

Cytogenetic and Molecular Findings in Children with Acute Lymphoblastic Leukemia: Experience of a Single Institution in Argentina

Mariela C. Coccé^a Cristina N. Alonso^b Jorge G. Rossi^c Andrea R. Bernasconi^c
Maria A. Rampazzi^a Maria S. Felice^b Patricia L. Rubio^b Silvia Eandi Eberle^b
Adriana Medina^b Marta S. Gallego^a

^aCytogenetics Laboratory, Genetics Department, and Departments of ^bHematology and Oncology and

^cImmunology and Rheumatology, Hospital de Pediatría 'Prof Dr. Juan P. Garrahan', Buenos Aires, Argentina

Key Words

Childhood acute lymphoblastic leukemia · Chromosomal abnormalities · FISH · RT-PCR

Abstract

The purpose of the current study was to evaluate the cytogenetic findings in 1,057 children with acute lymphoblastic leukemia (ALL) referred to the cytogenetics laboratory at the Hospital de Pediatría Dr. Juan P. Garrahan, between 1991 and 2014. Chromosomal abnormalities were evaluated by G-banding and FISH. Since December 2002, RT-PCR determinations were systematically carried out for *BCR-ABL1*, *KMT2A-AFF1*, *ETV6-RUNX1*, and *TCF3-PBX1* rearrangements in children, adding *KMT2A-MLLT3* and *KMT2A-MLLT1* in infants. The percentage of abnormalities detected by cytogenetics was 70.1%. Four novel abnormalities, t(2;8)(p11.2;p22), inv(4)(p16q25), t(1;7)(q25;q32), and t(5;6)(q21;q21), were found in this cohort. We compared cytogenetic and RT-PCR results for *BCR-ABL1*, *KMT2A-AFF1* and *TCF3-PBX1* rearrangements in 497 children evaluated by both methods. The results were highly concordant ($p < 0.7$), and interestingly, FISH was relevant to confirm G-banding findings that were discordant with RT-PCR studies. This study showed the importance of performing G-banding, FISH and RT-PCR simultaneously to improve the detection of chromosomal abnormalities con-

sidering their important value in the diagnosis and prognosis of childhood ALL patients. Finally, to the best of our knowledge, this is the first series of cytogenetic findings in children with ALL reported in Argentina.

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Acute lymphoblastic leukemia (ALL) is a neoplastic disease characterized by the abnormal proliferation of immature lymphoid cells. It is the most frequent hematologic malignancy diagnosed in children, and it represents ~25% of cancer diagnoses among children younger than 15 years of age [Howlander et al., 2015]. In Argentina, the frequency of acute leukemia in pediatric cancer is 36.7%, of which 79.2% corresponds to ALL [Moreno and Schwartzman, 2008].

The diagnosis of ALL is based on medical examination, flow cytometric immunophenotyping, cytochemical characteristics, and cytogenetic and molecular findings. ALL is classified as B-cell precursor acute lymphoblastic leukemia (BCP-ALL) and T-cell acute lymphoblastic leukemia (T-ALL), representing ~85 and 15% of the pediatric ALL cases, respectively. In addition, less than 5% of acute leukemias are classified as ambiguous lineage acute leukemia (ALAL). Several conventions have been used to define mixed phenotype leukemias, the most recent of which is

the WHO classification of tumor of hematopoietic and lymphoid tissues, which takes into account the expression of the most specific markers for lineage assignment [Swerdlow et al., 2008].

Nonrandom chromosomal abnormalities (CAs) have been reported in BCP-ALL and T-ALL. The Third International Workshop on Chromosomes in Leukemia [1983] was the first major study that demonstrated the independent prognostic significance of cytogenetic findings at diagnosis in ALL. Subsequently, several studies have been reported on CAs in childhood ALL, which support those first results and revealed important data of further recurrent structural and numerical CAs with a prognostic impact mainly in BCP-ALL. The most relevant examples of the prognostic impact of CAs are t(9;22)(q34;q11.2), t(4;11)(q21;q23) and near haploidy/low hypodiploidy, associated with poor prognosis, while high hyperdiploidy and t(12;21)(p13;q22) are associated with good prognosis. Conversely, in T-ALL, although recurrent CAs have been reported, they are mostly not associated with prognostic significance.

G-banding is the method commonly carried out to detect CAs. It is relatively inexpensive and covers the complete spectrum of karyotypic abnormalities; however, it is not always successful, and sometimes the chromosomes have a poor quality and indistinct banding for an accurate analysis. The use of FISH and molecular techniques, such as RT-PCR, have led to the detection of cryptic chromosomal rearrangements such as t(12;21)(p13;q22)/*ETV6-RUNX1* (former *TEL-AML1*), which is the most common chromosomal rearrangement in BCP-ALL.

Furthermore, some studies have applied spectral karyotyping or multi-FISH complemented by FISH with locus-specific probes, which are techniques that have proved very useful to clarify complex rearrangements. However, these methods are not routinely applied, since they are costly and labor-intensive procedures.

Recently, advances in molecular cytogenetics using array-based technologies have helped to detect additional submicroscopic DNA anomalies; however, this technology is not yet widely applied in clinical cancer diagnosis, mainly due to the inability to detect balanced chromosomal rearrangements.

The aim of this study is to report the CAs in 1,057 cytogenetically evaluable pediatric patients with ALL, studied at our cytogenetics laboratory during 23 years, and to correlate cytogenetic and molecular findings for *BCR-ABL1*, *KMT2A-AFF1*, *TCF3-PBX1* rearrangements in cases evaluated by both methods since the incorporation of RT-PCR in December 2002.

Materials and Methods

From January 1991 to August 2014, 1,482 bone marrow samples from ALL patients were referred to our laboratory for cytogenetic studies; in 1,057 (71.3%), a successful karyotyping was performed and this constitutes the cohort of the present study.

The mean age of the 1,057 patients was 6.5 years with a median age of 5.5 years (range: 10 days–16 years); 85 were infants (<1 year of age). The male to female ratio was 1.16 (569:488). Twenty-six patients (2.4%) had Down syndrome with constitutional trisomy 21, and one patient had a constitutional Robertsonian translocation der(13;15)(q10;q10).

Diagnosis of ALL was made following the European Group for the Immunological Classification of Leukemias (EGIL) recommendations until 2008 and subsequently by the WHO classification based on medical examination, flow cytometric immunophenotyping, cytochemical characteristics, and cytogenetic and molecular findings [Bene et al., 1995; Swerdlow et al., 2008].

Bone marrow smears were processed following standard laboratory protocols for cytochemical stains; these included myeloperoxidase (MPO) and Periodic Acid Schiff [Swirsky and Bain, 2006].

Immunophenotyping was performed on bone marrow blasts using the EGIL recommendations. Briefly, aliquots of $3-8 \times 10^5$ cells were stained in different tubes with fluorochrome conjugated monoclonal antibodies, appropriately combined, and analyzed by 3 color flow cytometry with a FacSort instrument (Becton Dickinson, San José, Calif., USA), using the CellQuest software. The antibodies used, conjugated either with fluorescein isothiocyanate, phycoerythrin or peridinin chlorophyll protein, were: CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD21, CD22, CD23, CD25, CD30, CD34, CD38, CD41, CD42, CD45, CD56, CD57, CD61, CD64, HLA-DR, anti μ chain from Becton Dickinson; from Dako, Denmark: CD79a, TdT and MPO, and from Immunotech, Marseille, France: CD19 Cy5, CD117.

For intracellular staining, cells were permeabilized and fixed using Intraprep Permeabilization reagent (Immunotech).

Cytogenetic studies were performed using G-banding, and when possible, 20 metaphases were analyzed in most cases. Cases with fewer than 20 normal metaphases were classified as unsuccessful and were not included in this cohort. Karyotypes were described according to the International System for Cytogenetic Nomenclature [ISCN, 2013].

In our institution, FISH has been carried out since 1998. Different types of commercially available probes have been used: chromosome painting probes, centromeric probes, subtelomeric probes, and locus-specific identifier probes. The locus-specific identifier panel (Abbott/Vysis) included *BCR-ABL1* dual color single fusion and dual color dual fusion translocation probes, *KMT2A* (former *MLL*) dual color break apart rearrangement probe, *ETV6-RUNX1* dual color extra signal translocation probe, *ETV6* dual color break apart rearrangement probe, *TCF3-PBX1* dual color dual fusion translocation probe, and *IGH* dual color break apart rearrangement probe. All specimens were scored following the scoring criteria described in the manufacturer's manual.

The cytogenetically cryptic abnormality t(12;21)(p13;q22)/*ETV6-RUNX1* was analyzed by FISH in only 22 cases until December 2002; afterwards, RT-PCR studies were systematically performed for all patients to evaluate *ETV6-RUNX1* rearrangement. Therefore, its frequency was calculated based on the total of the patients evaluated only by RT-PCR.

Table 1. Demographic data and frequency of genetic abnormalities in 874 BCP-ALL patients

Cytogenetic and molecular findings	n	Frequency, %	Sex, M/F	Median age
<i>Numerical abnormalities/ploidy</i>				
Normal (MN 46)	244	27.9	128/116	5 ys 5 mo
Hypodiploidy (MN 42–45)	19	2.2	5/14	5 ys 7 mo
Low hypodiploidy (MN 33–38)	5	0.6	2/3	10 ys 5 mo
Near haploidy (MN 24–29)	2	0.2	2/0	4 ys 4 mo
Low hyperdiploidy (MN 47–50)	85	9.7	51/34	6 ys 4 mo
High hyperdiploidy (MN 51–65)	227	26.0	116/111	4 ys 6 mo
High ploidy (MN 3n, 4n)	17	1.9	10/7	6 ys 5 mo
<i>Structural abnormalities</i>				
t(12;21)(p13;q22)/ETV6-RUNX1	75	15.2 ^a	38/37	4 ys 5 mo
11q23/KMT2A	72	8.2	33/39	5 mo
t(4;11)(q21;q23)/KMT2A-AFF1	42	4.8	19/23	4 mo
t(11;19)(q23;p13.3)/KMT2A-MLLT1	12	1.4	5/7	7 mo
t(9;11)(p22;q23)/KMT2A-MLLT3	9	1.0	4/5	5 mo
t(10;11)(p12;q23)/KMT2A-MLLT10	3	0.3	2/1	1 y 1 mo
t(1;11)(p32;q23)/KMT2A-EPS15	1	0.1	0/1	6 mo
inv(11)(q13q23)/KMT2A-BTBD18	1	0.1	1/0	9 mo
t(11;17)(q23;q21)/KMT2A-MLLT6	1	0.1	0/1	6 mo
t(11;22)(q23;q13)	1	0.1	0/1	7 mo
t(1;19)(q23;p13.3)/TCF3-PBX1	53	6.0	26/27	5 ys 5 mo
t(9;22)(q34;q11.2)/BCR-ABL1	26	3.0	21/5	9 ys 4 mo
iAMP21	7	0.8	1/6	6 ys 4 mo
t(5;14)(q31;q32)	3	0.3	2/1	4 ys 4 mo
t(8;14)(q11.2;q32)	1	0.1	0/1	4 ys 2 mo
t(14;19)(q32;q13.1)	1	0.1	0/1	12 ys 6 mo
dic(9;20)(p11;q11.1)	3	0.3	2/1	1 y 5 mo
dic(7;9)(p11;q11)	2	0.2	1/1	4 ys 3 mo
t(8;12)(q13;p13)	1	0.1	0/1	3 ys 2 mo
t(16;21)(p11.2;q22)	1	0.1	1/0	6 ys 7 mo
t(17;19)(q22;p13.3)	1	0.1	1/0	1 y 10 mo
t(6;7)(p12;p21)	1	0.1	1/0	5 ys 3 mo
t(9;15)(q32;q13)	1	0.1	1/0	7 ys 2 mo
Abnormal 9p	37	4.2	20/17	9 ys 6 mo
del(6q)	22	2.5	14/8	5 ys 6 mo
del(13q)	13	1.5	4/9	6 ys 7 mo

ys = Years; mo = months. ^a Percentages calculated in 491 cases evaluated by RT-PCR.

RT-PCR was carried out following BIOMED-1 guidelines and the determination of *BCR-ABL1* (p190), *BCR-ABL1* (p210), *KMT2A-AFF1*, *ETV6-RUNX1*, and *TCF3-PBX1* fusion genes were incorporated in December 2002 [van Dongen et al., 1999]. *KMT2A-MLLT3* and *KMT2A-MLLT1* were added in infants with ALL or when *KMT2A* abnormalities were suspected. In unusual cases, long-distance inverse PCR (LDI-PCR) was performed to further investigate the presence of *KMT2A* abnormalities [Meyer et al., 2005]. When cytogenetic and molecular studies were discordant, corresponding FISH tests were performed if samples were available.

Statistical Analysis

The χ^2 test was used to compare the percentage of successful karyotypes and the frequency of CAs in different periods of time.

To compare the cytogenetic and molecular findings in cases evaluated by both methods, the 2-sample proportion test was used; $p < 0.05$ was considered significant. The frequencies of recurrent CAs were estimated based on the total number of cases tested within each corresponding group.

Results

The immunophenotypic analysis revealed 874 BCP-ALL (82.6%), 160 T-ALL (15.0%) and 23 (2.4%) ALAL. Tables 1–3 show the demographic features (age and sex)

Table 2. Demographic data and frequency of genetic abnormalities in 160 T-ALL patients

Cytogenetic and molecular findings	n	Frequency, %	Sex, M/F	Median age
<i>Numerical abnormalities/ploidy</i>				
Normal (MN 46)	67	41.9	53/14	9 ys 6 mo
Hypodiploidy (MN 45)	4	2.5	2/2	6 ys 5 mo
Low hyperdiploidy (MN 47–50)	12	7.5	4/8	10 ys 5 mo
High hyperdiploidy (MN 51–65)	2	1.25	1/1	7 ys 10 mo
High ploidy (MN 4n)	1	0.6	1/0	11 ys 6 mo
<i>Structural abnormalities</i>				
t(11;14)(p13;q11.2)	5	3.1	5/0	12 ys 4 mo
t(8;14)(q24;q11.2)	5	3.1	3/2	1 y 4 mo
t(11;14)(p15;q11.2)	1	0.6	0/1	7 ys 8 mo
t(11;14)(p11.2;q11.2)	1	0.6	0/1	3 ys 1 mo
t(1;14)(p32;q11.2)	1	0.6	1/0	9 ys 6 mo
t(9;14)(p21;q11.2)	1	0.6	1/0	5 ys
inv(14)(q11.2q32)	1	0.6	1/0	6 ys 2 mo
del(14)(q11.2)	1	0.6		
inv(7)(p13q34)	2	1.25	2/0	5 ys 6 mo
7q32-7q34	5	3.1	4/1	12 ys 6 mo
t(3;12)(p21;p13)	1	0.6	0/1	3 ys 4 mo
t(7;12)(q36;p13)	1	0.6	0/1	3 mo
t(8;12)(q13;p13)	1	0.6	0/1	2 ys 4 mo
t(12;13)(p13;q14)	1	0.6	0/1	3 ys 4 mo
t(9;17)(q34;q23)	1	0.6	0/1	13 ys 11 mo
del(6q)	15	9.4	11/4	6 ys 6 mo
del(9p)	8	5.0	5/3	11 ys 6 mo
STIL/TAL1	19	21.1 ^a	14/5	9 ys 7 mo

ys = Years; mo = months. ^a Percentages calculated in 90 cases evaluated by RT-PCR.

Table 3. Demographic data and frequency of genetic abnormalities in 23 ALAL patients

Cytogenetic and molecular findings	n	Frequency, %	Sex, M/F	Median age
<i>Numerical abnormalities/ploidy</i>				
Normal (MN 46)	3	13.0	2/1	10 ys 2 mo
Hypodiploidy (MN 45)	4	17.4	2/1	4 ys 9 mo
Low hyperdiploidy (MN 47)	1	4.3	0/1	5 mo
High hyperdiploidy (MN 55)	1	4.3	1/0	9 ys 4 mo
High ploidy (MN 4n)	3	13.0	2/1	7 ys 7 mo
<i>Structural abnormalities</i>				
t(11;19)(q23;p13.3)/KMT2A-MLLT1	2	8.7	1/1	4 mo
t(9;11)(p22;q23)/KMT2A-MLLT3	1	4.3	0/1	6 mo
t(1;11)(p32;q23)/KMT2A-EPS15	1	4.3	0/1	6 ys 9 mo
t(10;11)(p12;q23)/KMT2A-MLLT10	1	4.3	1/0	5 mo
t(3;19)(p21;p13)	1	4.3	1/0	6 ys 9 mo

ys = Years; mo = months.

of the patients at diagnosis and the frequency of CAs and/or rearrangements detected by conventional cytogenetics, FISH and/or RT-PCR in BCP-ALL, T-ALL and ALAL patients.

Chromosomal Abnormalities and/or Rearrangements in 874 BCP-ALL Patients

Abnormal karyotypes were observed by G-banding in 630 of the 874 cases (72.1%) analyzed.

Numerical Chromosomal Abnormalities/Ploidy

Normal karyotypes were found in 244 cases by G-banding (27.9%); 54 were positive for *ETV6-RUNX1* by RT-PCR.

Hypodiploidy (modal number (MN) 42–45) was found in 19 cases (2.2%). Most of the cases (16/19) had an MN of 45, with loss of chromosomes 7, 13 or 20 and structural CAs of chromosomes 7, 9 and 12 the most frequent abnormalities found.

Low hypodiploidy (MN 33–38) and near haploidy (MN 24–29) were found in 5 and 2 cases, respectively. Patients with low hypodiploidy had 35 chromosomes and 37 chromosomes in 3 and 2 cases, respectively. One of the cases with MN 35 coexisted with a hyperdiploid line of MN 65.

The 2 cases with near-haploid karyotypes had 27 chromosomes and an extra copy of chromosome 18. Additional chromosomes were 2 chromosomes 21 and 1 marker in one case, and chromosomes 13, 16 and 20 in the other case.

Eighty-five patients (9.7%) showed low hyperdiploidy (MN 47–50) being 47 the most frequent MN ($n = 51$) followed by 48 ($n = 13$), 49 ($n = 11$), 50 ($n = 9$), and one case with a range of 47–50 chromosomes. In the different subgroups, trisomy or tetrasomy 21 and trisomy 8 were the CAs most frequently found. In the subgroup of 47 chromosomes, 2 cases each with trisomy 21, 2 cases with trisomy 2, 2 cases with trisomy 8 and 1 case with trisomy 16 were detected as a sole abnormality. In 11/85 cases, the chromosome morphology was substandard to identify structural abnormalities. In the remaining 74 cases, structural abnormalities were found in 55 cases (74.3%). The most frequent CAs were: abnormal 9p ($n = 37$), del(6q) ($n = 22$), abnormal 12p ($n = 13$), monosomy 13/abnormal 13q ($n = 12$), and abnormal 17p ($n = 3$). Nine cases with MN 47 and 2 cases with MN 48 were *ETV6-RUNX1* positive.

High hyperdiploidy, defined as having MN 51–65, was seen in 227 patients (25.9%). In 55 cases only the MN could be determined because the quality of the chromo-

somes was substandard to identify all extra chromosomes and/or structural abnormalities. Most of the remaining 172 cases had between 53 and 56 chromosomes with 56 as the most frequent MN. Chromosomes X, 4, 6, 10, 14, 17, 18, and 21 were the most frequent extra chromosomes. Additional structural CAs, in addition to the gain of whole chromosomes, were found in 86 cases (60.9%). The most common were partial duplications and triplications of 1q, deletions of 6q and isochromosomes of 17q and 7q.

High ploidy, including karyotypes near triploidy ($n = 9$), near tetraploidy ($n = 7$) and near pentaploidy ($n = 1$), was found in 17 patients (1.9%), with near triploidy representing 52.9% of all 17 cases. Structural CAs involved del(1p), del(6q) and additional material in 11q23 and 16q. The case involving 11q23 was negative for *KMT2A* rearrangement. Three cases with near tetraploidy were *ETV6-RUNX1* positive. Several cases were substandard G-banding and could not be evaluated for the presence of structural abnormalities.

Structural CAs

The *ETV6-RUNX1* rearrangement was the most frequent CA found in BCP-ALL patients. It was detected in 83 patients, 75 by RT-PCR and 8 by FISH, 2 of which showed loss of the green signal corresponding to the wild type of the *ETV6* allele. Sixty-two of the 83 patients (74.7%) had MN 46, 54 cases with normal karyotypes, followed by MN 47–48 ($n = 12$), MN 45 ($n = 4$), MN 81–106 ($n = 3$), and MN >50 ($n = 1$). Nine of the 83 patients (10.8%) had an abnormality in the short arm of chromosome 12.

Abnormalities of the 11q23 region involving the *KMT2A* gene were detected in 72 cases by G-banding, and/or FISH and/or RT-PCR. Among them, the following aberrations were found: t(4;11)(q21;q23)/*KMT2A-AFF1* ($n = 42$), t(11;19)(q23;p13.3)/*KMT2A-MLLT1* ($n = 12$), t(9;11)(p22;q23)/*KMT2A-MLLT3* ($n = 9$), t(10;11)(p12;q23)/*KMT2A-MLLT10* ($n = 3$), t(1;11)(p32;q23)/*KMT2A-EPS15* ($n = 1$), inv(11)(q13q23) ($n = 1$), t(11;17)(q23;q21)/*KMT2A-MLLT6* ($n = 1$), and t(11;22)(q23;q13) ($n = 1$).

Of the 42 cases with t(4;11)(q21;q23) and/or *KMT2A-AFF1* rearrangement, we found t(4;11) ($n = 37$), variant 3-way translocations ($n = 2$), ins(11;4)(q23;q21q25) ($n = 1$), and 2 cases with normal karyotypes. In all cases, *KMT2A-AFF1* rearrangement was detected by FISH and/or RT-PCR.

Twelve cases showed t(11;19)(q23;p13.3) and/or the *KMT2A-MLLT1* rearrangement. Of them, G-banding showed t(11;19) in 10 cases, but the *KMT2A-MLLT1* re-

arrangement was identified by FISH and RT-PCR in 2 additional cases. One of these cases had a complex karyotype that showed alterations of 11q23 and 19p13.3 regions, but did not have the typical t(11;19), and the other one had additional material on the short arm of chromosome 19 that could not be identified.

Translocation (9;11)(p22;q23) and/or *KMT2A-MLLT3* rearrangement were found in 9 cases. G-banding revealed t(9;11)(p22;q23) (n = 5) and one variant translocation (n = 1). Of the remaining 3 cases, one case showed a complex karyotype which involved only chromosome 9, and 2 cases showed normal karyotypes. In these 3 cases, the *KMT2A-MLLT3* rearrangement was detected by RT-PCR and/or FISH.

CAs 10;11 rearrangements and/or *KMT2A-MLLT10* were found in 3 cases; one case had t(10;11)(p12;q23), another case had a t(7;11)(q22;q23) and a cryptic ins(10;11)(p12;q23q23) was detected by FISH. The third case had an ins(10;11)(p12;q23q13). The transcript *KMT2A-MLLT10* was confirmed by RT-PCR in all cases.

Translocation (1;19)(q23;p13.3)/derivative 19 and/or *TCF3-PBX1* rearrangement were detected in 53 cases: 23 only by cytogenetics, 3 only by RT-PCR and 27, by both techniques. Eighteen out of the 50 cases detected by cytogenetics showed a balanced t(1;19)(q23;p13.3), and 32 cases showed the unbalanced der(19)t(1;19). One case had an abnormal karyotype with a 9p deletion as a sole abnormality, and FISH revealed a cryptic ins(19;1)(p13.3;q23q23). RT-PCR confirmed the presence of *TCF3-PBX1* rearrangement. Of note, in one case with t(1;19) and in 2 cases with derivative 19 detected by G-banding and confirmed by FISH, the presence of the *TCF3-PBX1* transcript was not detected by RT-PCR. One of these cases was hyperdiploid >50. The t(1;19) or the derivative 19 were the only abnormalities in 13 and 18 cases, respectively. Additional CAs to t(1;19) and the derivative 19 were unbalanced translocations (n = 3), marker chromosomes (n = 3), del(6q) (n = 3), i(9q) (n = 2), and i(7q) (n = 2). In the 3 cases with normal karyotypes and positive RT-PCR for *TCF3-PBX1*, we could not perform FISH analysis since there were no samples available.

Translocation (9;22)(q34;q11.2)/*BCR-ABL1* were observed in 26 patients, 6 only by G-banding and 19 by both, G-banding and RT-PCR. In addition, FISH revealed the *BCR-ABL1* fusion in interphase nuclei in one case with a normal karyotype and positive RT-PCR. Among the 25 cases detected by G-banding, 12 had the standard translocation t(9;22) as a sole abnormality, one case had a 3-way translocation which involved chromosomes 9, 17 and 22, and the remaining 12 cases showed additional ab-

normalities that included a second Philadelphia chromosome, an isochromosome of the derivative 9 of the t(9;22), several random translocations and marker chromosomes. Twenty-three karyotypes were pseudodiploid and 2 hyperdiploid.

Intrachromosomal amplification of chromosome 21 (iAMP21) was suspected in 7 patients in whom an abnormal chromosome 21 was observed by G-banding. In these cases, iAMP21 was confirmed by FISH using the *ETV6-RUNX1* probe that showed 5 or more *RUNX1* signals. The abnormal chromosome 21 included tandem triplication, rings, additional material in the 21 chromosome, and markers suspected of being iAMP21.

CAs of 14q32/*IGH@* rearrangements were found in 6 cases: t(5;14)(q31;q32) (n = 3), t(8;14)(q11.2;q32) (n = 1), t(14;19)(q32;q13.1) (n = 1), and one with additional material in 14q32 associated with a hyperdiploid karyotype. FISH showed split signal in all cases, but in the case with additional material in 14q32, the partner could not be identified.

In addition, there was a case with t(9;22)(q34;q11.2) associated with t(8;14)(q11.2;q32), which was included in the group of *BCR-ABL1* patients.

A dicentric chromosome between chromosome 9 and another chromosome was found in 5 cases: dic(9;20)(p11;q11.1) (n = 3) and dic(7;9)(p11;q11) (n = 2). All cases were confirmed by FISH using centromeric probes for the involved chromosomes. Two of the cases with dic(9;20) and one with dic(7;9) displayed dicentric chromosomes as the only abnormality. The remaining 2 cases coexisted with random CAs.

We found 6 rare, recurrent translocations, each of them in one patient. They were: t(8;12)(q13;p13), t(16;21)(p11.2;q22), t(17;19)(q22;p13.3), t(6;7)(p12;p21), t(8;14)(q11;q32), and t(9;15)(q32;q13).

Additional structural CAs were: abnormal 9p (n = 37), including del(9p), i(9q) and add(9p); del(6q) (n = 22); del(13q) (n = 12); abnormal 17p (n = 3), and random CAs. Most of them were present together in the same karyotype such as del(6q) and del(9p) or were associated with other CAs.

Chromosomal Abnormalities in 160 T-ALL Patients

Abnormal karyotypes were observed in 93 (58.1%) of the 160 patients with T-ALL.

Numerical CAs

Numerical CAs included: MN 45 (n = 4), MN 47–50 (n = 12), MN >50 (n = 2), and MN near tetraploidy (n = 1). The most common numerical CA was trisomy 8 (n = 4).

Table 4. Novel chromosomal abnormalities in our series

Chromosomal abnormalities	Immunophenotype	n	Sex, M/F	Age
t(2;8)(p11.2;p22)	BCP	1	0/1	7 ys 2 mo
inv(4)(p16q25)	BCP	1	1/0	16 ys 7 mo
t(1;7)(q25;q32)	BCP	2	2/0	15 ys 3 mo/11 ys 1 mo
t(5;6)(q21;q21)	T	1	1/0	13 ys 7 mo

ys = Years; mo = months.

Structural CAs

The most frequent CAs involved breakpoints at 14q11.2, 7q34 and 7q32. Among the 14q11.2 abnormalities we found t(11;14)(p13;q11.2) (n = 5), t(8;14)(q24;q11.2) (n = 5), and t(11;14)(p15;q11.2), t(11;14)(p11.2;q11.2), t(1;14)(p32;q11.2), t(9;14)(p21;q11.2), inv(14)(q11.2q32), and del(14)(q11.2) each in one case. The CAs involving chromosome 7 were: inv(7)(p13q34) (n = 2), abnormal 7q32 (n = 3), abnormal 7q34 (n = 1), and abnormal 7p13 (n = 1).

We also found 5 individual cases with rarely reported recurrent translocations: t(3;12)(p21;p13), t(7;12)(q36;p13), t(8;12)(q13;p13), t(9;17)(q34;q23), and t(12;13)(p13;q14). FISH revealed *ETV6* rearrangements in every case that involved the 12p13 region.

Additional structural CAs were: del(6q) (n = 15), del(9p) (n = 8), del(11q23) (n = 5), abnormal 12p (n = 4), abnormal 1q (n = 3), del(11q21) (n = 3), dup(1q) (n = 2), del(3q) (n = 1), del(17p) (n = 1), and other random CAs (n = 22).

Molecular studies in 90 T-ALL tested revealed 19 cases with *STIL-TAL1*. Among them we found: low hyperdiploidy MN 47–50 (n = 2), normal karyotypes (n = 8), pseudodiploid karyotypes with del(6q) (n = 3), t(11;14)(p13;q11.2) (n = 2), t(11;14)(p15;q11.2) (n = 1), t(8;14)(q24;q11.2) (n = 1), and random CAs (n = 2).

Chromosomal Abnormalities in 23 ALAL Patients

Twenty of the 23 patients (86.9%) with ALAL had an abnormal karyotype. Numerical abnormalities included: MN 45 (n = 4), MN 47 (n = 1), MN 55 (n = 1), and near tetraploidy (n = 3). Recurrent translocations found in pseudodiploid karyotypes included: t(11;19)(q23;p13.3) (n = 2), t(9;11)(p22;q23) (n = 1), t(1;11)(p32;q23) (n = 1), and t(3;19)(p21;p13) (n = 1). FISH analysis showed *KMT2A* rearrangement in a case with a normal karyotype in which RT-PCR revealed the *KMT2A-MLLT10* fusion. Other structural CAs were: del(6q) (n = 2), del(7q) (n = 1) and random CAs (n = 2).

Novel Chromosomal Abnormalities

In this series, we found 4 translocations which, to our knowledge, have not been previously described in the literature: t(2;8)(p11.2;p22) (n = 1), inv(4)(p16q25) (n = 1), t(1;7)(q25;q32) (n = 2), and t(5;6)(q21;q21) (n = 1). The first 3 CAs belong to BCP-ALL and the latter to T-ALL patients (table 4).

Correlation between Cytogenetic and Molecular Findings

The cohort was divided into 2 groups of 485 and 572 patients each, in order to compare the frequency of CAs in cases evaluated only by cytogenetic techniques or by both, cytogenetic and molecular studies. The frequency of CAs was 73.4% (356/485) and 77.1% (441/572) (p < 0.12) in each group of patients.

Furthermore, to compare cytogenetic and molecular results for *BCR-ABL1* (p190)/(p210), *KMT2A-AFF1*, and *TCF3-PBX1* rearrangements, we included 497/572 patients in whom cytogenetics (G-banding and/or FISH) and molecular studies were performed. Four cases had discrepant results in patients with t(4;11)(q21;q23) and t(1;19)(q23;p13.3)/derivative 19, identified by G-banding in 1 and 3 cases, respectively, in whom the fusion transcripts were not detected by RT-PCR. In the case with t(4;11)(q21;q23), the rearrangement was confirmed by LDI-PCR.

Discussion

Cytogenetic analyses have been very useful to identify acquired chromosomal aberrations and have contributed to the discovery of several genes whose fusion or activation has an important role in the neoplastic process. Approximately 75% of childhood ALL cases harbor CAs detected by conventional cytogenetics and FISH. However, the percentage varies from 55 up to 89% in different series

depending on the number of patients studied, the year they were reported, and the populations where they have been studied [Chessels et al., 1997; Andreasson et al., 2000; Forestier et al., 2000; De Braekeleer et al., 2010; Moorman et al., 2010; Gil et al., 2013].

We report the cytogenetic findings in 1,057 children with ALL studied in our center, spanning a period of 23 years. The percentage of successfully performed karyotyping improved progressively, with a mean of 60.5% in the first 12 years and 83.4 % in the last 11 years ($p < 0.00001$), mostly due to modifications introduced in the collection of bone marrow samples and in processing methods in order to optimize the results.

The use of FISH studies introduced in our laboratory in 1998 has been very useful to detect cryptic rearrangements, and the subsequent incorporation of the RT-PCR technique increased the detection of CAs from 73 to 77% in 572 patients in which all the techniques were applied ($p < 0.12$).

Among numerical recurrent abnormalities in BCP-ALL, MN 51–65 was the most common CA found in our series. The chromosomes involved and the percentage of cases with structural CAs (61.4%) are similar to data reported in other studies [Paulsson and Johansson, 2009]. Conversely, the frequency of low hypodiploidy (0.6%) and near haploidy (0.2%) was lower than the 1% reported in previous studies [Chessels et al., 1997; De Braekeleer et al., 2010; Moorman et al., 2010]. This may be due to the fact that the hypodiploid clone might be too small to be detected or may undergo endoreduplication, doubling the number of chromosomes, resulting in a near diploid or hyperdiploid karyotype.

Translocation (12;21)(p13;q22)/*ETV6-RUNX1* was found in 15% of the cases in a previous report [Alonso et al., 2012]; this percentage is lower than others reported in comparable studies, although geographic and/or ethnic variations in the frequency of this rearrangement has been described [Forestier et al., 2000; Douet-Guilbert et al., 2003; Moorman et al., 2010]. Of note, a FISH-detected deletion of the wild-type allele in 2 out of 8 positive cases, showing the importance of this technique in providing additional information that is given by molecular studies.

Chromosomal rearrangements involving the *KMT2A* gene were found in 8.2% of the cases (72/874) and increased to 68.2% (58/85) in infants. Translocation t(4;11)(q21;q23)/*KMT2A-AFF1* was the most frequent rearrangement found. Interestingly, one case had a translocation t(4;11) revealed by G-banding and confirmed by FISH, but the *KMT2A-AFF1* transcript was not detected by RT-PCR. In this case, molecular studies using LDI-

PCR revealed the presence of a reciprocal fusion gene that consisted of *AFF1* intron 10 fused with *KMT2A* intron 3; therefore, the corresponding fusion transcript would result in the fusion of *KMT2A* exon 3 to *AFF1* exon 11. This transcript is not detected by the standard primers used in routine RT-PCR studies, since it does not involve the classical breakpoint region of *KMT2A* [van Dongen et al., 1999].

Another interesting finding was an inv(11)(q12q23) detected by conventional cytogenetics and FISH. This led us to a novel fusion transcript identified by LDI-PCR which consisted of *KMT2A* fused with the *BTBD18* gene on chromosome region 11q12 [Alonso et al., 2010]. Therefore, our results confirm, as other authors have suggested, that the break apart dual color FISH probe is the most appropriate method to detect *KMT2A* rearrangements; it also detects 3' deletions and cryptic insertions. FISH for evaluation of *KMT2A* rearrangements should be used in all cases of acute leukemia with a normal karyotype or in those cases with suspected 11q23 abnormalities, and if necessary, further molecular cytogenetic and genomic PCR methods should be used [De Braekeleer et al., 2011].

iAMP21 with multiple copies of the *RUNX1* gene was identified in 0.9% of the patients in the present study. This rare entity is found in 1.5–4.5% of patients younger than 20 years of age (2% on average) [Harrison et al., 2014; Heerema et al., 2013]. As only structural rearrangements involving chromosome 21 that were suspected of amplification of the *RUNX1* gene by G-banding were studied by FISH, the frequency is lower than that found in other reports, where FISH screening was systematically performed for the detection of the cryptic t(12;21) with the *ETV6-RUNX1* probe.

In our study, rearrangements involving the immunoglobulin heavy chain locus (*IGH@*) were found in 0.8% of the cases; of them, 3 cases were translocation t(5;14)(q31;q32), and 2 of them were associated with hypereosinophilia, which is a characteristic feature described in these patients [Gallego et al., 2012]. Our findings confirm the low frequency of these rearrangements, the diversity of the partner chromosome regions such as 19p13 and 8q11, and the association of t(8;14)(q11;q32) with t(9;22)(q34;q11.2). Furthermore, we found another case with the association of t(8;14) with Down syndrome, as has been previously described.

The incidence of *IGH@* translocations remains unknown, and FISH has been recommended for its detection due to the cryptic nature of these abnormalities [Jeffries et al., 2014]. A recent study reported that adult pa-

tients with *IGH@* translocations have an adverse outcome, although this translocation is not an independent prognostic factor in children [Russell et al., 2014].

Interestingly, we found dic(9;20)(p11;q11.1) and dic(7;9)(p11;q11), all of which were confirmed by FISH. Our series has a lower frequency of dicentric chromosomes compared with previously reported data [Moorman et al., 2010]. In addition, even though aberrations involving the long arm of chromosomes 9 and 12 were suspected of being dicentric, we could not confirm such abnormalities.

Furthermore, in this series we found a 58.1% of CAs in T-ALL patients, which is in line with previous data reported [Schneider et al., 2000]. We also found rare, recurrent translocations and several common CAs in BCP, T and ALAL lineages. Interestingly, among BCP-ALL, we found a case of t(16;21)(p11.2;q22), which is the fourth case of this translocation in childhood ALL reported in the literature [Coccé et al., 2015].

Another finding in BCP-ALL in our series was the translocation t(9;15)(q34;q15). Rearrangements of band 15q13q15 are very rare in ALL, and it has been reported in 1% of the cases [Heerema et al., 2002].

We also found several cases involving region 19p13 besides t(1;19)(q23;p13.3) and t(11;19)(q23;p13.3), such as t(3;19)(p21;p13), t(17;19)(q22;p13.3) and 7 cases with additional material in 19p13. The 19p13 abnormality is a nonrandom CA in patients with ALL. Translocation t(3;19)(p21;p13) was found in this cohort in a patient with ambiguous lineage and, to our knowledge, it has been previously reported only in 2 children with ALL and in one adult with AML, suggesting that this CA could affect the germline [Zhu et al., 2007; Mitelman Database, 2015].

We also identified a patient with the rare translocation t(9;17)(q34;q23) in T cells, which to our knowledge, is the fourth case reported in T-ALL patients [Mitelman Database, 2015].

Actually, band 12p13 is one of the most common chromosomal regions involved in CAs in leukemia, mostly resulting in the *ETV6* gene rearrangement. Up to date, 48 chromosomal bands have been identified to be involved in *ETV6* translocations, insertions or inversions, and 30 *ETV6* partner genes have been molecularly characterized [De Brakeleer et al., 2012]. In the present series, we found 4 rare, recurrent translocations involving 12p13 rearrangement, namely t(3;12)(p21;p13), t(7;12)(q36;p13), t(12;13)(p13;q14), and t(8;12)(q13;p13) [Gallego et al., 2008]. They had been previously reported in 2, 2, 10, and 3 cases of ALL, respectively [Mitelman Database, 2015]. These translocations presented *ETV6* rearrangements and early T immunophenotype.

The 21 patients found with high ploidy (2%) confirm its low frequency in ALL and the heterogeneity regarding the immunophenotype of this subset, as reported in the literature [Lemez et al., 2010]. Deletions of 6q and 9p were found in all lineages, mainly associated with other CAs. However, del(9p) as a sole abnormality was found in 42.5% of the cases, suggesting that directly or indirectly it is involved in leukemogenesis.

The following novel CAs, not previously reported, were identified in the present study: t(2;8)(p11.2;p22) (n = 1), inv(4)(p16q25) (n = 1), t(1;7)(q25;q32) (n = 2), and t(5;6)(q21;q21) (n = 1) (table 4). We focused our search on the genes described in the breakpoints involved in these novel findings and their potential relation to cancer. We found the following genes: *CAPG* in 2p11.2; *DLC1*, *NAT2*, *MTSS1* and *TUSC3* in 8p22; *MED28* in 4p16; *ABL2* and *TPR* in 1q25; *RAB9BP1* in 5q21.2, and *PDSS2* in 6q21. All of them are associated with different types of cancers. It is remarkable to have found the *ABL2* gene in 1q25, which is a proto-oncogene whose protein is a non-receptor tyrosine kinase, and the *TPR* gene in the same region; its extreme 5' end fuses with several different kinase genes in some neoplasias and could be involved in leukemogenesis mechanisms [Huret et al., 2013]. Moreover, *ABL2* has been associated with the Ph-like subgroup of ALL, which is a targetable kinase-activating lesion [Roberts et al., 2014]. Further studies would determine if these CAs could be actionable targets for these lesions.

Another aim of this study was to compare cytogenetic and molecular results for *BCR-ABL1*, *KMT2A-AFF1* and *TCF3-PBX1* rearrangements. In the 497 patients in whom we could compare cytogenetic and RT-PCR studies, we had concurrent results with a few exceptions (p < 0.7). FISH was of benefit in detecting cryptic abnormalities such as *BCR-ABL1*, *KMT2A-MLLT1* and *KMT2A-MLLT3* rearrangements in cases with normal karyotypes. Furthermore, we need to point out that FISH confirmed the presence of t(4;11)(q21;q23) and t(1;19)(q23;p13.3)/derivative 19, identified by G-banding in 1 and 3 cases, respectively, in which the fusion transcripts could not be detected by RT-PCR. It could be possible that in these cases the fusion transcripts do not involve the classical breakpoints which are detected by the standard primers used in PCR [van Dongen et al., 1999], as it was confirmed by LDI-PCR in the case with t(4;11)(q21;q23).

In conclusion, this large series of cytogenetic studies has confirmed the results of previous data regarding type and frequency of CAs in pediatric ALL. In addition, more

novel CAs were found in this cohort which have not been previously described in ALL.

Finally, to the best of our knowledge, the present study is the first comprehensive series of cytogenetic findings in children with ALL reported in Argentina.

It shows, as in the case of previous reports [Soszynska et al., 2008; Olde Nordkamp et al., 2009; Harrison et al., 2010], that the detection of CAs using conventional cytogenetics is distinctly improved by FISH and/or RT-PCR methods. These techniques should be used simultaneously to improve accuracy in the identification of the main CAs, since they are useful tools with diagnostic and prognostic value in the management of children with ALL.

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Statement of Ethics

This study has been approved by the Garrahan Hospital Committee of Ethics.

Disclosure Statement

The authors declare neither competing financial interests nor conflicts of interest.

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