



Array comparative genomic hybridization and cytogenetic analysis in pediatric acute leukemias

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ABSTRACT

Most patients with acute lymphocytic leukemia (ALL) are reported to have acquired chromosomal abnormalities in their leukemic bone marrow cells. Many established chromosome rearrangements have been described, and their associations with specific clinical, biologic, and prognostic features are well defined. However, approximately 30% of pediatric and 50% of adult patients with ALL do not have cytogenetic abnormalities of clinical significance. Despite significant improvements in outcome for pediatric ALL, therapy fails in approximately 25% of patients, and these failures often occur unpredictably in patients with a favorable prognosis and “good” cytogenetics at diagnosis.

It is well known that karyotype analysis in hematologic malignancies, although genome-wide, is limited because of altered cell kinetics (mitotic rate), a propensity of leukemic blasts to undergo apoptosis in culture, overgrowth by normal cells, and chromosomes of poor quality in the abnormal clone. Array comparative genomic hybridization (aCGH—“microarray”) has a greatly increased genomic resolution over classical cytogenetics. Cytogenetic microarray, which uses genomic DNA, is a powerful tool in the analysis of unbalanced chromosome rearrangements, such as copy number gains and losses, and it is the method of choice when the mitotic index is low and the quality of metaphases is suboptimal. The copy number profile obtained by microarray is often called a “molecular karyotype.”

In the present study, microarray was applied to 9 retrospective cases of pediatric ALL either with initial high-risk features or with at least 1 relapse. The conventional karyotype was compared to the “molecular karyotype” to assess abnormalities as interpreted by classical cytogenetics. Not only were previously undetected chromosome losses and gains identified by microarray, but several karyotypes interpreted by classical cytogenetics were shown to be discordant with the microarray results. The complementary use

of microarray and conventional cytogenetics would allow for more sensitive, comprehensive, and accurate analysis of the underlying genetic profile, with concomitant improvement in prognosis and treatment, not only for pediatric ALL, but for neoplastic disorders in general.

KEY WORDS

Acute lymphoblastic leukemia, chromosomes, microarray, aCGH

1. INTRODUCTION

Conventional cytogenetics has played a pivotal role in the detection of recurrent chromosomal rearrangements in hematologic malignancies, aiding in diagnosis, prognosis, and identification of the genes involved. Current clinical detection of cytogenetic abnormalities is based primarily on karyotype or fluorescence *in situ* hybridization (FISH) analysis, or both¹. It is well known that karyotype analysis of hematologic malignancies, although genome-wide, is limited because of altered cell kinetics (mitotic rate), a propensity of leukemic blasts to undergo apoptosis in culture, overgrowth by normal cells, and chromosomes of poor quality in the abnormal clone². Fluorescence *in situ* hybridization expanded cytogenetics by identifying cryptic genetic lesions that are not seen by routine karyotyping, but that affect response to treatment. However, although FISH can be used on interphase (non-dividing) cells upon culture failure or normal karyotype, FISH analysis is not genome-wide; it is limited to specific loci of the abnormal clone, and it is highly dependent on clinical suspicion for the “choice” of locus to analyze.

Acute lymphoblastic leukemia (ALL) is the most common and among the most curable of pediatric cancers³. Today, 70%–80% of children with newly diagnosed ALL treated with contemporary intensive state-of-the-art protocols can be cured. Clonal chromosomal abnormalities can be identified in

approximately 90% of cases of childhood ALL. These changes may be of a structural or numeric type. Many established chromosome rearrangements have been described, and their associations with specific clinical, biologic, and prognostic features are well defined³. However, approximately 30% of pediatric and 50% of adult patients with ALL do not have cytogenetic abnormalities of clinical significance⁴. Despite significant improvements in outcome for pediatric ALL, therapy fails in approximately 25% of patients, and these failures often occur unpredictably in patients with a favorable prognosis and “good” cytogenetics at diagnosis⁵.

The generally accepted banding resolution for a routine blood karyotype is 550 bands per haploid genome^{6,7}, with a band equivalent to approximately 5–10 Mb of DNA; by contrast, a bone marrow karyotype has a resolution of only approximately 300–400 bands per haploid genome. Fluorescence *in situ* hybridization does allow for the detection of cryptic, submicroscopic chromosome rearrangements of approximately 3–5 Mb, but it is highly locus specific⁸. Cytogenetic microarrays use genomic DNA and thus do not depend on tissue culture for suitable cells. Their use has revolutionized conventional cytogenetics because of their significantly greater resolution, resulting in increased detection of constitutional chromosomal abnormalities in patients with congenital genetic anomalies^{9,10}.

Cytogenetic microarrays have not been extensively used in hematologic malignancies to look for acquired chromosome abnormalities because research has concentrated on gene expression microarrays to search for novel oncogenic pathways, prognostic factors, or mechanisms of drug resistance¹¹. However, clinical microarray platforms are currently being developed and evaluated by the Cancer Cytogenomics Microarray Consortium (<http://www.urmc.rochester.edu/ccmc/>).

The technique of array comparative genomic hybridization (aCGH), which uses genomic DNA, also allows for genome-wide screening for chromosome aberrations, but it is independent of the availability of suitable chromosomes in the specimen under investigation¹. A variation of FISH technology, aCGH uses DNA from a “normal” person as a control and compares DNA from a patient with congenital anomalies or a hematologic malignancy to detect imbalances across the entire genome. It generates, at resolutions of less than 1 Mb, a physical map of unbalanced chromosome copy number changes—that is, losses (deletions) and gains (duplications)—throughout the entire genome (analogous to a karyotype) and does not require prior knowledge of specific regions of interest (as in FISH). Reports on DNA microarray have indicated detection of additional abnormalities undetected or misidentified by conventional karyotype in approximately 35% of cases with uninformative cytogenetics¹.

However, DNA microarray also has limitations. Numerous hematologic malignancies have balanced chromosome rearrangements (such as translocations and inversions), which result in fusion genes—and associated proteins—with a known prognosis. Array CGH cannot detect these balanced chromosome rearrangements, because copy number changes are typically not involved, making continued use of routine conventional cytogenetics essential. As well, a bone marrow sample can contain an admixture of normal and abnormal cells, making conventional cytogenetic karyotype detection of the abnormal cell difficult if normal cells tend to overgrow in culture. In addition, DNA microarray can miss abnormalities if abnormal clones are present in fewer than 25% of cells¹, and the presence of small clonal populations may, therefore, also not be detected. In addition, the karyotypic evolution of primary compared with secondary abnormalities in cytogenetically related clones would not be discernible by microarray. All of the foregoing factors continue to uphold the essential nature of conventional cytogenetics.

In the present study, DNA microarray was applied to 9 retrospective cases of pediatric ALL either with initial high-risk features or with at least 1 relapse. The conventional karyotype at diagnosis was compared with the “molecular karyotype” at diagnosis to assess abnormalities as interpreted by classical cytogenetics.

2. METHODS

2.1 Patients

The 9 retrospective cases of pediatric ALL had sufficient stored diagnostic bone marrow DNA for a microarray analysis. The patients had various abnormal karyotypes that had been characterized by conventional cytogenetics and FISH. Table 1 summarizes the clinical data for these patients.

2.2 Cytogenetic Analysis

Standard banding procedures using Giemsa–trypsin–Wright staining were used for the direct and 24- and 48-hour bone marrow cultures. The level of banding resolution achieved was approximately 350–400 bands per haploid genome. Fluorescence *in situ* hybridization was used as required, per the manufacturer’s protocol (Vysis–Abbott, Abbott Park, IL, U.S.A.).

2.3 Microarray Analysis

High molecular weight DNA was isolated by manual phenol–chloroform extractions from frozen cell pellets of diagnostic bone marrow samples according to standard protocols. The DNA concentrations were determined using the NanoDrop (Thermo Scientific, Wilmington, DE, U.S.A.). This DNA was sent to

CombiMatrix Molecular Diagnostics (Irvine, CA, U.S.A.) for HemeScan aCGH analysis for hematologic malignancies, using either a bacterial artificial chromosome (BAC) or an oligonucleotide (oligo) microarray, or both. The BAC arrays have a resolution of approximately 1 Mb; oligo arrays have a greater resolution of approximately 400 Kb¹⁰.

3. RESULTS

Table II describes the microarray and conventional karyotype for each patient, according to the 2009 International System of Cytogenetic Nomenclature¹². Copy number changes, as detected by HemeScan, that overlap regions known to show copy number changes in the phenotypically normal population were interpreted as polymorphisms and are not reported, although a disease association cannot be conclusively ruled out.

Patient 1 was diagnosed with B-cell ALL. The conventional karyotype was cytogenetically interpreted to be missing normal chromosomes Y, 8, 9, 21, and 22, and to have an additional chromosome 19 and three unidentified marker chromosomes. However, those changes were not detected by microarray analysis. The BAC HemeScan showed a copy number gain of region 1q21.1→q44, resulting in trisomy for most of the long arm of chromosome 1. The dup(1) was not identified by routine chromosome analysis, but in retrospect may be represented by one of the marker chromosomes. In addition, the microarray also showed an interstitial deletion of chromosome 9q22.31→q33.2, but not the entire chromosome. This change again suggests that the remainder of chromosome 9 may be present in the marker chromosomes. In addition, the marker chromosomes are likely composed of material from chromosomes Y, 8, 21, and 22. The HemeScan also did not detect the “additional”

TABLE I Clinical data for pediatric patients with acute lymphoblastic leukemia (ALL)

Pt	Sex	Age at diagnosis (months)	Diagnosis	White blood cell count ($\times 10^9/L$)	Relapses	Status
1	Male	67	B-cell ALL	5.3	Bone marrow and testicular, 49 months after diagnosis; 2nd bone marrow, 54 months after diagnosis	Alive post allogeneic SCT; no evidence of disease at 125 months after diagnosis
2	Male	19	T-cell ALL	296.6	None	Alive; no evidence of disease at 21 months after diagnosis; continues on maintenance therapy
3	Male	36	B-cell ALL	22.74	Central nervous system, 31 months after diagnosis; bone marrow, 45 months after diagnosis; allogeneic SCT in 2nd remission	Died of disease at 55 months after diagnosis
4	Male	75	Acute leukemia of ambiguous lineage (T/myeloid)	8.9	Bone marrow, 9 months after diagnosis	Alive post allogeneic SCT; no evidence of disease at 30 months after diagnosis
5	Female	24	B-cell ALL	3	Central nervous system, 28 months after diagnosis	Alive; no evidence of disease at 78 months after diagnosis
6	Male	14	B-cell ALL	25.1	Central nervous system, 18 months after diagnosis; bone marrow, 35 months after diagnosis	Died of disease at 41 months after diagnosis
7	Male	66	B-cell ALL	40.5	Central nervous system, 42 months after diagnosis	Alive; no evidence of disease at 82 months after diagnosis
8	Male	102	B-cell ALL	28.6	Bone marrow and central nervous system, 4 months after diagnosis	Died of disease at 5 months after diagnosis
9	Female	10	B-cell ALL	236.6	Bone marrow, 26 months after diagnosis	Alive post allogeneic SCT; no evidence of disease at 52 months after diagnosis

Pt = patient; SCT = stem-cell transplantation.

chromosome 19, which could represent material from the missing chromosomes and coincidentally resemble a chromosome 19.

Patient 2 was diagnosed with T-cell ALL. The conventional karyotype showed a normal male karyotype. Microarray analysis with the BAC HemeScan was also normal. However, oligo HemeScan analysis showed a loss of copy number on chromosome 22q11.22, which encompassed the *VPREB1* gene (Figure 1). An oligo HemeScan of peripheral blood DNA from this patient showed a normal copy number at this locus, confirming that the deletion was related to the neoplasm. The *VPREB1* gene is involved in B cell development, and deletions of this gene have been associated with high-risk disease¹³. The significance of this deletion in T-cell ALL is not known.

Patient 3 was diagnosed with B-cell ALL. The conventional karyotype at diagnosis showed an ALL hyperdiploid pattern with trisomies of chromosomes X, 4, 6, 8, 14, and 17; tetrasomy for chromosome 21; and a marker chromosome. The G-band→FISH analysis confirmed a balanced $t(17;19)(q22;p13)$, with the marker being the $der(17)t(17;19)$ (Figure 2, Table II). The BAC HemeScan detected only an increased copy number of chromosomes X, 4, 6, 8, 14, 17, and 21. The patient was in remission and did well for approximately 2.5 years. A central nervous system relapse occurred at 31 months post diagnosis, and a bone marrow relapse, at 45 months post diagnosis. The patient received an allogeneic stem cell transplant in second remission, but relapsed again in the bone

marrow post transplant. He died of his disease at 55 months post diagnosis.

Patient 4 was diagnosed with biphenotypic acute leukemia. The conventional karyotype had a modal chromosome number of 47, resulting from the presence of an additional chromosome 16. A ring chromosome 7 [r(7)] was also present, with breakpoints at 7p13 and 7q36, resulting in monosomy for most of the short arm of chromosome 7. No other abnormalities were detected. However, the BAC HemeScan yielded a more complex result. A gain of copy number for all of chromosome 16 was confirmed, as was copy number loss of 7p15.3→pter and 7q36→qter, respectively consistent with trisomy 16 and a r(7). However, copy number loss of 2q36→qter, 13q21.2→q33.2, and 21q11.1→q21.3, and copy number gain of 6p24→pter were also noted. A small clonal population with trisomy 8 may also have been present. These latter abnormalities were not detected by conventional karyotype.

Patient 5 was diagnosed with B-cell ALL. The conventional karyotype showed a complex rearrangement involving chromosomes 7, 9, and 20. The net genetic imbalance of this rearrangement was interpreted as monosomy for chromosomes 9p12→pter and 20q11.2→qter. The oligo HemeScan detected copy number loss of 30.05 Mb of distal 9p21.1→pter, including the *CDKN2A* (*p16*) gene, and 31.9 Mb of 20q11.21→qter. The cyclin-dependent kinase inhibitor Cdkn2A (*p16*) functions as a tumor suppressor; it is upregulated in cellular senescence and often deleted in ALL¹⁴.

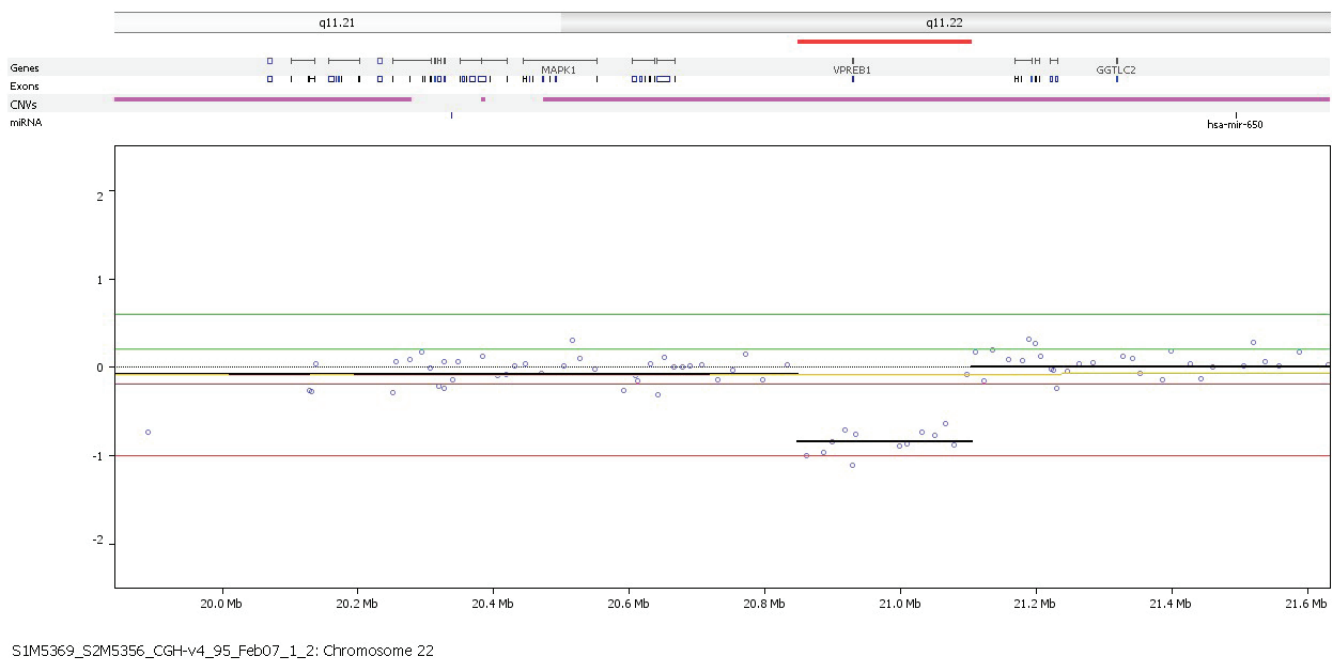


FIGURE 1 Oligo HemeScan (CombiMatrix Diagnostics, Irvine, CA, U.S.A.) bone marrow results for patient 2, showing copy number loss of *VPREB1* (red line).

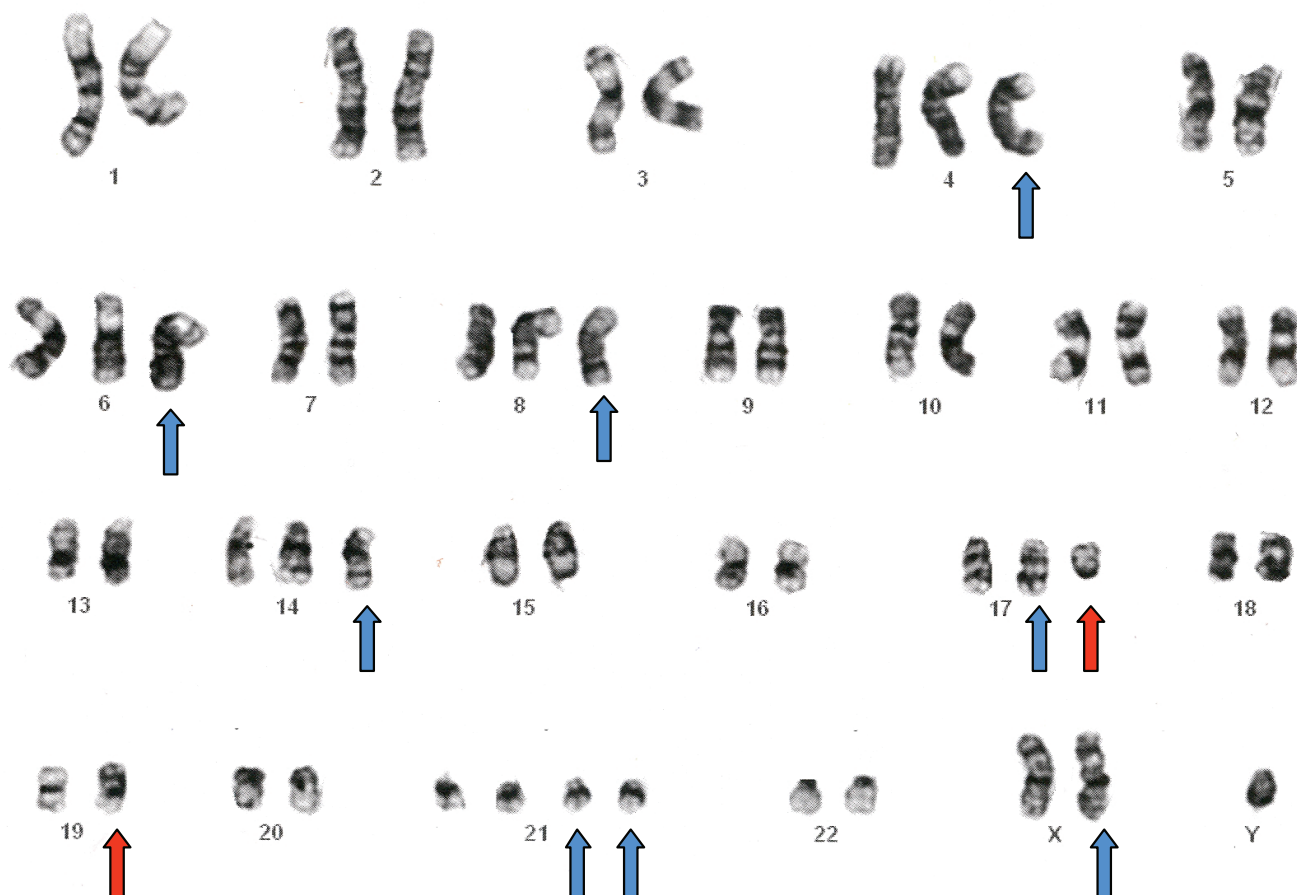


FIGURE 2 Karyotype of patient 3 at follow-up: 54,XY,+X,+4,+6,+8,+14,+17,i(17;19)(q22;p13),+21,+21.

Patient 6 was diagnosed with B-cell ALL. The conventional karyotype showed an apparently normal male karyotype. However, the oligo HemeScan showed a 3.17 Mb deletion of chromosome 17q24.3, including the *SOX9* gene, and a 500 Kb deletion of 22q11.22, including the *PRAME* gene. *Prame* is a melanoma antigen¹⁵. Deletions of the *PRAME* gene have been reported in B-cell chronic lymphocytic leukemia and are of unknown clinical significance¹⁶. Interaction between *PRAME* and *SOX9* has been reported in malignancy¹⁵. This patient relapsed and is now deceased.

Patient 7 was diagnosed with B-cell ALL. The conventional karyotype for this patient was a failure, because the bone marrow did not grow in culture. No cells were available for FISH analysis. However, using reverse-transcriptase polymerase chain reaction, this patient was found to have a *TEL/AML1 (ETV6/RUNX1)* gene fusion. The oligo HemeScan showed a complex abnormal male karyotype with multiple copy number losses including 1q (6 Mb), 2p (1 Mb), 3q (3 Mb), 7q (200 Kb), discontinuous 8p (approximately 1 Mb), discontinuous 9p (6 Mb), 9q (1 Mb), 10p (approximately 1 Mb), 11p (2.5 Mb), 12p (26 Mb), 13q (8 Mb), 14q (12 Mb), 15q (2 Mb), 16p (2.5 Mb), 16q (1 Mb), 19p (approximately 3 Mb), and 20q (approximately 2 Mb). No chromosomal material with copy number gain was

observed. Loss of 12p13.33–p11.23 (0–26,681,811 bp) necessarily included the *TEL (ETV6)* gene (Database of Genomic Variants: <http://projects.tcag.ca/variation>)¹⁷.

Patient 8 was diagnosed with B-cell ALL. The conventional karyotype shows a complex rearranged male karyotype, including a duplication of 1q21→q25 and monosomy for 9p22→pter. The oligo HemeScan showed multiple complex genomic changes, including a discontinuous 14-Mb gain of 1q21.1→q24.1 and a 7.7-Mb loss of 17q. There was discontinuous segmental monosomy of 9p. The 17q deletion was not detected by conventional karyotype. Gain of 1q has been reported to be sufficiently mutagenic to favour leukemogenesis¹⁸. This patient had a central nervous system and bone marrow relapse and is now deceased.

Patient 9 was also diagnosed with B-cell ALL. The conventional karyotype showed a complex rearranged female karyotype. The net genetic imbalance of the rearrangements was interpreted as monosomy for 7p and 12p, with concomitant deletion of the *ETV6* gene as verified by G-band→FISH (Table II). The oligo HemeScan detected a discontinuous 9-Mb deletion of 12p13.2→p12.3 and 12p11.23→p11.2, with the break-point within exon 2 of the *ETV6* gene. In addition, a 1.1-Mb bi-allelic deletion of chromosome 14q32.33 was found, but the del(7p) not detected by microarray.

TABLE II Microarray abnormalities detected by HemeScan^a and conventional cytogenetics in diagnostic bone marrow DNA of pediatric patients with acute lymphoblastic leukemia

Pt	2009 ISCN aCGH karyotype	2009 ISCN G-band karyotype
1	^b arr 1q21.1q44 (RP11-436I6→RP11-88N11)x3, 9q22.31q33.2 (RP11-1007A10→RP11295D11)x1	45,X,-Y,-8,-9,+19,-21,-22,+mar1-3[4]/46,XY[11]
2	^b (a) arr(1-22)x2,(XY)x1 ^c (b) arr 22q11.22(20,847,913-21,105,096)x1 ^{cd} (c) arr(1-22)x2,(XY)x1	46,XY[50]
3	^b arr(X)x2,(4,6,8,14,17,21)x3	54,XY,+X,+4,+6,+8,+14,+17,+21,+21,+mar[8]/46,XY[4]. ish t(17;19)(q22;p13)(wcp17+;wcp17+)[4]
4	^b (complex) arr(6p,16p,16q)x3,(2q,7p,13q,21q)x1	47,XY,idic r(7)(p13q36),+16[10]/46,XY[17]
5	^c arr 9pter→p21.1(0→30,053,691)x1, 20q11.21→qter(30,518,953→62,435,964)x1	45,XX,dic(7;20)(p11.2;q11.2)t(7;9)(p11.2;p12)dic(9;20) (p11.2;q11.2), der(9)t(7;9)(p11.2;p12)dic(9;20)(p11.2;q11.2)[8]/46,XY[23]
6	^c arr 17q24.3(65,054,881-68,227,532)x1; 22q11.22(21,073,347-21,573,549)x1	46,XY[25]
7	^c (complex) arr(1q,2p,3q,8p,9p,9q,10p,11p,12p,13q,14q)x1	Fail
8	^c (complex) arr(1q,17p)x3,(2p,7p,7q,8p,8q,9p,9q,10q,11p, 14q,15q,16q,17q,18q,20q,21q)x1	46,XY,dup(1)(q21q25),add(9)(p22),der(9;14)(q10;q10), del(12)(q?22),add(20)(q?11.2),+mar[11]/46,XY[1]
9	arr 12p13.2p12.3(11,801,644-15,922,374)x1, 12p11.23p11.2(26,463,250-29,386,296)x1 14q32.33(105,054,774-106,177,900)x0	46,XX,der(7)t(7;9)(p11.2;p13),der(9)t(7;9)del(7)(p11.2), del(12)(p12),der(15)t(?3;15)(q13;q22)[12]/46,XX[22]. ish der(7)(wcp9+),der(9)(wcp9+),del(12)(TEL-)[4]

^a CombiMatrix Diagnostics, Irvine, CA, U.S.A.^b Bacterial artificial chromosome HemeScan.^c Oligonucleotide HemeScan.^d Peripheral blood DNA.

Pt = patient; ISCN = International System for Human Cytogenetic Nomenclature; aCGH = array comparative genomic hybridization.

4. DISCUSSION AND CONCLUSIONS

Genomic DNA microarray analysis using the HemeScan (CombiMatrix Molecular Diagnostics) was performed for 9 patients with pediatric ALL. The microarray results were compared with the standard results obtained by conventional chromosome karyotyping and FISH. Microarray revealed imbalances that were not readily identifiable or were likely misidentified by routine metaphase chromosome analysis.

Conventional karyotype and microarray analysis were concordant in only 2 of the 9 cases examined (patients 3 and 5). Patient 3 showed the presence of multiple gains of entire chromosomes by both cytogenetics and microarray. However, cytogenetics also identified a balanced t(17;19). The cytogenetic interpretation of monosomy of 9p and 20q was also detected by microarray in patient 5. The loss of *CDKN2A* at

9p21.3 was implied by the karyotype and confirmed by microarray.

As expected, microarray provided additional information, with the identification of cryptic rearrangements—such as the *VPREB1* deletion in patient 2 and the *SOX9* and *PRAME* deletions in patient 6—that can be considered clinically relevant. Patients 2 and 6 both had normal male karyotypes by conventional cytogenetics. Based on HemeScan microarray, patients 1 (BAC), 4 (BAC), 8 (oligo), and 9 (oligo) require a revision of their karyotype.

Patient 7, with a failed conventional karyotype, now had a complex molecular karyotype, based on multiple chromosome deletions. However, this patient was shown, by reverse-transcriptase polymerase chain reaction, to have a *TEL/AML1* gene fusion and, by oligo microarray, a deletion of the non-translocated *TEL* (*ETV6*). The *TEL/AML1* gene fusion is associated

with a good prognosis in ALL, with or without deletion of the second *TEL* locus. The prognostic significance of the other abnormalities is not known¹⁹.

This initial study has demonstrated that DNA microarray is a reliable method for the identification of cytogenetically visible and cryptic imbalances in pediatric ALL. The complementary use of aCGH and conventional cytogenetics can provide further information regarding relevant and repeated mutations in ALL. Clinical analysis could then be focused on those specific mutations with impact on diagnosis, prognosis, and treatment.

Based on our experience with these 9 cases, we propose the following algorithm for the cytogenetic evaluation of hematologic malignancies:

1. Conventional karyotype
2. Complementary microarray analysis
3. If required, FISH for microarray-identified abnormalities to confirm or revise the conventional karyotype

The complementary use of microarray and conventional cytogenetics would allow for more sensitive, comprehensive, and accurate analysis of the underlying genetic profile with concomitant improvement in prognosis and treatment, not only for pediatric ALL, but for neoplastic disorders in general.

5. ACKNOWLEDGMENTS

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6. CONFLICT OF INTEREST DISCLOSURES

No financial conflict of interest exists.

7. REFERENCES

1. Rabin KR, Man TK, Yu A, *et al.* Clinical utility of array comparative genomic hybridization for detection of chromosomal abnormalities in pediatric acute lymphoblastic leukemia. *Pediatr Blood Cancer* 2008;51:171–7.
2. Kearney L, Horsley SW. Molecular cytogenetics in haematological malignancy: current technology and future prospects. *Chromosoma* 2005;114:286–94.
3. Harrison CJ. The detection and significance of chromosomal abnormalities in childhood acute lymphoblastic leukaemia. *Blood Rev* 2001;15:49–59.
4. Strefford JC, Worley H, Barber K, *et al.* Genome complexity in acute lymphocytic leukemia is revealed by array-based comparative genomic hybridization. *Oncogene* 2007;26:4306–18.
5. Carroll WL, Bhojwani D, Min DJ, Moskowitz N, Raetz EA. Childhood acute lymphoblastic leukemia in the age of genomics. *Pediatr Blood Cancer* 2006;46:570–8.
6. Canadian College of Medical Geneticists (CCMG). *CCMG Practice Guidelines for Cytogenetic Analysis: B. Recommendations for the Indications, Analysis and Reporting of Constitutional Specimens (Peripheral Blood, Solid Tissues)*. Ottawa, ON: CCMG; 2010. [Available online at: [http://www.ccmg-ccgm.org/pdf/policy/2010/\(5\)%20CCMG_PractGuide_CYTO_BCconstit_24Feb2010.pdf](http://www.ccmg-ccgm.org/pdf/policy/2010/(5)%20CCMG_PractGuide_CYTO_BCconstit_24Feb2010.pdf); cited June 2, 2010]
7. Shaffer LG, American College of Medical Genetics Professional Practice and Guidelines Committee. American College of Medical Genetics guideline on the cytogenetic evaluation of the individual with developmental delay or mental retardation. *Genet Med* 2005;7:650–4.
8. Baldwin EL, Lee JY, Blake DM, *et al.* Enhanced detection of clinically relevant genomic imbalances using a targeted plus whole genome oligonucleotide microarray. *Genet Med* 2008;10:415–29.
9. Canadian College of Medical Geneticists (CCMG). *CCMG Position Statement: Use of Array Genomic Hybridization Technology in Constitutional Genetic Diagnosis in Canada*. Ottawa, ON: CCMG; 2009. [Available online at: <http://www.ccmg-ccgm.org/pdf/policy/aCGH%20for%20pch%20sept%2009.pdf>; cited June 2, 2010]
10. Miller DT, Adam MP, Aradhya S, *et al.* Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Leukemia* 2006;20:840–6.
11. Paulsson K, Heidenblad M, Strömbeck B, *et al.* High resolution genome-wide array-based comparative genome hybridization reveals cryptic chromosome changes in AML and MDS cases with trisomy 8 as the sole cytogenetic aberration. *Leukemia* 2006;20:840–6.
12. Shaffer LG, Slovak ML, Campbell LJ, eds. *ISCN 2009: An International System for Human Cytogenetic Nomenclature (2009): Recommendations of the International Standing Committee on Human Cytogenetic Nomenclature*. Basel, Switzerland: S. Karger AG and Cytogenetic and Genome Research; 2009.
13. Den Boer ML, van Slegtenhorst M, De Menezes RX, *et al.* A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. *Lancet Oncol* 2009;10:125–34.
14. Kim M, Yim SH, Cho NS, *et al.* Homozygous deletion of *CDKN2A* (p16, p14) and *CDKN2B* (p15) genes is a poor prognostic factor in adult but not in childhood B-lineage acute lymphoblastic leukemia: a comparative deletion and hypermethylation study. *Cancer Genet Cytogenet* 2009;195:59–65.
15. Passeron T, Valencia JC, Namiki T, *et al.* Upregulation of *SOX9* inhibits the growth of human and mouse melanomas and restores their sensitivity to retinoic acid. *J Clin Invest* 2009;119:954–63.
16. Gunn SR, Bolla AR, Barron LL, *et al.* Array CGH analysis of chronic lymphocytic leukemia reveals frequent cryptic monoallelic and biallelic deletions of chromosome 22q11 that include the *PRAME* gene. *Leuk Res* 2009;33:1276–81.
17. The Centre for Applied Genomics (TCAG). Database of genomic variants [Web resource]. Toronto, ON: TCAG; 2009. [Available at: <http://projects.tcag.ca/variation/>; cited June 2, 2010]
18. Djordjević V, Dencić-Fekete M, Jovanović J, *et al.* Pattern of 1q trisomy in hematological malignancies: a single institution experience. *Cancer Genet Cytogenet* 2008;186:12–18.

19. Stams WA, Beverloo HB, den Boer ML, *et al.* Incidence of additional genetic changes in the *TEL* and *AML1* genes in DCOG and COALL-treated t(12;21)-positive pediatric ALL, and their relation with drug sensitivity and clinical outcome. *Leukemia* 2006;20:410–16.

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