Cytogenetic and Fluorescence in situ Hybridization Profile of Pediatric Acute Lymphoblastic Leukemia in a University Hospital in South India

Latha Sneha Magatha^a Julius Xavier Scott^a Gayathri Subramaniam^b
Thirugnanasambandan Chandrasekaran^a Solomon Franklin Durairaj Paul^b
Teena Koshy^b

^aDivision of Pediatric Hemato-oncology, Department of Pediatrics, Sri Ramachandra Institute of Higher Education and Research, Chennai, India; ^bDepartment of Human Genetics, Sri Ramachandra Institute of Higher Education and Research, Chennai, India

Highlights of the Study

- We report the frequency of cytogenetic abnormalities in children with acute lymphoblastic leukemia referred to a university hospital.
- In our study, 29.5% of the patients had chromosome anomalies and 46.4% had nonrandom abnormalities detected by interphase fluorescent in situ hybridization.
- Gain of *RUNX1*, *KMT2A*, and *ETV6/RUNX1* fusion was observed in a significant number of patients.

Keywords

Pediatric acute lymphoblastic leukemia · Cytogenetics · BCR/ABL1 · ETV6/RUNX1 · KMT2A rearrangement · TCF3-PBX1

Abstract

Objective: The purpose of this study was to evaluate the cytogenetic and fluorescent in situ hybridization (FISH) profile in children with acute lymphoblastic leukemia (ALL), referred to a university hospital in a 5-year 6-month period.

Subjects and Methods: Cytogenetic analysis of the bone marrow aspirate specimens of 91 patients was performed by standard Giemsa (G)-banding and interphase FISH (iFISH).

Results: The frequency of chromosomal abnormalities detected by G-banding was 29.5%, and the frequency of non-random abnormalities with independent prognostic significance identified by iFISH was 46.4%. The abnormality with

the highest frequency was gain of *RUNX1* ($n = 18$, 21.4%), followed by *ETV6/RUNX1* fusion ($n = 7$, 8.3%), and gain of *KMT2A* ($n = 6$, 7.1%). Additionally, rarely reported gains of *ETV6*, *PBX1*, and *ABL1* were observed at a frequency of 6% ($n = 5$), and the deletion of *ETV6* and *TCF3* was seen at a frequency of 3.6% ($n = 3$) and 2.3% ($n = 2$), respectively. A 10-year old with intrachromosomal amplification of chromosome 21 was also observed. **Conclusions:** This study strengthens and widens the current knowledge of the cytogenetic landscape of pediatric ALL.

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Introduction

Acute lymphoblastic leukemia (ALL) is the most frequent hematologic malignancy detected in the pediatric population and accounts for approximately 25% of can-

cer diagnoses among children younger than 15 years of age [1, 2]. This neoplasia is characterized by the abnormal proliferation of immature lymphoid cells, and the diagnosis is based on medical examination, flow cytometric immunophenotyping, cytochemical characteristics, and genetic findings [1].

In genetic analyses, conventional Giemsa (G)-banding is commonly used to detect chromosomal abnormalities in ALL [3]. However, this method has a few limitations; sometimes poor-quality chromosomes with indistinct banding for an accurate analysis are obtained, other times chromosomes may not be available for analysis and hence must be designated as a culture failure. Additionally, identification of certain subtle rearrangements, such as translocation of *ETV6/RUNX1*, is not possible, and this would require the application of interphase fluorescent in situ hybridization (iFISH) for detection [4, 5]. Hence, chromosomal analysis with iFISH is more effective in diagnosing certain cryptic translocations [6]. In most countries, it has been established that genetic abnormalities can be identified in 75%–80% of childhood ALL by standard chromosomal and iFISH analyses [2, 7]. Both these approaches group patients into favorable and unfavorable prognostic subgroups; the former includes hyperdiploidy and translocation (t) 12; 21 and the latter includes hypodiploidy, amplification of chromosome 21, t (9; 22), and *KMT2A* rearrangements.

Global studies also suggest that the frequency of genetic abnormalities in childhood ALL vary by ethnicity and the geographic region [8]. However, there remain limited figures from India; the reported data showing a higher proportion of children with unfavorable cytogenetics [9]. Since information on the cytogenetic profile among children belonging to south India is fairly scarce, it is still unclear whether these children present aberrations typically associated with poor or favorable prognoses more frequently than other ethnicities. The aim of this study was to report the chromosome abnormalities in cytogenetically evaluated pediatric patients with ALL, treated at a single university hospital in Chennai between October 2014 and April 2020.

Materials and Methods

This study was conducted in the Division of Pediatric Hemato-Oncology, Department of Pediatrics in collaboration with the Department of Human Genetics, Sri Ramachandra Institute of Higher Education and Research (Chennai, India). All the patients younger than 18 years at the time of diagnosis and undergoing treatment for ALL were included in this study. Patients presenting with leukemia other than progenitor B-cell ALL were excluded.

Fig. 1. Trend in percentage of successfully performed karyotyping.

For patients who had a relapse of ALL, only the findings at initial diagnosis were considered. The study was approved by the Institutional Ethics Committee (reference no: IEC-NI/14/JAN/38/03), and an informed assent was obtained from the parents.

Bone marrow aspiration was performed and processed for karyotyping by standard methods [10]. At least 10 metaphases were analyzed, and any numerical or structural chromosome abnormalities observed were reported in accordance with the ISCN 2020 [11]. FISH using commercial probes was performed as per the manufacturer's protocol and included the LSI™ *ETV6/RUNX1* dual color (DC), dual fusion (DF) translocation probe, LSI™ *BCR/ABL* DC DF translocation, LSI™ *TCF3/PBX1* DC DF translocation, and the LSI™ *MLL* DC, break apart rearrangement probes (Abbott Molecular, IL, USA). Analysis was conducted following the American College of Medical Genetics technical standards and guidelines for iFISH [12]. Two hundred interphase nuclei were scored for each probe by 2 independent analysts. If there were discrepant results, additional nuclei were examined by a third technologist. Probes were validated and cutoff values were determined in-house, before clinical use. To establish a FISH probe's limit of detection, the upper limit of the abnormal signal pattern in normal cells was calculated. Analysis of 200 nuclei, from at least 20 individuals who had no indication of having the condition of interest, was recorded. The normal cutoff value was calculated using the statistical method of confidence interval around the mean. A cutoff of 5% for abnormal signal pattern with a confidence limit of a 95% was considered acceptable for each FISH probe used.

Standard descriptive statistics was used to analyze the data. The categorized data were expressed as both number and percentage.

Results

In this 5.5-year period, a total of 91 patients underwent cytogenetic investigation, either chromosome analysis (7.7%, $n = 7$) or iFISH (49.4%, $n = 45$) alone or in combi-

Table 1. Abnormal karyotypes were observed by G-banding in our series ($n = 13$)

Patient, n	Age, years	Sex	Karyotype
1	17	M	92,XXYY[10]
5	11	M	46,XY,del(11)(q13q23)[10]
18	4	F	45,XX,-7,add(14)[10]
28	4	M	46,XY,del(9)(q12q21)[4]/46,XY[13]
33	13	M	46,XY,t(9;22)(q34;q11)[10]
34	5 mo	M	49,XY,+7,+8,+12[11]/46,XY[5]
45	14	F	46,XY,del(9)(p12)[15]
50	4	M	58~61,XXY,t(1;19)(q23;p13),del(12)(p12),+2[10],+3[5],+4[11],+5[10],+6[11],7[7],+8[7],+10[9],+11[9],+12[8],+13[5],+14[11],+15[6],+16[1],+17[11],+18[6],+21[12],+21[12],+21[2] [cp12]/46,XY[8]
61	11	F	45,X,-X,t(9;12)(p24;q21),t(12;17)(p13;q12)[10]/44,X,-X,t(9;12)(p24;q21),t(12;17)(p13;q12),-22[6]
64	4	F	46,XX,add(12)(p13)[5]/46,XX[7]
74	3	F	52~55,XX[10]
89	1	F	45,XX,dic(7;9)(p11;p11),der(8)?t(7;8)(p12-22;p21)[20]
91		M	42~56,XY[3]/46,XY[17]

G, Giemsa.

nation (42.9%, $n = 39$). The mean age of patients at the time of diagnosis was 6.8 ± 4.7 years, with the minimum age at diagnosis being 5 months and the maximum 17 years. Sixty-four patients (70.3%) were below the age of 10 years and 27 (29.7%) were above the age of 10 years. A majority of the patients were males (65%, $n = 59$). While the total leukocyte count at diagnosis ranged from 1,500/ mm^3 to 1,91,200/ mm^3 , the platelet count ranged from 12,000/ μL to 5,00,000/ μL .

The percentage of successfully performed karyotyping improved progressively, with a success rate of 31.3% ($n = 10$ of 32) in 2014, 69.2% ($n = 3$ of 4) in 2019, and 100% ($n = 5$ of 5) by 2020 (Fig. 1). This is credited to the modifications in processing methods to optimize the results. The use of FISH, introduced in our laboratory in 2014, was very useful to detect cryptic rearrangements and was used as an alternate test in cases where metaphase chromosomes were not obtained for analysis (culture failure). Using this approach, we were able to improve our reporting rate from 50% ($n = 9$ of 18) in 2014 to 87.5% ($n = 6$ of 7) in 2016 and 100% by 2020 ($n = 5$ of 5).

Cytogenetic Profile

Karyotyping was performed in 46 patients at diagnosis and was successful in 44 patients (95.6%). An abnormal chromosome study was observed in 13 patients (29.5%). Low hypodiploidy, defined with the modal number 33–38 and near haploidy (modal number 24–29), was not observed in this study population. Karyotypes with structural abnormalities, either isolated or in combination

Table 2. Frequency of abnormalities detected by iFISH ($n = 84$)

Loci	Frequency, n (%)	Sex, M/F	Median age, years
Fusions/rearrangements			
<i>ETV6/RUNX1</i>	7 (8.3)	3/4	4
<i>BCR/ABL1</i>	4 (4.8)	4/0	9.5
<i>KMT2A</i>	3 (3.5)	2/1	11
<i>TCF3/PBX1</i>	3 (3.5)	0/3	2
Loss/deletion			
<i>ETV6</i>	3 (3.6)	1/2	4
<i>TCF3</i>	2 (2.3)	2/0	4
Gain			
<i>RUNX1</i>	18 (21.4)	12/6	3.5
<i>KMT2A</i>	6 (7.1)	6/0	4.5
<i>ETV6</i>	5 (6.0)	3/2	3
<i>PBX1</i>	5 (6.0)	4/1	4

iFISH, interphase fluorescent in situ hybridization.

with numerical abnormalities/ploidy, observed by G-banding are summarized in Table 1.

Interphase Fluorescent in situ Hybridization

iFISH was performed for 84 patients (92.3%), and nonrandom abnormalities were observed in 39 patients (46.4%). Table 2 describes the frequency of these abnormalities detected by iFISH. Gains and losses of the targeted loci were observed, either as isolated findings or in combination with the fusion/rearrangements. Of the

Table 3. Distribution of cytogenetic and iFISH findings ($n = 39$)

Patient ID	iFISH results			Karyotype findings
	probe	pattern	interpretation	
3	<i>BCR/ABL</i> DC DF	1G1O2F (100%)	Fusion	NA
5	<i>MLL</i> DC BA	1G1O1F (100%)	Rearrangement of <i>KMT2A</i>	46,XY,del(11)(q13q23)[10]
7	<i>BCR/ABL</i> DC DF	1G1O2F (50%)	Fusion	NA
8	<i>BCR/ABL</i> DC DF	1G1O2F (100%)	Fusion	NA
31	<i>ETV6/RUNX1</i> DC DF	1G1O2F (100%)	Fusion	NA
33	<i>BCR/ABL1</i> DC DF	1G1O2F (40%)	Fusion	46,XY,t(9;22)(q34;q11)[10]
35	<i>ETV6/RUNX1</i> DC DF	1G1O2F (100%)	Fusion	46,XY[11]
38	<i>ETV6/RUNX1</i> DC DF	1G1O2F (100%)	Fusion	NA
46	<i>MLL</i> DC BA	1G1O1F (100%)	Rearrangement of <i>KMT2A</i>	NA
47	<i>BCR/ABL1</i> DC DF	1G1O2F (70%)	Fusion	NA
48	<i>BCR/ABL1</i> DC DF	1G1O2F (70%)	Fusion	NA
	<i>TCF3/PBX1</i> DC DF	1G2O (23%)	Loss of <i>TCF3</i>	
50	<i>TCF3/PBX1</i> DC DF	1G3O (77%)/2G3O (23%)	Gain of <i>PBX1</i> and loss of <i>TCF3</i>	58~61,XXY,t(1;19)(q23; p13),del(12)(p12),+2[10],+3[5],+4[11],+5[10],+6[11],7[7],+8[7],+10[9],+11[9],+12[8],+13[5],+14[11],+15[6],+16[1],+17[11],+18[6],+21[12],+21[12],+21[2] [cp12]/46,XY[8]
	<i>ETV6/RUNX1</i> DC DF	2G4O (80%)	Gain of <i>RUNX1</i>	
51	<i>BCR/ABL1</i> DC DF	1G1O2F (55%)	Fusion	NA
	<i>TCF3/PBX1</i> DC DF	1G2O1F (70%)	Fusion	
54	<i>TCF3/PBX1</i> DC DF	2G3O (15%)	Gain of <i>PBX1</i>	NA
	<i>ETV6/RUNX1</i> DC DF	2G3O (85%)	Gain of <i>RUNX1</i>	
56	<i>TCF3/PBX1</i> DC DF	2G3O (76%)/2G2O	Gain of <i>PBX1</i>	NA
	<i>ETV6/RUNX1</i> DC DF	3G4O (100%)	Gain of <i>ETV6</i> and <i>RUNX1</i>	
58	<i>ETV6/RUNX1</i> DC DF	2G4O (85%)	Gain of <i>RUNX1</i>	NA
59	<i>BCR/ABL1</i> DC DF	3G2O (20%)	Gain of <i>BCR</i>	NA
	<i>ETV6/RUNX1</i> DC DF	3G4O (35%)	Gain of <i>ETV6</i> and <i>RUNX1</i>	
	<i>MLL</i> DC BA	3F (45%)	Gain of <i>KMT2A</i>	
62	<i>ETV6/RUNX1</i> DC DF	2G4O (85%)	Gain of <i>RUNX1</i>	NA
63	<i>TCF3/PBX1</i> DC DF	1G2O1F (100%)	Fusion	46,XX[10]
64	<i>ETV6/RUNX1</i> DC DF	1O2F (100%)	Fusion and concomitant loss of the other <i>ETV6</i> allele	46,XX,add(12)(p13)[5]/46,XX[7]
65	<i>ETV6/RUNX1</i> DC DF	1G2O1F (100%)	Single fusion and gain of <i>RUNX1</i>	46,XX[15]
67	<i>ETV6/RUNX1</i> DC DF	1G2O1F (85%)	Single fusion and gain of <i>RUNX1</i>	46,XY[25]
	<i>MLL</i> DC BA	1G1O1F (20%)	Rearrangement of <i>KMT2A</i>	
68	<i>ETV6/RUNX1</i> DC DF	2G3O (75%)	Gain of <i>RUNX1</i>	46,XY[15]
70	<i>ETV6/RUNX1</i> DC DF	2G4O (22%)	Gain of <i>RUNX1</i>	46,XY[20]
	<i>MLL</i> DC BA	3F (25%)	Gain of <i>KMT2A</i>	

Table 3 (continued)

Patient ID	iFISH results			Karyotype findings
	probe	pattern	interpretation	
71	<i>TCF3/PBX1</i> DC DF	1G2O1F	Fusion	46,XX[14]
72	<i>BCR/ABL1</i> DC DF	2G3O (65%)	Gain of <i>ABL1</i>	46,XY[12]
	<i>ETV6/RUNX1</i> DC DF	2G4O (68%)	Gain of <i>RUNX1</i>	
	<i>MLL</i> DC BA	3F (64%)	Gain of <i>KMT2A</i>	
73	<i>BCR/ABL1</i> DC DF	2G3O (45%)	Gain of <i>ABL1</i>	46,XY[20]
74	<i>ETV6/RUNX1</i> DC DF	2G4O (100%)	Gain of <i>RUNX1</i>	52~55,XX[10]
75	<i>ETV6/RUNX1</i> DC DF	2G3O (66%)	Gain of <i>RUNX1</i>	46,XY[11]
76	<i>BCR/ABL1</i> DC DF	2G3O (25%)	Gain of <i>ABL1</i>	NA
	<i>ETV6/RUNX1</i> DC DF	2G4O (22%)	Gain of <i>RUNX1</i>	
77	<i>ETV6/RUNX1</i> DC DF	2G4O (100%)	Gain of <i>RUNX1</i>	46,XY[11]
	<i>MLL</i> DC BA	3F (80%)	Gain of <i>KMT2A</i>	
	<i>TCF3/PBX1</i> DC DF	2G3O (80%)	Gain of <i>PBX1</i>	
78	<i>BCR/ABL1</i> DC DF	3G2O (40%)	Gain of <i>BCR</i>	NA
	<i>ETV6/RUNX1</i> DC DF	2G4O (35%)	Gain of <i>RUNX1</i>	
	<i>MLL</i> DC BA	3F (25%)	Gain of <i>KMT2A</i>	
79	<i>TCF3/PBX1</i> DC DF	3G2O (57%)	Gain of <i>TCF3</i>	NA
81	<i>ETV6/RUNX1</i> DC DF	1G2O1F (100%)	Single fusion and gain of <i>RUNX1</i>	46,XY[20]
82	<i>ETV6/RUNX1</i> DC DF	1G7O (100%)	iAMP21	NA
83	<i>BCR/ABL1</i> DC DF	4G3O (100%)	Gain of <i>ABL1</i> and <i>BCR</i>	NA
85	<i>ETV6/RUNX1</i> DC DF	2G4O (100%)	Gain of <i>RUNX1</i>	46,XX[20]
86	<i>ETV6/RUNX1</i> DC DF	1G4O (100%)	Loss of <i>ETV6</i> and gain of <i>RUNX1</i>	46,XY[20]
91	<i>BCR/ABL1</i> DC DF	2G3O (65%)	Gain of <i>ABL1</i>	52~66,XX[2]/46,XY[18]

BA, break apart; DC, dual color; DF, dual fusion; F, fusion; G, spectrum green; iAMP21, intrachromosomal amplification of chromosome 21; O, spectrum orange; iFISH, interphase fluorescent in situ hybridization.

21 patients detected with gains of *RUNX1*, *KMT2A*, *ETV6*, *PBX1*, and *TCF3* (either isolated or in combination), 10 of them had a normal karyotype with no cells showing high hyperdiploidy (HHD). While for 9 patients, a karyotype was not available and HHD could not be confirmed, for 2 patients, HHD was observed. Also, as per the guidelines of Harrison et al. [13] patient # 82 was classified as intrachromosomal amplification of chromosome 21 (iAMP21) using the established criteria of 3 or more additional *RUNX1* signals (5 or more signals per cell in total) with a FISH probe targeting the *RUNX1* gene.

Among the fusions, the translocation of chromosomes 12 and 21, *ETV6/RUNX1* fusion, was the most frequent rearrangement found in our series. The expected iFISH signal pattern of t(12;21)(p13;q22) translocation consists of 1 orange (*RUNX1*), 1 green (*ETV6*), and 2 fusions (der [12] and der [21]). It was detected in 7 patients (8.3%), identified solely by iFISH. In addition to having the *ETV6/RUNX1* fusion, 1 patient had an abnormality in the short arm of chromosome 12 with additional chromosome material of unknown origin present on the p arm. A high frequency (4.8%, $n = 4$) of the translocation of chromosomes 9 and 22 (*BCR-ABL1* fusion) was also seen; 1 patient by

iFISH and G-banding and in 3 patients by iFISH alone. In a nucleus containing a simple balanced t(9;22), 1 orange and 1 green signal from the normal 9 and 22 chromosomes and 2 orange/green (yellow) fusion signals, one each from the derivative 9 and 22 chromosomes, will be observed (1O1G2F). Gain of the *BCR* and *ABL1* loci, seen as either an ES (extra green or orange) signal pattern, was observed in 3 (3.6%) and 5 (6%) patients, respectively.

Abnormalities of the 11q23 region involving the *KMT2A* gene were detected in 3 patients (3.3%) by iFISH alone. Interphase nuclei with one fusion signal and one separate orange and green signal (1G1R1F) represents a translocation with *KTM2A* involvement. One patient with this rearrangement also had a deletion in the long arm of chromosome 11, possibly the one not involved in the rearrangement.

TCF3-PBX1 fusion or t(1;19)(q23;p13.3) by iFISH was detected in 3 patients (3.5%). Abnormal hybridization would result in 1 green, 1 orange, and 2 fusion (1G1O2F) signals. One patient (#50), in whom the translocation was detected in the karyotype, had an atypical iFISH signal pattern interpreted as gain of *PBX1* and loss of *TCF3*. The distribution of the abnormal cytogenetic and iFISH findings is summarized in Table 3.

Discussion

Fifty-five to eighty-nine percent of pediatric patients with ALL have chromosome abnormalities detected by conventional cytogenetics and FISH; the varying percentage is dependent on the number of patients studied, the year they were reported, and the populations where they have been studied [14–19]. As established in these studies, to improve the survival of children with ALL, it is important to evaluate the cytogenetic profile. We report the cytogenetic findings in 91 children with ALL spanning a period of 5 years and 6 months referred to our university hospital located in south India.

The frequency of the *BCR/ABL1* positive cases at 4.8% ($n = 4$) was similar to that observed in other populations reporting 5–10% [20], highlighting the fact that analysis of this fusion transcript should be mandatory in all cases of ALL as these patients may benefit from the addition of tyrosine kinase inhibitors. The frequency of *ETV6/RUNX1* at 8.3% ($n = 7$) was higher than that observed in 2 studies from India reporting a frequency of 4.3%–7.3% [21, 22]. A worldwide variation in the incidence of *ETV6/RUNX1* fusion has been reported with the frequency ranging from 13 to 25% [23]. *KMT2A* gene rearrange-

ments are associated with a poor prognosis and are generally seen in infants <1 year of age [22]. In contrast, in this study, *KMT2A* rearrangement was detected in 3 patients (3.5%) aged 2, 11, and 14 years. Previous studies in India have reported either an absence of this transcript or a low frequency ranging from 1.5% to 3.1% [22].

FISH analysis is used in many laboratories to confirm the presence of previously characterized chromosome abnormalities or to rule out suspected cryptic aberrations, thus compensating the shortcomings of karyotyping including culture failure. In cases where we failed to obtain metaphases for analysis, iFISH allowed us to screen large numbers of nondividing cells. In addition, with the inclusion of FISH we were also able to detect losses and gain of loci targeted by the FISH probes.

Similar to our findings, Harewood et al. [24] reported clusters of *RUNX1* signals visible at interphase by FISH in the largest series of 20 ALL patients. However, compared to their patient series, ours differed in 2 factors; the karyotypes in all but one were normal, and the median age of our patient series was younger, 3.5 years compared to their 9 years. Mikhail et al. [25] had a study population that appeared similar to our series in that they were all 6 years old or less and karyotypes were normal in 3 of them, even though one harbored a t(12;21) translocation. These authors also demonstrated that the detection of extra *RUNX1* signals by FISH was associated with overexpression of *RUNX1* measured by real-time quantitative RT-PCR. Since we did not include RT-PCR in our investigations we cannot confirm that is applicable to our patients. Of interest was one of the patients (#82) with 7 signals for *RUNX1* which was interpreted as iAMP21 as per the guidelines of Harrison et al. [26]. iAMP21 is defined as gain of at least 3 copies of part of chromosome 21 incorporating *RUNX1* [2], (5 or more signals per cell in total) with a FISH probe targeting the *RUNX1* [26]. However, it is of interest to consider whether amplification within a single chromosome has the same effect as an acquired gain of chromosome 21, and to what extent the structure and expression of the gene are altered in these different situations and directly contributes to the etiology of ALL.

KMT2A rearrangement most often results from translocation or partial tandem duplication, although recent published reports suggest a different mechanism by which *KMT2A* might participate in leukemogenesis, *KMT2A* amplification [27]. Homogeneously staining regions or ring chromosomes are the most common manifestations of gain/amplification seen in a karyotype, which we did not observe in our 2 patients. Gain of *KMT2A* and/or amplifications have been reported primarily in myeloid

malignancies, therapy-related acute myeloid leukemia, or myelodysplasia [28]. In this context, our findings in B-lineage ALLs seem noteworthy. The same could be said for the *ABL1* gene, which was the next most frequent gain seen in our patient series. The presence of just one extra signal for *ABL1* and *BCR* seen in 5 and 3 patients, respectively, was interpreted as gain of these loci. However, to the best of our knowledge, there have been limited reports of such findings in pediatric ALL. Considering that there was no visible evidence of double minutes in the available karyotype, it would be worthwhile to characterize these findings with molecular techniques like microarray.

The deletion of the normal *ETV6* allele was detected in 2 patients with the *ETV6/RUNX1* gene fusion and 1 patient with a normal karyotype. Raynaud et al. [29] indicated that the association between the t(12;21) and the deletion of the nontranslocated allele of *ETV6* is among the most frequent abnormalities observed in B-lineage ALLs and provides a further proliferative advantage to leukemic cells. However, there are limited reports of gain of *ETV6*, *TCF3* deletion, or gain of *PBX1* with ALL. Thus, this could be unique findings in our population and warrants exploration in a larger patient series.

Conclusion

There still exists a need to address racial and ethnic gaps in the cytogenetic profile of childhood ALL, the most common pediatric cancer. In this study, though the number of patients tested was small, our findings are comparable to other studies and, more importantly, add to the limited data from India.

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Statement of Ethics

The parents of the subjects gave their written informed consent and that the study protocol was approved by the Institute's Committee on Human Research (reference no: IEC-NI/14/JAN/38/03).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Latha Sneha Magatha, Julius Xavier Scott, and Thirugnana-sambandan Chandrasekaran performed clinical investigations, shared clinical data, and wrote clinical parts of the manuscript. Gayathri Subramaniam, Solomon Franklin Durairaj Paul, and Teena Koshy performed the cytogenetic and iFISH investigations and compiled the data. Teena Koshy drafted the manuscript. All authors read and approved the final manuscript.

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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