Clinical and Cytogenetic Features of Pediatric dic(9;20)(p13.2;q11.2)-Positive B-Cell Precursor Acute Lymphoblastic Leukemias: A Nordic Series of 24 Cases and Review of the Literature

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Although dic(9;20)(p13.2;q11.2) is a characteristic abnormality in childhood B-cell precursor acute lymphoblastic leukemias (BCP ALL), little is known about its clinical impact or the type and frequency of additional aberrations it may occur together with. We here review the clinical and cytogenetic features of a Nordic pediatric series of 24 patients with dic(9;20)-positive BCP ALL diagnosed 1996–2006, constituting 1.3% of the BCP ALL, as well as 47 childhood cases from the literature. Consistent immunophenotypic features of the Nordic cases included positivity for HLA-DR, CD10, CD19, CD20, and CD22 and negativity for T-cell and myeloid markers; no detailed immunophenotypes were reported for the previously published cases. In the entire cohort of 71 cases, the modal chromosome distribution was 45 (62%), 46 (21%), 47 (7%), 48 (4%), 49 (3%), 44 (1%), and 50 (1%). Additional changes were present in 63%, the most frequent of which were homozygous loss of *CDKN2A* (33%) and gains of chromosomes 21 (28%) and X (10%). The median patient age was 3 years, the female/male ratio was 2.0, the median white blood cell count was 24×10^9/l, 11% had central nervous system involvement, and 5% had a mediastinal mass at diagnosis. Risk group stratification was nonstandard risk in 79%. The event-free survival and overall survival at 5 years for the 24 Nordic cases was 0.62 and 0.82, respectively. Thus, although relapses are quite common, postrelapse treatment of many patients is successful. This article contains Supplementary Material available at http://www.interscience.wiley.com/jpages/1045-2257/suppmat.

INTRODUCTION

The dic(9;20)(p11-13;q11) was first reported as a nonrandom chromosome abnormality in B-cell precursor acute lymphoblastic leukemia (BCP ALL) in the mid 1990s (Rieder et al., 1995; Slater et al., 1995) and since then, close to 60 dic(9;20)-positive BCP ALL have been described (Heerema et al., 1996; Clark et al., 2000; Raimondi et al., 2003; van

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Zutven et al., 2005; Schoumans et al., 2006; Mitelman et al., 2007a). Although the dic(9;20) was first reported in three adult BCP ALL (Rieder et al., 1995), subsequent studies have shown that this abnormality is more characteristic for pediatric ALL; in fact, ~90% of the reported cases have been children or adolescents, with dic(9;20) occurring in ~2% of childhood and ~0.5% of adult BCP ALL (Mitelman et al., 2007a). However, considering that dic(9;20) is a subtle abnormality that easily may be mistaken for monosomy 20 and/or del(9p) when using chromosome banding alone, many cases undoubtedly go undetected unless fluorescence in situ hybridization (FISH) analyses are performed (Rieder et al., 1995; Slater et al., 1995; Heerema et al., 1996; Clark et al., 2000). Thus, the true prevalence of this change remains unknown.

Very little is known about the molecular genetic consequences of dic(9;20) and of the type and frequency of additional genetic changes in pediatric BCP ALL with dic(9;20). Previous FISH analyses, using chromosome painting and centromeric probes, have confirmed the dicentric nature of the abnormality (Rieder et al., 1995; Slater et al., 1995; Heerema et al., 1996; Clark et al., 2000). We recently characterized seven dic(9;20)-positive pediatric BCP ALL by the use of tiling resolution array-based comparative genomic hybridization (array CGH) and showed that the breakpoints clustered to sub-bands 9p13.2 and 20q11.2; thus, the aberration should be designated dic(9;20)(p13.2;q11.2) (Schoumans et al., 2006). The breakpoints were not identical, however, suggesting that the functional outcome of the aberration is loss of genetic material rather than a consistent gene rearrangement (Schoumans et al., 2006), a conclusion reached also by Strefford et al. (2007) in their array CGH analysis of dic(9;20)-positive cases. As regards additional genetic changes in ALL with dic(9;20), only a few have been reported as recurrent, namely duplication of the dic(9;20), deletions involving 13q, and, in particular, gain of chromosome 21 (Slater et al., 1995; Clark et al., 2000). Furthermore, the *CDKN2A* gene at 9p21 has been shown to be homozygously deleted in some of the cases (Andreasson et al., 2000; Van Zutven et al., 2005; Schoumans et al., 2006; Strefford et al., 2007), and t(9;22)(q34;q11), involving either the der(9) of the dic(9;20) or the other chromosome 9 homologue, has been identified in one pediatric and three adult cases (Rieder et al., 1995; Jarošová et al., 2003; Wetzler et al., 2004; Song et al., 2007).

The clinical and prognostic implications of dic(9;20) are to a large extent unknown. Slater et al. (1995) noted that most patients were females and that many of them presented with nonstandard risk clinical features, including high white blood cell (WBC) counts and extra-medullar leukemia (EML), but because of the low number of cases and the short follow-up time, the prognostic significance of the cytogenetic change could not be determined. Clark et al. (2000), who also identified a similarly skewed sex ratio and a relatively high median WBC count, concluded that, although relapses were not uncommon, the overall survival (OS) appeared to be good, but again emphasized the need for larger series and longer follow-up. In contrast, among the seven dic(9;20)-positive cases reported by Raimondi et al. (2003), two had died and a third was alive with therapy-related acute myeloid leukemia.

To investigate further the clinical and cytogenetic features of dic(9;20)-positive pediatric BCP ALL, we here describe a series of 24 Nordic cases, diagnosed between 1996 and 2006, and review of previously reported cases.

MATERIALS AND METHODS

Patients from the Nordic Countries, Risk Stratification, and Therapy

Between January 1, 1996 and December 31, 2006, 1,827 infants, children, and adolescents were diagnosed with BCP ALL in the Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden). During this time period, two different—but closely related—protocols have been used (NOPHO-ALL-1992 and NOPHO-ALL-2000; the former is described in detail by Gustafsson et al. (2000), whereas the latter has not been published). The dic(9;20) has not been a risk stratifying aberration in any of the two protocols. The choice of treatment intensity for patients with dic(9;20)-positive ALL was made according to age, WBC count, the presence of EML and morphologic response during induction therapy. The risk groups and the main treatment features of the two treatment protocols are summarized in Supplementary Table 1.

Karyotyping and FISH Analyses

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 in Sweden and since 2000 in all five Nordic countries). To retrieve as many dic(9;20)-positive ALL as possible, cases with either probable dic(9;20) or with abnormalities suggesting the

TABLE 1. Clinical Features and Karyotypes of the Pediatric dic(9;20)-Positive BCP ALL

Reference/case No.	Age (years)/sex	WBC ($\times 10^9$/l)	EFS (months)	Survival (months)	Karyotype (including data from FISH and array CGH analyses)[a]
Slater et al. (1995)					
1	4/F	145	24	57+	45,XX,dic(9;20)(p1?3;q11)
2	4/F	31	24+	24+	46,XX,dic(9;20)(p1?3;q11),+21/46,idem,add(14)(p11)/47,idem,+dic(9;20)(p1?3;q11)
3	2/M	23	6+	6+	45,XY,dic(9;20)(p1?3;q11)
4	3/F	16	49	94+	45,XX,dic(9;20)(p1?3;q11),del(13)(q1?q3?)/45,idem,del(9)(q22q32-33)[b]
5	4/F	54	24	124+	45,XX,dic(9;20)(p1?3;q11),del(13)(q14q34)
6	1/F	117	62+	62+	45,XX,dic(9;20)(p1?3;q11)
7	3/M	5	40+	40+	46,XY,dic(9;20)(p1?3;q11),+21
8	13/F	18	124+	124+	45,XX,dic(9;20)(p1?3;q11)/46,idem,+dic(9;20)(p1?3;q11)
9	6/F	42	25	61+	45,XX,dic(9;20)(p1?3;q11)
Heerema et al. (1996)					
1	1/M	210	31+	31+	45,XY,dic(9;20)(p11;q11)/44,idem,-22
2	3/F	22	37+	37+	48,XX,+8,dic(9;20)(p11;q11),+10,+21,dmin/49,idem,+X/49,idem,+18/47,idem,del(4)(p14),-17,der(18)t(17;18)(q11;q23)
3	12/F	57	24	38	50,XX,+2,del(2)(p21)x2,del(5)(q22q33),del(9)(p22),dic(9;20)(p11;q11),+16,+22,+del(22)(q11),+mar
4	7/M	5.8	NA	NA	46,XY,add(1)(q32),-5,add(7)(p22),dic(9;20)(p11;q11),add(12)(p13),+2mar
Andreasson et al. (2000)					
218	2/M	500	NA	NA	45,XY,**del(9)(p21p21),**dic(9;20)(p11;q11)/45,idem,del(6)(q21q25)[c,d]
Clark et al. (2000)					
1	1/F	134	51+	51+	45,XX,dic(9;20)(p11-13;q11)
2	2/F	4	22	41+	45,XX,dic(9;20)(p11-13;q11)
3	2/F	145	47	49	45,XX,dic(9;20)(p11-13;q11)
4	2/M	32	5+	5+	45,XY,dic(9;20)(p11-13;q11)
5	5/M	21	46+	46+	45,XY,dic(9;20)(p11-13;q11)/46,idem,+X
6	2/F	38	80+	80+	46,XX,dic(9;20)(p11-13;q11),+21
7	5/M	11	15+	15+	46,XY,dic(9;20)(p11-13;q11),+21
8	2/F	25	26+	26+	46,XX,dic(9;20)(p11-13;q11),+21/48,idem,+2mar
9	5/F	6	28+	28+	45,XX,dic(9;20)(p11-13;q11)
10	2/M	28	28	63	45,XY,dic(9;20)(p11-13;q11)
11	17/M	6	33+	33+	45,XY,dic(9;20)(p11-13;q11)/46,idem,+mar
12	2/F	80	70+	70+	45,XX,inv(7)(p11q22),dic(9;20)(p11-13;q11)
13	14/F	11	5+	5+	46,XX,add(8)(p11),dic(9;20)(p11-13;q11),+21
14	6/F	536	43+	43+	45,XX,dic(9;20)(p11-13;q11),del(11)(q14q23)
15	4/M	23	60+	60+	47,X,-Y,dic(9;20)(p11-13;q11),+21,+2r/48,idem,+21
16	2/F	56	49+	49+	49,XX,+X,dic(9;20)(p11-13;q11),+10,+21,+21/49,idem,del(17)(p13)
19	11/F	2.4	6+	6+	45,XX,dic(9;20)(p11-13;q11)/46,idem,+20/52,idem,+6,+8,+9,+12,+18,+22/47,idem,+9,+20/47,idem,+dic(9;20)(p11-13;q11),+20
Heerema et al. (2000)					
5	NA/M	NA	NA	NA (alive)	48,XY,+7,+8,dic(9;20)(p12;q11),del(13)(q14q22),add(17)(q25),+21

(Continued)

TABLE 1. Clinical Features and Karyotypes of the Pediatric dic(9;20)-Positive BCP ALL (Continued)

Reference/case No.	Age (years)/sex	WBC ($\times 10^9$/l)	EFS (months)	Survival (months)	Karyotype (including data from FISH and array CGH analyses)[a]
Jarošová et al. (2003)					
9	10/F	NA	10	16	45,XX,dic(9;20)(p12;p11),t(9;22)(q34;q11),dup(20)(p11p13)
18	5/F	NA	13+	13+	45,XX,dic(9;20)(p12;q1?1)/45,idem,der(9)t(9;18)(p2?2;q2?2)
Raimondi et al. (2003)					
48	2/F	73	48[e]	48+	45,XX,dic(9;20)(p11;q11)
49	3/M	4.3	41	NA (dead)	45,XY,dic(9;20)(p11;q11)
50	11/F	4.9	173+	173+	45,XX,dic(9;20)(p11;q11)
51	2/M	12	125+	125+	45,XY,dic(9;20)(p11;q11)
52	3/F	2.3	13[e]	NA (dead)	45,XX,dic(9;20)(p11;q11)
53	1/F	108	54+	54+	45,XX,dic(9;20)(p11;q11),add(11)(q24),der(14)t(11;14)(q24;q32)
54	15/F	16	42+	42+	45,XX,t(2;14)(p11;q32),dic(9;20)(p11;q11)
Kuchinskaya et al. (2005)					
1	0/F	415	16+	16+	45,XX,dic(9;20)(p11-13;q11)
Van Zutven et al. (2005)					
7	2/F	NA	NA	NA	47,XX,dic(9;20)(p11;q11),+21,+mar/48,idem,+21
12	3/F	NA	NA	NA	46,XX,+X,**del(9)(p21p21)**,dic(9;20)(p13;?q11)[d]
15	1/F	NA	NA	NA	45,XX,**del(9)(p21p21)**,dic(9;20)(p12;q11)/44,idem,-X[d]
16	1/M	NA	NA	NA	45,XY,**del(9)(p21p21)**,dic(9;20)(p1?3;q1?1)[d]
Strefford et al. (2007)					
6,789	4/F	NA	NA	NA	46-47,XX,**del(6)(q11q22)**,+7,**del(9)(p21p21)**,dic(9;20)(p13;q11),+mar[d]
Present Nordic series					
1	3/F	121	123+	123+	45,XX,dic(9;20)(p13;q11)
2	1/M	22	92+	92+	46,XY,dic(9;20)(p13;q11),+21/46,idem,add(X)(p22)/47,idem,+X
3	2/M	112	33	33+	45,XY,dic(9;20)(p13;q11)
4	1/M	14	30	30+	45,XY,dic(9;20)(p13;q11),inc
5	3/F	80	74+	74+	45,XX,dic(9;20)(p13;q11)
6	3/F	26	72+	72+	45,XX,dic(9;20)(p13;q11)
7	2/F	46	77+	77+	45,XX,**del(9)(p21p21)**,dic(9;20)(p13.2;q11.2)[d,f]
8	1/F	14	64+	64+	46,XX,**del(4)(p11p12)**,t(7;22)(p15;q11),dic(9;20)(p13.2;q11.2)[f]
9	4/F	8.9	69+	69+	47,XX,+2,dic(9;20)(p13;q11),+21
10	15/M	1.7	64+	64+	45,XY,dic(9;20)(p?;q?)
11	2/M	35	25	47	45,XY,dic(9;20)(p13;q11)
12	1/F	94	48+	48+	45,XX,dic(9;20)(p13.2;q11.2),**del(10)(q11q11)**[f]
13	3/F	2.6	42+	42+	47-48,XX,+X,**der(7)del(7)(p11p14)del(7)(p22)**,dic(9;20)(p13.2;q11.2),t(11;17)(q21;q23),+21,+mar[f]
14	2/M	2.9	29	29+	46,XY,dic(9;20)(p13;q11),+21,inc
15	4/F	34	46+	46+	48-50,XX,+X,+X,+X,dic(9;20)(p13.2;q11),+21,+21
16	5/F	77	39+	39+	47,XX,dic(9;20)(p13;q11),del(11)(q13q23),+21,+21
17	14/M	8.4	32+	32+	46,XY,t(2;12)(p13;q21),dic(9;20)(p13.2;q11),+**20**[f]
18	3/M	59	13	13+	44-47,XY,add(3)(q27),t(6;11)(p21;p15),+del(9)(q22q34),dic(9;20)(p13;q11)x2,del(13)(q?)

(Continued)

TABLE 1. Clinical Features and Karyotypes of the Pediatric dic(9;20)-Positive BCP ALL (Continued)

Reference/case No.	Age (years)/sex	WBC ($\times 10^9$/l)	EFS (months)	Survival (months)	Karyotype (including data from FISH and array CGH analyses)[a]
19	0/F	303	3[g]	3	45,XX,dic(9;20)(p13;q11)
20	2/F	19	22	22+	45,XX,dic(9;20)(p13;q11)
21	3/M	69	7	20	45,XY,dic(9;20)(p13;q11)
22	6/F	7.3	15+	15+	49,XX,del(2)(p21p25),+**8**,dic(9;20)(p13.2;q11.2),**del(15)(q26q26)**,+**20**,+21,+21[f]
23	3/F	0.9	13+	13+	46,XX,dic(9;20)(p13.2;q11.2),**del(12)(q23q24)**,+21[f]
24	13/F	3.4	3+	3+	46,XX,dic(9;20)(p13;q11),+21

CGH, comparative genomic hybridization; EFS, event-free survival; F, female; FISH, fluorescence in situ hybridization; M, male; NA, not available.
[a]Abnormalities in addition to dic(9;20) identified/refined by FISH and/or array CGH are indicated in bold type.
[b]Karyotype at second relapse (45,XX,-20 at diagnosis and first relapse).
[c]Karyotype at relapse (karyotypic failure at diagnosis).
[d]The del(9)(p21p21) was identified using FISH for the *CDKN2A* gene, which hence is homozygously deleted.
[e]Developed treatment-related acute myeloid leukemia.
[f]Previously reported in Schoumans et al. (2006).
[g]Died of causes unrelated to leukemia (accident).

presence of this aberration, such as monosomy 20 and deletion of 9p (Clark et al., 2000), and with cells in fixative, surplus to the initial cytogenetic investigations, were screened by FISH using the LSI 9p21/CEP-9 dual color and the CEP-20 probes according to the manufacturer's instructions (Vysis, Stockholm, Sweden); in total, 37 such cases were analyzed. None of these had been confirmed to harbor the dic(9;20) using G-banding alone, but seven cases had previously been evaluated by array CGH (Schoumans et al., 2006). In addition, 50 high hyperdiploid cases (all with disomy 20) were screened in order to ascertain whether dic(9;20) might be present in this particular cytogenetic subgroup. The LSI 9p21/CEP-9 probes were also applied to confirmed dic(9;20)-positive ALL to investigate the possibility of homozygous *CDKN2A* deletions; sufficient amount of material for this analysis was available in 10 cases. The project was approved by the Research Ethics Committee at Karolinska Institutet.

Statistical Methods

The SPSS software 11.0 for Macintosh was used for the statistical analyses. The probability of event-free survival (EFS) was calculated using the Kaplan-Meier method. In the analysis of EFS, events comprised induction failures, death in remission, relapse, and the occurrence of a second malignancy. In the OS analysis, death was the endpoint. Patients in continuous first complete remission were followed between 3 and 123 months (median 56 months); last date of follow-up was April 19, 2007.

Patients Retrieved from the Literature

The Mitelman Database of Chromosome Aberrations in Cancer (Mitelman et al., 2007a) was used to retrieve previously published pediatric (<18 years) dic(9;20)-positive BCP ALL. Data on age, gender, WBC, EFS, OS, and karyotypes, including findings from FISH and array CGH analyses, were extracted from the original articles.

Cytogenetic Features Ascertained

The cytogenetic features ascertained were (1) modal chromosome numbers (only the most basic clone and the lowest mode in cases with modal number spans), (2) incidence of secondary changes in addition to the dic(9;20), (3) types (balanced and unbalanced) of additional aberrations; one Nordic case with an incomplete karyotypes was excluded

TABLE 2. Cytogenetic Features of Pediatric dic(9;20)-Positive BCP ALL

Cytogenetic features	Nordic cases ($n = 24$)	Literature cases ($n = 47$)	Total ($n = 71$)
Modal chromosome number	44 (4%)	44 (0%)	44 (1%)
	45 (50%)	45 (68%)	45 (62%)
	46 (25%)	46 (19%)	46 (21%)
	47 (13%)	47 (4%)	47 (7%)
	48 (4%)	48 (4%)	48 (4%)
	49 (4%)	49 (2%)	49 (3%)
	50 (0%)	50 (2%)	50 (1%)
Secondary changes			
Yes	58%	66%	63%
Unbalanced only	69%	90%	84%
Unbalanced + balanced	31%	3%	11%
Balanced only	0%	6%	5%
No	42%	34%	37%

in this analysis, and (4) genomic imbalances, i.e., whole or partial chromosome gains/losses (in relation to the nearest ploidy level); identical imbalances were registered only once per case.

RESULTS

Patients

Among the 1,827 infants, children, and adolescents diagnosed with BCP ALL in the Nordic countries 1996–2006, G-banding together with FISH analyses identified the dic(9;20) in 24 (1.3%) cases. None of the 50 high hyperdiploid ALL investigated by FISH had dic(9;20). Age, gender, WBC, EFS, OS, and karyotypes of the 24 cases are given in Table 1. Searching the literature, a total of 47 children/adolescents (<18 years) with dic(9;20)-positive BCP ALL were identified (Table 1). Thus, a total of 71 pediatric BCP ALL with dic(9;20) were available for analysis.

Cytogenetic Features

Modal chromosome numbers and incidences/types of additional abnormalities

These basic cytogenetic features are summarized in Table 2. As seen, the most common modal chromosome numbers were 45 and 46. No case had less than 44 and no case had more than 50 chromosomes (except one with 52 chromosomes in a subclone; Table 1). Aberrations in addition to dic(9;20) were found in 63% of dic(9;20)-positive BCP ALL. Among the cases with additional changes, the vast majority (84%) had unbalanced aberrations only.

Genomic imbalances

The genomic imbalances generated by additional changes are depicted in Figure 1. The most common imbalances—identified cytogenetically—were gains of chromosomes 21 (20/71; 28%) and X (7/71; 10%). FISH for *CDKN2A* revealed homozygous loss of this gene in one of the Nordic ALL [previously reported in Schoumans et al. (2006) as case 7]; the remaining nine tested cases had, as expected, hemizygous deletions. Eight of the 49 previously reported cases had been analyzed by FISH for *CDKN2A* alterations (Andreasson et al., 2000; Kuchinskaya et al., 2005, Van Zutven et al., 2005, Strefford et al., 2007), and five of these had homozygous deletions. Thus, 6 (33%) of 18 dic(9;20)-positive ALL tested displayed homozygous loss of this gene (Table 1).

Clinical Features

Age and gender

The median age in the Nordic cases was 3 years (range 0–15 years) and the female/male (F/M) ratio was 1.7. The median age and F/M ratio in the previously reported cases were 3 years (0–17 years) and 2.1, respectively. In the total cohort of 71 patients, the median age was 3 years and the F/M ratio was 2.0 (Table 1). As seen in Figure 2, there is a pronounced age peak at 1–3 years.

Extra-medullar leukemia

Among the 24 Nordic patients, one had a mediastinal mass, one had CNS involvement, and one (out of 10 boys) had testicular leukemia. This type of information has been reported for only 14 patients in the literature (Slater et al., 1995; Heerema et al., 1996; Kuchinskaya et al., 2005), of which three had CNS involvement and one had a mediastinal mass. Thus, CNS involvement has been reported in 4/38 (11%) and a mediastinal mass in 2/38 (5%) cases.

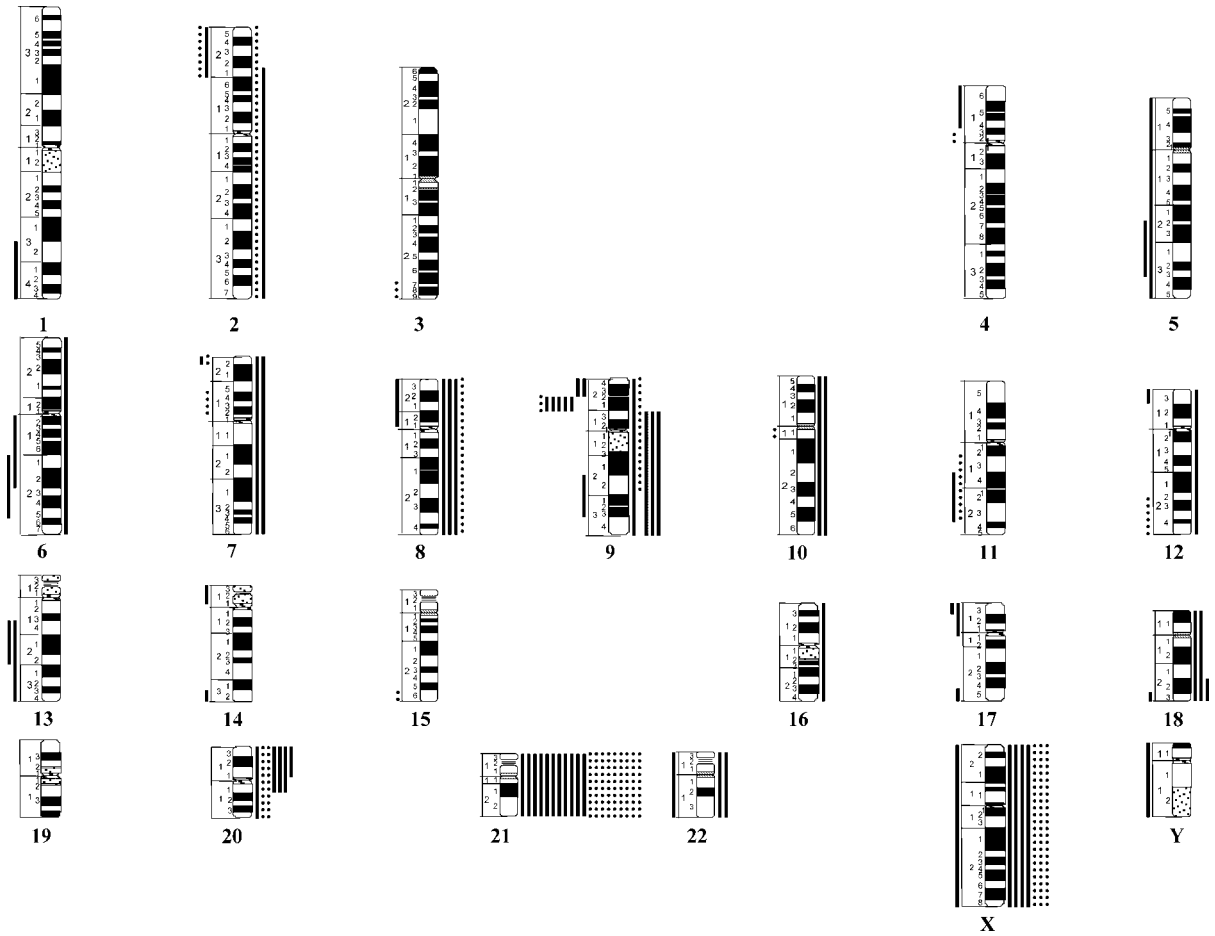

Figure 1. Map of genomic imbalances generated through additional chromosome changes in dic(9;20)-positive pediatric BCP ALL. Gains are indicated to the right and losses to the left side of each chromosome. Solid and dashed lines represent cases from the literature and from the Nordic countries, respectively.

White blood cell counts

The median WBC count in the Nordic cases as well as in the previously published cases was 24 $\times$ 10^9/l (ranges 0.9–303 $\times$ 10^9/l in the Nordic cases and 2.3–536 $\times 10^9$/l in the published cases; Table 1).

Immunophenotypic features

Detailed immunophenotypic data were available for 9 of the 24 Nordic cases, all of which were positive for HLA-DR, CD10, CD19, CD20, and CD22 but negative for T-cell and myeloid markers. No detailed immunophenotypes were reported for the previously published cases.

Risk stratification

Among the 24 Nordic cases, the risk group distribution was as follows: five standard/standard intensity (SR), 10 intermediate/intermediate intensity (IR), eight high/intensive (HR), and one infant. Data on risk stratification have been given for 15 previously reported cases (Slater et al., 1995; Heerema et al., 1996, 2000; Kuchinskaya et al., 2005), of which only 3 were standard risk. Thus, 31/39 (79%) cases were nonstandard risk.

Event-free survival

The predicted EFS at 5 years for the 24 Nordic cases were 0.62 (standard error, SE 0.11) (Fig. 3). At last follow-up, a total of eight events have occurred—one patient, an infant, died in complete remission of causes unrelated to leukemia (accident) and seven patients had recurrence of ALL after 7–33 months (median 25 months); these seven patients had been treated as SR (1 case), IR (3 cases), and HR (3 cases). The relapses were located in the bone marrow (BM) in four patients, in the CNS in 3, and in the testis in one patient. EFS for the previously published BCP ALL is given in Table 1. Ten patients (25% of the 40 informative cases) have relapsed between 10 and

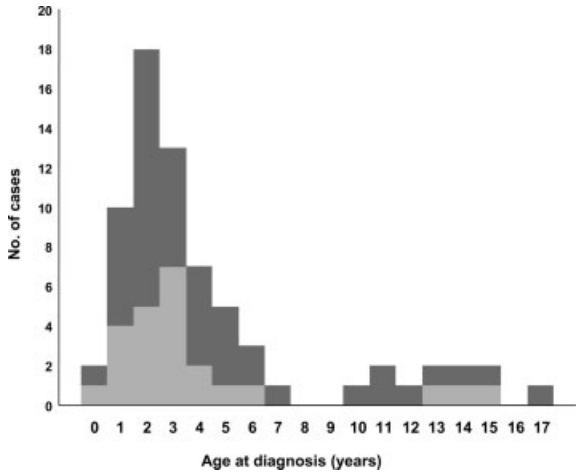

Figure 2. Age distribution of pediatric dic(9;20)-positive B-cell precursor acute lymphoblastic leukemia. Light grey (Nordic cases); dark grey (cases from the literature).

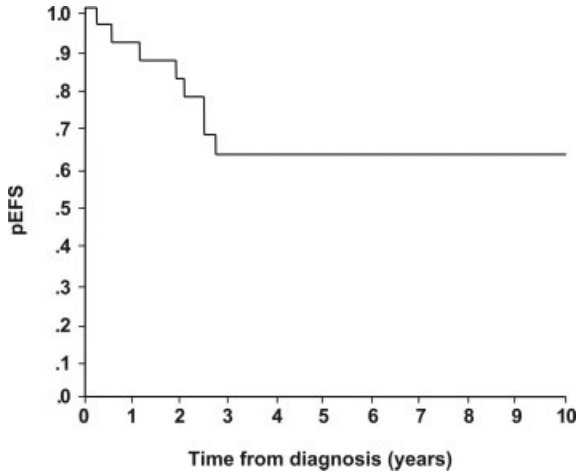

Figure 3. Predicted EFS (pEFS) for 24 Nordic children with dic(9;20)-positive B-cell precursor acute lymphoblastic leukemia.

49 months (median 24.5 months). Site of relapse was reported in six of them (4 BM and 2 CNS).

Overall survival

Of the 24 Nordic patients, 3 (13%) have died—two from leukemia after 20 and 47 months and one in first complete remission after 3 months (see above); the predicted OS at 5 years was 0.82 (SE 0.10) (Fig. 4). OS for the previously reported patients is given in Table 1. Six (15%) of 40 cases with information on survival have died after 16–63 months (median 43.5 months; data on the time of death lacking for two of the six patients).

DISCUSSION

The main cytogenetic features of dic(9;20)-positive pediatric BCP ALL are that all cases have

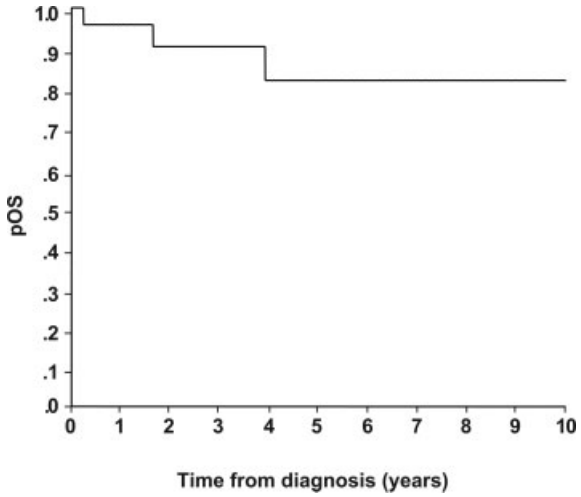

Figure 4. Predicted OS (pOS) for 24 Nordic children with dic(9;20)-positive B-cell precursor acute lymphoblastic leukemia.

modal chromosome numbers between 44 and 50, additional chromosomal changes are present in 60%, most such secondary aberrations are unbalanced, and that the additional abnormalities are nonrandom. The clinical implications of dic(9;20) include an age-peak at 2–3 years, a female predominance, and a median WBC count of 24 $\times$ 10^9/l resulting in most patients being stratified into nonstandard risk. Furthermore, the outcome does not appear to be particularly favorable.

The fact that dic(9;20) is the sole change in ~40% of the cases (Table 2) and that it is not associated with other well-known ALL-associated translocations, except for t(9;22) in one case (Table 1), strongly suggests that it may be considered a primary abnormality in childhood BCP ALL. Indirect support for this conclusion is the finding that the vast majority of the additional changes are genomically unbalanced (Table 2), which is typical for secondary neoplasia-associated abnormalities (Johansson et al., 1996). However, in contrast to most other primary leukemia-associated aberrations (Mitelman et al., 2007b), the dic(9;20) does not seem to result in a fusion gene (Schoumans et al., 2006; Strefford et al., 2007). Instead, loss of genetic material may be the functionally important outcome. It is in this context noteworthy that *CDKN2A* was homozygously deleted in one third of all cases analyzed by FISH, without any G-banding evidence for deletions involving the homologous 9p. However, whether loss of function of this gene is pathogenetically and/or clinically important in dic(9;20)-positive ALL, remains to be elucidated. Other common additional chromosomal changes included gains of X and 21 (Fig. 1), both of which

are frequent in other subtypes of BCP ALL as well (Johansson et al., 2004). As for numerical aberrations in general (Heim, 1992), the molecular genetic consequences of these changes are unknown, although gene dosage effects are likely, as has been suggested also by several global gene expression analyses of ALL with nonrandom trisomies and tetrasomies (Yeoh et al., 2002; Gruszka-Westwood et al., 2004; Andersson et al., 2005).

The median age of the patients was 3 years, with a clear incidence peak at 1–3 years (Fig. 2). Hence, the dic(9;20) contributes, together with t(12;21)(p13;q22) and high hyperdiploidy (Forestier and Schmiegelow, 2006), to the characteristic childhood peak of BCP ALL. Considering that there is ample evidence that both t(12;21) (Ford et al., 1998; Wiemels et al., 1999a,b; Mori et al., 2002) and high hyperdiploidy (Yagi et al., 2000; Panzer-Grümayer et al., 2002; Taub et al., 2002; Maia et al., 2003, 2004) often—perhaps always—arises in utero, it is tempting to speculate that also dic(9;20) has a prenatal origin. To the best of our knowledge, no studies have as yet addressed this possibility. Since dic(9;20) is not associated with a specific gene fusion, it may be difficult to obtain conclusive evidence that this aberration is generated prenatally. However, molecular genetic analyses of clonotypic *IGH* rearrangements in neonatal blood spots or FISH studies of *CDKN2A* deletions in chord blood cells could prove fruitful.

There was a notable predominance of females with dic(9;20)-positive ALL, with a F/M ratio of 2.0. Such a gender preference in childhood ALL is highly unusual. In fact, an assessment of all cytogenetically abnormal pediatric B-lineage ALL reported in the literature (Mitelman et al., 2007a) reveals that only cases with t(11;19)(q23;p13) had a similar F/M ratio, namely 1.9. The F/M ratios for other common genetic subtypes were 1.4 for t(4;11)(q21;q23), 1.1 for hypodiploidy, 1.0 for t(1;19)(q23;p13), 0.8 for high hyperdiploidy (51–65 chromosomes), 0.8 for >65 chromosomes, 0.8 for t(9;22)(q34;q11), 0.7 for t(8;14)(q11;q32), 0.7 for t(12;21)(p13;q22), and 0.3 for t(8;14)(q24;q32). The fact that dic(9;20) is more common in girls is intriguing and presently unexplained but should—together with the possibility of a prenatal origin—be addressed in future etiologic studies.

The present compilation clearly shows that dic(9;20) is associated with a relatively high median WBC count (24×10^9/l). This, together with other poor risk factors such as CNS involvement, explains why most cases have been classified as nonstandard risk and, hence, have received intensive treatment. This notwithstanding, the pEFS at 5 years for the Nordic cases was only 0.62 (Fig. 3), which is clearly lower than, for example, the 0.80 and 0.83 seen for t(12;21) and high hyperdiploidy, respectively (Forestier et al., 2007). Of the previously published cases informative in this respect, 25% had relapsed (Table 1), providing further evidence for a relatively poor EFS in this cytogenetic ALL subset. Most of the relapses occurred early, either during treatment or shortly thereafter; no relapse more than 49 months after diagnosis has been reported (Table 1). Thus, the relapse pattern is quite different from the one observed in t(12;21)-positive ALL, a subgroup characterized by late relapses in many studies (Nakao et al., 1996; Harbott et al., 1997; Seeger et al., 1998; Tsang et al., 2001; Forestier et al., 2007). In spite of the relatively frequent occurrence of relapses, the OS at 5 years was 0.82 for the Nordic patients (Fig. 4), and 5 of the 10 relapsed patients in the literature were alive at the time of reporting (Table 1). This indicates that postrelapse treatment of many patients with dic(9;20) is often successful.

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