

Cytogenetic Patterns in *ETV6/RUNX1*-Positive Pediatric B-Cell Precursor Acute Lymphoblastic Leukemia: A Nordic Series of 245 Cases and Review of the Literature

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Between 1992 and 2004, 1,140 children (1 to <15 years) were diagnosed with B-cell precursor acute lymphoblastic leukemia (ALL) in the Nordic countries. Of these, 288 (25%) were positive for t(12;21)(p13;q22) [*ETV6/RUNX1*]. G-banding analyses were successful in 245 (85%); 43 (15%) were karyotypic failures. The modal chromosome numbers, incidence, types, and numbers of additional abnormalities, genomic imbalances, and chromosomal breakpoints in the 245 karyotypically informative cases, as well as in 152 previously reported cytogenetically characterized t(12;21)-positive ALLs in the same age group, were ascertained. The most common modal numbers among the 397 cases were 46 (67%), 47 (16%), 48 (6%), and 45 (5%). High-hyperdiploidy, triploidy, and tetraploidy were each found in ~1%; none had less than 40 chromosomes. Secondary chromosomal abnormalities were identified by chromosome banding in 248 (62%) of the 397 ALLs. Of these, 172 (69%) displayed only unbalanced changes, 14 (6%) only balanced aberrations, and 26 (10%) harbored both unbalanced and balanced abnormalities; 36 (15%) were uninformative because of incomplete karyotypes. The numbers of secondary changes varied between 1 and 19, with a median of 2 additional aberrations per cytogenetically abnormal case. The most frequent genomic imbalances were deletions of 6q21-27 (18%), 8p11-23 (6%), 9p13-24 (7%), 11q23-25 (6%), 12p11-13 (27%), 13q14-34 (7%), loss of the X chromosome (8%), and gains of 10 (9%), 16 (6%), and 21 (29%); no frequent partial gains were noted. The chromosome bands most often involved in structural rearrangements were 3p21 (2%), 5q13 (2%), 6q12 (2%), 6q14 (2%), 6q16 (2%), 6q21 (10%), 6q23 (6%), 6q25 (3%), 9p13 (2%), 11q13 (2%), 11q23 (2%), 12p11 (6%), 12p12 (7%), 12p13 (25%), 21q10 (6%), and 21q22 (6%). Considering that the t(12;21) is known to arise in utero and that the postnatal latency period is protracted, additional mutations are most likely necessary for overt ALL. The frequently rearranged chromosome regions may harbor genes of importance for the transformation and/or progression of an initial preleukemic t(12;21)-positive clone. © 2007 Wiley-Liss, Inc.

INTRODUCTION

The cytogenetically cryptic t(12;21)(p13;q22) was first reported by Romana et al. (1994) as a fortuitous FISH finding when investigating pediatric acute lymphoblastic leukemias (ALLs) harboring 12p deletions and was soon afterward shown to result in fusion of the *ETV6* (*TEL*) and *RUNX1*

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TABLE 1. Frequencies of t(12;21)(p13;q22) in Pediatric B-Cell Precursor Acute Lymphoblastic Leukemia

% t(12;21)	Origin	Reference	% t(12;21)	Origin	Reference
0 (0/29)	Spain	García-Sanz et al., 1999	22 (11/51)	Italy	Lanza et al., 1997
8.5 (6/71)	Mexico	Pérez-Vera et al., 2005	23 (16/69)	France	Cayuela et al., 1996
8.7 (4/46)	India	Inamdar et al., 1998	23 (22/96)	France	Baruchel et al., 1997
9.7 (7/72)	Japan	Eguchi-Ishimae et al., 1998	23 (44/188)	USA	Rubnitz et al., 1997
12 (9/77)	Japan	Kobayashi et al., 1996	24 (24/98)	Israel	Avigad et al., 1999
13 (4/31)	UK	Spathas et al., 1999	24 (22/91)	The Netherlands	Hubeek et al., 2001
16 (14/86)	Japan	Nakao et al., 1996	25 (94/372)	Austria	Attarbaschi et al., 2004
17 (10/58)	Sweden	Andreasson et al., 2000	25 (288/1140)	Nordic countries	Present series
18 (12/67)	Hong Kong	Tsang et al., 2001	26 (9/34)	USA	Fears et al., 1996
19 (7/36)	Taiwan	Liang et al., 1996	26 (37/144)	Czech Republic	Trka et al., 1998
19 (23/120)	Japan	Takahashi et al., 1998	26 (77/299)	USA	Loh et al., 2006
19 (15/80)	USA	Jamil et al., 2000	27 (45/166)	USA	Borowitz et al., 1998
19 (7/36)	Spain	Martinez-Ramirez et al., 2001	28 (35/126)	USA	Shurtleff et al., 1995
19 (95/504)	USA	Uckun et al., 2001	28 (14/50)	Canada	Alessandri et al., 2002
19 (11/58)	Brazil	Zen et al., 2004	28 (37/134)	Turkey	Ozbek et al., 2003
20 (12/60)	Brazil	Magalhães et al., 2000	32 (22/68)	USA	McLean et al., 1996
20 (139/693)	UK	UK Childhood Cancer Investigators, 2000	33 (22/66)	Australia	Amor et al., 1998
20 (25/122)	Hungary	Pajor et al., 2001	34 (17/50)	France	Raynaud et al., 1996a
20 (11/56)	France	Douet-Guilbert et al., 2003	34 (20/58)	Belgium	Ameys et al., 2000
22 (8/36)	France	Romana et al., 1995b	35 (44/125)	France	Cavé et al., 1997
22 (63/286)	Germany/Italy	Borkhardt et al., 1997	41 (22/54)	UK	Codrington et al., 2000

(*AML1*) genes (Golub et al., 1995; Romana et al., 1995a). Today, the t(12;21) is known to be the most common translocation in B-lineage childhood ALL, occurring in ~25% of such cases, with a median age of 4 years (Johansson et al., 2004; Forestier and Schmiegelow, 2006) and possibly with a geographic frequency heterogeneity (Table 1). In contrast, it is rarely observed in adult ALL (Cayuela et al., 1996; McLean et al., 1996; Raynaud et al., 1996b; Jabber Al-Obaidi et al., 2002).

Except for one T-ALL (Ma et al., 2001), all reported t(12;21)-positive ALLs have had a B-cell precursor (BCP) phenotype, characteristically positive for HLA-DR, CD10, CD13, CD19, CD24, CD33, CD34, CD40, CD45, and CDw65 and negative for CD9, CD20, and CD86 (Romana et al., 1995b; Cayuela et al., 1996; Baruchel et al., 1997; Borkhardt et al., 1997; Borowitz et al., 1998; De Zen et al., 2000; Alessandri et al., 2002). The aberrant expression of CD13, CD33, and CDw65 is quite characteristic; in fact, these myeloid markers in BCP ALLs have been shown to be significantly associated with the presence of *ETV6/RUNX1* (Baruchel et al., 1997; Borkhardt et al., 1997; Borowitz et al., 1998; Rafi et al., 2000; Tsang et al., 2001; Attarbaschi et al., 2004). The fact that t(12;21)-car-

rying ALLs have quite characteristic immunophenotypes and also specific gene expression profiles (Ross et al., 2003; Andersson et al., 2005a,b; Kuchinskaya et al., 2005) suggests that the *ETV6/RUNX1* fusion gene confers a strong impact on crucial genetic pathways.

The prognostic implication of t(12;21) has—and continues to be—debated. Soon after the identification of *ETV6/RUNX1*, Shurtleff et al. (1995) reported an “excellent prognosis,” although the 5-year event free survival in their study did not differ significantly from t(12;21)-negative B-lineage cases. Since then, numerous studies have corroborated the early findings, indicating a favorable outcome for patients with this ALL subtype (McLean et al., 1996; Borkhardt et al., 1997; Rubnitz et al., 1997, 1999; Loh et al., 1998; Avigad et al., 1999; Maloney et al., 1999; Zuna et al., 1999; Jamil et al., 2000; Uckun et al., 2001; Ozbek et al., 2003). However, several groups have not observed any significant survival differences between t(12;21) and non-t(12;21) ALLs; furthermore, frequent late relapses as well as similar incidences of this fusion gene in ALLs at diagnosis and relapse have also been noted, suggesting that *ETV6/RUNX1* is not an independent good-risk marker and emphasizing the need for long follow-up periods (Nakao et al.,

1996; Harbott et al., 1997; Lanza et al., 1997; Satake et al., 1997; Takahashi et al., 1998; Kempfski et al., 1999; Seeger et al., 1999; Codrington et al., 2000; Hann et al., 2001; Hubeek et al., 2001; Pajor et al., 2001; Tsang et al., 2001). Recently, a prospective analysis showed that the survival rate in t(12;21)-positive BCP ALLs was significantly better when compared with negative cases; however, this did not hold true in a multivariate analysis—other factors, in particular age and leukocyte counts, were found to be independent predictors of outcome (Loh et al., 2006). Thus, it seems safe to conclude that the jury is still out on this issue.

Although the *ETV6/RUNX1* fusion undoubtedly is important in leukemogenesis, considering its high incidence in BCP ALL (Table 1) and its negative correlation with other well-known ALL-associated genetic abnormalities such as high hyperdiploidy (>50 chromosomes), *BCR/ABL1* and *TCF3/PBX1* fusions, and *MLL* rearrangements (Cayuela et al., 1996; Fears et al., 1996; Borkhardt et al., 1997; Raimondi et al., 1997; Rubnitz et al., 1997; Eguchi-Ishimae et al., 1998; Raynaud et al., 1999; Andreasson et al., 2000; Tsang et al., 2001; Uckun et al., 2001; Attarbaschi et al., 2004), it is equally clear that it is not sufficient for overt leukemia. First, several studies have shown that the *ETV6/RUNX1* fusion may arise already in utero, probably in a committed B-cell progenitor (Hotfilder et al., 2002; Mori et al., 2002; Castor et al., 2005), but that there is a postnatal—often protracted—preleukemic phase before the diagnosis of ALL (Ford et al., 1998; Wiemels et al., 1999a,b; Maia et al., 2001, 2004; Hjalgrim et al., 2002; McHale et al., 2003; Broadfield et al., 2004). Second, the *ETV6/RUNX1* chimera can be found in normal cord and peripheral blood samples at frequencies 100-fold greater than the risk of the corresponding leukemia (Eguchi-Ishimae et al., 2001; Mori et al., 2002). Third, various mouse models have shown that although the fusion gene promotes B-cell development, albeit with an incomplete differentiation block, it is not sufficient for ALL induction unless complementary mutations are present (Andreasson et al., 2001; Bernardin et al., 2002; Morrow et al., 2004; Tsuzuki et al., 2004; Fischer et al., 2005). The available data therefore strongly indicate that an initial t(12;21)-positive preleukemic clone, which perhaps always arises prenatally, must acquire additional mutations before full leukemic transformation.

Most studies of the secondary leukemia-promoting genetic changes have focused on 12p deletions, other structural rearrangements involving 12p, and

gains of chromosome 21 material, aberrations often identified by chromosome banding, comparative genomic hybridization, and, in particular, by FISH analyses in *ETV6/RUNX1*-positive leukemia (Shurtleff et al., 1995; Fears et al., 1996; Kobayashi et al., 1996; Raimondi et al., 1997; Amor et al., 1998; O'Connor et al., 1998; Codrington et al., 2000; Jarošová et al., 2000; Rafi et al., 2000; Martínez-Ramírez et al., 2001; Mathew et al., 2001; Tsang et al., 2001; Uckun et al., 2001; Nordgren et al., 2002; Douet-Guilbert et al., 2003; Stams et al., 2006). Apart from the fact that the chromosome 21 gains may involve either the normal homologue or the der(21) (Kempfski et al., 1999; Loncarevic et al., 1999; Andreasson et al., 2000; Ma et al., 2001), the latter occasionally forming an isodervative chromosome 21 (Martineau et al., 1996; Andreasson et al., 1997; Ameye et al., 2000; Alvarez et al., 2005), little is known about their pathogenetically important outcome. In contrast, the 12p deletions have been characterized in more detail. FISH analyses have revealed occasional intragenic deletions of the nontranslocated *ETV6*, strongly indicating that this gene is the target of the 12p aberrations (Raynaud et al., 1996a; Cavé et al., 1997; Kempfski et al., 1999). It has also been clearly shown the *ETV6* loss occurs as a secondary event, with deletions being found in subclones only (Raynaud et al., 1996a; Romana et al., 1996; Eguchi-Ishimae et al., 1998; Maia et al., 2001), varying in size between diagnostic and relapse samples, or not being present at relapse but at diagnosis (Ford et al., 2001; Konrad et al., 2003; Zuna et al., 2004). In addition, the *ETV6* gene has been reported to be retained in t(12;21)-positive cells found in Guthrie cards (Mori et al., 2002). Loss of the nonrearranged *ETV6* has hence been implicated as one—perhaps the most—important secondary event in the transformation of preleukemic cells with t(12;21) to overt leukemia.

Surprisingly few studies have specifically addressed the types and frequencies of abnormalities other than 12p aberrations and chromosome 21 gains in *ETV6/RUNX1*-positive ALLs, although deletions of 6q and 9p, including the *CDKN2A* gene, losses of chromosomes X, 8, 12, and 5, and trisomy for chromosomes 4, 10, and 16 have been noted by several investigators (Kempfski et al., 1999; Loncarevic et al., 1999; Raynaud et al., 1999; Andreasson et al., 2000; Tsang et al., 2001; Uckun et al., 2001; Attarbaschi et al., 2004; Alvarez et al., 2005; Martineau et al., 2005). Since such secondary chromosome changes may be of both clinical and pathogenetic importance, we have ascertained the

cytogenetic patterns, i.e., modal chromosome numbers, incidence, types, and numbers of additional abnormalities, genomic imbalances, and chromosomal breakpoints, in ALLs with the *ETV6/RUNX1* fusion diagnosed between 1992 and 2004 in the Nordic countries. In addition, information on all previously reported cases (Mitelman et al., 2006) has been reviewed.

MATERIALS AND METHODS

Patients

Between January 1, 1992, and December 31, 2004, 2,350 infants, children, and adolescents were diagnosed with ALL in the Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden). This cohort includes all children below the age of 15, whereas some older patients may have been treated in nonpediatric hematology departments. Thus, the major subgroup comprising children aged 0–<15 years is all-inclusive and hence truly population-based. Chromosome banding analyses were performed using standard methods in 15 cytogenetic laboratories in the Nordic countries, and all abnormal karyotypes have been centrally reviewed annually since 1996 (Sweden)/2000 (all five Nordic countries).

For the purpose of this report, the following inclusion criteria were applied: (1) BCP immunophenotype, (2) age between 1 and <15 years, and (3) known *ETV6/RUNX1* status, as determined by FISH and/or RT-PCR. Screening for t(12;21) has generally been performed prospectively from 1997 in Sweden and from 2000 in the other Nordic countries; positive cases prior to this time period have been identified in retrospective analyses. A few ALLs not screened for the presence of t(12;21) were nevertheless included in the study as negative for this translocation, namely, those shown to be positive for *MLL* rearrangements or *BCR/ABL1* and *TCF3/PBX1* fusions; these genetic changes are, as referred to earlier, not present in *ETV6/RUNX1*-positive ALLs.

The Mitelman Database of Chromosome Aberrations in Cancer (Mitelman et al., 2006) was used to retrieve previously reported t(12;21)-positive ALLs, applying the same inclusion criteria, i.e., BCP ALL diagnosed in children 1–<15 years of age (cases from the Nordic countries were excluded to avoid recording the same case twice).

Cytogenetic Patterns Ascertained

The cytogenetic features ascertained and reviewed in the Nordic cases as well as in the pub-

lished *ETV6/RUNX1*-positive ALLs were (1) modal chromosome numbers (only the most basic clone and the lowest mode in cases with modal number spans were registered), (2) incidence of secondary changes in addition to the t(12;21), (3) types (balanced and unbalanced) and numbers of additional aberrations (cases with incomplete karyotypes were excluded in these analyses), (4) genomic imbalances, i.e., whole or partial chromosome gains/losses, of secondary changes (in relation to the nearest ploidy level), and (5) chromosomal breakpoints involved in additional structural abnormalities. Identical imbalances or breakpoints were registered only once per case, and when a case harbored different imbalances involving the same chromosome, the largest imbalance was recorded.

Both FISH and RT-PCR analyses have been used to identify the *ETV6/RUNX1* fusion, in the present Nordic series as well as in the cases retrieved from the literature. Considering that FISH—but not RT-PCR—can detect cytogenetically cryptic secondary 12p and 21q abnormalities, only aberrations of these chromosome arms identified by G-banding were ascertained to avoid a frequency bias of such changes in relation to the other secondary abnormalities found by chromosome banding.

RESULTS

Patients

Among the 2,350 infants, children, and adolescents diagnosed with ALL in the Nordic countries between 1992 and 2004, 1,140 (49%) were between the age of 1 and 15 years, had a BCP ALL, and were known to be either *ETV6/RUNX1*-positive or negative. The entire patient cohort had a median age of 4.3 years and a male/female ratio of 1.14. The t(12;21) was found in 288 (25%) of the 1,140 cases, with a median age and male/female ratio of 4.3 years and 1.32, respectively; 245 (85%) of these were cytogenetically informative whereas 43 (15%) were karyotypic failures.

Searching the literature (Mitelman et al., 2006), 152 children aged 1–<15 years with cytogenetically characterized *ETV6/RUNX1*-positive BCP ALLs were identified. Thus, the karyotypic patterns of a total of 397 cases were available for analysis.

Cytogenetic Features

Modal chromosome numbers

The most common modal numbers among the 397 cases were 46 (67%), 47 (16%), 48 (6%), and 45

TABLE 2. Modal Chromosome Numbers in t(12;21)-Positive B-Cell Precursor Acute Lymphoblastic Leukemias in Children Aged 1 to <15 Years

Modal number	Nordic cases, n = 245 (%)	Published cases ^a n = 152 (%)	Total (n = 397)
<40	0 (0)	0 (0)	0 (0)
40	0 (0)	1 (0.7)	1 (0.3)
41	1 (0.4)	0 (0)	1 (0.3)
42	0 (0)	0 (0)	0 (0)
43	1 (0.4)	1 (0.7)	2 (0.5)
44	2 (0.8)	0 (0)	2 (0.5)
45	14 (5.7)	7 (4.6)	21 (5.3)
46	170 (69)	96 (63)	266 (67)
47	36 (15)	27 (18)	63 (16)
48	11 (4.5)	11 (7.2)	22 (5.5)
49	5 (2.0)	0 (0)	5 (1.3)
50	0 (0)	1 (0.7)	1 (0.3)
51–57	4 (1.6)	1 (0.7)	5 (1.3)
58–80	1 (0.4)	3 (2.0)	4 (1.0)
>81	0 (0)	4 (2.6)	4 (1.0)

^aRetrieved from Mitelman et al. (2006); previously published Nordic cases are excluded.

(5%). High-hyperdiploidy, triploidy, and tetraploidy were each found in ~1%; none had less than 40 chromosomes (Table 2).

Incidence of secondary changes in addition to t(12;21)(p13;q22)

Of the 245 cytogenetically characterized ALLs with t(12;21) diagnosed in the Nordic countries between 1992 and 2004, G-banding analysis revealed chromosomal abnormalities in 130 (53%). The corresponding frequency among the cases previously reported by others was 78% (118/152). Taken together, 248 (62%) of the 397 *ETV6/RUNX1*-positive ALLs reviewed were karyotypically abnormal, i.e., displayed chromosome aberrations in addition to the cryptic t(12;21).

Types and numbers of additional abnormalities

The majority of the 248 cases with secondary changes harbored unbalanced abnormalities only (172/248; 69%). The remaining cases had either only balanced aberrations (14/248; 6%) or both unbalanced and balanced changes (26/248; 10%); 15% (36/248) were uninformative because of incomplete karyotypes. The numbers of secondary changes displayed a geometric-like distribution (Fig. 1), varying between 1 and 19 with a median of 2 additional aberrations per cytogenetically abnormal case.

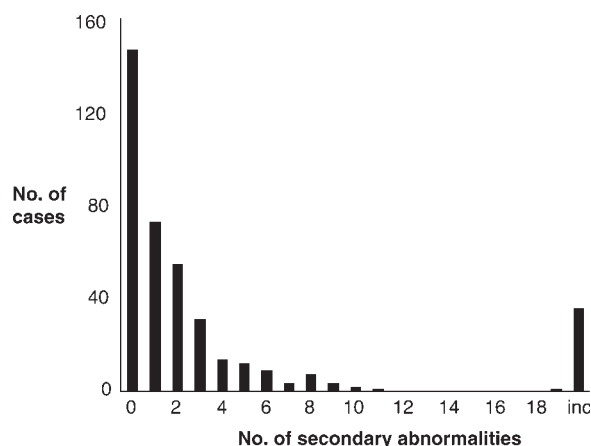


Figure 1. Numbers of secondary changes per case among 397 cytogenetically characterized t(12;21)-positive childhood ALLs. Uninformative cases, i.e., those with incomplete (inc) karyotypes, are presented separately. The figure is based on the present Nordic series as well as on previously reported cases.

Secondary genomic imbalances

The most common genomic imbalances among the 248 cases with secondary changes were partial losses from 6q, 8p, 9p, 11q, 12p, 13q—in particular involving chromosome bands 6q21-27 (18%), 8p11-23 (6%), 9p13-24 (7%), 11q23-25 (6%), 12p11-13 (27%), 13q14-34 (7%)—loss of the whole X chromosome (8%), and gains of 10 (9%), 16 (6%), and 21 (29%); no frequent partial gains were noted (Fig. 2). Loss of the X chromosome was, with one exception, seen only in females.

Chromosome bands involved in secondary aberrations

All chromosomes except the Y were found to be structurally rearranged in one or several of the t(12;21)-positive cases with secondary abnormalities, but the breakpoint distribution was clearly nonrandom. The chromosome bands most often involved in structural rearrangements were 3p21 (2%), 5q13 (2%), 6q12 (2%), 6q14 (2%), 6q16 (2%), 6q21 (10%), 6q23 (6%), 6q25 (3%), 9p13 (2%), 11q13 (2%), 11q23 (2%), 12p11 (6%), 12p12 (7%), 12p13 (25%), 21q10 (6%), and 21q22 (6%) (Fig. 3).

DISCUSSION

The salient results of the present review of cytogenetically characterized *ETV6/RUNX1*-positive childhood BCP ALLs were that ~60% of the cases harbor karyotypically identifiable chromosomal changes in addition to the cryptic t(12;21), that the vast majority are either pseudodiploid or near-diploid (Table 2), that most secondary changes are genomically unbalanced, and that the numbers

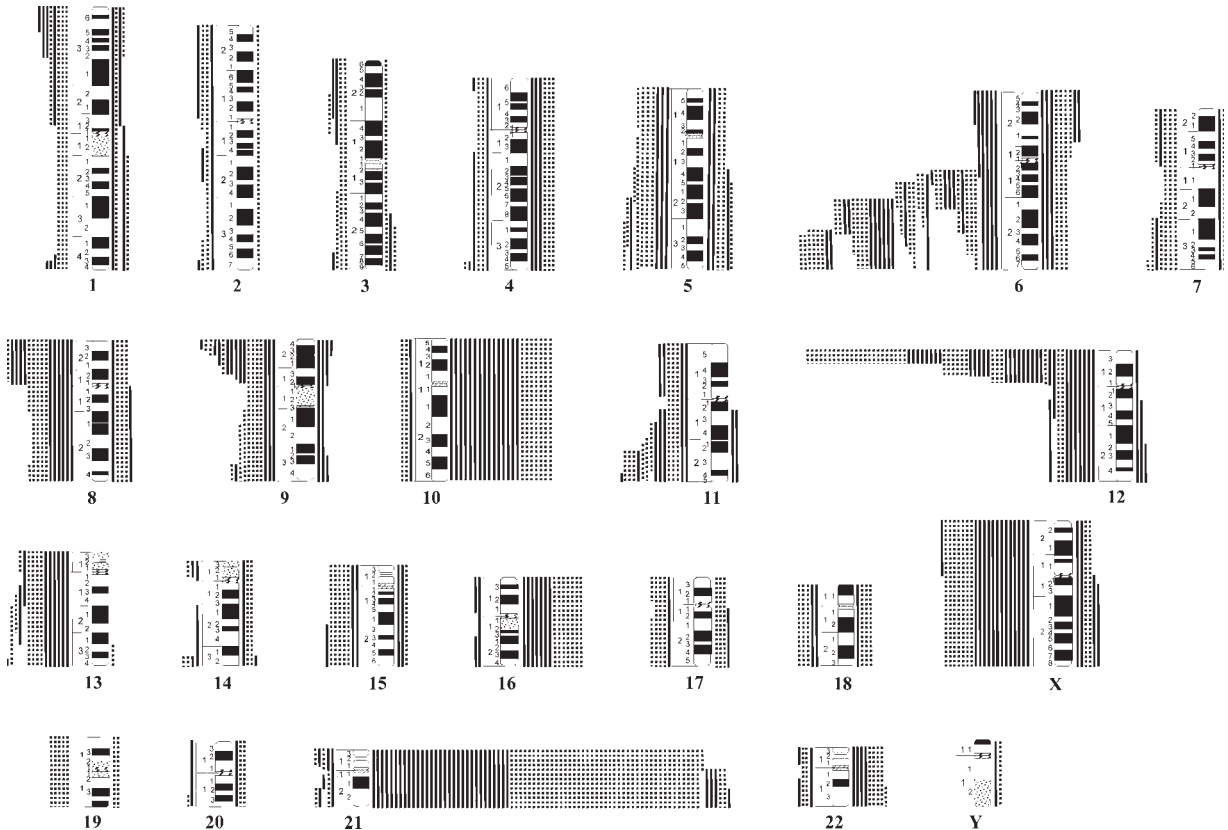


Figure 2. Map of karyotypic imbalances in 248 childhood ALLs with chromosomal aberrations in addition to the t(12;21)(p13;q22). Gains are indicated to the right and losses to the left side of each chromosome. Solid and dashed lines represent cases from the Nordic countries and the literature (Mitelman et al., 2006), respectively.

of additional aberrations display a geometric-like distribution (Fig. 1). Furthermore, several frequent genomic imbalances (Fig. 2) and chromosomal breakpoints (Fig. 3), apart from the well-known 12p rearrangements and chromosome 21 gains, were identified.

Secondary changes were found in 53% of the Nordic ALLs, a proportion clearly lower than the 78% in the non-Nordic cases reported in the literature. Although this could indicate geographic heterogeneity, several other possibilities should be considered before drawing such a conclusion. First, the incidence of cytogenetically abnormal ALLs with t(12;21) in the database (Mitelman et al., 2006) is likely to be an overestimate; there may well be a bias toward reporting mainly cases with additional changes. Furthermore, methodological issues such as complementary FISH analyses, number of metaphases analyzed, and sizes of the patient cohorts investigated should also be taken into consideration. The variable incidences of additional chromosomal changes in *ETV6/RUNX1*-

positive ALLs, ranging from 29% (Eguchi-Ishimae et al., 1998) to 100% (Nordgren et al., 2002; Pérez-Vera et al., 2005), must therefore be interpreted with caution. It should also be emphasized that the frequency in this Nordic series was based on chromosome banding analyses only; if findings from locus-specific FISH studies had been included, the incidence would have been higher. Taken together, we argue that the different frequencies of secondary aberrations in previous series of ALLs with *ETV6/RUNX1* reflect the eyes of the beholder as well as the investigatory tools used rather than biologically important geographic and/or ethnic differences.

The modal number distribution identified herein, with the vast majority being pseudodiploid or near-diploid (Table 2), agrees well with previously published data (Borkhardt et al., 1997; Loncarevic et al., 1999; Maloney et al., 1999; Raynaud et al., 1999; Pajor et al., 2001; Tsang et al., 2001; Uckun et al., 2001; Nordgren et al., 2002). Only one of the Nordic cases was triploid and none was tetraploid;

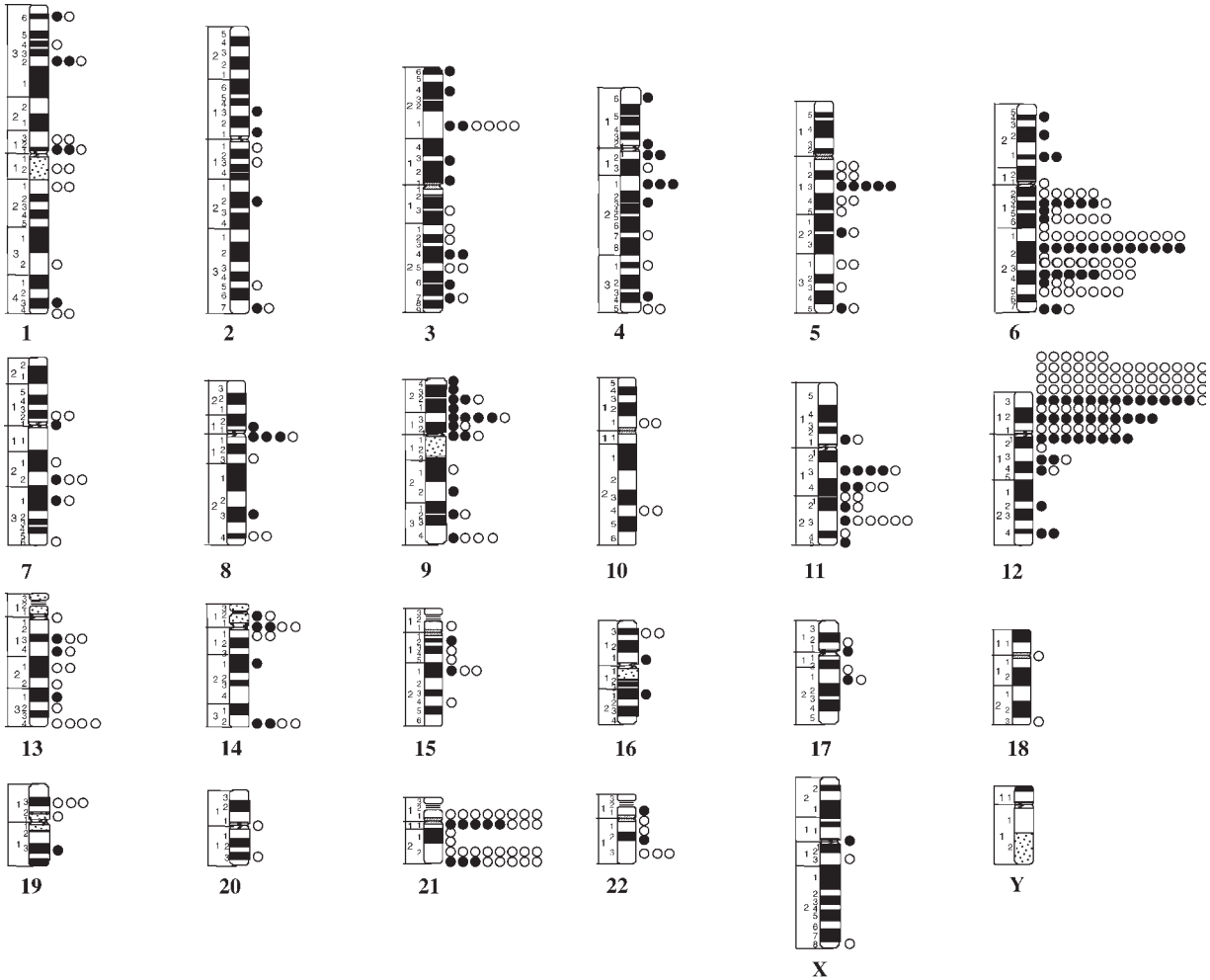


Figure 3. Breakpoint map of secondary structural chromosomal abnormalities in 248 childhood ALLs with aberrations in addition to the $t(12;21)(p13;q22)$. Filled and unfilled circles represent cases from the Nordic countries and the literature (Mitelman et al., 2006), respectively.

however, ~2 to 3% of cases retrieved from the literature harbored more than 57 chromosomes (Table 2). Such modes have indeed been noted in a few instances (Borkhardt et al., 1997; Loncarevic et al., 1999; Raynaud et al., 1999; Andreasson et al., 2000; Douet-Guilbert et al., 2003; Martineau et al., 2005), and—very recently—two groups reported a significant association between near-tetraploidy and *ETV6/RUNX1* positivity in pediatric ALLs (Attarbaschi et al., 2006; Raimondi et al., 2006). Hence, nondiploidy does definitely not exclude the presence of $t(12;21)$.

More than two thirds of the 248 cases with secondary changes harbored unbalanced abnormalities only. Although not emphasized previously in $t(12;21)$ -positive ALLs, this finding was not unexpected, considering that most chromosomal abnormalities occurring in addition to leukemia-specific

gene fusions are known to be genomically unbalanced (Johansson et al., 1996). The reasons why secondary chromosome aberrations generally are unbalanced are unknown, and so are their molecular genetic consequences. Obviously, gains and losses of oncogenes and tumor suppressor genes are one possibility. However, global gene dosage effects may equally well be the pathogenetically important outcome of the secondary changes. Gene expression analyses, in which *ETV6/RUNX1*-positive ALLs generally cluster closely together (Ross et al., 2003; Andersson et al., 2005a,b; Kuchinskaya et al., 2005), should be able to address the latter possibility.

The numbers of additional abnormalities displayed a geometric-like distribution (Fig. 1). Similar distributions—which suggest that each aberration has the same probability to occur and

that each acquisition is independent of earlier changes—have been identified in several other tumor types (Höglund et al., 2001). Although this type of pattern does not favor karyotypic instability playing a major role in the cytogenetic evolution of *ETV6/RUNX1*-carrying cells, it does not exclude karyotypic complexity. In fact, quite a large number of the cases harbored three or more chromosomal aberrations (Fig. 1). It should therefore be stressed that also cytogenetically very complex ALLs may be positive for t(12;21), as was also recently shown by Martineau et al. (2005).

The most common genomic imbalances identified in the present review were, as expected, loss from 12p and gain of chromosome 21 (Fig. 2), occurring in almost 30% of cytogenetically abnormal cases. These changes are so common that their presence in a pediatric ALL can be taken as strong circumstantial evidence for a cryptic *ETV6/RUNX1* fusion. In fact, 80–90% of pediatric BCP ALLs with 12p rearrangements, as ascertained by molecular genetic methods, and ~50% of cases with trisomy 21 as the sole change harbor the 12;21-translocation (Cavé et al., 1997; O'Connor et al., 1998; Karman et al., 2006). Other frequent imbalances (Fig. 2) included deletions of 6q, 9p, 11q, and 13q, losses of X, 8, and 13, and gains of 4, 10, and 16, abnormalities reported to be common in previous studies as well (Kempinski et al., 1999; Loncarevic et al., 1999; Raynaud et al., 1999; Andreasson et al., 2000; Tsang et al., 2001; Uckun et al., 2001; McHale et al., 2003; Attarbaschi et al., 2004; Alvarez et al., 2005; Martineau et al., 2005). The frequent occurrence of 9p deletions, often resulting in loss of *CDKN2A* and *PI4ARF* at 9p21, is noteworthy considering that *ETV6/RUNX1* has been shown to cooperate with loss of these genes to transform lymphoid progenitors in a mouse model (Bernardin et al., 2002).

Except the Y, all chromosomes have been shown to be structurally rearranged in t(12;21)-positive cases with secondary abnormalities, with a seemingly nonrandom involvement of 6q, 9p, 11q, 12p, and 21q (Fig. 3). Most of these regions have been noted in previous studies as well (Raynaud et al., 1999; Alvarez et al., 2005), strongly suggesting that they are particularly break-prone in ALLs with *ETV6/RUNX1* and/or contain genes that if rearranged or otherwise deregulated are intimately involved in the leukemogenic process. However, apart from the 12p abnormalities, whose pathogenetically important outcome most likely is loss of the wild-type *ETV6*, the genes targeted through

these rearrangements are unknown. Detailed molecular (cyto)genetic analyses of the various frequent breakpoints may well turn out to be fruitful because additional mutations undoubtedly are necessary for overt *ETV6/RUNX1*-positive ALL to occur.

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