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Genome-Wide Single-Nucleotide Polymorphism Array Analysis Improves Prognostication of Acute Lymphoblastic Leukemia/Lymphoma

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Chromosomal abnormalities are important for the risk stratification of acute lymphoblastic leukemia/lymphoma (ALL). However, approximately 30% of pediatric and 50% of adult patients lack abnormalities with clinical relevance by traditional cytogenetic analysis. We integrated cytogenetic, fluorescence *in situ* hybridization, and whole-genome single-nucleotide polymorphism array results from 60 consecutive clinical ALL cases. By cytogenetic and/or fluorescence *in situ* hybridization analyses, recurring abnormalities with clinical relevance were observed in 33 B-cell ALL (B-ALL), including t(9;22), hyperdiploidy, *KMT2A* translocation, *ETV6-RUNX1*, intrachromosomal amplification of chromosome 21, near haploidy or low hypodiploidy, and t(8;22). Single-nucleotide polymorphism array analysis found additional aberrations with prognostic or therapeutic implication in 21 B-ALL and two T-cell ALL, including *IKZF1* deletion, intrachromosomal amplification of chromosome 21 (one case with a normal karyotype), low hypodiploidy (two cases with a normal karyotype), and one case each with fusion genes *ETV6-NTRK3*, *CRLF2-P2RY8*, *NUP214-ABL1*, and *SET-NUP214*. *IKZF1* deletion was noted in nine B-ALL with t(9;22), one B-ALL with t(4;11), five B-ALL with a normal karyotype, and three B-ALL with nonrecurring karyotypic abnormalities. Combining single-nucleotide polymorphism array with chromosome and fluorescence *in situ* hybridization assays, the detection rate for clinically significant abnormal results increased from 56% to 75%. Whole-genome single-nucleotide polymorphism array analysis detects cytogenetically undetectable clinically significant aberrations and should be routinely applied at diagnosis of ALL. *(J Mol Diagn 2016, 18: 595—603; http://dx.doi.org/10.1016/j.jmoldx.2016.03.004)*

Identification of recurring cytogenetic abnormalities has had a major effect on risk classification of acute lymphoblastic leukemia/lymphoma (ALL).[1—4] Current therapeutic protocols consider a number of these abnormalities when assigning patients to the high- or low-risk categories. For example, hyperdiploidy (>50 chromosomes) and *ETV6-RUNX1* fusion gene confer the most favorable prognosis, whereas near haploidy, low hypodiploidy or near triploidy, t(9;22), and *KMT2A* (*MLL*) translocation are associated with poor prognosis in B-cell ALL (B-ALL).[1—3] In T-cell ALL (T-ALL), t(11;14)(p13;q11), del(17p), and a complex karyotype are associated with a poor overall survival.[4]

Karyotype and fluorescence *in situ* hybridization (FISH) analyses are the standard cytogenetic assays for identifying genomic abnormalities. However, because of inadequate specimens and absent or few mitotic cells, approximately 16% to 30% of the ALL cases yield no or inadequate cytogenetic results. Among those with a cytogenetic result, 15% to 25% have a normal karyotype.[2,5,6] Recent application of whole-genome single-nucleotide polymorphism (SNP) arrays, multiplex ligation-dependent probe amplification, and next-generation sequencing has revealed a plethora of submicroscopic genomic abnormalities that specifically target genes involved in key signaling pathways and are associated with patient outcome.[7—13] For example, submicroscopic deletions of genes *CDKN2A/B*, *ETV6*, *IKZF1*, and

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PAX5 have been frequently observed in B-ALL, and deletions of *IKZF1*, *RB1*, *EBF1*, and pseudoautosomal region 1 have been associated with an unfavorable prognosis.

High-resolution genome-wide SNP array analysis allows determination of both copy number aberrations and copy neutral loss of heterozygosity (CN-LOH) and therefore is able to provide important insights into the often complex and unique genomic profiles for many patients. SNP array analysis requires only a small amount of DNA and can provide useful information when specimens are limited or metaphase cells are not available. In recent years, SNP arrays have been used for detecting genomic abnormalities in ALL and proved to facilitate diagnosis, risk stratification, and determination of efficient therapeutic regimens.[9–14] Despite these advantages, genome-wide SNP arrays have not been routinely used for ALL by clinical laboratories. This study retrospectively reviewed results of cytogenetics, FISH, and whole-genome SNP array assays in 60 consecutive clinical ALL cases from a single institution. We found that SNP array improved the detection rate of genomic abnormalities (90% by cytogenetics and FISH versus 100% by SNP array) and provided additional prognostic and therapeutic information (56% by cytogenetics and FISH versus 75% by cytogenetics, FISH, and SNP array). These results indicate that whole-genome SNP arrays should be incorporated as standard of care for ALL patients.

Materials and Methods

Patient Samples

Beginning in March 2014, we incorporated SNP array analysis as part of our standard clinical testing for patients with newly diagnosed ALL. We retrospectively reviewed cytogenetic, FISH, and SNP array results for consecutive ALL cases from the Clinical Cytogenetics Laboratory at the University of Michigan Health System between March 2014 and June 2015. Sixty consecutive patients had SNP array results and 59 also had concurrent cytogenetic and/or FISH analyses. Cytogenetic analysis was not performed in two patients with T-ALL; FISH analysis was not performed in four patients with B-ALL and two patients with T-ALL. This study was approved by the institutional review board at the University of Michigan.

Cytogenetic and FISH Analyses

At least 20 G-banded metaphase cells were obtained from overnight and/or 24-hour cultures using standard techniques. Cases with <15 analyzable normal metaphase cells were considered inadequate. FISH analysis was performed using probe sets, including BCR-ABL1 dual fusion, KMT2A breakapart, ETV6-RUNX1 fusion, and centromere probes for chromosomes 4, 10, and 17 (Abbott Molecular, Des Plaines, IL) for pediatric patients with B-ALL and BCR-ABL1 and KMT2A probes for adults with B-ALL and most patients with T-ALL. Some of the array results were confirmed by FISH analysis using probes commonly used in the clinical cytogenetics laboratory. Karyotypic and FISH results were interpreted according to the International System for Human Cytogenetic Nomenclature. FISH images were captured using a Leica DMRA microscope with the Cytovision Imaging system (Leica Microsystems, Buffalo Grove, IL).

SNP Array Analysis

Genomic DNA was extracted from leukemic blood, bone marrow, or fluidic samples using the QIAamp DNA mini-extraction kit (Qiagen, Germantown, MD), according to the manufacturer's instructions. Genome-wide SNP array analysis was performed using the Affymetrix CytoScan HD platform with approximately 2.7 million probes according to the manufacturer's protocols (Affymetrix, Santa Clara, CA) as published previously.[15]

SNP array data were analyzed by the Affymetrix ChAS software version 2.1 (Affymetrix). Plots of two parameters, the $\log_2$ ratio and the allele peaks, providing information regarding copy number and genotype, respectively, were examined by visual inspection. All genomic positions were based on the hg19 (2009) build of the human genome sequence (*http://genome.ucsc.edu/cgi-bin/hgGateway*, last accessed March 17, 2016). Results were compared to databases of known common copy number variations seen in healthy controls, including the Toronto database of genomic variants (*http://dgv.tcag.ca/dgv/app/home*, last accessed March 17, 2016), International Standards for Cytogenomic Arrays database (*http://dbsearch.clinicalgenome.org*, last accessed March 17, 2016), and Children's Hospital of Philadelphia copy number variation database (*http://cnv.chop.edu*, last accessed March 17, 2016); common population variants were excluded from the results. Gains or losses >35 markers within or including a known clinically significant cancer-related gene or >1 Mb outside the known clinical oncology significant regions and loss of heterozygosity >10 Mb are reported.

Results

A total of 60 consecutive ALL cases were received between March 2014 and June 2015 in the Clinical Cytogenetics Laboratory for clinical SNP array analysis. Among those, 27 (45%) were from pediatric patients (0.5 to 18 years old), 13 (21.7%) were from young adults (19 to 30 years old), and 20 (33.3%) were from adults (31 to 70 years old). Fifty-three patients (88.3%) were diagnosed as having B-ALL and seven (11.7%) as having T-ALL.

Karyotypic abnormalities were found in 47 of 58 patients (81%), and FISH abnormalities were found in 42 of 54 patients (78%) (Table 1), among whom recurring abnormalities with clinical relevance were observed in 33 patients with B-ALL. These abnormalities included t(9;22)(q34;q11.2), hyperdiploidy, *KMT2A* translocation, *ETV6-RUNX1* fusion, intrachromosomal amplification of