The Incidence Peaks of the Childhood Acute Leukemias Reflect Specific Cytogenetic Aberrations

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Summary: The correlation between age and karyotype was studied in 1425, 0 to 14.9 years old children who were diagnosed with acute lymphoblastic leukemia (ALL) or acute myeloblastic leukemia. Almost 80% of the non-Down B-cell precursor ALL cases in the 2 to 7 years frequency peak group who had aberrant cytogenetic results had either a high-hyperdiploid clone (51 to 61 chromosomes) or a translocation t(12;21)(p13;q22). Among B-cell precursor ALL cases, high white blood cell counts correlated with earlier age at diagnosis ($r_S = -0.23$; $P < 0.001$) being most evident for 11q23/*MLL*-aberrations, translocation t(12;21)(p13;q22), and high-hyperdiploidy. Among acute myeloblastic leukemia patients, frequency peaks were found for those with *MLL*/11q23 rearrangements (peak: first year), Down syndrome (peak: second to third year), or cytogenetic abnormalities other than translocations t(8;21), t(15;17), and inv(16)/t(16;16) (peak: first to third year). The epidemiology of the cytogenetic subsets of acute leukemias questions whether age as a disease-related prognostic parameter has any relevance in childhood leukemia clinical research beyond being a surrogate marker for more important, truly biologic features such as cytogenetic aberrations and white cell count at diagnosis. Further research is needed to explore whether the 2 to 7 years age incidence peak in childhood ALL harbor yet unidentified cytogenetic subsets with the same natural history as the high-hyperdiploid and t(12;21)-positive leukemias.

Key Words: leukemia, cytogenetics, epidemiology

(*J Pediatr Hematol Oncol* 2006;28:486–495)

Childhood acute myeloblastic leukemia (AML) and the 6 times more common acute lymphoblastic leukemia (ALL) are clonal diseases driven by mutations. Age at diagnosis has played a role in the exploration of the epidemiology of these diseases and in the risk grouping and treatment stratification.[1,2] However, the background for the linkage between age and the clinical course of these diseases has been unclear. The age-related incidence pattern of ALL has been interpreted to support a prenatal origin.[1] Furthermore, a prenatal origin of many cases of ALL and AML has been established by the demonstration of clone-specific 11q23-, t(12;21)-, and t(8;21)-translocations, hyperdiploidy, or clone-specific immune gene rearrangements in Guthrie cards.[3–8] Based on these and other findings, the natural history of the majority of childhood ALL cases has been proposed to reflect in utero emergence of preleukemic cells and crucial-but rare-postnatal interactions between these, the immune system, and common childhood infections.[9] In contrast, little is known about the etiology of AML, except for those with secondary leukemia and those with a known predisposing disorder, such as Down syndrome.[10,11]

In Europe and the United States, 85% to 90% of ALL cases have a B-cell precursor phenotype (pre-B) with a characteristic incidence peak in the age group 2 to 7 years. Smaller incidence peaks have also been demonstrated for childhood T-cell ALL and for AML.[12] Most of the pre-B ALL cases carries either of 2 cytogenetic changes: (A) the chromosomal translocation t(12;21)(p13;q22) is present in 20% to 25% of cases and involves the *RUNX1* gene on chromosome region 21q22 and the *ETV6* gene on chromosome region 12p13 that encode transcriptional factors essential for normal fetal hemopoiesis.[13] The translocation is cryptic by G-band karyotyping, because it involves translocation of 2 white bands, (B) the high-hyperdiploid karyotype with a modal chromosome number above 50 are present in approximately 30% of the patients, and characteristically involves trisomies of chromosomes X, 4, 6, 10, 14, 17, 18, and 21,[14,15] that in most cases arise by simultaneous chromosomal gains in a single abnormal mitosis.[16] Because the t(12;21)-translocation is cryptic by standard G-band karyotyping, its diagnosis generally relies on targeted reverse transcriptase-polymerase chain reaction (RT-PCR) and/or fluorescent in situ hybridization (FISH) analyses.[15,17] High-hyperdiploid leukemic clones are sometimes overlooked by standard G-band karyotyping due to selective growth of normal hemopoetic cells or poor metaphase quality. However, detection rates can be raised by improved G-band karyotyping, conventional or extended FISH analyses through high-resolution

Received for publication March 21, 2006; accepted June 16, 2006.
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Support by the Danish Cancer Society (grant no: 9710030), the Danish Childhood Cancer Foundation, and the Swedish Childrens Cancer Fund.
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comparative genomic hybridization (CGH) and array CGH, and flow cytometric DNA-index analyses.[15,17–19]

To improve the understanding of childhood acute leukemia epidemiology and to clarify the cytogenetic backbone for etiologic research, in the present study we have analyzed data from the Nordic Society of Paediatric Haematology and Oncology (NOPHO) population-based cytogenetic registry to explore the age-related incidence patterns of cytogenetically defined subsets of ALL and AML.

PATIENTS AND METHODS

The NOPHO population-based leukemia register contains children diagnosed before the age of 15 years in Denmark, Finland, Iceland, Norway, and Sweden. Of these, the study included 1075 cases of ALL and 350 cases of AML diagnosed January 1993 to December 2003.

ALL Patients

For the ALL cohort (Table 1), we included: (A) all patients with T-cell ALL and (B) all patients with B-cell precursor ALL, who had the *ETV6/RUNX1* [t(12;21)(p13;q22)] gene fusion status explored by FISH and/or RT-PCR.

The frequency of non-Down pre-B ALL cases tested for the *ETV6/RUNX1* translocation was significantly skewed toward the later study period due to the increased awareness in recent years of the cryptic t(12;21)-translocation, being 21% in 1993 to 1996, 65% in 1997 to 2000, and 79% in 2001 to 2003. In addition, the preferred method to screen for t(12;21) has drifted from RT-PCR to interphase and metaphase FISH as the knowledge of the possible biologic and clinical importance of secondary aberrations, such as 12p deletion and *AML1* amplification, has evolved.[15,20,21] On the basis of immunophenotyping [according to the European Group for the Immunological Characterization of Leukemias (EGIL)[22]] and the various performed cytogenetic analyses (eg, chromosome banding, FISH, Southern blot, high-resolution CGH, and RT-PCR) 10 ALL subgroups were defined: (1) Down syndrome; (2) T-cell ALL; translocations involving (3) *MLL* (11q23-translocations); (4) *BCR/ABL*[t(9;22)(q34;q11)]Ph-pos.; (5) *TCF3/PBX1* [t(1;19)(q23;p13)]; (er disse grupper[3–5] kun G-bånd eller også FISH/RT-PCR; and (6) *ETV6/RUNX1*[t(12;21)(p13;q22)]; (7) high-hyperdiploidy with 51 to 61 chromosomes; (8) all other clonal abnormalities including hypodiploid, pseudodiploid, near triploid, and near-tetraploid cases; (9) normal diploid karyotypes; and (10) no cytogenetic result either because of too few metaphases, or the leukemic bone marrow not being cytogenetically analyzed. Unless otherwise stated, all the analyses and figures were based on the results obtained by RT-PCR/FISH for t(12;21)(p13;q22) and by G-band karyotyping. In addition to these, DNA measurement with flow cytometry has been applied at an increasing frequency to detect high-hyperdiploidy, which in a matched comparison is equal to a flow-cytometric DNA index >1.10).[19] Except for the low frequency of Down

TABLE 1. Cytogenetic Subgroups in 1075 Children With ALL

Subgroup No.	Characteristics	All Patients/2nd-7th Year of Life (%/%)	All Patients Median WBC (Range)	All Patients Median Age (Range) M/F	White Cell Count Groups Median Age (N) M/F		
					WBC ≤ 10	WBC 10-49	WBC ≥ 50
1	DS	16/14 (1.5/1.9)	17 (2-122)	5.41 (1.33-11.0) 9/7	5.62 (7) 4/3	4.44 (6) 2/4	5.52 (3) 3/0
2	T-cell	96/36 (8.9/4.8)	110 (1-815)	8.66 (1.14-14.67) 67/29	9.23 (13) 8/5	7.31 (19) 13/6	8.76 (64) 46/18
3	MLL/11q23	30/8 (2.8/1.1)	126 (1-950)	0.61 (0.03-3.72) 14/16	2.70 (2) 2/0	0.60 (7) 3/4	0.57 (21) 9/12
4	Ph-pos	25/13 (2.4/1.7)	43 (1-284)	5.98 (1.40-14.96) 14/11	8.22 (7) 4/3	4.12 (8) 4/4	7.12 (10) 6/4
5	t(1;19)	23/16 (2.1/2.1)	15 (1-113)	5.01 (1.34-14.27) 11/12	6.54 (9) 4/5	4.57 (10) 6/4	4.05 (4) 1/3
6	t(12;21)	244/207 (22.7/27.5)	8.5 (0.8-225)	4.29 (1.19-13.85) 139/105	4.79 (128) 70/58	3.72 (86) 50/36	3.39 (30) 19/11
7	51-61 chr	259/219 (24.2/29.1)	6 (0.5-171)	4.03 (0.76-14.68) 133/126	4.33 (160) 72/88	3.22 (80) 46/34	2.78 (19) 15/4
8	Other	151/87 (13.9/11.6)	9 (0.8-623)	5.43 (0.55-14.92) 72/79	5.48 (78) 40/38	5.13 (40) 13/27	5.29 (33) 19/14
9	Diploid	146/92 (13.6/12.2)	5 (0.5-366)	4.82 (0.26-14.82) 80/66	5.27 (90) 44/46	4.62 (42) 28/14	4.50 (13) 7/6
10	Unsuccessful	85/60 (7.9/8.0)	5 (0.2-296)	3.96 (0.16-14.39) 52/33	4.02 (54) 36/18	3.06 (19) 9/10	3.45 (12) 7/5
Total	—	1075/752 (100/100)	9 (0.2-950)	4.45 (0.03-14.96) 591/484	4.77 (548) 284/264	3.87 (317) 174/143	4.58 (209) 132/77

In 1 case (infant with diploid karyotype) WBC data is missing.
F indicates female; N, number of patients; M, male; 2nd-7th year, second to seventh year of life; %, percentage of total number of patients; WBC, white blood cell count ($\times 10^9$/L); DS, Down syndrome.
For groups 3, 4, 5, 7, 8, 9 and 10 the results are based on G-band karyotyping. For group 6 it is based on FISH/RT-PCR for the t(12;21)-translocation.

syndrome in the present material (1.5% vs. 2.1% in the total Nordic ALL material 1993 to 2003[23]) no significant differences in sex, age, white blood cell count (WBC), and presence of central nervous system leukemia were found in the NOPHO non-B ALL cohort 1993 to 2003 between patients that were (n = 1075) or were not (n = 926) tested for the *ETV6/RUNX1*[t(12;21)(p13;q22)] translocation, and the age incidence curves of these 2 groups are superimposable (data not shown).

AML Patients

All primary AML patients diagnosed 1993 to 2003 were included and divided into 8 subgroups: (1) AML in Down syndrome; translocations involving (2) 11q23 [*MLL*] except for t(9;11)(q21;q23); (3) t(9;11)(q21;q23); (4) t(8;21)(q22;q22), inv(16)(p13q22)/t(16;16)(p13;q22) or t(15;17)(q22;q12-21); (5) +8 as the sole abnormality; (6) any other clonal abnormality; (7) normal diploid karyotypes; and (8) no cytogenetic result, either because of too few metaphases, or the leukemic bone marrow not being analyzed. All patients were classified by the G-band karyotyping results, except for one for whom an *MLL*/11q23-rearrangement was identified by FISH.

Definition of clonality and description of the cytogenetic abnormalities in both ALL and AML followed the recommendations of International System for Human Cytogeneic Nomenclature.[24]

RESULTS

ALL

The age-related frequency peak of childhood ALL (Fig. 1) is dominated by a few well-defined cytogenetic subsets, first of all the t(12;21)(p13;q22)-translocation and high-hyperdiploid ALL, that with respect to their modal chromosome number are virtually exclusive of one another (Figs. 2A, 3). In contrast, the *MLL*/11q23-rearranged ALL cases (n = 30, all identified by G-band karyotyping) have their highest frequency during the first year of postnatal life, whereas the frequency is low and evenly distributed during childhood for t(1;19)[*TCF3/PBX1*] and t(9;22)[*BCR/ABL*] (n = 23 and 25, respectively, all but one in each group identified by G-band karyotyping, the last by RT-PCR) (Fig. 2B). Cases with other cytogenetic abnormalities by G-band karyotyping (n = 150) showed a moderate frequency peak for children less than 7 year at diagnosis (median age: 4 y) (Fig. 2C). The structural cytogenetic aberrations in 2.0 to 7.0 years age group are listed in Table 2. Ten cases in this group had a der/dic/t(9;20) and additional 3 had −20 with a deletion of chromosome arm 9p or a marker chromosome. Noteworthy, 15 cases with a modal number above 61 chromosomes, out of which 2 were near-tetraploid and 6 were near-triploid, were evenly distributed between 2 and 15 years of life (data not shown).

Cases with either a normal diploid G-band karyotype (n = 145) or no results due to too few metaphases or the leukemic clone not being analyzed (n = 85) (Fig. 2D) demonstrate a moderately increased frequency during the second to seventh year of life (median age at 4.8 and 4.0 y, respectively), where the frequency on average is approximately 3 times as high as in older age groups. For all the

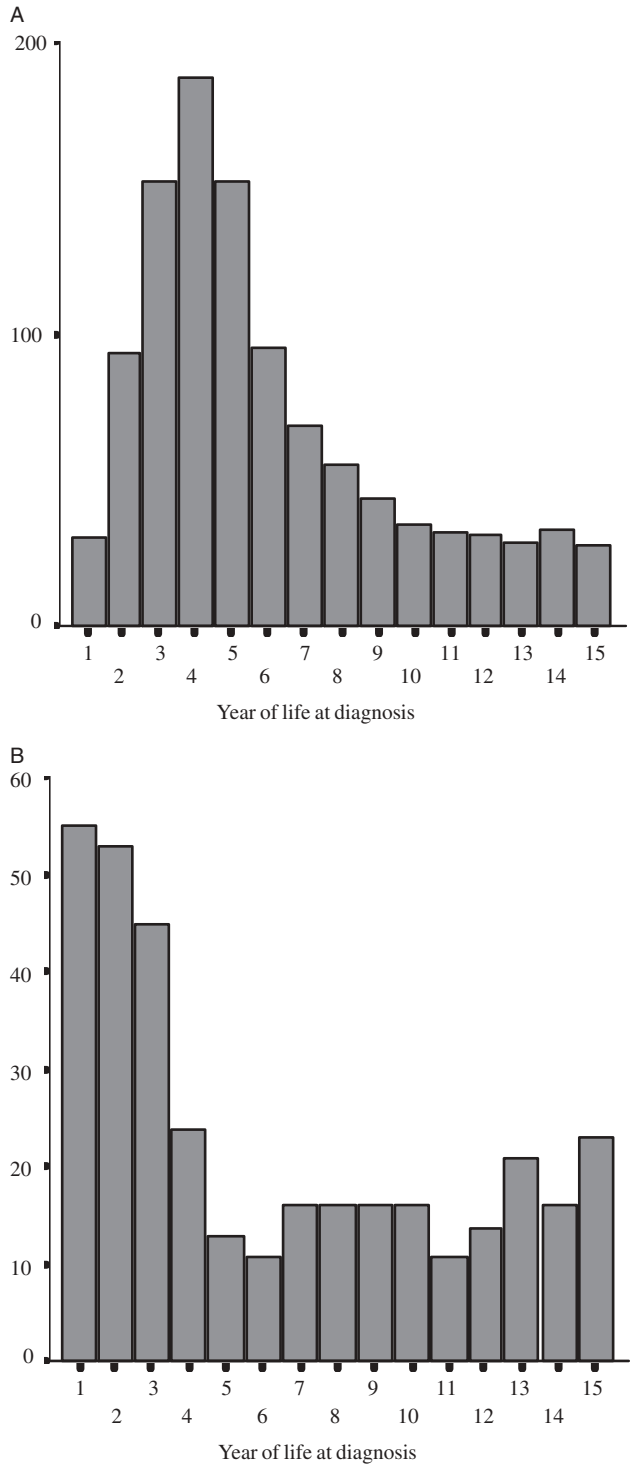

FIGURE 1. A, Age distribution for 1076 Nordic children with ALL who have been explored for translocation t(12;21) by RT-PCR or FISH. B, Age distribution for 350 Nordic children with de novo AML.

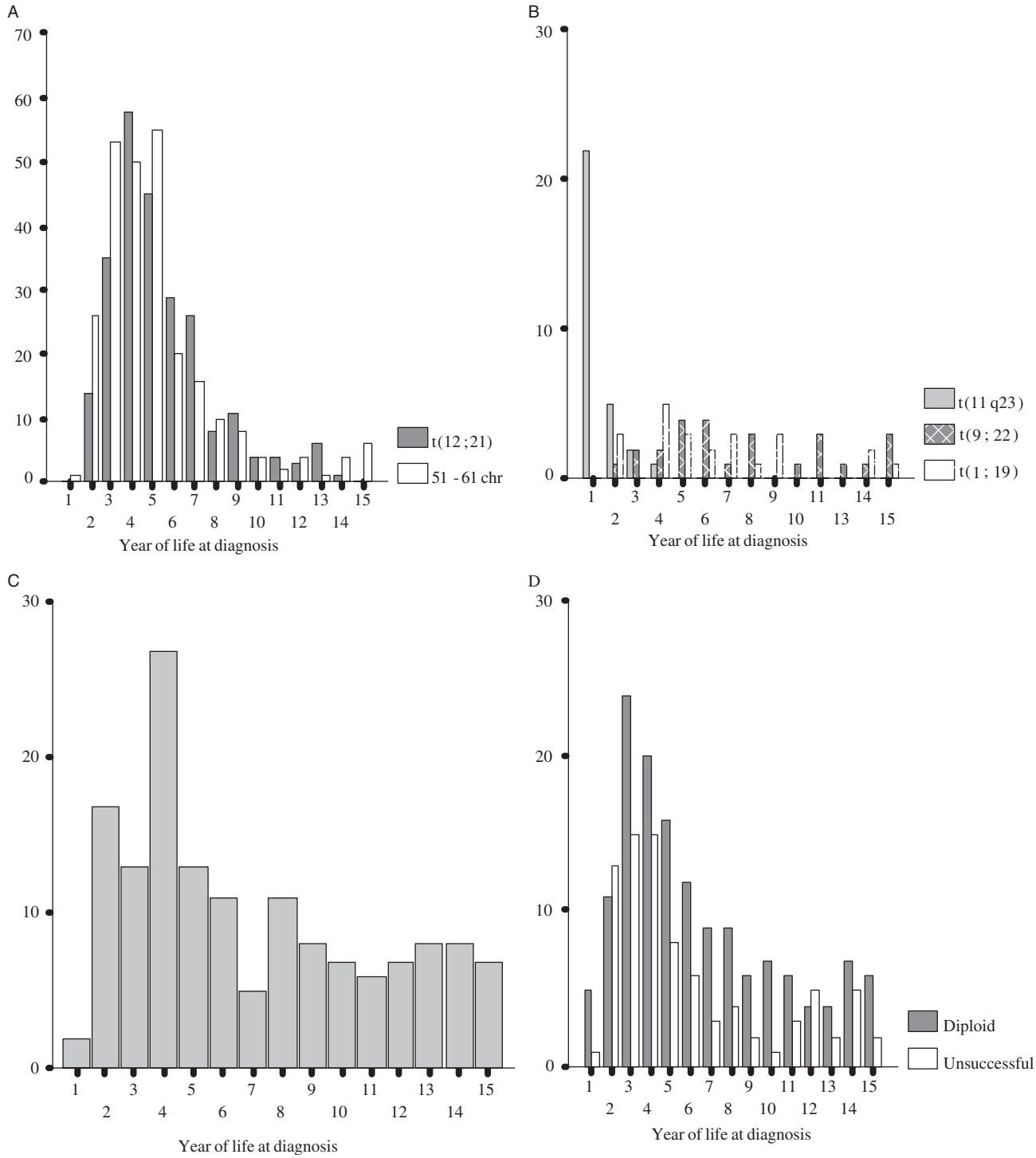

FIGURE 2. A, Age distribution for children with translocation t(12;21) (n = 244) identified by RT-PCR or FISH or a modal chromosome number of 51 to 61 by G-band karyotyping (n = 260). B, Age distribution for children with translocations *MLL*/11q23-translocation (n = 30), t(9;22) (n = 25, one detected by RT-PCR), or t(1;19) (n = 23, one detected by RT-PCR). C, Age distribution for children with cytogenetic abnormalities by G-band karyotyping other than those in A and B (n = 150). D, Age distribution for children with a normal diploid karyotype (n = 145) or no cytogenetic result by G-band karyotyping, either because of too few metaphases or the leukemic bone marrow not being analyzed (n = 85).

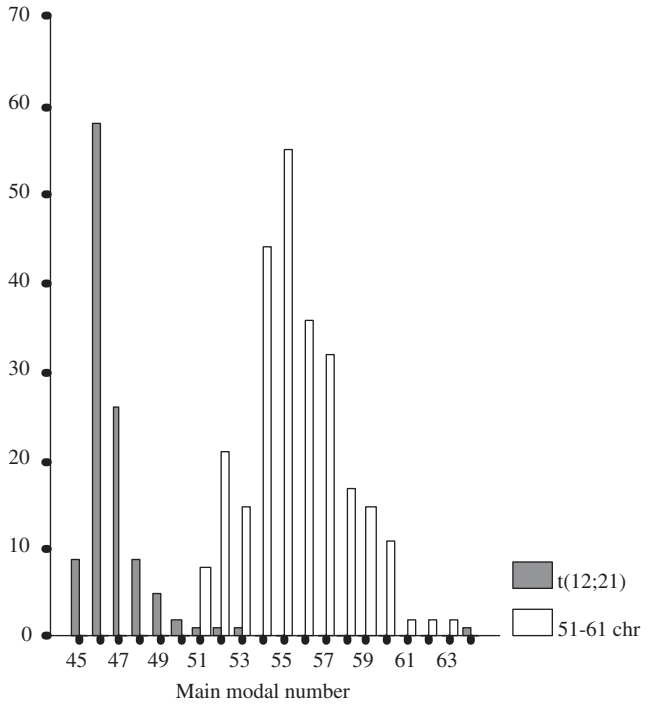

FIGURE 3. Frequency distribution of the modal chromosome number for high-hyperdiploidy by G-band karyotyping (51 to 61 chromosomes) or translocation t(12;21) identified by RT-PCR or FISH.

ALL patients in the study cohort, additional cytogenetic information was available with CGH in 11%, with flow cytometric DNA-index analyses in 27%, and with targeted FISH for 11q23, t(12;21), t(9;22), and/or t(1;19) in 29 of the cases. Among the 85 patients in the "no result" group, targeted analysis for t(12;21) with FISH discovered one or more additional chromosomes 21 in 15 of the cases (17%), and flowcytometry and/or CGH revealed 5 cases (6%) with a DNA-index between 1.10 and 1.35, which in this material corresponds to a chromosome modal number of 51 to 61. Furthermore, among the 145 cytogenetically normal diploid cases by G-band analysis, targeted FISH analysis demonstrated 10 cases (7%) with one or more extra chromosomes 21, and CGH and DNA-index showed a high-hyperdiploid karyotype in 3 cases (2%).

Among the cases with translocation t(12;21) or a high-hyperdiploid karyotype by G-band karyotyping, their maximum frequencies in the 2 to 7 years age group were at least 10 times higher compared with the older and younger age groups (Fig. 2). Cases with 51 to 61 chromosomes have their peak just after the fourth year of postnatal life, starting at the second year and declining gradually until the seventh year. Thus, 75% of all high-hyperdiploid cases occurred in the 2.0 to 7.0 years age group. For the t(12;21)-translocation positive patients, the maximum frequency occurs approximately 3.5 months later, starting during the second year with 79% of all cases occurring in the 2.0 to 7.0 years age group.

TABLE 2. Structural Genetic Aberrations Found in ALL for Group 8–"Other Abnormalities"—by G-band Karyotyping and Diagnosed During the Second to Seventh Year of Life

add(1)(p36)	add(9)(p24)
add(1)(q11)	del(9)(p12)
del(1)(p11)	del(9)(p13)
?del(1)(?p36.3q44)	del(9)(p13)
del(1)(q43q43)	del(9)(p21)
der(1)t(1;17)(p36;q21)	del(9)(p22)
dic(1;12)(q21;p13)	del(9)(p22)
dup(1)(q23q32)	der(9)del(9)(p22)del(9)(q34)
t(1;12)(q21;p13)	der(9)t(9;10)(p1?;?)
t(1;9)(p31?;p13?)	der(9)t(9;11)(q34;p1?5)
add(2)(q31)	der(9)t(9;13)(?;?)
add(2)(q37)	der(9;20)(q10;q10)
t(2;18)(p15;q21)	?dic(9;20)(p11-13;q11)
t(2;3)(p12;q27)	dic(9;20)(p11;q11)
t(2;3)(p2?4;q2?1)	dic(9;20)(p11;q11)
t(2;7)(p21;p13)	dic(9;20)(p11;q11)
add(3)(p11)	dic(9;20)(p11;q11)
add(3)(q12)	dic(9;20)(p11-13;q11)
del(3)(p12)	dic(9;20)(p11-13;q11)
del(3)(p23)	i(9)(q10)
der(3)t(2;3)(?;?)	t(9;20)(p11-13;q11)
der(3)t(3;8)(p12;q22)	?add(10)(q22)
der(3;6)(q10;p10)	del(10)(q24)
del(4)(q27q35)	der(10)t(6;10)(q1?;p1?)
del(4)(q27q35)	add(11)(p15)
der(4)t(4;10)(q3?;?)	del(11)(q13q23)
del(5)(q13q33)	del(11)(q23)
del(5)(q33)	t(11;17)(q21;q23)
t(5;11)(q2?;q2?)	add(12)(p11)
t(5;13)(q31;q21-q22)	der(12)t(3;12)(q10;p10)
t(5;15)(p13;q13)	der(12)t(7;12)(q11;p13)
add(6)(p25)	t(12;14)(p13;q11)
der(6)t(6;?17)	del(13)(q12q14)
i(6)(p10)	add(14)(p11)
t(6;7)(q21;?q3?)	inv(14)(q11q32)
t(6;8)(q21;q2?2)	add(16)(p13)
?der(7;12)(q10;q10)	t(16;21)(p11;q22)
?dic(7;9)(p11;p11)	add(17)(p11)
add(7)(p13-15)	der(17)t(6;17)
der(7)(?)	der(17)t(9;17)(q12;p13)
der(7;16)(?;?)	i(18)(q10)
inv(7)(q22q35)	add(19)(p13) or add(19)(q13)
t(7;22)(p15;q11)	der(20)t(9;20)(q22;q11.2)
t(7;9)(q11;p21)	add(21)(q22)
der(8)t(3;8)(p12;q22)	?i(22)(q10)
der(8)t(4;8)(q?;p?)	add(X)(p22)
t(8;10)(p2?1;q2?4)	add(X)(q28)

These 2 cytogenetic aberrations were detected in 61% of the non-Down pre-B cases during the second to seventh year of life (Table 1). When including the additional information gained by CGH and DNA-index analyses these 2 groups comprised 78% of all the pre-B ALL cases with an aberrant cytogenetic result in the 1 to 7 years of age group.

Among B-cell precursor ALL cases, high WBCs correlated with earlier age at diagnosis ($r_S = -0.23$; $P < 0.001$). Among the t(12;21)-positive patients, the WBC at diagnosis was 8.5×10^9/L (range: 0.8 to 225×10^9/L) and inversely correlated to the age ($r_S = -0.34$; $P < 0.001$) (Table 1). A male predominance was present irrespective of the WBC with an overall male:female ratio of 1.3 (Table 1). For the

high-hyperdiploid group, the median WBC at diagnosis was 6.0×10^9/L (range: 0.5 to 171×10^9/L) with a similar inverse correlation between WBC and age at diagnosis ($r_S = -0.35$; $P < 0.001$) (Table 1). The male:female ratio showed a female predominance in the lower WBC group and a male predominance in the higher WBC group (Table 1). Five trisomies were present in at least 50% of the 260 high-hyperdiploid cases being trisomies X (60%), 4 (53%), 6 (63%), 14 (50%), and 21 (85%). All the classic trisomies (X, 4, 6, 10, 14, 17, 18, and 21) demonstrated peak frequencies in the 2 to 7 years age groups that were 15 to 20 times higher than among 10.0 to 14.9 year old children. Furthermore, except for one trisomy 14, none of these trisomies occurred among the 30 infants.

Overall, there was a male preponderance in this childhood ALL cohort being most pronounced for pre-B cases with a WBC $> 10 \times 10^9$/L (male:female 1.2). For cases with a WBC $\leq 10 \times 10^9$/L, males dominated only for the t(12;21)-positive cases (ratio: 1.3) and for those with an unsuccessful karyotype (ratio: 2.1), but not for the high-hyperdiploid cases (ratio: 0.8) or for the remaining cases (ratio:1.0).

For T-cell ALL there was only a moderate increase in frequency during later childhood (median age: 8.7 y) as previously published.[12]

In the subset of patients selected for the present study no clear relation between age and incidence could be demonstrated for children with Down syndrome (Fig. 5). Their median age at diagnosis was 5.4 years. Included in this group of patients are 1 case with translocation t(9;22)(q34;q11) and 1 case with translocation t(12;21).

AML

The cytogenetic subgroups in AML (Table 3) also show specific age-related incidence patterns (Fig. 1B). In the first year of life, 36% of the non-Down AML cases are *MLL*-rearranged, and an additional 42% have other cytogenetic aberrations, but notably none had the classic AML translocations: t(8;21), t(15;17), or inv(16) (Fig. 4). During the first 3 years of life 23% are *MLL*-rearranged, and an additional 47% had abnormalities other than t(8;21), t(15;17), and inv(16) (Fig. 4 and Table 3). Overall, the AML cases have their highest frequency during the first 3 years of life (Fig. 1), which is most pronounced for the aforementioned 11q23 [*MLL*]-rearranged leukemias, and those with abnormalities other than +8 (as the only abnormality) and translocations t(8;21), t(15;17), and inv(16) (Fig. 4 and Table 3). In contrast, cases with the AML specific translocations t(8;21), t(15;17), and inv(16), those with a gain of a whole chromosome 8 as the sole aberration, and those with no cytogenetic results or a normal diploid karyotype showed a fairly even distribution during childhood (Fig. 4).

Forty-seven of the AML patients (13%) had Down syndrome. Their frequency peaked during the second and third year of postnatal life, and only 1 child was older than 5 years (11 y), and this patient had a t(8;21)-translocation.

Excluding those with translocation t(9;11), patients with 11q23-rearranged AML demonstrated a significant inverse correlation between WBC and age at diagnosis ($r_S = -0.43$; $P = 0.02$). This was not found for any other subset of AML. The male:female ratio differed significantly among the subgroups with a male preponderance among t(9;11)-positive patients and a female predominance among other 11q23-aberrant cases (Table 3).

DISCUSSION

Many previous epidemiologic studies of childhood ALL have roughly subdivided the cases into infant leukemias characterized by an inferior outcome, a frequency peak group in the 2 to (eg,) 7 years age group with a favorable outcome, and the remaining inhomogeneous group of patients that have a fairly stable incidence during childhood. Among the latter are T-cell leukemias and certain more rare subsets [eg, t(9;22), t(1;19), and hypodiploid ALL] usually found to have a higher than average risk of relapse depending on the therapy given. The picture is somewhat less clear-cut for childhood AML.

The present study adds to the growing epidemiologic and biologic knowledge that demonstrate that the childhood acute leukemias encompass clinically related, but cytogenetically distinct diseases, and that cytogenetic subgrouping is important to understand the diversity in their etiology, epidemiology, and natural history. Furthermore, the results of this study question if age as a disease-related prognostic parameter has any relevance in childhood leukemia research beyond being a surrogate marker of more important truly biologic features.

Infant ALL

Although 11q23-translocated leukemias are also seen in older children and even in the elderly,[25] it is clearly the leukemia of infancy. The *MLL*/11q23-translocations, of which t(4;11)(q21;q23) and t(11;19)(q23;p13) are the most common, have a distinct incidence peak during the first year of life, where the 11q23-aberrant leukemias are the second most common malignancy. When these leukemias appear in very early infancy their prenatal origin is well proven, and close to 100% concordance rate in monozygous twins (with identical *MLL-rear*rangements) indicates, that when these mutations are present at birth, they almost invariably lead to overt leukemia.[26] In contrast, it is unknown in which time frame the 11q23 translocations have evolved, when such leukemias occur in adults.

ALL Incidence Peak Group

Most cases of childhood ALL occur between 2 and 7 years of age, where the incidence in the Nordic countries may be up to 10 times higher than in the older age group.[12] Supported both by epidemiologic studies and by their favorable prognosis it has long been suspected, that these patients constitute a special entity, and consequently age has by many collaborative groups been included in the risk stratification and treatment assignment.

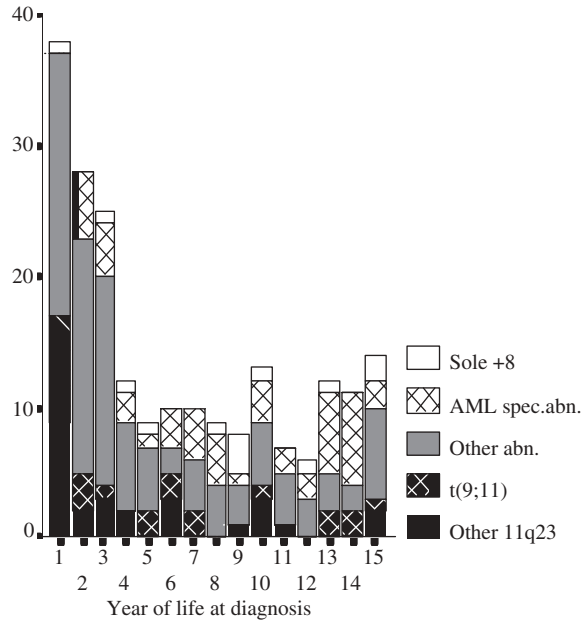

FIGURE 4. Age distribution for non-Down syndrome children with AML and cytogenetic aberrations; that is, trisomy 8 (n = 13), the AML-specific abnormalities [t(8;21), t(15;17), and inv(16)/t(16;16) (n = 46)], t(9;11) (n = 24), other 11q23/*MLL* translocations (n = 26, one detected by RT-PCR), and other clonal abnormalities by G-band karyotyping (n = 103). Patients with normal diploid karyotypes (n = 76), or no cytogenetic result either because of too few metaphases or the leukemic bone marrow not being analyzed (n = 15) are not shown.

The frequencies by which the different cytogenetic aberrations occur in this series are in agreement with earlier Nordic studies.[2,14,15,17,27] Furthermore, in this geographic region the age incidence peak is prominent and stable over time.[12] Despite the skewed distribution of targeted analysis for the cryptic t(12;21), the age-related frequency distribution and the clinical characteristics indicate that the ALL cohort included in this study is representative for the Nordic patient population. In the beginning of the study period, RT-PCR were in most cases applied to detect the t(12;21) translocation, but with the growing awareness of the possible impact of additional aberrations,[20,21] FISH analysis has taken over as the preferred method. Targeted analyses for the frequently recurring translocations (eg, involving *MLL, RUNX1, BCR, ABL, ETV6, TCF3, and PBX1*) give, apart from knowledge of their presence, also information about hidden trisomies for chromosomes 1, 9, 11, 12, 19, 21, 22. The most frequent additional finding was +21q22 that often reflects a gain of one or more whole chromosomes 21, which occurs both in undetected high-hyperdiploid ALL and in low-hyperdiploid cases, where trisomy 21 also is the most common aberration.[14]

The percentages of t(12;21)-positive and high-hyperdiploid (51 to 61 chr) cases in this series (23% and 24%, respectively) are in concordance with most, but not all, childhood series from the developed countries,[13,28–30] whereas both these cytogenetic subsets are very rare in adults.[31,32] In this respect it is of interest that most of the noninfant cases of ALL, that have been demonstrated to be initiated in utero, are characterized by either harboring the t(12;21)-translocation or to be high-hyperdiploid.[3–8]

The crucial first and second hits in the high-hyperdiploid cases are less clear than for translocation t(12;21). The classic high-hyperdiploid cases have a peak modal number of 55 chromosomes with trisomies X, 4, 6, 10, 14, 17, 18, and 21 being the most frequent, and the remaining trisomies being far more rare.[14,15] The incidence pattern of the frequent trisomies differ somewhat, chromosome 21, X, and 6 being the most frequent, and some of the incidence peaks being more prominent than others. However, it remains to be explored whether such dissection of the trisomies have any biologic significance, and if there is a biologic and prognostic profile directly linked to the gene dosage effects of the trisomies that can justify treatment stratification on the basis of the specific pattern of trisomies.

A significant proportion of the incidence peak in the 2 to 7 year age group is not explained by t(12;21)-positive and high-hyperdiploid leukemias, and although the frequencies by which the different cytogenetic aberrations occur in this series for ALL (and for AML) are very much in agreement with earlier Nordic studies, it is well known that some specific cytogenetic subsets could often are missed routine G-band karyotyping.[29] Because all patients in the present study were screened by FISH

TABLE 3. Cytogenetic Subgroups by G-band Karyotyping in 350 Children With AML

Subgroup	Characteristic	N (%)	Ratio M/F	Years of Life N (%)			
				1	2	3	4-15
1	AML in Down syndrome	47 (13.4)	0.52	7 (12.7)	17 (32.1)	14 (31.1)	9 (4.6)
2	Other 11q23	26 (7.4)	0.44	9 (16.4)	2 (3.8)	3 (6.7)	12 (6.1)
3	t(9;11)	24 (6.9)	1.18	8 (14.5)	3 (5.7)	1 (2.2)	12 (6.1)
4	t(8;21)/t(15;17)/inv(16)	46 (13.1)	1.0	0 (0)	5 (9.4)	4 (8.9)	37 (18.8)
5	Sole +8	13 (3.7)	0.44	1 (1.8)	0 (0)	1 (2.2)	11 (5.6)
6	Other abn.	103 (27.4)	1.02	20 (36.4)	18 (34.0)	16 (35.6)	49 (24.9)
7	Diploid	76 (21.7)	1.45	7 (12.7)	7 (13.2)	5 (11.1)	57 (28.9)
8	No result	15 (4.3)	0.36	3 (5.5)	1 (1.9)	1 (2.2)	10 (5.1)
Total	—	350 (100.0)	0.89	55 (100.0)	53 (100.0)	45 (100.0)	197 (100.0)

F indicates female; N, number of patients; M, male; %, percentage of total.

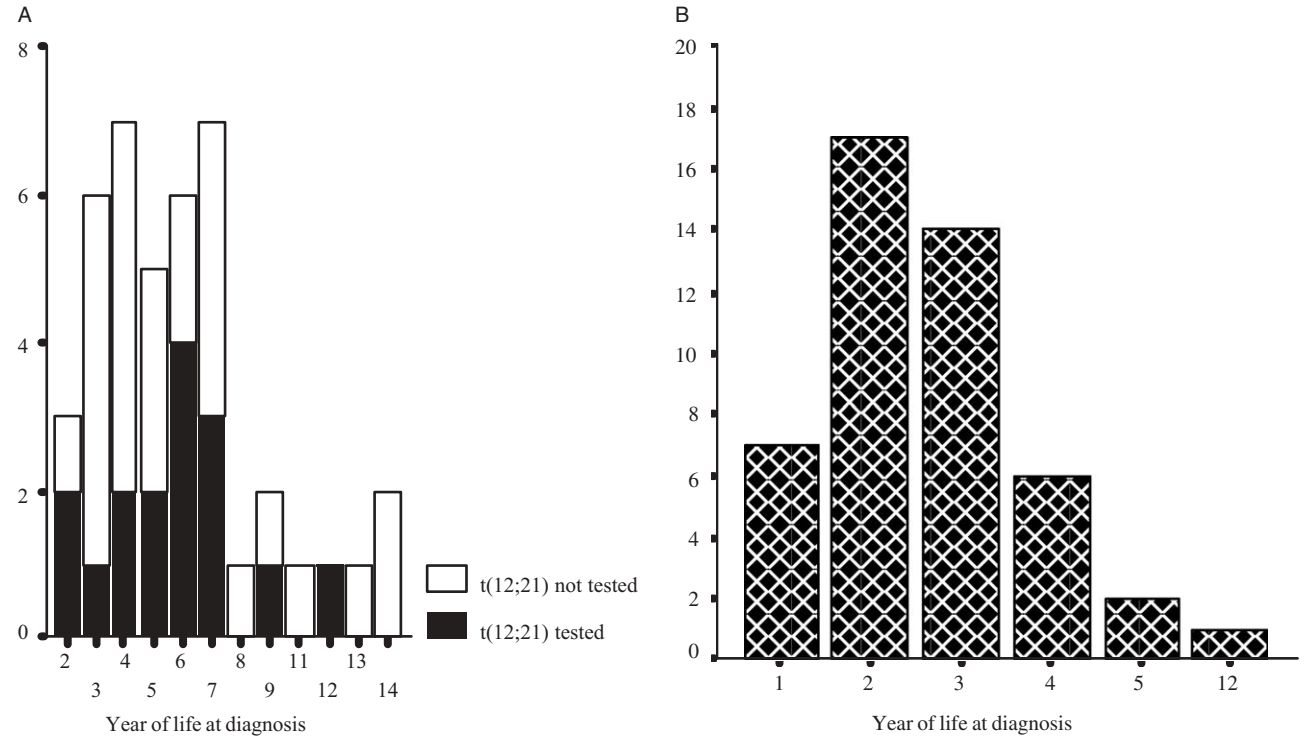

FIGURE 5. Age distribution for Down syndrome children with ALL (n = 42, 5A) and AML (n = 47, 5B). In (A) all children with Down syndrome diagnosed January 1993 to December 2003 has been included whether they were (black, n = 16) or were not (white, n = 26) explored for translocation t(12;21) by RT-PCR or FISH. The only patient in (B) above 5 years of age had a t(8;21)-translocation.

and/or RT-PCR for t(12;21) [*ETV6*/*RUNX1*], we expect the detection rate for this subset to be near complete. In contrast, G-banded karyotyping is subjective, and the bone-marrow karyotypes, especially for the hyperdiploid cases, are often of poor quality. Accordingly, several studies that use techniques that are not dependent on cell culture have revealed high-hyperdiploidy at a higher rate than that yielded by standard G-band karyotyping, especially among leukemias classified as diploid or unsuccessful.[15,17,19,29] On the other hand it is possible, that the incidence peak group could harbor smaller subsets of ALL with biologic and clinical characteristics similar to those of t(12;21)-positive and high-hyperdiploid ALL, such as an in utero initiation and a favorable outcome, not least because the incidence peak in it self has been taken as support for the prenatal origin of childhood ALL.[1] Because the incidence peaks for the patients that in the present study is classified in group 8 (''other clonal abnormalities''), 9 (''normal diploid karyotypes''), and 10 (''no cytogenetic result'') are less prominent than that of translocation t(12;21) and high-hyperdiploidy, such subsets could well have an incidence curve that differ from these 2 dominating groups of B-lineage ALL. Thus, among the patients in the 2 to 7 years age group that did not have translocation t(12;21) or a high-hyperdiploid karyotype, some recurrent chromosomal aberrations were detected, the most significant being der/dic/t(9;20). The abnormality is underdiagnosed with G-banded karyotyping and sometimes described as 45,XX/XY, −20, +mar, or with a del(9p), where −20 is the indicator of this aberration.[33] These patients, share many of the characteristics of high-hyperdiploid and t(12;21)-positive leukemias, including a median age at diagnosis of 4 years and probably a favorable prognosis.[34] Thus, to explore the cytogenetic pattern of the incidence peak not explained by translocation t(12;21) and high-hyperdiploidy there is a need for large prospective studies that apply FISH techniques including multicolor FISH, CGH including CGH array, multiplex PCR, and flow cytometric DNA-index analyses.

ALL in Down Syndrome

In the present study we found a lower incidence of Down syndrome in the translocation t(12;21)[*ETV6*/*RUNX1*] tested group compared with that of the Nordic population-based registration (1.5% vs. 2.1%).[23] This could be a coincidence or it could reflect a low frequency of targeted *ETV6*/*RUNX1* analyses done in Down syndrome patients. Judging from the initial panel of targeted analysis done in Down syndrome leukemia most physicians tend to expect their patients to have AML and-if not-that the otherwise well-known cytogenetic subsets of childhood ALL [such as the t(12;21)-translocation] are rare in patients with Down syndrome, and thus not worth

exploring. In an ongoing large international study the cytogenetic aberrations of Down syndrome childhood acute leukemias is being explored, and the frequency of t(12;21) is close if not similar to the frequency found in non-Down syndrome patients (Erik Forestier, personal communication).

AML

As for ALL, childhood AML encompasses several well-defined cytogenetic subgroups, that each have their characteristic age incidence curve. Thus, the incidence peak of AML in the first 3 years of life primarily reflects AML in Down syndrome, *MLL*/11q23-translocations [most commonly t(9;11)], and a group of leukemias with other structural abnormalities, although rarely translocations t(8;21), t(15;17), or inv(16).[27] In contrast, the classic AML specific abnormalities like t(8;21), t(15;17), and inv(16) demonstrate a fairly even distribution during childhood. This is noteworthy, because an in utero occurrence of t(8;21) has been demonstrated both for children that did and did not develop t(8;21)-positive AML.[5,35] Because, a healthy carrier-state of t(8;21) has been demonstrated both for newborns[35] and for patients cured for t(8;21)-positive AML,[36] it is possible that these translocations can evolve throughout life and persist in a nonmalignant carrier state. In contrast, the t(9;11)-positive AML have many clinical similarities with the incidence peak cases of ALL, including their incidence peak in the first years of life, and their high cure rate with chemotherapy.[37,38] Whether they also have a prenatal origin remains to be determined.

AML in Down Syndrome

The incidence patterns of AML in children with Down syndrome makes it the second largest single group (13% of all patients) in the Nordic AML cohort, which is somewhat higher than the frequency reported by other groups.[27,38–41] With its clear peak in the second year of life and the in utero origin of the crucial GATA1-mutations on chromosome X, it is being explored whether AML in Down syndrome could be a useful model giving general insight into leukemogenesis of childhood AML.[11] Accordingly, an international collaboration has been initiated to explore the incidence pattern for phenotypic and cytogenetic subsets groups of AML in Down syndrome (Erik Forestier, personal communication).

The heterogeneity in the frequency distribution, clinical characteristics, and prognosis of the cytogenetic subsets of ALL and AML emphasizes the importance of detailed karyotyping of childhood acute leukemias to improve our understanding of their pathogenesis and natural history, which could lead to refined stratification of therapy. Although, G-band karyotyping remains the golden standard, there is a need for routine use of other techniques that do not require cell cultures. This may include RT-PCR, FISH, and high resolution CGH. The development of the gene array-techniques will also be valuable to clarify the patterns of cytogenetic imbalances and their affect on gene expression.[18,42]

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