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Clinical Utility of Array Comparative Genomic Hybridization for Detection of Chromosomal Abnormalities in Pediatric Acute Lymphoblastic Leukemia

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Abstract

Background—Accurate detection of recurrent chromosomal abnormalities is critical to assign patients to risk-based therapeutic regimens for pediatric acute lymphoblastic leukemia (ALL).

Procedure—We investigated the utility of array comparative genomic hybridization (aCGH) for detection of chromosomal abnormalities compared to standard clinical evaluation with karyotype and fluorescent in-situ hybridization (FISH). Fifty pediatric ALL diagnostic bone marrows were analyzed by bacterial artificial chromosome (BAC) array, and findings compared to standard clinical evaluation.

Results—Sensitivity of aCGH was 79% to detect karyotypic findings other than balanced translocations, which cannot be detected by aCGH because they involve no copy number change. aCGH also missed abnormalities occurring in subclones constituting less than 25% of cells. aCGH detected 44 additional abnormalities undetected or misidentified by karyotype, 21 subsequently validated by FISH, including abnormalities in 4 of 10 cases with uninformative cytogenetics. aCGH detected concurrent terminal deletions of both 9p and 20q in three cases, in two of which the 20q deletion was undetected by karyotype. A narrow region of loss at 7p21 was detected in two cases.

Conclusions—An array with increased BAC density over regions important in ALL, combined with PCR for fusion products of balanced translocations, could minimize labor- and time-intensive cytogenetic assays and provide key prognostic information in the approximately 35% of cases with uninformative cytogenetics.

Keywords

Array comparative genomic hybridization (aCGH); acute lymphoblastic leukemia (ALL)

Introduction

Specific chromosomal abnormalities in acute lymphoblastic leukemia are powerful independent predictors of prognosis, and significantly impact choice of therapy.(1) Current

clinical detection of abnormalities relies primarily on karyotype and FISH, but both these techniques have limitations. They are both time-consuming and highly variable in quality between laboratories. Moreover, karyotype may be uninformative because of poor leukemic cell growth *in vitro*, leading to failed culture in nearly 20% and normal cell overgrowth in an additional 20% of cases.(2) There is wide variation among cytogenetic laboratories in cell culture media, duration of culture, and cytotechnologist technique and aptitude in interpreting banding patterns. Advantages of aCGH as a complementary diagnostic tool in pediatric ALL include significantly higher resolution than routine karyotype; genome-wide scope in contrast to the targeting of select lesions by FISH; and lack of requirement for *in vitro* culture prior to analysis. aCGH is rapidly becoming established as a clinical diagnostic tool in the area of constitutional genetic defects,(3) but in the field of oncology, it has been used thus far primarily as a research tool, for the discovery of novel oncogenic pathways and/or prognostic factors.(4) In this study, we assessed its utility as a clinical tool, comparing its strengths and weaknesses to the current standard methodology of karyotype and FISH by comparing aCGH findings to the clinical cytogenetic findings reported for diagnostic pediatric ALL samples.

Methods

Patients and samples

We analyzed 50 diagnostic bone marrow aspirates obtained from pediatric patients diagnosed with pre-B or T-cell ALL at Texas Children's Hospital between August 2003 and September 2006, which constitute all samples collected during this time period for which sufficient excess material was available for array CGH analysis. Excess sample was pelleted at the time of diagnosis and stored at -80°C for later use.

Karyotyping and FISH

G-band karyotyping and FISH were performed by a single clinical cytogenetics laboratory for diagnostic purposes, prior to and independent of this research study, following standard clinical protocols. Routine methods were used for cell culture, harvest, slide preparation and G-banding, according to the method of Barch(5) with modifications as recently described in (6). At least 20 mitoses per case were karyotyped where possible (34 of 50 cases), with 6-19 mitoses per case in the remainder due to insufficient mitotic cell yield. Abnormalities were classified according to ISCN 1995 and 2005.(7;8) FISH panel screening was performed for all pre-B ALL cases for trisomies 4, 10, and 17; TEL-AML1; BCR-ABL1; MLL rearrangement; and 9p21 deletion (p14, p15, and p16 genes). For T-cell ALL cases, FISH panel screening was performed for TEL-AML1, MLL rearrangement, and 9p21 deletion (all probes from Vysis, Downers Grove, IL). FISH testing was performed according to the manufacturer's instructions. Additional Vysis probes were selected to screen cases with a normal karyotype and to characterize additional abnormal karyotypic findings as clinically indicated; these included probes for IgH, c-MYC, EGR1 (5q31), centromeres, specific subtelomeres, and whole chromosome paints.

aCGH

Genomic DNA from tumor and opposite-sex normal reference samples was extracted by standard techniques (DNeasy blood and tissue kit, Qiagen, Valencia, CA), and then labeled and hybridized to the SpectralChip 2600 array (PerkinElmer, Waltham, MA). A subset of five samples were subsequently run again, with same-sex normal reference samples, to demonstrate that X chromosome copy number could be accurately determined under this condition, which it was. Each sample was run in duplicate, with tumor labeled with Cy3 and reference with Cy5, and with the dye assignment reversed. Hybridization was performed according to the Spectral Genomics protocol for 16 hours at 37°C. Following post-hybridization washes, the slides were imaged using an Axon 4000B scanner and GenePix Pro 6.0 scanning software.

Data analysis was performed using Bioconductor project packages.⁽⁹⁾ ArrayQuality was used to assess hybridization quality and detect array outliers. The marray package was used to perform print-tip Lowess and scaled normalization. The Gain and Loss Analysis of DNA (GLAD) algorithm was used to define gains and losses and remove outlying BAC clones.⁽¹⁰⁾ A calling algorithm then converted BAC calls to the corresponding cytoband calls for comparison with cytogenetic results. The algorithm performed the following steps: (1) ordered the BAC clones according to chromosomal locations; (2) scanned genome for gains or losses; (3) once three consecutive BAC clones with the same change were detected, the corresponding cytoband was marked with the appropriate change; (4) the resulting cytoband results from aCGH were then compared to cytogenetic results. aCGH calls were only scored for changes detected by three consecutive BAC clones.

Results

Demographic features of the 50 cases are summarized in Table I. Most characteristics were representative of all pediatric ALL cases seen at this institution, and of pediatric ALL in general,⁽¹¹⁾ with the exception of age and initial blast percentage. Age at diagnosis was slightly lower in the study cases than in the remainder of the institutional population (4.8 vs 6.5 years, $p < 0.01$), and initial blast percentage in the marrow was slightly higher (76.7 vs 68.9 percent, $p < 0.01$). These differences are small and likely attributable to the relatively small number of samples in each group. Of note, the mean initial WBC for study cases fell above 50,000, the cut-off between standard-and high-risk ALL, whereas the mean for the remaining institutional cases fell below 50,000. However, this difference between means did not reach statistical significance (65.6 vs 36.1, $p < 0.09$), and cases with an initial WBC $< 50,000$ were still well-represented in the study sample, accounting for approximately 70% of cases.

A detailed comparison of findings in each case by aCGH compared to karyotype and FISH is presented in Table II. There were a total of 184 findings described in the original clinical karyotype reports. Six trisomies from these reports were found to be misidentified by karyotype on subsequent FISH testing. Nineteen findings were balanced translocations, which cannot be detected by aCGH since the method detects copy number alterations but not spatial rearrangements. Of the remaining karyotypic findings, aCGH detected 126 of 159 findings, for an overall sensitivity of 79%. Discordant findings are summarized in Table III.

Findings detected exclusively by karyotype

The largest category of abnormalities detected by karyotype but not aCGH were 19 balanced translocations. Karyotype also reported eight whole chromosome or chromosome arm findings which did involve copy number alterations and yet escaped detection by aCGH. Four of these findings (cases 21, 52, 82, and 85) were likely missed due to sample mosaicism, because the copy number changes occurred in subclones constituting only a mean of 11% of cells (range 2-24%). This is consistent with previous reports that aCGH detects only changes which occur in at least 20-40% of the cell population.(12) Two other copy number changes detected by karyotype alone (cases 21 and 52) showed a subthreshold trend toward a call by aCGH, presumably due to relatively poor array signal quality. Two cases had loss of the Y chromosome detected by karyotype but not by aCGH (cases 40 and 41), likely due to the sparse Y chromosome coverage on the SpectralChip 2600 array with only 4 BAC probes. Loss of Y has no known prognostic significance in ALL.

Less significant findings by karyotype included 12 small regions of copy number change; and 13 areas of additional unidentified chromosomal material, a nonspecific observation which does not convey prognostic information. Most of these cases involved complex derivative karyotypes (e.g. cases 2, 16, 82, and 86), in which the lack of net copy number change as detected by aCGH is likely due to the fact that the rearrangements detected by karyotype were balanced.

Findings detected exclusively by FISH

As expected, FISH detected balanced translocations such as TEL-AML1 fusion and MLL rearrangement which aCGH could not detect. aCGH also missed several small gains and losses detected by FISH, such as deletion of TEL (12p13) and the p15/p16 locus (9p21). These gains and losses escaped detection because the SpectralChip 2600 array has insufficient BAC coverage of these loci, but in principle, a leukemia-specific diagnostic chip could be designed with enhanced coverage at sites of recurrent abnormalities in order to achieve sensitivity equivalent to FISH, as has been done in the development of BAC array platforms used in other clinical diagnostic settings such as evaluation for constitutional genetic defects associated with congenital anomalies and/or developmental delay.(13;14)

Findings detected exclusively by aCGH

aCGH detected 17 whole chromosome gains (trisomies), 13 partial chromosome gains, and nine partial chromosome losses which were undetected or misidentified by karyotype due to culture failure, poor mitotic yield, or poor morphology (Table III). aCGH also corrected six incidences of a trisomy identified in error by karyotype (Figure 1). In each of these six cases, aCGH detected trisomy in a different chromosome from the one reported by karyotype. FISH confirmed the trisomic and disomic chromosomes as reported by aCGH in every case. A total of 21 discordant findings had sufficient sample available for follow-up testing, and in all of these cases, FISH testing confirmed aCGH findings.

Ten cases had normal or unsuccessful cytogenetics, and in four of these cases, aCGH demonstrated that abnormalities were indeed present. Two exhibited hyperdiploidy (cases 37 and 48), one demonstrated gain of a region including c-MYC (case 45), and one showed

losses at 7p21 and 11q14-11q23 (case 42). A subset of the trisomies in cases 37 and 48 were identified by the standard clinical FISH panel performed for ALL, but in each case several additional trisomies were identified by aCGH.

Concurrent terminal deletions at 9p and 20q were a recurrent finding in three cases (cases 29, 73, and 86). Combined loss of both 9p and 20q has been reported as a rare recurrent abnormality in ALL as the result of a dicentric (9;20).(15;16) Case 73 did indeed demonstrate a dicentric (9;20), but the other two cases did not, with the cryptic 20q loss detectable only by aCGH. Loss of 9p21 was detected by FISH in all three cases, and by karyotype in two. It is possible that concurrent losses at 9p and 20q occur more frequently than has been reported, but are often missed by karyotype except when arising due to a dicentric (9;20). The 9p proximal breakpoints in all three cases occurred at the same BAC (Figure 2), which spans the region 9p12-9p13.3, and matches the breakpoint region identified in a previous report describing seven cases with dic(9;20).(17) The 20q proximal breakpoints for two of the cases (29 and 86) were identical, while the third occurred 2.6 Mb downstream. These breakpoints localized to 20q11.23, which lies 5 to 8 Mb downstream of the area previously reported at 20q11.2.(17)

Loss of 7p21 was identified exclusively by aCGH in two cases (cases 42 and 43) with a 2.6 Mb area of deletion in common. Losses involving 7p have been previously reported to occur in approximately 2% of pediatric ALL and to have independent adverse prognostic significance,(18) but a specific critical region on 7p has not been identified to date. In a case series which included 41 cases with losses of 7p, nearly all the losses included the 7p21 region described here.(18)

Very high hyperdiploidy

Two cases with very high hyperdiploidy created a challenge for the aCGH copy number assignment algorithm (cases 5 and 48, each with over 60 chromosomes). In the setting of multiple trisomies and tetrasomies, the aCGH algorithm in these cases erroneously assigned copy number of two instead of three to trisomies. It was necessary to utilize karyotype and/or FISH data in order to correctly assign a copy number of two. Subsequent assignment of copy number gain and loss, after this “calibration,” proceeded accurately without the need for further intervention. This “calibration” issue, particularly common in ALL due to the frequency of high hyperdiploidy, has been reported by others as well with both CGH and oligonucleotide array platforms, and appears to require correlation with karyotype for accurate copy number assignment.(19;20)

Discussion

aCGH showed 79% sensitivity to detect karyotypic findings other than balanced translocations, and in several discrepant cases, it proved to be more accurate than karyotype, correcting morphologic misidentifications (cases 5, 16, 31, 69, 91, and 93). Such karyotypic misidentification is not uncommon, since ALL chromosome morphology tends to be exceedingly poor. ALL blasts are also difficult to grow *in vitro*, and aCGH demonstrated advantages here as well, in providing information in cases for which karyotyping was

unsuccessful or produced normal results due to nonleukemic cell overgrowth (cases 37, 42, 45, and 48).

In clinical settings with a significant number of failed cultures, normal karyotypes, and chromosome morphology misidentifications, aCGH could provide valuable prognostic information that would otherwise be lost. In the current study, aCGH identified abnormalities in four of the 10 cases from this center with uninformative cytogenetics. The Pediatric Oncology Group cytogenetics reference laboratory reported normal or failed cytogenetics in approximately 35% of cases (A. Carroll, personal communication, July 2007). Current cytogenetic analysis in pediatric ALL is performed in decentralized laboratories, which as a group are likely to yield uninformative cytogenetics in at least 35% of cases, if not more. Thus, aCGH could provide the sole cytogenetic information available to guide risk stratification in a substantial proportion of cases, if adopted on a widespread basis.

Since aCGH cannot detect either balanced translocations or subclones that constitute fewer than approximately 20-40% of the sample, it does not appear suitable as a single diagnostic test for ALL. An efficient model for evaluation of new ALL cases might include aCGH using a chip with enriched coverage of areas frequently altered in ALL; PCR-based assays for the common prognostically important balanced translocations (t(12;21), t(1;19), and t(9;22)); FISH for MLL rearrangement (since the multiple potential fusion partners make PCR impracticable); and calculation of DNA index by flow cytometry. Karyotype analysis would be performed only for those cases with normal findings by aCGH, PCR and FISH; and for cases with a DNA index indicating high hyperdiploidy corresponding to 60 chromosomes, due to the difficulty of accurate copy number assignment by aCGH in this setting. Use of aCGH in tandem with FISH, flow cytometry, and PCR, could thus eliminate the need for karyotype analysis in the majority of cases and significantly improve abnormality detection rates in ALL, hence allowing appropriate risk stratification of patients who might otherwise be inadvertently over- or undertreated.

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Figure 1. aCGH can correct misidentification of chromosome morphology by karyotype. **A:** Karyotype for case 69 indicated high hyperdiploidy including trisomy 14. **B:** aCGH findings were concordant except for detection of trisomy 18, and no trisomy 14. **C:** Subsequent FISH testing confirmed presence of two copies of chromosome 14 (using a two-color 14q34 IgH break-apart probe) and three copies of chromosome 18 (using a chromosome 18 centromeric probe).

Figure 2.

Location of the bacterial artificial chromosome (BAC) clones covering the breakpoints on chromosomes 9 and 20 in cases 29, 73, and 86 (based on information from the UCSC Human Genome Browser, March 2006 assembly, at <http://genome.ucsc.edu/cgi-bin/hgGateway>).

TABLE I
Comparison of study and total population characteristics

Characteristics of the study cases, compared with all other new pediatric ALL cases diagnosed at the study institution during the same time period. Features which significantly differ between the study and remaining population are indicated by an asterisk (two-sample t-test, $p < 0.05$).

		Study, n (%)	Institution, n (%)	p-value
Gender	Male	26 (52)	110 (55)	0.70
	Female	24 (48)	90 (45)	
Diagnosis	Pre-B	48 (96)	186 (93)	0.44
	ALL			
	T ALL	2 (4)	14 (7)	

	Mean (range)	Mean (range)	
Age (years)	4.8 (1-15)	6.5 (0.50-20)	0.01*
Initial WBC	65.6 (0.4-800)	36.1 (0.2-855)	0.09
Initial blasts (%)	76.7 (33-94)	68.9 (16-94)	0.01*

TABLE II

Comparison of karyotype, FISH, and aCGH findings

Findings by karyotype and FISH are listed in the two left-hand columns, and any additional findings detected only by aCGH are listed in the two right-hand columns. Boldface font indicates those findings by karyotype and FISH which were also detected by aCGH. The superscript ⁽³⁾ indicates findings with a discrepancy between aCGH and karyotype, for which confirmatory FISH testing was performed. In all such cases, the FISH testing confirmed that aCGH was correct.

Case	Karyotype	FISH	Additional CGH findings	
			Gain	Loss
02	45,XY,der(7)add(7)(p22),der(12)(t(12;22)(p11.2;q11.2))[12/25]	(TELx1)		
05	63,XY,+X,-4,+5,+6,+8,+10,+11,+12,+14,+16,+17,+18,+20 ^a ,+21,+22[19/20]	(4cenx3),(10cenx3),(17cenx3)[89%],(BCR _x 3),(MLLx3)[88%],(TELx3),(AMLIx4)[95%]		
16	51-52,XX,+4,+4,t(9;22)(q34;q11.2),+21,der(22)t(9;22)(q34;q11.2),+1-2mar[cp4/7]	(ABL con BCRx1-2)[96%],(4cenx2-4)[91%], (AMLIx3)[84%]	14 ^a	
21	47,XX,t(9)(q10),add(21)(p11.2),+21c[cp6/25]	(9cenx1)[24%],(ABLx3)[19%],(AMLIx3)[84%]		
24	46,XY,t(7)(q10)[11/20]	t(7)(q10)		
27	52,XY,+X,+4,+6,+14,+21,+21[4/21]	(4cenx3)[67%],(AMLIx3-4)[73%]		
28	45,XY,-4 ^a ,add(9)(p13)x2,add(15)(p13)[9/11]	(4cenx1)[89%],(9p21x0)[67%]		1q42-44 ^a incomplete loss of 4 (4p14-p16 preserved) ^a
29	45,XY,t(7;9)(p11;q11),-9[5/12]	(9p21x1)[33%]		20q ^a
31	55-58,XY,+X,t(1:9)(q23;p13),+1,+4,+6,t(7)(q10),+8 ^a ,+10,+14,+17,+18,+21,+21[11/20]	t(1:19),(4cenx3),(10cenx3)[94%],(AMLIx4)[92%]		
33	46,XX[15]	(TEL con AML1x1)[84%]		
37	46,XY[20]	(4cenx3)[59%],(AMLIx3)[76%]	X ^a ,18 ^a	
38	46,XX,der(22)t(1:22)(q25;q13)[9/13]	der(22)t(1:22)	1q21-24	
40	45,X,-Y,del(9)(p13)[3/8]	(9p21x1)[89%]	11q23-25	
41	45,X,-Y,del(9)(p13)[19/20]	(9p21x1)[92%]		
42	46,XX inc [6 poor quality cells]	(9p21x?1-2)[27%]		7p21, 11q14-q23 ^a
43	47,XX,t(2;9)(p13;p13),+21c[20/22]	t(2;9),(AMLIx3)[88%]		7p21
44	54,XX,+X,-del(4)(q12),+9,+del(10)(q22),+14,+18,+21,+21[cp13/20]	(4cenx3),(10cenx3)[80%],(AMLIx4)[79%],(ABLx3)[77%]	10q22>tel ^a 17q22>tel ^a	
45	Unsuccessful culture	Normal FISH panel	8q21>tel ^a	
48	46,XY[18]	(4cenx3-4),(10cenx3),(17cenx3)[86%],(9cenx3-4),(9p21x3-4)[71%],(ABLx3-4),(BCR _x 3-4)[81%],(MLLx3)[61%],(TEL con AML1x2)[78%]	2,3,5,6,7,8,12,14,16,18,20,21	
52	47,XY,+5[10/17]/48,idem,+11[2/17]	(5p15.2x3)[66%],(TELEX1)[96%],(MLLx3)[3%],(1cenx3)[2%]	6q16-26 ^a	
64	46,XY,t(5;16)(q13;q23-q24),t(9;10)(q34;q22),?add(12)(p11.2)[11/18]	t(5;16),(TEL con AML1x1)[88%],(TEL con AML1x2)[7%]		
65	46,XX,t(1:19)(q23;p13)[4/18]	t(1:19)	1q44, 8q24.3, 10p15, 10q26, 13q33-34, 16p13	

Case	Karyotype	FISH	Additional CGH findings	
			Gain	Loss
66	46,XX,t(8;10)(q24.1;q22)[3/21]	t(8;10)		
67	47,XX,+10,add(12)(p?11.2)[cp9/16]	(10cenx3)[67%],(TELx1),(TEL con AML1x1),(AML1x1)[87%]		
68	54,XX,+X,+4,+10,+11,+14,+18,+21+21[18/20]	(4cenx3),(10cenx3),(MLLx3)[89%],(AML1x4)[93%]		
69	50-52,XX,+X,+4,+6,+14 ^a ,+17,+21[cp6/25]	(4cenx3),(17cenx3)[75%],(AML1x3)[70%],(9p21x1)[7%]	18 ^a	
70	46,XX[28]	(TEL con AML1x1)[82%]		
71	46,XY,t(7;15)(q11.2;q1?);?(9;12)(p2?p11.2),add(13)(p11.2)[cp19/34]	t(7;15)t(9;12),(9p21x1)[30%],(TEL con AML1x1)[82%]		7q1.1
72	47,XX,+X,t(4;11)(q21;q23),t(7)(q10)[15/20]	(MLLx1),(MLL-spv1)[94%]		
73	47,XX,+7,dic(9;20)(p11-13;q11),+mar[2/23]	dic(9;20),(9p21x1)[81%]		
74	46,XY[20]	(9p21x0)[91%]		
75	46,XY[30]	Normal FISH panel		
76	47,XX,Xc?;t(4;11;5)(q21;q23;q31)[17/20]	der(5)t(4;11;5),der(4)t(4;11;5),(MLLx1),(MLL-spv1)[94%]		
77	56,XX,+X,+4,+6,+8,+10,+14,+17,+18,+21,+21[20]	(4cenx3),(10cenx3),(17cenx3)[85%],(AML1x3-4)[92%]		
79	57,XX,+X,+5,+6,+9,+10,+11,+14,+17,+18,+21,+21[8/20]	(10cenx3),(17cenx3)[83%],(9p21x3)[81%],(ABLx3)[81%],(MLLx3)[82%],(AML1x4)[100%],(TELx3)[21%]		
81	48,X,+16,+21,+21,-Y[1/20]	(TELx1),(AML1x4),(TEL con AML1x1)[91%]		
82	47,XY,der(7;7)(?;?7pter->p11.2;?)(7;9)(q10;q10),+11,add(12)(p13)[6/20]	der(7;2)t(7;9),(9p21x1)[82%],(MLLx3)[6%]		
84	46,XX,t(7)(q10)[19/20]	Normal FISH panel		
85	46,XY,t(7)(q10)[2/20]	(7q31x3)[12%]		
86	47,XX,add(6)(p11.2-12),add(9)(p12-13),del(15)(q11.2),del(17)(p11.2),+21[3/24]	(9p21x1)[87%],(AML1x3)[84%]		20q ^a
87	46,XX[30]	Normal FISH panel		
88	46,XX,t(1;19)(q23;p13)[19/20]	Normal FISH panel	1q ^a	
89	47,XX,+21q[28]	(AML1x3)[100%]		
90	55,XY,+X,+6,+8,+10,+14,+17,+18,+21,+21[4/20]	(10cenx3)[71%],(17cenx3)[50%],(AML1x4)[91%]		
91	57,XY,+X,+4,+6,+8,+11,+14 ^a ,+15 ^a ,+17,+19 ^a ,+21,+21[7/20]/57,iden,add(1)(q43) or dup(1)(q25q45)[10/20]	(4cenx3)[90%],(10cenx3)[4%],(17cenx3)[76%],(MLLx3)[81%],(AML1x4)[86%]	18 ^a	
92	46,XY,dic(9;20)(p11-13;q11),+21[5/20]	dic(9;20),(9p21x0-1)[83%],(AML1x3)[91%]		
93	55,XY,+X,+4,+6,+10,+14,+16 ^a ,+17,+18,+21[16/20]	(4cenx3),(10cenx3),(17cenx3)[90%],(AML1x3)[93%]		
94	49,XX,+6,del(9)(q13q22),dup(9)(q21q22),+10,+21[8/20]	del(9)(q13q22),dup(9q),(TELx1)[87%],(AML1x3-4),(TEL con AML1x1)[91%],(10cenx3)[37%]		9p22
95	46,XX,t(7)(q10),der(19)t(1;19)(q23;p13)[9/20]	(7cenx3)[40%]		
96	47,XY,t(4;11)(q21;q23),+8[20]	der(4)t(4;11),ins(4;11),der(11)t(4;11),5' MLL x1[86%],(MLL-spv1)[75%]		

TABLE III

Discrepancies between aCGH and karyotype

	Number of findings	Cases, with number of findings per case indicated in parentheses if >1
Changes detected by karyotype and not by aCGH		
Whole chromosome gain <i>erroneously</i> reported by karyotype (subsequent FISH confirmed aCGH)	6	5, 31, 69, 91 (2), 93
Balanced translocations	19	2, 16 (2), 29, 31, 38, 43, 64 (2), 65, 66, 71 (2), 72, 76, 82, 88, 95, 96
Whole chromosome or chromosome arm gains or losses	8	21 (2), 40, 41, 52 (2), 82, 85
Smaller region of gain or loss	12	2 (2), 21, 71, 82 (2), 86 (3), 91, 94 (2)
Additional, unidentifiable chromosomal material	13	2, 16, 21, 28 (2), 64, 67, 71, 73, 82, 86 (2), 91
Changes detected by aCGH and not karyotype		
Whole chromosome gains	17	16, 37 (2), 48 (12), 69, 91
Partial gains	13	38, 40, 44 (2), 45, 52, 65 (6), 88
Partial losses	9	28 (2), 29, 42 (2), 43, 71, 86, 94