RESEARCH COMMUNICATION

Karyotypic Evolution: Cytogenetics Follow-Up Study In Childhood Acute Lymphoblastic Leukemia

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Abstract

Forty seven children affected with acute lymphoblastic leukemia (ALL) were cytogenetically investigated at diagnosis and all through different stages of the disease (remission and relapse). A clonal karyotypic abnormality was found in 32% at diagnosis (mainly comprised of cALLa+). A hyperdiploid mode with chromosome counts ranging from 47-58, was found to be most prominent among cALLa+ patients. The most common numerical aberrations were gain of chromosomes 2, 5, and 21. The structural aberrations at diagnosis were found to be del(9)(p22), inv(9)(p11q13) and del(19)(p12). None of the children showed ph+ chromosome. A good prognosis was found in cALLa+ children with an abnormal karyotype at diagnosis and of these children, those who showed karyotypic instability , had a significantly longer first remission time. The karyotypic evolution through remission(s) and relapse(s) revealed the occurrence of structural alterations , including changes in chromosomes 3, 6, 9, 21 and 22. However, irrespective of the karyotypic clonal nature at diagnosis, chromosome 9 was the most commonly involved chromosome through the course of disease.

Key Words: Acute lymphoblastic leukemia (ALL) - karyotypic evolution - pseudopolyploidy.

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Introduction

The majority of childhood haematopoeitic malignancies are classified as ALL, based upon the morphology of the leukemic cells. Clonal chromosomal abnormalities, including numerical and structural , have been described for childhood ALL, which are correlated with both prognosis and other clinical features.

In the case of numerical aberrations the reports have rarely showed gain or loss of a single autosome or sex chromosome. However the involvement of chromosomes 2, 4, 5, 6, 8, 10, 13, 14, 17, 18, 20, and 21 was observed (Oshimura and Sandberg, 1976; Bloomfield et al.,1981; Sandberg, 1980; Angioni et al., 1994; Secker-Walker, 1990). The most frequent reported recurrent structural chromosomal abnormalities in ALL are: t(1;19)(q34;p13), t (4;11)(q21;q23), t(8;14)(q24;q32) , t(9;22)(q34;q11), deletion of long arm of chromosome 6, and deletion of short arms of chromosomes 9 and 12 (Sandberg, 1980; Petkovi et al., 1996; Katz et al., 1991; Sessarego et al., 1991; Filatov et al., 1996; Theodossiou et al., 1992; Sharma et al., 2001). Some of these abnormalities could be used as diagnostic and/or prognostic parameters. For example, it is suggested that hyperdiploid clones (>50 chromosomes) are associated with a good prognosis and such patients have a more favorable response to chemotherapy (Secker-Walker, 1990; Katz et al., 1991). Particular structural chromosome alterations, such as deletions and translocations, have also been shown to have a prognostic significance (Secker-Walker, 1990; So and Wong, 2001). In addition, cytogenetic studies are useful in evaluating remission status and understanding of the genetic alterations in malignancy.

In this paper we report the results of cytogenetic studies in 47 ALL children. The aim of this study was to define the frequency and types of chromosomal aberrations in our population of children in different stages of the

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disease, in order to draw a frame of karyotypic evolution.

Materials and Methods

Forty seven children affected with acute lymphoblastic leukemia (ALL) have been cytogenetically investigated. Stimulated (+PHA) cultures were prepared from peripheral blood cells according to the standard protocol. Direct and short-term cultures of bone marrow cells and unstimulated peripheral blood were prepared pre-therapy, during remission and relapse periods according to the standard protocols [Ford et al., 1958; Arakaki and Sparkes, 1963; Seabright, 1971]. After slide preparation and using banding techniques (G- and C-banding), chromosomes were analyzed according to ISCN [ISCN, 1995].

Results

According to the cytogenetic data, type of disease, and on the basis of cell surface markers, ALL children have been classified into 3 groups as follows:

1st. 15 children with abnormal karyotypes at diagnosis, either with only abnormal cell line (AA) or accompanied by a diploid clone (AN).

2nd. 22 children with normal karyotypes at diagnosis who developed abnormal clone later (NA).

3rd. 10 children with normal karyotypes at diagnosis without further evidence of karyotypic alterations.

The distribution of the patients, including the number in each sex, is given in table 1.Tables 2, 3 and 4 present the type of disease, age, sex, clinical status and the cytogenetic results for all patients. As these tables show, all the patients have been cytogenetically analyzed at diagnosis with the exception of case numbers 11 and 15 who were in relapse and case numbers 29, 36, and 37 who were in remission.

Discussion

With regard to clonal karyotypic mode, in 60% of cALLa+ patients , the abnormal cell lines (AN) revealed to be predominant ; 20% showed completely abnormal cell lines (AA), and 20% revealed predominantly diploid cell line (NA) accompanied by a few abnormal cells. From two cALLa- cases, one showed AN cell lines at diagnosis and the other which was cytogenetically analyzed in relapse, the cell population was only presentative of abnormal cell lines (AA) (Table 2) . When the total patients with non-T, non-B ALL were considered, irrespective of the presence or absence of cALLa, 64% were AN; 18% were AA, and 18% had NA cell lines at diagnosis.

These figures are closer to the findings of Oshimura, et al [Oshimura and Sandberg, 1976] than to those of Cimino, et al [Cimino et al., 1979].

Regarding the modal chromosome count in patients with null ALL and abnormal karyotypes, the present study reveals that 80% (8 out of 10) of the patients with cALLa had a hyperdiploid mode and 20% (2 out of 10) had a pseudodiploid mode,whereas the only patient with null ALL (cALLa-) had a hypodiploid cell line (table 3).

In order to compare the present series with previous reports, the frequency of abnormal cell lines in the unselected patients with non-T, non-B ALL (irrespective of the presence or absence of cALL antigen) must be considered. In the present series at diagnosis 73% (8 out of 11) revealed a hyperdiploid cell line, with the chromosome count ranging from 47 to 63; 18% (2 out of 11) showed a pseudodiploid pattern; and 9% (case no.12) showed a hypodiploid pattern (44-45 chromosomes) . The present investigation suggests that the hyperdiploid mode was the most prominent among cALLa+ patients, with non-T, non-B ALL, specifically cALLa+.

The preferential order of the abnormal cell lines was found to be hyperdiploid, pseudodiploid, and hypodiploid. The only cALLa- patient, who showed a hypodiploid mode at diagnosis, revealed to have a hyperdiploid clone (AA) post therapy (table 2).

Previous reports by Oshimura and Bloomfield also

Table 1. Distribution of 47 Children Affected with ALL.

KARYOTYPE	Null - ALL (cALLa+)	Null - ALL (cALLa-)	T- Cell ALL	B - Cell ALL	Undiff. ALL	Total
Group A	10 (3M, 7F)	2 (1M, 1F)	1 (1M)	-	2 (2M)	15
Group B	14 (9M, 5F)	-	4 4M	1 (1M)	3 (2M, 1F)	22
Group C	6 (3M, 3F)	-	3 3M	-	1 (1M)	10
Total	30 (15M, 15F)	2 (1M, 1F)	8 (8M)	1 (1M)	6 (5M, 1F)	47

M= Male F= Female

Table 2. Clinical and laboratory findings in ALL children

Case No	Disease Type	Age/Sex (year)	Spec	Tissue	Disease Stage	Normal	Metaphases			
							Ps	Ho	He	Pp
1	Null ALL (cALLa+)	4/F	1st	BM	Diag.	5			15	
				BL		14			7	4
			2nd	BM	Rem.	34			3	5
			3rd	BM	Rem.	26				4
			4th	BM	Rem.	28		5		
			5th	BM	Rem.	34		4		5
			6th	BM	Rem.	23		2		
			7th	BM	Rem.	23				
			8th	BM	Rem.	15	3			4
2	Null ALL (cALLa+)	2.8/F	1st	BM	Diag.				20	
				BL					10	
			2nd	BM	Rem.	27				2
			3rd	BM	Rem.	24				8
			4th	BM	Rem.	23				2
			5th	BM	Rem.	18				
			6th	BM	Rem.	22				4
			7th	BM	Rem.	30				
3	Null ALL (cALLa+)	3/F	1st	BM	Diag.	6	16			
				BL		5	16			
			2nd	BM	Rem.	21				
			3rd	BM	Rem.	19		3		3
			4th	BM	Rem.	22	2			4
			5th	BM	Rem.	20		2		4
			6th	BM	Rem.	29		2		
			7th	BM	Rem.	26		4		
4	Null ALL (cALLa+)	10.1/F	1st	BM	Diag.	4			18	
			6th	BM	Rem.	14				
5	Null ALL (cALLa+)	3.1/M	1st	BM	Diag.	35			77	
			2nd	BM	Rem.	16		6		5
			3rd	BM	Rem.	35			1	7
			4th	BM	Rem.	19			2	
			5th	BM	Rem.	23				2
6	Null ALL (cALLa+)	6.6/F	1st	BM	Diag.				22	
			2nd	BM	Rem.	34				17
			3rd	BM	Rem.	16	3	3	6	9
			4th	BM	Rem.	23				
			5th	BM	Rem.	20				
			6th	BM	Rem.	20				
			7th	BM	Rem.	17	2			5
7	Null ALL (cALLa+)	0.10/M	1st	BM	Diag.	23	2		1	
			2nd	BM	Rem.	31			2	5
			3rd	BM	Rem.	18		2		
			4th	BM	Rem.	22				
8	Pre - Leukemia	1.6/F	1st	BM	Diag.	10				
				BL		10				2
			2nd	BM	Rem.	8			22	3
				BL		14			4	
			3rd	BM	Rem.	17			14	5
			4th	BM	Rem.	21	2			
			5th	BM	Rem.	27				
			6th	BM	Rem.	33			2	4

Case No	Disease Type	Age/Sex (year)	Spec	Tissue	Disease Stage	Normal	Metaphases Ps	Ho	He	Pp
			7th	BM	Rem.	26				3
			8th	BM	Rem.	26				
			9th	BM	Rem.	14			2	4
			10th	BM	Rel.	2				25
			11th	BM	Rem.	23				4
9	Null ALL (cALLa+)	1.6/F	1st	BM	Diag.	4			9	
			2nd	BM	Rem.	28				3
			3rd	BM	Rem.	21			5	4
			4th	BM	Rem.	20			3	2
			5th	BM	Rem.	22			2	
			6th	BM	Rem.	18				2
			7th	BM	Rem.	21				
10	Null ALL (cALLa+)	1.8/M	1st	BM	Diag.	9			3	
			2nd	BM	Rem.	23				5
			3rd	BM	Rem.	26				2
			4th	BM	Rem.	25	2	2		
			5th	BM	Rem.	16		6		
			6th	BM	Rem.	12		6		
11	Null ALL (cALLa+)	3.4/F	1st	BM	2nd Rel.				10	
12	Null ALL (cALLa-)	7.10/M	1st	BM	Diag.	14		24		
				BL		11		15		
			2nd	BM	Rem.	9		3	11	
			3rd	BM	Rem.	29		4	2	5
			4th	BM	Rem.	33	5	1	2	8
			5th	BM	Rem.	19		4		2
			6th	BM	Rem.	24				
			7th	BM	Rem.	24		2		
			8th	BM	Rem.	7	2	1		
13	T-Cell ALL	5.7/M	1st	BL	Diag.	13	2			
			2nd	BM	Rem.		29			4
			3rd	BM	Rem.		33	2		
			4th	BM	Rem.		23	5	2	5
			5th	BM	Rem.		18			3
			6th	BM	Rem.	4	18			3
			7th	BM	Rem.	10				
14	ALL	6.14/M	1st	BL	Diag.	11			9	
			2nd	BM	Rem.	15				
			3rd	BM	Rem.	17		2		
15	ALL	2.6/M	1st	BM	Rel.	10			8	3
16	Null ALL (cALLa+)	5.5/M	1st	BM	Diag.	15				
			2nd	BM	Rem.	20				2
			3rd	BM	Rem.	20				
			4th	BM	Rem.	27	2			4
			5th	BM	Rem.	23	2			2
			6th	BM	Rem.	18				
17	Null ALL (cALLa+)	4.6/M	1st	BM	Diag.	15				
			2nd	BM	Rem.	21				
			3rd	BM	Rem.	17		3		
			4th	BM	Rem.	22	2			3
			5th	BM	Rem.	8		4		
18	Null ALL (cALLa+)	8.7/M	1st	BM	Diag.	14	2			
			2nd	BM	Rem.	31				

Case No	Disease Type	Age/Sex (year)	Spec	Tissue	Disease Stage	Normal	Metaphases			
							Ps	Ho	He	Pp
			3rd	BM	Rem.	27				4
			4th	BM	Rem.	28				2
			5th	BM	Rem.	22				
			6th	BM	Rem.	24				2
			7th	BM	Rem.	24				
19	Null ALL	7.6/M	1st	BM	Diag.	17				2
	(cALLa+)		2nd	BM	Rem.	15				5
			3rd	BM	Rem.	25			3	
			4th	BM	Rem	20				3
			5th	BM	Rem.	21		2	4	5
20	Null ALL	10.3/F	1st	BM	Diag.	12				
	(cALLa+)		2nd	BM	Rem.	27	4		2	
			3rd	BM	Rem.	19		2		3
			4th	BM	Rem.	21	6			4
			5th	BM	Rem.	10				
21	Null ALL	2.0/M	1st	BM	Diag.	21				
	(cALLa+)		2nd	BM	Rem.	17		3		3
			3rd	BM	Rem.	24				3
22	Null ALL	13.8/M	1st	BM	Diag.	16		2		
	(cALLa+)		2nd	BM	Rem.	11				2
			3rd	BM	Rem.	14				
23	Null ALL	5.6/F	1st	BM	Diag.	20				3
	(cALLa+)		2nd	BM	Rem.	18				
			3rd	BM	Rem.	24				
24	Null ALL	3.0/M	1st	BM	Diag.				15	
	(cALLa+)		2nd	BM	Rem.				25	
			3rd	BM	Rel.				28	3
			4th	BM	Rem.				28	6
			5th	BM	Rem.				18	
25	Null ALL	3.1/F	1st	BM	Diag.	18				
	(cALLa+)		2nd	BM	Rem.	20				7
			3rd	BM	Rem.	13	2		3	
			4th	BM	Rem.	26				3
			5th	BM	Rem.	17				5
			6th	BM	Rem.	24				4
			7th	BM	Rem.	22			2	3
			8th	BM	Rem.	20				
26	Null ALL	4.6/M	1st	BM	Diag.	10				
	(cALLa+)		2nd	BM	Rem.	22				3
			3rd	BM	Rem.	22		2		3
			4th	BM	Rem.	21	2			
			5th	BM	Rem.	19		3		3
27	Null ALL	8.6/F	1st	BM	Diag.	17				
	(cALLa+)		2nd	BM	Rel.	16	6		2	3
			3rd	BM	Rel.	18				2
			4th	BM	Rel.	23				
			5th	BM	Rem.	21				
28	Null ALL	1.10/F	1st	BM	Diag.	22				
	(cALLa+)		2nd	BM	Rem.	20		2		4
			3rd	BM	Rem.	15		2		
			4th	BM	Rem.				3	3
			5th	BM	Rem.	17				2
			6th	BM	Rem.	18			2	4
			7th	BM	Rem.	21		2		

Case No	Disease Type	Age/Sex (year)	Spec	Tissue	Disease Stage	Normal	Metaphases Ps	Ho	He	Pp
29	Null ALL	6.7/M	1st	BM	Rem.	17				2
	(cALLa+)		2nd	BM	Rem.	21	2			3
30	B-Cell	11.0/M	1st	BM	Diag.	10				
	ALL		2nd	BM	Rel.	4			13	2
			3rd	BM	Rel.	14				2
			4th	BM	Rem.	29			2	
			5th	BM	Rel.	14		2	7	4
31	T-Cell	5.2/M	1st	BM	Diag.	22				
	ALL		2nd	BM	Rem.	20	3			
			3rd	BM	Rem.	9		3		3
			4th	BM	Rem.	23				
32	T-Cell	5.5/M	1st	BM	Diag.	21				
	ALL		2nd	BM	Rem.	10				
			3rd	BM	Rem.	27				3
			4th	BM	Rem.	32				2
			5th	BM	Rem.	25				
			6th	BM	Rem.	24				
			7th	BM	Rem.	15			2	2
33	T-Cell	13.4/M	1st	BM	Diag.	15				
	ALL		2nd	BM	Rem.	22			6	3
			3rd	BM	Rem.	14				
			4th	BM	Rem.	26				3
			5th	BM	Rem.	20		2		3
34	T-Cell	8.5/M	1st	BM	Diag.	20				
	ALL		2nd	BM	Rem.	18				
			3rd	BM	Rem.	20		4		
			4th	BM	Rem.	23				
			5th	BM	Rel.	30		8		
35	ALL	10.8/M	1st	BM	Diag.	19				
			2nd	BM	Rem.	23	2			
			3rd	BM	Rem.	20		2		4
			4th	BM	Rel.	24				
			5th	BM	Rem.	9	4	3		
36	ALL	4.5/F	1st	BM	Rem.	22				3
37	ALL	5.10/M	1st	BM	Rem.	22				
			2nd	BM	Rem.	28	2			
			3rd	BM	Rem.	21	3			
			4th	BM	Rel.	25				
			5th	BM	Rem.	21				

M=Male F=Female BM= Bone Marrow BL= Blood
Diag.= Diagnosis Rem.= Remission Rel.= Relapse Ps= Pseudodiploid
Ho= Hypodiploid He= Hyperdiploid Pp= Polyploid Spec.= Specimen

revealed a significant increase in hyperdiploid mode [Oshimura and Sandberg, 1976; Bloomfield et al.,1981]. On contrast, a report by Secker-Walker indicates that 40% of ALL children showed a pseudodiploid mode, but the range of hyperdiploidy was 22% to 26% [Secker-Walker, 1990].

It is suggested that patients with hyperdiploid karyotypes (chromosome number > 51) appear to have a good prognosis and more favorable response to chemotherapy, whereas patients with hypodiploid (<45) and pseudodiploid karyotypes have a poor response [Secker-Walker, 1990; Katz et al., 1991; Secker-Walker et al., 1982; Williams et al., 1986].

Regarding the numerical aberrations, previous reports have shown the involvement of different chromosomes. Secker-Walker showed that among patients with 47 chromosomes, trisomy of # 8, 16, 21 and 22 were most common, whereas in all hyperdiploid cases, the most commonly gained were chromosomes 4, 6, 8, 10, 14, 17, 18, 21, and 22[Secker-Walker, 1990]. Petkovic, et al, also showed gain of chromosomes 4, 6,13, 14, 21, and 22 [Petkovi et al., 1996]. The present study indicates that the most common numerical aberrations in cALLa+ patients included gain

Table 3. Frequency of Abnormal Cell lines in ALL Children

Type of Disease	Number of cases	Abnormal		Nature of Abnormal Cell Lines		
		No.	%	Hyperdip.	Hypodip.	Pesudodip.
Non-T, Non-B ALL (cALLa+)	30*	10	34	8	-	2
Non-T, Non-B ALL (cALLa-)	2**	1 (Diag.)	100	-	1	-
		1 (Rel.)	-	1	-	-
T-Cell ALL	8	1	12.5	-	-	1
B-Cell ALL	1	-	-	-	-	-
Undifferentiated ALL	6***	1 (Diag.)	50	1	-	-
		1 (Rel.)	-	1	-	-
TOTAL	47 41 at diagnosis	15	32	11 (73%)	1 (7%)	3 (20%)

* 1 case with a normal cell line studied in remission.
** 1 case studied in relapse.
*** 3 cases with normal cell lines studied in remission and 1 case in relapse.The rest studied at diagnosis.

of chromosomes 2 (30%), 5 (30%), and 21 (30%). This data agrees with that of Oshimura, et al, who also found gain of chromosomes 2, 5 and 21 in one female patient with a modal chromosome count of 53 [Oshimura and Sandberg, 1976]. It also seems that gain of chromosome 21 to be most frequent among ALL children. Since Down' s syndrome patients have an increased risk of developing childhood leukemia, the observation of trisomy 21 in leukemic cells requires more attention and investigation.

Bloomfield , et al , suggested that all chromosomes except the Y chromosome were involved in those cases with non-T, non-B ALL [Bloomfield et al.,1981]. In contrast, loss of the Y chromosome is also reported in a few papers [Secker-Walker, 1990; Whang-Peng et al., 1976; Zuelzer et al., 1976]. In present study the only case (no.12) with null ALL (cALLa-) and a hypodiploid mode (AN) at diagnosis, showed the loss of chromosomes 20 and Y either as a sole anomaly or included in complex aberrations.

Previous reports have shown that the most frequent recurrent structural chromosomal abnormalities found in ALL are: t (1;19) (q34;p13), t (4;11)(q21;q23), t (8;14)(q24;q32), t (9;22) (q34;q11), t (11;14) (p13;q13), deletion of long arm of chromosome 6, and deletion of short arms of chromosomes 9 and 12 [Sandberg, 1980; Petkovic et al., 1996; Katz et al., 1991; Sessarego et al., 1991; Filatov et al., 1996; Theodossiou et al., 1992; Sharma et al., 2001]. Many of these chromosomal translocations have been shown to be of prognostic significance and have been incorporated into risk-classification systems [So and Wong, 2001] . As it is shown in table 4, the present investigation did not reveal the occurrence of any structural aberrations (i.e. 6q-and different translocations) mentioned above at diagnosis. However we faced with chromosomal deletions in relatively same chromosomes and

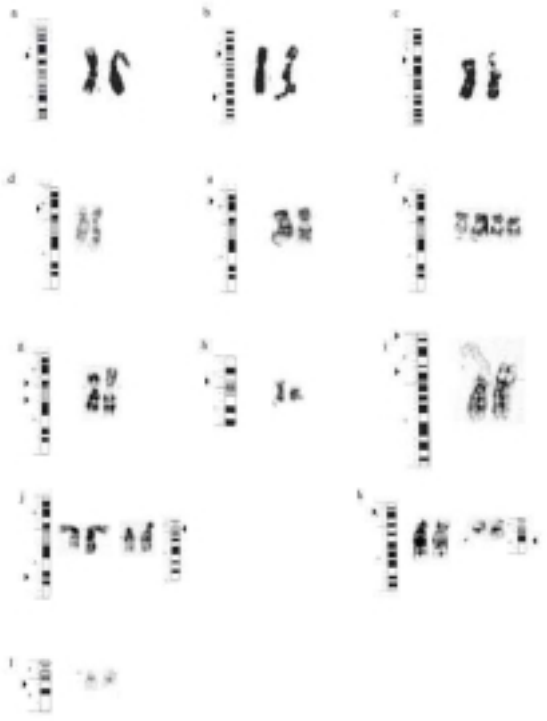

Figure 1. Partial Karyotypes Illustrating the Structural Chromosome Abnormalities.
Arrowheads indicate breakpoints. a: ctb(1)(p22), b: ctb(2)(p13)(q31), c: ctb(3) (p13), d: del(9)(p13), e and f: del(9)(p22), g: inv(9)(p11q13), h: del(19)(p12), i: trp(6)(p21p25), j: t(9;15)(q32;p11), k: t(5;21)(p15;q22), l: del(22)(q11).
The alterations including inversion 9 are considered as NORMAL VARIANTS.

also at the same regions at different stages of the disease (Figures 1-3). Chromosome deletions were found in 20% (2 out of 10) of cALLa+ patients at diagnosis.This anomaly had affected 9p22 in case no.3 with an AN cell line, and 19p12 in case no.7 with an AN karyotypic pattern. This patient also had an inversion in 9 (p11q13).

Previous studies have shown the occurrence of structural aberrations in chromosome 9 in ALL patients [Secker-Walker, 1990; Petkovic et al., 1996; Katz et al., 1991; Sessarego et al., 1991; Swan, 2001; Riesch et al., 2001; Ferro et al., 1991]. The involvement of the short arm of chromosome 9 was reported by Kowalczyk and Sandberg, as a translocation or deletion; in a few ALL cases [Kowalczyk and Sandberg, 1993] . Although further studies confirmed the involvement of 9p in pediatric leukemias, additional patients have been shown with different hematological disorders and partial deletion of 9p, suggesting that such rearrangement is not specific for ALL [Pollak and Hagemeijer, 1987].

The Philadelphia chromosome is the most common chromosomal aberration in ALL.

Nearly 20% of adult and 5% of children with typical Ph have the same cytogenetic appearance in chronic myeloid leukemia (CML) [Sverre and Mitelman, 1987]. It is suggested that such patients to have a reduced survival as compared with Ph negative patients [Third International workshop on chromosome and leukemia 1980, 1981]. It is now considered that the genes encoding for the methylthioadenosine phosphorylase (MTAP), interferon α and β to be located on 9p21-22.These genes are detected in a high percentage of ALL patients, not necessarily with cytogenetic evidence of 9p involvement [Sessarego et al., 1991]. Therefore it may be concluded that 9p rearrangement might play an important role in nucleic acid metabolism, cellular proliferation and differentiation. As it is offered by Diaz, et al, loss of a hypothetical leukemia suppressor

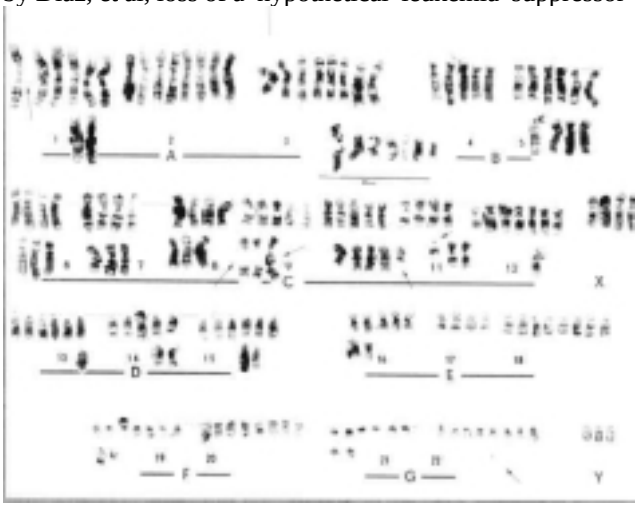

Figure 2. 182,XXXXYYY, del(10)(q22), del(11)(p11), del(22)(q11), +7 mars.

G-banded karyotype of an ALL case (no:12, cALLa-), including normal
variants: 1qh+ and 9qh+ in the 4th bone marrow.

Figure 3. A schematic diagram comparing breakpoints in present study with previous reports. Circles indicate breakpoints in present study and arrows indicate previously reported breakpoints.

gene close to the mentioned genes may be an important event in leukemogenesis [Diaz et al., 1990]. Considering the above evidence, our study may suggest 9p21 and 9p22 as vulnerable points in childhood ALL cases with non-T, non-B ALL.To best of our knowledge, inversion of the chromosome 9 is a rare rearrangement in children ALL and has been reported by Swan in a 16-year-old Caucasian male [Swan, 2001].

Among the previously reported alterations, the t (1;19) is a non random rearrangement associated with ALL observed in 5% - 6% of reported childhood ALL, and 3% of adult ALL [Hunger et al., 1997]. Translocations involving chromosome 19p 13have frequently been reported in childhood ALL. The E2A gene encoding the immunoglobulin kapa chain enhancer binding factors E12 and E47, has been localized to this region [Maloney et al., 1998]. In the present study one of the cALLa+ patients (case no.7) revealed to have such a breakpoint at diagnosis.

The present investigation also revealed the presence of markers in 40% (4 out of 10) of the cALLa+ patients, which could be considered as the result of structural aberrations.

In the case of evidence for karyotypic evolution the present data reveal that the nature of karyotypic evolution was different in patients with an abnormal karyotype at diagnosis (Group A) than in cases with a normal karyotype (Group B). In group A, the modal chromosome count showed a tendency to decrease , or chromosomes were found to have structural aberrations with pseudodiploidy. In this group loss of the chromosomes 20 and Y in a cALLa- patient were identified as a sole numerical anomaly. The most frequent chromosomes involved in structural abnormalities were found to be chromosomes 9 and 22 (table 4).

Among group B, however, the chromosome count mainly showed a tendency to increase, including numerical and structural anomalies. In this group gain of chromosomes 3,15 and loss of chromosomes 9, 19 were identified as

Table 4. Karyotypic Evolution in ALL Children.

Case No.	Patient Group	Type of Disease	Specimen	Clinical Status	Structural Abnormality
3	A	Null-All cALLa+	1st	Diagnosis	del(9)(p22)[Sandberg, 1980]
			4th	Remission	del(9)(p22)[Bloomfield et al.,1981] / 9p-[Angioni et al., 1994]
6	A	Null-All cALLa+	7th	Remission	del(22)(q11)[Filatov et al., 1996]
7	A	Null-All cALLa+	1st	Diagnosis	inv(9)(p11q13)[Sandberg, 1980] / del(19)(p12)[Sandberg, 1980]
			2nd	Remission	inv(9)(p11q13)[Secker-Walker, 1990] / del(9)(q33)[Secker-Walker, 1990]
			3rd	Remission	inv(9)(p11q13)[Secker-Walker, 1990]
			4th	Remission	inv(9)(p11q13)[Secker-Walker, 1990]
8	A	Null-All cALLa+	3rd	Remission	del(5)(q32)[5] / del(15)(q22)[Secker-Walker, 1990]
9	A	Null-All cALLa+	5th	Remission	del(1)(q25)[Bloomfield et al.,1981] / del(7)(q22)[Bloomfield et al.,1981]
12	A	cALLa-	4th	Remission	del(22)(q11)[13] / del(10)(q22)[Sessarego et al., 1991] del(11)(p11)[Sessarego et al., 1991]
13	A	T-Cell ALL	2nd	Remission	del(9)(q32)[Bloomfield et al.,1981] t(9:15)(q32;p11)[Sverre and Mitelman, 1987]
20	B	Null-All cALLa+	2nd	Remission	del(21)(q22)[Oshimura and Sandberg, 1976]
			4th	Remission	del(21)(q22)[Petkovi et al., 1996] / t(5;21)(p15;q22)[Bloomfield et al.,1981]
25	B	Null-All cALLa+	2nd	Remission	inv(9)(p11q13)[Katz et al., 1991]
			3rd	Remission	trp(6)(p)[Sandberg, 1980]
			4th-6th	Remission	inv(9)(p11q13)[So and Wong, 2001]
26*	B	Null-All cALLa+	3rd	Remission	del(3)(p13)[Sandberg, 1980] / del(6)(q21)[Sandberg, 1980] del(12)(p13)(q24)[Sandberg, 1980]
27	B	Null-All cALLa+	2nd	Relapse	del(21)(q22)[Sandberg, 1980]
28	B	Null-All cALLa+	6th	Remission	del(1)(q22)[Angioni et al., 1994] / del(3)(p13)[Angioni et al., 1994]
29	B	Null-All cALLa+	2nd	Remission	del(19)(p)[Bloomfield et al.,1981]
30	B	B-Cell ALL	5th	Relapse	del(4)(q31)

* An individual metaphase with 409 chromosomes included 1q-, del(3)(q13), inv(9)(p11q13), and del(12)(q24) was also observed in 5th bone marrow in remission. Numbers in brackets show number of metaphases.

numerical anomalies, whereas chromosomes 3, 6, 9, and 21 were found to be frequently involved in structural abnormalities. As we have previously mentioned, patients with Down's syndrome have an increased risk for developing leukemia, which shows the role of chromosome 21 in hematopoeitic malignancies. Several genes have been located on chromosome 21 and are believed to be involved in cell growth and differentiation. It is suggested that 50-100 genes are located in the critical region on chromosome band 21q22 (especially ETS and ERG) [Thompson et al., 1991; Baialardo et al., 1996]. As it is shown in table 4 such a breakpoint was found in 2 patients in the form of del (21)(q22) and/or t(5;21)(p15;q22). Regarding the karyogram of patient (case no.25) with 409 chromosomes (pseudopolyploidy, ~22n), this is the highest chromosome number which has been ever reported so far (Figure 4). A previous report by Sandberg has shown 360 chromosomes (16n: 45X8) in the bone marrow of a patient affected with Turner's syndrome [Sandberg, 1980].

As figure 4 shows, in this karyotype the highest number is represented by chromosome 15 (N=22). The distribution of the other chromosomes are as: 3, 4 (N=21); 2, 9 (N=20); 1, 10, 13 (N=19); 12, 16, 21 (N=18); 6, 18 (N=17); 19, 22 (N=16); 5 (N=15); 11, 20 (N=14) ; 17 (N=13) ; and 11, X, 14 (N=12) . A clear observation is an unbalanced distribution of chromosomes 9 and X that revealed to be eight inverted and twelve normal 9 chromosomes, and interestingly the presence of only twelve copies of chromosome X. However, we have faced with a ladder type increasing of chromosome number ranging from 12 to 22 (Figure 4 and table 4).

Altogether, the present series reveal that 40% (13 out of 32) of patients with childhood ALL had karyotypic evolution, which is higher than the figure of 33% reported by Cimino, et al [Cimino et al., 1979]. Although, the hematological and clinical features are absent during the remission period, the occurrence of alterations, could attract the performance of cytogenetics investigations at this particular stage of disease.

Figure 4. 409, XXXXXXXXXXX, 1q- , del(3)(q13), inv(9)(p11q13), del(12)(q24) ,+8 mars. G-banded karyotype of an ALL case (no:25, cALLa+) in the 5th bone marrow.

As the result of cytogenetics evolution, the observed breakpoints in the present study (table 4), relatively correspond with the molecular findings in regards of gene luci map (Figure 3). This might lead to the better understanding of the disease in direction of the appropriate therapeutic applications for clinical management and prevention. Therefore, linking cytogenetics with molecular genetics could reveal the involvement of more genomic regions as candidates for leukemogenesis.

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