpectrumOrange *RUNX1* probe. In normal interphase nucleus with the use of *ETV6*(*TEL*)/*RUNX1*(*AML1*) ES probe, the expected pattern is two orange and two green signals (2O2G). The expected FISH signal pattern of t(12;21)(p13;q22) translocation consists of one fusion (*ETV6–RUNX1*) on the der(21), one green (normal *ETV6* allele), one large orange (normal *RUNX1*) and one smaller orange (*RUNX1*) on the der(12).

Reverse Trancriptase-PCR (RT-PCR)

RNA extraction from bone marrow tissues and cDNA synthesis were performed by using QIAmp RNA Blood Mini Kit (Cat. no. 52304) and kit Roche Transcriptor First Strand cDNA Synthesis Kit (Cat. no. 04379012001) respectively, according to manufacturer's recommendations. Nested and shifted amplifications were conducted using previously standardized PCR primers and conditions for detection of translocation t(12;21)(p13;q22) [16]. ABL1 gene was used as control as suggested by Dongen et al. [16]. All PCR products were run on 3 % agarose gel electrophoresis.

Results

A total of 165 pediatric ALL cases were screened by FISH technique using *ETV6/RUNX1* ES dual color translocation probe. *ETV6* and/or *RUNX1* gene abnormalities were

observed in 42 (25.5 %) patients. Structural changes were detected in 33 (78.6 %) and numerical abnormalities in 9 (21.4 %) of abnormal karyotypes by FISH. The results are summarized in Table 1. The clinical features of these patients were showed Table 2. t(12;21)(p13;q22) translocation (*ETV6–RUNX1* fusion) signal pattern was observed in 14 cases (8.4 %) of 165 pediatric ALL patients. Among these 14 cases, typical t(12;21)(p13;q22) translocation signal pattern was determined in 8 (4.8 %) cases. Atypical FISH signal patterns in addition to *ETV6–RUNX1* fusion were observed in 6 (3.6 %) patients and detected signal patterns of these cases were as follows; deletion of the normal *ETV6* allele in 2 cases (1.21 %), deletion of the normal *ETV6* allele and monosomy 21 in 1 case (0.6 %), amplification of *RUNX1* allele in 2 cases (1.21 %) and double *ETV6–RUNX1* fusion combined with *ETV6* deletion in 1 case (0.6 %). In cases with *ETV6–RUNX1* fusion, the presence of *ETV6–RUNX1* mRNA transcript was detected by RT-PCR.

Other atypical FISH abnormalities without the *ETV6–RUNX1* fusion were determined in 28 of the ALL cases (16.9 %). These abnormalities were classified as amplification of the *RUNX1* allele in 10 cases (6.1 %), tetrasomy 21 in 2 cases (1.21 %), trisomy 21 in 3 cases (1.8 %), polyploidy in 1 case (0.6 %), near triploidy in 2 cases (1.21 %), *ETV6* deletion in 3 cases (1.8 %), *ETV6* deletion with *RUNX1* amplification in 2 cases (1.21 %), hyperdiploidy in 2 cases (1.21 %), *ETV6* amplification with *RUNX1* amplification in 1 case (0.6 %), *RUNX1* deletion in 1 case (0.6 %) and diminished *ETV6* signal in 1 case (0.6 %).

These FISH results were used for the follow-up of residual disease in eight patients with only *ETV6–RUNX1* fusion and 17 patients with atypical signal patterns. Patients were followed up for 5–45 months. Twenty-three patients became FISH negative after induction. There were, however, 1 positive case for FISH, relapsed and exitus (Case #18). Also, during both the diagnosis and the follow-up, the RT-PCR results of all of the patients were found to be correlated with their FISH results.

Conventional cytogenetic analysis was used to successfully determine the karyotype in 28 (66.7 %) of the 42 cases. While 12 cases (42.9 %) had a normal karyotype, 16 of the cases (57.1 %) were found to have an abnormal karyotype. The abnormal karyotypes included the following: hyperdiploidy (2 cases), hypodiploidy (2 cases), complex karyotype (3 cases), near triploidy (3 cases), trisomy 21 (3 cases), polyploidy (1 case) and structural chromosome abnormalities (2 cases). While trisomy 21 was the only cytogenetic abnormality that was detected in the patients with this karyotype, tetrasomy 21 was observed in the patients with the hyperdploidy karyotype.

Discussion

Genetic abnormalities play an essential role in diagnosis and management of patients with childhood ALL. Cytogenetic analysis of ALL is difficult due to the low mitotic index and poor quality of metaphases. Conventional cytogenetic analysis, complemented by FISH, is highly effective in the accurate detection of childhood ALL related chromosomal abnormalities [9]. Because the translocation t(12;21)(p13;q22) is invisible by conventional cytogenetic analysis, diagnosis is based on molecular methods. The fusion gene can be visualized by FISH using specific dual colour probes. Detection of this fusion gene is important as it has been found to occur in approximately 25 % of childhood B-lineage ALL. This translocation has been associated with good prognosis.

In our present study, we used FISH and RT-PCR techniques for detection of *ETV6–RUNX1* fusion in 165 patients with childhood ALL. We found *ETV6–RUNX1* fusion with an incidence of 8.5 % (14/165) in these patients. This incidence was similar to that reported in India by Inamdar et al. (7 %) [17], but lower to that reported by Nordic countries (25 %) [18], Chinese (17 %) [19, 20], US (22 %) [8], Brazil(40 %) [21], UK(22 %) [22], Germany and Italy (18.9 %) [23]. The difference might be explained by geographical heterogenity in the frequency of this fusion t(12;21) of childhood ALL.

Also, the incidence of additional abnormalities in *ETV6* and *RUNX1* genes in patients with t(12;21) positive ALL were detected as 42.9 % (6 out of 14 patients). Deletion of the unrearranged *ETV6* allele and amplification of *RUNX1* gene were observed most frequently (14.3 % each) followed by unrearranged *ETV6* deletion combined with monosomy 21, and an double *ETV6–RUNX1* fusion accompanying *ETV6* deletion (Table 2). In previous studies, inconsistent results on the prognostic effects of additional genetic changes were reported. For example, Chung et al. reported that no significant differences in the clinical features and outcome according to the presence or absence of additional genetic changes [24]. In another study, native *TEL* deletions in *TEL-AML1* + childhood ALL patients are associated with better prognosis among *TEL-AML1* + childhood ALL cases [25]. In contrast, Attarbaschi et al. reported that *TEL* deletions, trisomy 21 and an additional der(21)t(12;21) were detected in 55, 14 and 15 % in patients with *TEL/AML1* fusion, respectively and found that *TEL/AML1* + patients with a *TEL* deletion seem to fare actually worse than those without it [26]. In our study, three patients showed *ETV6* gene deletion without *ETV6–RUNX1* fusion. One of these patients had del(12)(p12) karyotype was observed by conventional cytogenetics (case #32). On the other hand, we determined two cases with

Table 1 Clinical, cytogenetic and FISH findings of childhood ALL patients

Case no	Diagnosis	Age/sex	FISH results	Interpretation	RT-PCR results	Cytogenetic results	Follow up FISH results	Clinical findings
1	B-ALL	4/M	1F2O1G [93 %]	ETV6/RUNX1 (+)	+	46, XY [9]	2O2G [100 %]	Alive at 26 months, no relaps
2	B-ALL	4/M	1F2O1G [97 %]	ETV6/RUNX1 (+)	+	40–45, XY, −19 [4] [cp6]/ 46, XY [9]	2O2G [100 %]	Alive at 25 months, no relaps
3	B-ALL	1/M	1F2O1G [90 %]	ETV6/RUNX1 (+)	+	–	2O2G [100 %]	Alive at 18 months, no relaps
4	B-ALL	5/M	1F2O1G [90 %]	ETV6/RUNX1 (+)	+	47, XY, t(3;12)(q11;p13), +10, t(15;19) (q15;q13) [6]/46, XY [5]	2O2G [100 %]	Alive at 15 months, no relaps
5	B-ALL	7/M	1F2O1G [95 %]	ETV6/RUNX1 (+)	+	46, XY [8]	2O2G [100 %]	Alive at 15 months, no relaps
6	B-ALL	3/M	1F2O1G [90 %]	ETV6/RUNX1 (+)	+	46, XY [8]	2O2G [100 %]	Alive at 15 months, no relaps
7	B-ALL	2/M	1F2O1G [95 %]	ETV6/RUNX1 (+)	+	–	2O2G [100 %]	Alive at 14 months, no relaps
8	B-ALL	3/F	1F2O1G [90 %]	ETV6/RUNX1 (+)	+	46, XX, del(6)(q21;q23) [6]/46, XX [13]	2O2G [100 %]	Alive at 13 months, no relaps
9	B-ALL	3/M	1F2O [71 %]	ETV6/RUNX1 (+)/ETV6 del	+	46, XY [9]	–	Alive at 30 months, no relaps
10	B-ALL	4/F	1F2O [95 %]	ETV6/RUNX1 (+)/ETV6 del	+	–	2O2G [100 %]	Alive at 17 months, no relaps
11	B-ALL	4/F	1F2O1G [48 %]/ 1F3O1G [27 %]	ETV6/RUNX1 (+)/RUNX1 amp	+	–	2O2G [100 %]	Alive at 17 months, no relaps
12	B-ALL	7/F	1F3O1G [84 %]	ETV6/RUNX1 (+)/RUNX1 amp	+	–	2O2G [100 %]	Exitus
13	B-ALL	2/M	1F1O [90 %]	ETV6/RUNX1 (+)/ETV6 del/ Monosomy 21	+	42, XY, −18, −19, −20, −21 [1]/46, XY [5]	2O2G [100 %]	Alive at 40 months, no relaps
14	B-ALL	6/M	2F4O2G [89 %]	ETV6/RUNX1 × 2/ETV6 del	+		2O2G [100 %]	Alive at 45 months, relaps, SCT
15	B-ALL	11/F	5O2G [85 %]/4O2G [13 %]	RUNX1 amplification	−	46, XX [9]	2O2G [100 %]	Alive at 40 months, no relaps
16	B-ALL	6/M	3O2G [28 %]/4O2G [72 %]	RUNX1 amplification	−	–	2O2G [100 %]	Alive at 27 months, no relaps
17	B-ALL	2/F	4O2G [93 %]	RUNX1 amplification	−	46, XX [8]	–	Alive at 19 months, no relaps
18	B-ALL	16/F	4O2G [95 %]	RUNX1 amplification	−	47, XX, +21 [12]	4O2G [95 %]	Down syndrome, relaps, exitus
19	B-ALL	6/F	4O2G [74 %]/3O2G [6 %]	RUNX1 amplification	−	46, XX [15]	2O2G [100 %]	Alive at 13 months, no relaps
20	B-ALL	5/M	6O2G [10 %]/5O2G [17 %]/4O2G [23 %]/ 3O2G [10 %]	RUNX1 amplification	−	–	2O2G [100 %]	Alive at 17 months, no relaps
21	B-ALL	4/F	5O2G [32 %]/4O2G [46 %]/3O2G [49 %]	RUNX1 amplification	−	–	–	Alive at 11 months, no relaps
22	B-ALL	8/M	5O2G [95 %]	RUNX1 amplification	−	–	–	Alive at 5 months, no relaps
23	B-ALL	3/F	3O2G [73 %]	RUNX1 amplification	−	46, XX [8]	2O2G [100 %]	Alive at 25 months, no relaps
24	B-ALL	16/M	3O2G [78 %]	RUNX1 amplification	−	46, XY [25]	2O2G [100 %]	Alive at 35 months, no relaps
25	B-ALL	6/M	1O2G [13 %]	RUNX1 deletion	−	–	2O2G [100 %]	Alive at 33 months, no relaps

Table 1 continued

Case no	Diagnosis	Age/sex	FISH results	Interpretation	RT-PCR results	Cytogenetic results	Follow up FISH results	Clinical findings
26	B-ALL	5/M	3O2G [40 %]	Trisomy 21	–	46, XY, −7, +21 [7]/46, XY [6]	–	Alive at 18 months, no relaps
27	B-ALL	1/M	3O2G [92 %]	Trisomy 21	–	47, XY, +21 [15]	–	Alive at 16 months, no relaps
28	B-ALL	2/M	3O2G [92 %]	Trisomy 21	–	54, XY, +X, dup(1)(q31;q43), +4, +6, +10, +14, +17, +18, +21 [6] /46,XY [2]	–	Alive at 18 months, no relaps
29	B-ALL	4/M	4O2G [95 %]	Tetrasomy 21	–	57, XY, +3, +4, +5, +8, +9, +11, +14, +15, +17, +21, +21 [8]	–	Alive at 7 months, no relaps
30	B-ALL	10/M	4O2G [96 %]	Tetrasomy 21	–	59, XY, +X, +Y, +3, +4, +6, +8, +10, +14, +14, +17, +18, +21, +21 [8] / 46, XY [6]	2O2G [100 %]	Alive at 36 months, no relaps
31	B-ALL	5/F	2O1G 1 [81 %]	ETV6 deletion	–	–	–	No follow up
32	B-ALL	14/F	2O1G 1 [63 %]	ETV6 deletion	–	46, XX, del(12)(p12) [8]/ 46, XX [4]	2O2G [100 %]	Alive at 35 months, no relaps
33	B-ALL	5/F	2O1G 1 [56 %]	ETV6 deletion	–	–	–	Alive at 45 months, relaps??
34	B-ALL	5/M	4O1G [62 %]/3O1G [12 %]/4O2G [11 %]/	ETV6 del/RUNX1 amp	–	46, XY [8]	2O2G [100 %]	Alive at 18 months, no relaps
35	B-ALL	2/M	4O1G [89 %]	ETV6 del/RUNX1 amp	–	46, XY [4]	–	Alive at 20 months, no relaps
36	B-ALL	2/M	3O3G [38 %]/4O3G [12 %]/2O4G 28 %]/4O2G [22 %]	ETV6 amp/RUNX1 amp	–	–	2O2G [100 %]	Alive at 35 months, no relaps
37	B-ALL	5/M	3O2G [4 %]/3O3G [22 %]/3O4G [63 %]	Polyploidy	–	46, XY, inv(9)(p11;q13) [11]	–	Alive at 33 months, no relaps
38	B-ALL	6/F	4O4G [53 %]	Near triploidy	–	79, XXXX, del(17)(p12) [3]/46, XX, del(17)(p12) [15]	–	Alive at 27 months, no relaps
39	B-ALL	9/M	6O2G [48 %]/5O2G [41 %]	Hyperdiploidy/ RUNX1 amp	–	57, +X, +der(1)t(1;9)(p31;p21), +del(6), (q21;q23), +12, +14, +15, −16, +17, +19, +20, +21, +add(22)(q13), +mar [13]/46, XY [2]	–	Relaps, Exitus

Table 1 continued

Case no	Diagnosis	Age/sex	FISH results	Interpretation	RT-PCR results	Cytogenetic results	Follow up FISH results	Clinical findings
40	B-ALL	6/M	4O2G [72 %]/ 3O2G [18 %]	Hyperdiploidy/RUNX1 amp	–	56,XY, +X, +4, +del(6)(q21), +8, +9, +11, +14, +15, +20, +21 [11]/46, XY [22]	–	Alive at 11 months, no relaps
41	T-ALL	4/F	4O4G [24 %]	Near triploidy	–	61–68, XXX, del(6)(q21) [10]/46, XX [8]	–	Alive at 11 months, no relaps
42	B-ALL	16/M	2O1G 1dimG [75 %]	Diminished ETV6 signal	–	43, XY, t(6;9;12)(q13;p22;p13), −15, 21 [2]/45, XY, t(6;9;12)(q13;p22;p13),-6 [2] /46, XY [7]	–	Alive at 33 months, no relaps

ETV6 deletion and *RUNX1* amplification (case #34–35) and one case with amplifications of *ETV6* and *RUNX1* (case #36). These deletions and amplifications had not been detected by conventional cytogenetics. The deletion of untranslocated *TEL* allele accompanying the *TEL/AML1* fusion in ALL patients has been frequently reported [26, 27]. The deletion of the normal *TEL* allele appears to be significant in terms of understanding the progression of leukemia with t(12;21), but impact on prognosis has not yet been clarified. The *TEL* deletion without *TEL/AML1* fusion has been reported rarely. Lee et al. showed the cryptic deletion of *TEL* gene in the absence of *TEL/AML1* rearrangement in three adult ALL patients (4.0 %) [28]. To clearly identify the incidence and significance of this abnormality, much more study is needed.

Amplification of *RUNX1* gene has been described in 20 ALL pediatric patients in our study. Only, nine patients had three or more copies of *RUNX1* gene without polysomy of chromosome 21 (21.4 %). This incidence was higher than that reported by Gmidene et al. (10.5 %) [29]. Most of our patients achieved complete remission periods vary between 5 and 45 months except of four cases. Indeed, one the patients with four copies of *RUNX1* gene, had Down syndrome (Case #18). This patient relapsed 42 months after diagnosis and patient died due to developing sepsis. Children with Down syndrome have an increased risk for developing B-cell precursor ALL and an poor outcome due to a high relapse rate and the increased adverse effects of chemotherapy [30]. Two patients had *RUNX1* gene amplification together with *ETV6–RUNX1* fusion (Case #11 and 12). One of these patients died from fungal pneumonia during induction therapy (Case #12). The one patient relapsed and subsequently died after stem cell transplantation (SCT), had five and six copies of *RUNX1* gene with near triploid karyotype (Case #39). In spite of limited number of patients, one could speculate that coincidence of fusion of *ETV6–RUNX1* and amplification of *RUNX1* gene might be unfavorable.

In cases 2, 4 and 8, in addition to the t(12;21) positive signal pattern that was detected by FISH, chromosomal abnormalities were also detected by conventional cytogenetic analysis. Each of these patients underwent a complete period of remission, which varied between 13 and 25 months. Based on these findings, it can be speculated that these chromosomal abnormalities do not improve the prognosis associated with the t(12;21) translocation. In order to gain a better understanding of how the prognosis for the t(12;21) translocation is affected by these chromosomal abnormalities, further studies are needed.

Chromosome 6 abnormalities were observed most frequently in conventional cytogenetic analysis in our patient cohort. Trisomy of chromosome 6 was detected in two cases without t(12;21) translocation (cases #28 and #30)

Table 2 The clinical features of patients with *ETV6* and *RUNX1* abnormalities

	ETV6–RUNX1 positive ($n = 14$)	RUNX1 amplification ($n = 10$)	Polysomy of chr. 21 ($n = 10$)	ETV6 deletion ($n = 3$)	Additional cytogenetics abnormalities ($n = 5$)
Age					
0–5	10	3	4	–	2
5–10	4	4	6	2	2
>10	–	3	–	1	1
Sex					
Male	10	4	8	–	5
Female	4	6	2	3	–
WBC (BIN/mm^3)					
<10.000	5	3	7	2	4
10–20.000	5	3	2	1	–
>20.000	4	4	1	–	1
Immunophenotype					
Pro-B	–	2	–	–	–
Pre-B	14	8	9	3	5
T-Cell	–	–	1	–	–
Follow-up (min–max)	13–45 months	5–40 months	7–36 months	35–45 months	18–35 months

with hyperploidy including chromosome 21 numerical abnormality. These cases were at remission and have been followed up for 18 and 36 months. Chromosome 6 long arm deletions were detected in four cases. Chromosome 6 long arm deletion was observed in a case (Case #8) with t(12;21) positive FISH signal pattern. This case was in remission at 13 months. Remaining three cases with chromosome 6 long arm deletions (Cases #39, #40, #41) had hyperdiploidy including chromosome 21 numerical abnormality. One of these cases was relapsed and subsequently died, while remaining two cases were in remission at 11 months. In case 42 with a diminished *ETV6* signal, t(6;9;12)(q13;p22;p13) translocation was detected by conventional cytogenetics. This finding suggests that diminished *ETV6* signal observation might be an indicator of variant *ETV6* translocations. In conclusion, long term clinical evaluations of further cases are needed to unravel the prognostic importance of chromosome 6 aberrations in childhood ALL cases with *ETV6–RUNX1* fusion.

Chromosome analysis was failed in 12 cases with atypical signal patterns. On the other hand, we used FISH results for minimal residual disease (MRD) follow-up in 17 cases with atypical signal patterns. Our FISH results was determined as compatible with clinical course of patients. As a result, interphase FISH analysis with *ETV6*(*TEL*)/*RUNX1*(*AML1*) *ES* dual-color translocation probe system allows us to detect abnormalities in *ETV6* and *RUNX1* genes, as well as to detect *ETV6–RUNX1* fusion. Also, detection of atypical FISH signal patterns of *ETV6* and *RUNX1* genes is important for follow-up of pediatric ALL patients.

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Conflict of interest The authors declare that they have no conflict of interest.

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