CME/SAM

Whole-Genome Single Nucleotide Polymorphism Array Analysis Is Complementary to Classical Cytogenetic Analysis in the Evaluation of Lymphoid Proliferations

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ABSTRACT

Objectives: *To explore how much additional information single nucleotide polymorphism (SNP) arrays provide and whether they could partially replace classical cytogenetics.*

Methods: *Twenty-six lymphoid proliferations with available cytogenetic studies were analyzed with the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA).*

Results: *Eleven of 26 cases demonstrated complete concordance between cytogenetics and SNP analysis, and 10 of 26 cases demonstrated partial concordance. Five discordant cases had copy number abnormalities (CNAs) with cytogenetics not identified with SNP arrays. While SNP analysis showed CNAs not apparent by cytogenetics in eight cases and helped clarify the karyotype in six cases, cytogenetics demonstrated CNAs not seen by SNP analysis in 15 cases as well as balanced translocations in 12 cases.*

Conclusions: *The combination of cytogenetics and SNP analysis results in a higher overall yield in identifying numerical chromosomal abnormalities than either technique alone.*

Upon completion of this activity you will be able to:
- discuss the clinical utility of karyotypic analysis in lymphoid neoplasms.
- examine the potential role of whole-genome single nucleotide polymorphism array analysis in the evaluation of lymphoid proliferations.
- describe the advantages and disadvantages of array-based karyotyping compared to classical cytogenetics.

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Cytogenetic analysis is of great utility in the evaluation of hematopoietic neoplasms, providing both diagnostic and prognostic information that guides therapeutic decisions. In clinical practice, cytogenetic evaluation currently relies primarily on directed fluorescence in situ hybridization (FISH) studies and, for a more global analysis, classical karyotypic analysis, which requires fresh tissue or viable stored cells. However, comparative genomic hybridization and, in more recent years, array-based karyotyping, including both single nucleotide polymorphism (SNP) arrays and array-based comparative genomic hybridization (aCGH), have been widely used to evaluate a variety of hematopoietic neoplasms.[1-20] These high-throughput techniques have been used to demonstrate additional clinically relevant copy number abnormalities (CNAs), many of which are not identifiable with classical cytogenetic and conventional directed FISH

studies.[1,2,5,7,11-14,16,17,19,21,22] In some neoplasms, including myelodysplastic syndromes, lymphoblastic leukemia/lymphoma, and follicular lymphoma (FL), CNAs detected by array-based karyotyping have provided additional prognostic information or have identified small regions containing genes that may be therapeutic targets in the future.[1-3,6,8,9,12,13,21,23,24]

Whole-genome SNP arrays and aCGH have some advantages over classical cytogenetics in that these arrays can evaluate the entire genome at a higher resolution than classical G-banded karyotypes and do not require dividing cells.[25,26] Array-based karyotyping may be particularly useful in the evaluation of lymphoid neoplasms, many of which lack fresh tissue or yield an insufficient number of metaphase cells for conventional cytogenetic studies. Although targeted FISH studies can be helpful in evaluating lymphoid neoplasms that lack material for metaphase karyotyping, overall cytogenetic complexity has also been shown to be of prognostic significance in various lymphomas, including diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), and splenic marginal zone lymphoma.[3,27-31] Therefore, array-based karyotypic techniques could play an important role in providing a more global analysis of the cytogenetic complexity of lymphoid neoplasms in routine clinical practice. In addition, SNP arrays allow the detection of copy-neutral loss of heterozygosity/uniparental disomy (UPD), which cannot be detected by classical cytogenetics or FISH studies and may also be of clinical significance.[25,26]

However, array-based karyotyping techniques may be less sensitive than metaphase cytogenetics, in some circumstances requiring up to 30% neoplastic cells to reliably detect abnormalities, with an even higher percentage needed to detect CNAs in any subclones that might be present.[25,26,32] Because SNP arrays and aCGH measure the mean DNA copy number of the cells analyzed, these platforms also cannot fully distinguish and characterize tumor subclones, which can be readily identified with metaphase cytogenetics.[25,26,32] Currently available SNP arrays and aCGH also cannot detect balanced chromosomal translocations, which may be detected with classical cytogenetics, as well as by directed FISH studies.[33]

The extent to which the advantages of high-throughput array-based karyotyping platforms are balanced by their disadvantages relative to classical cytogenetics in achieving a genome-wide assessment of CNAs and overall cytogenetic complexity remains uncertain. Therefore, 26 lymphoid proliferations with available frozen tissue samples and previously performed classical cytogenetic studies were analyzed using the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA) to determine how well the array-based karyotype correlated with conventional cytogenetics. Selected cases with apparently discrepant CNAs were further validated with FISH studies.

Materials and Methods

Case Selection

Twenty-six lymphoid proliferations were identified in the archives of the Division of Hematopathology, University of Pittsburgh School of Medicine, in which classical cytogenetics had been performed and where frozen tissue samples were available (20 lymph nodes, five extranodal tissues, and one spleen). The specimens included five reactive lymphoid proliferations; eight FLs; six DLBCLs; two extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue (MALT); one lymphoplasmacytic lymphoma; one monomorphic posttransplant lymphoproliferative disorder, DLBCL type; two classical Hodgkin lymphomas (CHLs); and one nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL). The classical cytogenetic studies and any available FISH studies performed on all cases at the time of diagnosis were reviewed, with a second review of the G-banded karyotypes when required.

SNP Array Analysis

DNA was extracted from the frozen lymphoid tissue samples using a Qiagen tissue kit (Qiagen, Valencia, CA). Genomic DNA (500 ng) was digested with Nsp I and Sty I for two hours at 37°C and then purified and ligated with primer adaptors at 16°C for 12 to 16 hours. Amplicons were generated by performing polymerase chain reaction (PCR) using primers provided by the manufacturer (Affymetrix) on the ligation products using the following program: 94°C for three minutes and then 35 cycles of 94°C for 30 seconds, 60°C for 45 seconds, and 65°C for one minute. This was followed by extension at 68°C for seven minutes. The PCR products were purified and digested with DNase I for 35 minutes at 37°C to fragment the amplified DNA. The fragmented DNA was labeled with biotinylated nucleotides through terminal deoxynucleotide transferase for four hours at 37°C. Fragmented DNA (250 µg) was hybridized with a preequilibrated Affymetrix Genome-Wide Human SNP Array 6.0 at 50°C for 18 hours. Washing and scanning of SNP 6.0 chips was performed following the procedures provided by Affymetrix.

Cell intensity (CEL) files generated from the Affymetrix Genome-Wide Human SNP Array 6.0 were analyzed with Genotyping Console version 4.1.3 (Affymetrix) for quality control. Samples with a quality control call of more than 80% were further analyzed. The CEL files were then analyzed in Genotyping Console and Partek Genomics Suite version 6.6 (Partek, St Louis, MO) to assess for CNAs. Regions of CNAs were subsequently filtered by limiting to regions 1 Mb or more. Normal copy number variants were excluded using the Database of Genomic Variants (http://dgv.tcag.ca).[34]

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Fluorescence In Situ Hybridization

To confirm abnormalities detected on SNP array analysis that were not seen by classical cytogenetic studies, we performed FISH on 5-µm formalin-fixed, paraffin-embedded (FFPE) whole-tissue sections from 10 cases using the following probes: *BRAF* (7q34) and *CITED2* (6q23.3) (Invitrogen, Grand Island, NY). The signal patterns from a minimum of 200 cells were scored, and cutoffs to determine positive samples were established for each probe based on evaluation of FFPE whole-tissue sections of benign tonsil tissue. To confirm abnormalities detected by classical cytogenetic studies that were not seen on SNP array analysis, we performed FISH on 5-µm FFPE whole-tissue sections from seven cases using the following probes: Vysis LSI N-MYC (2p24)/CEP2; Vysis LSI EGR1 (5q31)/LSI D5S23, D5S721 (5p15.2), and CEP6 (D6Z1); Vysis LSI MYC (8q24) and CEP11 (D11Z1); Vysis LSI ETV6 (12p13)/RUNX1 (21q22) ES Dual Color; and Vysis D13S319 (13q14.3)/13q34 (Abbott Molecular, Des Plaines, IL). The signal patterns from a minimum of 200 cells were scored, and cutoffs to determine positive signal patterns were established for each probe based on published literature and on individual laboratory experience with clinical samples.[35]

Results

Cases With Complete Concordance Between Classical Cytogenetics and SNP Array Analysis

Eleven cases (five reactive lymphoid proliferations, two DLBCLs, one MALT, two CHLs, and one NLPHL) had complete concordance between the metaphase karyotype and SNP array analysis **Table 1**. Of these 11 cases, 10 demonstrated a normal karyotype with classical cytogenetic studies and SNP array analysis. The 11th case, a MALT lymphoma, demonstrated deletion 17p and trisomy 18 with both classical cytogenetics and SNP analysis **Image 1**. Although by metaphase cytogenetics, the 17p deletion in this case appeared to involve the *TP53* gene region at 17p13.1, SNP analysis showed that the deletion only involved a region spanning from 17p12 to 17p11.1. FISH studies performed at diagnosis also showed no evidence of loss of *TP53*.

Table 1
Comparison of Classical Cytogenetic and SNP Array Analysis in 26 Lymphoid Proliferations

Characteristic	Number
Concordant CNA	11[a]
Partially concordant CNA	
Extra abnormalities on cytogenetics only	2
Extra abnormalities on SNP only	0
Extra abnormalities on both cytogenetics and SNP	8
Discordant CNA	5[b]
Cases with balanced translocations	12
Cases with cytogenetics clarified by SNP	6

CNA, copy number abnormality; SNP, single nucleotide polymorphism.
[a] Ten of 11 concordant cases had a normal cytogenetic karyotype and SNP analysis and included six of the lymphomas.
[b] Five of five discordant cases had an abnormal cytogenetic karyotype and normal SNP analysis.

Cases With Partial Concordance Between Classical Cytogenetics and SNP Array Analysis

Ten cases demonstrated partial concordance between classical cytogenetics and SNP analysis **Table 2**. Additional abnormalities were seen with classical cytogenetic studies in

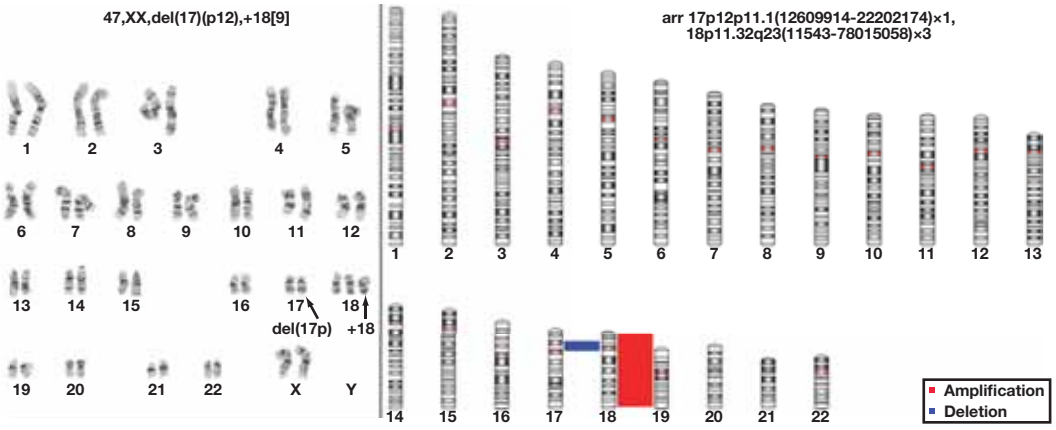

Image 1 Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) with basic concordance between classical cytogenetics and single nucleotide polymorphism (SNP) array analysis. SNP analysis, however, clarified that the 17p deletion identified with classical cytogenetic studies spanned 17p12p11.1 and did not involve the *TP53* gene region (17p13.1).

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Table 2
Cases With Partial Concordance Between Classical Cytogenetic and SNP Array Analysis[a]

Case No.	Diagnosis	Classical Cytogenetic Karyotype	SNP Array Analysis
1	LPL	46,XY[2]/45,XY,t(1;2)(p10;q10),**i(6)(p10)**, -11,del(13)(q14q22),del(17)(p11.2)[1]/45, XY,add(2)(p25),**i(6)(p10),-11**[1]	arr **6p25.3-q11.1(149661-61968257)x3,*6q11.1q27(61968257-171051006)x1*, 11q13.4q25(72011235-134944771)x1**
2	MALT	47,XY,**dup(1)(q21q32),+3**[15]/47,idem, t(6;7)(q21;q34)[3]/46,XY[2]	arr **1p11.2q24.3(121396050-172176162)x3,3p26.3q29(60345-197896119)x3**
3[b]	FL	45-47,XX[13],+X[6],*-2*,**-5**,add(7)(q22)[8], +der(7)t(7;?)(p11.2;?)[13],**+der(7;16) (p10;q10)**[10],***-8***[17],**add(11)(p15)**[15], **add(13)(q34)**[15],t(14;18)(q32;q21)[17], del(15)(q22q24)[16],**-16,-16**,-17, +marx4~7[cp19]/46,XX[1]	arr 2q35q37.3(216419183-243089457)x3,**5q13.3q15(73935454-92547659) x1,5q21.1q22.1(99453962-110843699)x1,5q33.3q35.1(158069468-169874373)x1**,7p14.2p14.1(35262996-41803032)x1,*7q11.22q36.3 (71803432-159127005)x3*,**8p23.3p11.21(31254-40677145)x1**, 10q23.1q25.1(84713590-106990759)x1,**11p15.3p14.3(11037781-24050331)x1**,11p14.3p11.12(24050331-50290497)x3,**13q33.3q34 (108276010-115108398)x1,16p11.1q21(35087891-65589712)x1, 16q22.2q23.1(71967433-74460998)x1,16q23.1(74460998-77108621)x4, 16q23.1q23.2(77108621-81025149)x1,16q23.2q23.3(81025149-82246166)x4, 16q23.3q24.1(82246166-84734140)x1,16q24.1(84734140-86779900)x4, 16q24.1q24.3(86779900-90287536)x1**,17p13.3p13.1(526-8675507)x3, 17p12q11.2(12426775-26617556)x3,17q21.33q25.3(48527871-81049727)x3
4	FL	47,XX,t(14;18)(q32;q21),**+der(18)t(14;18) (q32;q21)**[15]/48,idem,**+12**[4]/46,XX[1]	arr 5q33.3(156973168-158437089)x1,*6q23.3q24.1(136995379-141093268)x1*, **12p13.33q24.33(150442-133778190)x3,18p11.32q21.33(11543-60853655)x3**
5[b]	FL	85~89,XXYY,add(1)(p12),add(1)(q21),del(1) (q32q42),-4,**-5**,del(5)(p14p14),inv(5) (q13q15)x1~2,+6,del(6)(q15q25)x1~2, -10,-10,-11, **der(13)t(1;13)(q21;q34)x2**, t(14;18)(q32;q21)x2,-15,-16, +rx1~2, +mar1[cp15]/ 46,XY[4]	arr **5q15q21.3(94812357-106407261)x1,5q22.2q23.3(112740225-127887761)x1**, *7q11.23q22.1(77188561-100008951)x3*,**13q22.2q34(76950238-115108398)x1**
6[c]	FL	48,XX,t(3;15)(p10;q10),**+7,+8**,t(14;18) (q32;q21)[20]	arr 6q23.3q24.1(137283484-142430852)x1,***7p22.3q36.3(43259-159127005)x3*, 8p23.3q24.3(31254-146298156)x3**
7[b]	DLBCL	50~51,XX,+X,+X,**add(1)(p36.3)**,*+5*, **del(6)(q23q27),+11**,+11,del(11) (p11.2p15),der(14)t(14;?)(q32;?), **ins(17;?)(q21;?)**[cp14]/ 46,XX[3]	arr **1p36.32p36.31(2478089-6158903)x1**,1q32.3q42.2(212900341-249224389)x3, 1q43q44(237221961-249224389)x3,6q12q14.3(66409383-87509388)x1, 6q16.3q21(105375244-109853810)x1,**6q27(16466703-168443024)x1, 11p15.5q25(198510-134944771)x3,17q12q24.1(36524460-63176130)x3**
8[b]	M-PTLD	49~50,XX,**psudic(1;7)(q44;q11.2)**, t(3;3)(q12;q27),**+5**,der(5;19)(p10;q10), **+7,i(7)(q10)**,der(7;9)(p22;q11),der(7;10) (p10;q10),**-8,add(8)(q11.2)**,*-9*,der(9;13) (q11;p11.2),**+11,der(11)t(11;19) (p11.2;p13.1),+13,del(13)(q12q14), +18**,del(18)(q21q23)[8],**+20**,*+21*, add(21)(p11.2),**del(22)(q13)**,+der(?) (?::11q13->11qter),+mar[cp11]	arr 1q42.15q42.2(227475613-234060570)x3,**1q42.2q44(234060570-249224389)x1**, 2q21.2q22.1(133913639-140379338)x1,**5p15.33p11(15532-46261157)x3, *7p22.3q36.3(43259-159127005)x3*,8q11.21q21.12(52056853-79636171)x1, 8q21.3q23.3(92242608-114024889)x1**,10q25.2q26.3(114208836-135238532)x1,**11q13.1q25(63429218-134944771)x3,13q11q12.3 (19026949-31556718)x3,13q12.3q21.31(31556718-64367195)x1, 13q21.31q34(64367195-115108398)x3,18p11.21q23(15383054-78015058)x3**, 19p13.3p13.12(90910-14382443)x1,19p11q13.43(24476319-59097855)x3, **20p13q13.33(61305-62956154)x3,22q12.2q13.33(31556805-51234456)x1**
9	DLBCL	49,X,+X,-Y,t(2;10)(q11.2;q22),**+5,der(5) t(5;6)(p13;p12)x2,-6,+7,+11**,t(14;18) (q32;q21),+1~2r[cp4]	arr **5p15.33p14.1(15532-28595655)x1,5p14.1p13.3(28595655-32624404)x3, 5q11.1q21.1(49564713-100695378)x3,5q23.2q35.3(125311919-180790321)x3, 6p25.3p12.3(149661-47957622)x3,*6q11.1q27(62247670-171051006)x1*, 7p22.3p22.1(43259-7057542)x3,7p21.2q11.23(15031892-76280309)x3, *7q33q36.3(136922905-159127005)x3*,11p15.5p15.2(198510-16109161)x3, 11p15.1(16428179-20745137)x3,11p14.1q25(30168613-134944771)x3**, 17q23.1q23.2(57611971-61032813)x3
10[b]	DLBCL	88<4n>,XXXX,*i(2)(p10)*,**+6,der(6;15) (p10;q10)x2**,dup(7)(p11.2p13)x2,*-11*, del(16)(q22)x2,-18,der(19)t(18;19)(q12; p13.3)x2,-22[8]/46,XX[4]	arr 5q23.2q35.3(126154917-180352580)x3,**6p21.32p21.2(32682793-37070412) x3**,7p21.1p14.2(17889322-37106419)x3,16p13.3p11.1(60777-35171022)x3, 16q12.2q24.3(53295973-90287536)x3,22q12.3q13.33(37387356-51234456)x3

DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; LPL, lymphoplasmacytic lymphoma; MALT, extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue; M-PTLD, monomorphic posttransplant lymphoproliferative disorder, DLBCL type; SNP, single nucleotide polymorphism.

[a] Abnormalities with at least partial concordance between classical cytogenetics and SNP analysis are in bold. Abnormalities confirmed by fluorescence in situ hybridization (FISH) studies are italicized.

[b] SNP array analysis helped to clarify the metaphase karyotype.

[c] FISH studies to confirm the deletion of 6q23 detected with SNP analysis showed loss of signal in interphase cells just below the numerical cutoff for false positive for the *CITED2* probe.

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two of 10 cases, while in eight of 10 cases, varied CNAs were detected with both metaphase cytogenetics and SNP array analysis. In addition, 8 of 10 cases contained balanced translocations identified by classical cytogenetics that would not be identified with SNP analysis. SNP array analysis helped to clarify the karyotype in five cases, all of which had complex karyotypes (cases 3, 5, 7, 8, and 10). In case 7, SNP analysis clarified the structural abnormality on chromosome 17 seen in the metaphase karyotype **Image 2**. SNP array analysis suggested that the insertion in chromosome 17 suspected with classical cytogenetics actually represented an interstitial duplication of 17q12q24.1. SNP analysis also detected the deletion at chromosome 1p36, a portion of the deletion 6q, and the gain of chromosome 11 seen with metaphase cytogenetics, but failed to identify trisomy 5 or any abnormalities of chromosome 14. In the other four cases, SNP array analysis helped to the clarify breakpoints of many deletions and duplications. For example, the breakpoint of the add(13)(q34) in case 3 was refined to 13q33.3 with SNP analysis, and the breakpoint of the der(13)t(1;13)(q21;q34) in case 5 was suggested to be 13q22.2 with SNP array analysis. In case 10, the duplication involving chromosome 7 identified by classical cytogenetics [dup(7)(p11.2p13)] appeared to involve a much larger area spanning 7p21.1p14.2 with SNP analysis. SNP array analysis also suggested the possible origin of marker chromosomes in three cases (cases 3, 5, and 8).

FISH studies using probes for *CITED2* at 6q23.3 and *BRAF* at 7q34 were performed on eight cases with partially concordant karyotypes to confirm the findings seen on SNP array analysis (Table 2). The FISH studies confirmed the gains at 7q34 seen on SNP analysis in all five cases evaluated with the *BRAF* probe. The deletions at 6q23 seen on SNP analysis were also confirmed by FISH for *CITED2* in three of four cases analyzed. In the one case with a 6q23 deletion detected with SNP analysis that was not confirmed by FISH (case 6), the percentage of interphase cells with loss of signal was just below the numerical cutoff for false positives for the *CITED2* FISH probe. Although not definitive, this finding suggests some degree of mosaicism in this lymphoma and that perhaps the proportion of neoplastic cells with deletion 6q was higher in the frozen tissue used for SNP analysis and lower in the paraffin-embedded tissue section used for FISH studies. In case 8, which had a complex composite karyotype, a deletion of 6q23 was identified by FISH studies, which was not seen with either classical cytogenetics or SNP array analysis. Additional FISH studies were also performed in four cases to confirm selected numerical abnormalities detected by classical cytogenetics that were not seen with SNP array analysis (Table 2). These studies confirmed all seven numerical abnormalities evaluated.

Cases With Discordant Classical Cytogenetics and SNP Array Analysis

Five cases demonstrated discordant karyotypes with classical metaphase cytogenetics and SNP array analysis **Table 3**. In all cases, numerical abnormalities detected by classical cytogenetics were not seen with SNP analysis. In two cases (cases 3 and 5), the numerical abnormalities detected by classical cytogenetic studies were seen in less than one-third of metaphase cells, but in the other three cases, the numerical

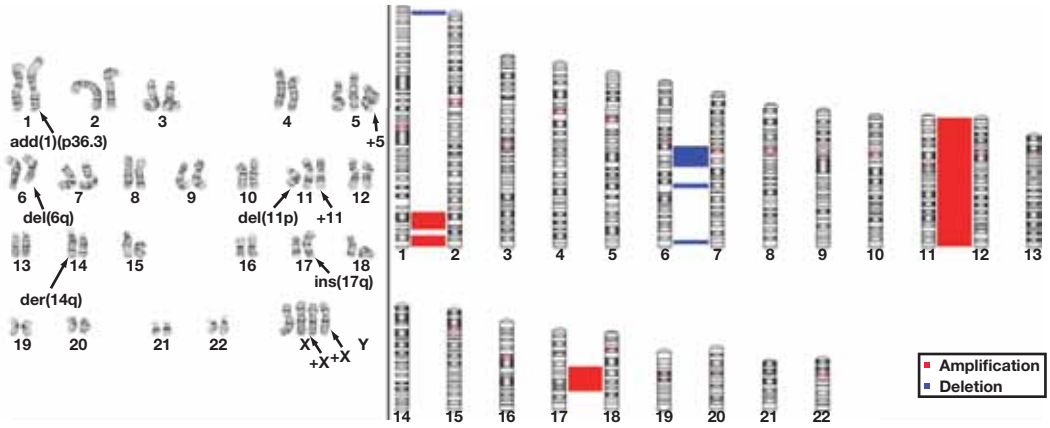

Image 2 Diffuse large B-cell lymphoma with partial concordance between classical cytogenetics and single nucleotide polymorphism (SNP) array analysis (case 7). Metaphase cytogenetics demonstrated 50~51,XX,+X,+X,add(1)(p36.3),+5,del(6)(q23q27),+11,+11,del(11)(p11.2p15),der(14)t(14;?)(q32;?),ins(17;?)(q21;?)[cp14]/46,XX[3]. SNP analysis detected the deletion at chromosome 1p36, a portion of the deletion 6q, and the gain of chromosome 11 but did not identify the trisomy 5 or any abnormalities of chromosome 14. SNP array analysis did help to clarify the structural abnormality involving chromosome 17 and suggested an interstitial duplication of 17q12q24.1.

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Table 3
Cases With Discordant Classical Cytogenetic and SNP Array Analysis[a]

Case No.	Diagnosis	Classical Cytogenetic Karyotype	SNP Array Analysis
1	FL	47,XX,+X,der(10)t(1;10)(q12;q26),*del(13)(q12q21.3)*[12]/47,idem,del(6)(q21),add(7)(q32)[2]/46,XX[5]	arr(1-22)x2
2	FL	49,XX,+X,der(1)dic(1)t(1;?)(?::1p13->1q21::?),del(2)(p21),del(2)(q35),-3,add(4)(q25),t(5;15)(q13;q26), add(9)(p11.2),add(10)(q24),*add(13)(q32),*-13,t(14;18)(q32;q21),-15,add(16)(p11.2),i(17)(q10),add(19 (p13.3),*+21*,+1~4mar[7]/ 46,XX[12]	arr(1-22)x2
3	FL	47,XY,t(14;18)(q32;q21),+mar[cp6]/46,XY[10]	arr(1-22)x2
4	FL	46,XY,add(1)(p36.3),t(14;18)(q32;q21)[1]/46,sl,t(8;9)(q24.3;p13)[11]/48,sdl1,+X,+12[3]/46,XY[6]	arr(1-22)x2
5	DLBCL	44~47,XX,inv(6)(p11.2q23),t(14;18)(q32;q21),+21 or i(21)(q10)[cp3]/88~92,idemx2[cp3]/46,XX[1]	arr(1-22)x2

DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; SNP, single nucleotide polymorphism.
[a] Fluorescence in situ hybridization (FISH) confirmed selected classical cytogenetic findings in both cases studied. Abnormalities confirmed by FISH are italicized.

abnormalities were in a much larger proportion of metaphases. FISH studies were performed on cases 1 and 2 to confirm selected abnormalities that were identified by classical cytogenetics in 63% and 37% of metaphases, respectively. These studies confirmed deletion 13q in cases 1 and 2 and trisomy 21 in case 2. In addition, classical cytogenetics identified balanced translocations in four cases that would not have been detected with SNP array analysis.

Discussion

Karyotypic analysis of lymphoid neoplasms provides important information that may aid in diagnosis and prognostication, as well as guide treatment decisions.[36] Many lymphomas are associated with specific chromosomal translocations, such as t(14;18)(q32;q21) in FL and t(11;14)(q13;q32) in MCL, or variably specific numerical chromosomal abnormalities that can aid in diagnosis.[36-38] In addition, some discrete CNAs such as del 17p and del 11q in CLL/SLL, as well as the degree of global cytogenetic complexity, can provide prognostic information in some lymphoid neoplasms.[3,29-31]

Currently, in most clinical laboratories, karyotypic analysis involves classical metaphase cytogenetics for a more global analysis and FISH studies for more directed evaluation of chromosomal translocations and selected numerical abnormalities. Although classical cytogenetic studies can be of great utility in the evaluation of lymphomas, their use in this setting is limited in many institutions because of the requirement for fresh tissue and viable, dividing cells, as well as the expertise necessary for analysis and interpretation of G-banded karyotypes.[36] Classical cytogenetic studies have a relatively low resolution, with estimates ranging from 5 to 10 Mb based on the quality of the metaphases obtained, and sometimes not all abnormal chromosomes can be identified with certainty, leading to their designation simply as "marker chromosomes."[39,40] These limitations can lead to clinically significant chromosomal abnormalities being missed. Due to these varied limitations, FISH studies, which may be performed on FFPE tissue sections, have become increasingly important in evaluating lymphomas. However, since FISH studies are generally performed in a more targeted fashion, a comprehensive screen for chromosomal abnormalities cannot be easily obtained using this technique, and some prognostically significant abnormalities may be missed with limited FISH panels.[39,41] In addition, some clinically significant chromosomal abnormalities may have atypical breakpoints that are missed by commercial FISH probes, such as with some 11q deletions that have been described in CLL/SLL.[26,41] Truncation artifact when FFPE tissue sections are used is another major confounding factor complicating FISH analysis of chromosomal deletions.

In recent years, array-based karyotypic platforms have provided an alternate technique for whole-genome copy number analysis and in some studies have demonstrated clinically important CNAs not detectable by conventional cytogenetic studies.[1,2,5,7,11-14,16,17,19,21,22,38,41] In the clinical setting, DNA microarrays are widely used for the evaluation of individuals with developmental disabilities or congenital anomalies, where these platforms are considered a first-tier diagnostic test.[42] The role of DNA microarrays in the evaluation of hematologic malignancies is less well defined. However, commercially available aCGH or SNP array assays are available for the evaluation of some hematologic malignancies, including CLL/SLL, DLBCL, and myelodysplastic syndromes. Particularly for CLL/SLL, comparisons with FISH studies have shown improved detection of prognostically significant numerical abnormalities with aCGH, as well as recurring numerical chromosomal abnormalities that are not evaluated with standard FISH panels.[14,43] Whole-genome SNP arrays and aCGH have some advantages over classical cytogenetics in that they do not require dividing cells and may therefore be particularly useful in the evaluation of lymphoid neoplasms, many of which lack fresh tissue or do not produce an adequate number of metaphases for karyotyping.[25,26] The resolution of array-based karyotypic platforms is also much higher than metaphase cytogenetics, with some platforms

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detecting CNAs of less than 100 kb.[32,39,44] In addition, SNP arrays allow the detection of UPD that may be clinically significant, which is not possible with classical cytogenetic or FISH studies.[25,26,41] However, evaluation for UPD in tumor samples is best performed with a matched normal DNA sample to reduce false discovery, which is not always available and was a limitation of the current study.[45] Another limitation of array-based karyotyping is that SNP arrays and aCGH are somewhat less sensitive than metaphase cytogenetics or FISH in many circumstances, since they generally require 10% to 30% neoplastic cells to reliably detect CNAs.[25,26,32] Currently available array-karyotypic platforms also cannot detect balanced chromosomal translocations, which are particularly important in the evaluation of lymphoid neoplasms.

Given the advantages and disadvantages of array-based karyotyping, it has been unclear to what extent currently available high-resolution arrays would supplement or replace classical cytogenetic studies in the analysis of CNAs in lymphoid neoplasms. We found that SNP array analysis overall had a relatively good concordance with classical cytogenetics, demonstrating complete or partial concordance in 21 of 26 cases, including 16 of 21 lymphoma cases. However, all but one of the completely concordant cases showed no CNAs. Of the 10 cases with partial concordance between classical cytogenetics and SNP analysis, additional abnormalities were identified with SNP array analysis in eight cases and with classical cytogenetics in all 10 cases. In addition, five lymphoma cases in our study demonstrated discordant classical cytogenetics and SNP array findings, with SNP analysis failing to demonstrate any CNAs. There are several possible explanations for the discordant results in our study. First, given the higher resolution of SNP arrays, some CNAs detected were below the resolution of metaphase cytogenetics in eight of 26 cases. As noted previously, the sensitivity of SNP analysis may be lower than that of metaphase cytogenetics, with typical SNP arrays requiring up to 30% neoplastic cells to detect CNAs.[25,26,32] Therefore, SNP array analysis of lymphomas with a high proportion of admixed nonneoplastic cells may show a poor sensitivity compared with classical cytogenetics. One limitation of our study is that in using archived frozen tissue, it was impossible to enrich the neoplastic cell population, and the proportion of neoplastic cells in the frozen samples could not be assessed. The sensitivity of SNP analysis may be improved with positive or negative selection of neoplastic cells using magnetic or flow cytometric cell-sorting technologies. Neoplastic cell enrichment has been shown to improve the sensitivity of array-based karyotypic techniques in some studies.[20]

Another explanation that could explain the discrepant results in our study is the presence of intratumoral heterogeneity, which is well recognized in lymphomas.[46-48] Classical cytogenetic studies enrich for cell populations that are able to divide well in culture and may not necessarily represent the dominant clone in the tumor. Therefore, SNP arrays may not detect all clonal abnormalities seen by metaphase cytogenetics if they are not present in the dominant clone in the tissue sample.[25,26] FISH studies performed on selected cases in our study also provide some evidence of intratumoral heterogeneity. In one case with a 6q23 deletion identified by SNP analysis (case 6), FISH studies demonstrated a loss of signal in interphase cells that was just below the numerical cutoff for false positives for the FISH probe. This finding suggests that there may be some degree of mosaicism in this lymphoma and that perhaps the proportion of neoplastic cells with deletion 6q was higher in the frozen tissue used for SNP array analysis than in the samples used for metaphase cytogenetics and FISH.

One advantage of the high resolution of SNP arrays is that this technique can help to clarify the numerical chromosomal abnormalities seen in the metaphase karyotype, particularly if the resolution of the metaphases is not optimal.[32] In our study, SNP analysis refined the classical cytogenetic findings in six cases. The utility of this platform was exemplified in one case in which classical cytogenetic studies were thought to demonstrate deletion 17p involving the *TP53* gene at 17p13.1. However, SNP array analysis determined that the 17p deletion did not include the *TP53* gene region, which was also confirmed by FISH studies. SNP analysis can also help to clarify apparent structural abnormalities associated with CNAs. For example, in a second case, what was thought to be an insertion at 17q21 on classical cytogenetics was found on SNP analysis to be an interstitial duplication of 17q12q24.1.

Although SNP array analysis showed relatively good concordance with classical cytogenetics, could identify additional cryptic CNAs, and helped to clarify the metaphase karyotype, our study also showed that classical cytogenetic studies revealed additional CNAs and important balanced translocations that were not detected with the SNP arrays. Classical cytogenetic studies identified CNAs not detected by SNP arrays in almost 60% of total cases and in 87% of cases with a cytogenetic abnormality, which is due at least in part to the lower sensitivity of SNP array analysis. The metaphase karyotypes also revealed balanced translocations in almost half of all cases and in 75% of cases with any cytogenetic abnormality. These balanced translocations would not be detectable with currently available SNP arrays.[33] Therefore, classical cytogenetic studies continue to provide important karyotypic information, including numerical abnormalities that would be missed by even high-resolution array-based karyotypic platforms.

In conclusion, SNP array analysis of lymphoid proliferations provides additional copy number information that is not seen with classical cytogenetic studies and may help to clarify the metaphase karyotype. However, SNP arrays do not replace classical cytogenetic studies, which remain

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important in identifying numerical chromosomal abnormalities as well as balanced chromosomal translocations. Although the role of array-based karyotypic techniques in the routine clinical evaluation of lymphomas is currently not well defined, our study shows that SNP arrays are a useful supplement to classical cytogenetics and more directed FISH studies. Given that overall cytogenetic complexity has been shown to be of prognostic significance in some lymphomas and selected CNAs are associated with specific types of lymphomas or have prognostic significance, SNP or aCGH platforms may be useful in the routine evaluation of at least selected lymphomas with a normal karyotype, when there are insufficient metaphase cells for classical cytogenetics, or when fresh cells are not available for classical cytogenetic studies.[3,29-31] The higher resolution of these array-based platforms may also help clarify the classical chromosome analysis, particularly of lymphomas with more complex karyotypes. It has also been suggested in the literature that DNA microarrays improve detection of clinically significant CNAs that may be missed by commercial FISH probes.[26,41] Further studies are needed to investigate methods to improve the sensitivity of SNP array analysis, to better understand when these studies will be most cost-effective, and to evaluate if newer technologies such as whole-genome next-generation sequencing can actually replace classical cytogenetics, FISH, and SNP testing.

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