

# Clinical Significance of Previously Cryptic Copy Number Alterations and Loss of Heterozygosity in Pediatric Acute Myeloid Leukemia and Myelodysplastic Syndrome Determined Using Combined Array Comparative Genomic Hybridization plus Single-Nucleotide Polymorphism Microarray Analyses

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The combined array comparative genomic hybridization plus single-nucleotide polymorphism microarray (CGH+SNP microarray) platform can simultaneously detect copy number alterations (CNA) and copy-neutral loss of heterozygosity (LOH). Eighteen children with acute myeloid leukemia (AML) (n = 15) or myelodysplastic syndrome (MDS) (n = 3) were studied using CGH+SNP microarray to evaluate the clinical significance of submicroscopic chromosomal aberrations. CGH+SNP microarray revealed CNAs at 14 regions in 9 patients, while metaphase cytogenetic (MC) analysis detected CNAs in 11 regions in 8 patients. Using CGH+SNP microarray, LOHs > 10 Mb involving terminal regions or the whole chromosome were detected in 3 of 18 patients (17%). CGH+SNP microarray revealed cryptic LOHs with or without CNAs in 3 of 5 patients with normal karyotypes. CGH+SNP microarray detected additional cryptic CNAs (n = 2) and LOHs (n = 5) in 6 of 13 patients with abnormal MC. In total, 9 patients demonstrated additional aberrations, including CNAs (n = 3) and/or LOHs (n = 8). Three of 15 patients with AML and terminal LOH > 10 Mb demonstrated a significantly inferior relapse-free survival rate (P = 0.041). This study demonstrates that CGH+SNP microarray can simultaneously detect previously cryptic CNAs and LOH, which may demonstrate prognostic implications.

**Keywords:** Leukemia, Myeloid, Acute; DNA Copy Number Variations; Loss of Heterozygosity; Comparative Genomic Hybridization; Single-Nucleotide Polymorphism Microarray

## INTRODUCTION

Although chromosomal aberrations demonstrate important prognostic impacts in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), a significant proportion of patients do not demonstrate cytogenetic aberrations on metaphase cytogenetic (MC) analysis (1, 2). In addition, patients with similar cytogenetic aberrations demonstrate heterogeneous clinical characteristics. Although molecular genetic assays have revealed numerous genetic aberrations, such as internal tandem duplications (ITD) in *FLT*, and *CEBPA* and *NPM1* mutations, clinical heterogeneity among patients with similar MC results (especially those with normal cytogenetics) has not been fully elucidated (3-7).

Despite the established clinical implications of MC, several limitations are also apparent: low resolution, reliance on cell division, and the inability to detect copy-neutral genetic aber-

rations. Array comparative genomic hybridization (CGH) does not require cell division and demonstrates much greater resolution than MC, allowing for a more accurate quantification of copy number alterations (CNAs) in malignant cells. Another novel array-based technique—single-nucleotide polymorphism (SNP) microarray—can detect copy-neutral loss of heterozygosity (CN-LOH), which reportedly plays a role in oncogenesis by duplicating oncogenes, inhibiting tumor suppressors, and stimulating improper epigenetic programming, and has been recently described in myeloid malignancies (8, 9).

Array CGH and SNP microarray have been used to study MDS, myeloproliferative disorders (MPD), and AML (mainly in adult patients), and new cryptic lesions have been detected in many patients with normal or abnormal karyotypes using MC analysis (10-12). Furthermore, previously cryptic CNAs and CN-LOH reportedly demonstrate prognostic significance in adult AML and MDS (10, 11). However, whole-genome scanning using ar-

ray CGH and SNP microarray are insufficient for studying pediatric AML and MDS, and their clinical and biological implications still need to be determined for use in pediatric populations.

The combination array CGH plus SNP microarray (CGH+SNP microarray) platform can simultaneously detect CNAs and CN-LOH at high-resolution without running 2 separate microarray experiments. In our present study, we report the simultaneous detection of cryptic CNAs and CN-LOH using CGH+SNP microarray and characterize the clinical significance of these sub-microscopic defects in childhood AML and MDS.

## MATERIALS AND METHODS

### Patients

In total, 18 patients with AML (n = 15) or MDS (n = 3) who were < 18 yr at the time of diagnosis were included in this study. All patients were diagnosed between 2008 and 2011 at Asan Medical Center. DNA was extracted from frozen bone marrow (BM) (n = 17) or peripheral blood (PB) samples (n = 1) that were collected at diagnosis (n = 15) or relapse/persistence (n = 3) and used in subsequent CGH+SNP microarray analyses.

The clinical characteristics of the study population are listed in Table 1. The median age at diagnosis was 11 yr (range: 0.5-17 yr), and 7 male and 11 female patients were included in our cohort. Patients were categorized according to the World Health

Organization (WHO) classification system (13). Nine patients having AML with recurrent genetic abnormalities, 2 patients having AML with myelodysplasia-related changes (AML with MDS), 4 patients having uncategorized AML, and 3 patients having MDS were included.

The clinical characteristics and treatment outcomes of our patients are summarized in Table 2. In the AML group (n = 15), 6 patients remained alive and categorized as in complete remission (CR) after receiving allogeneic hematopoietic stem cell transplantation (HSCT), 2 patients were alive and in CR following the administration of only chemotherapy, 3 patients died of relapse after receiving HSCT, 1 patient died of pulmonary hemorrhage after receiving HSCT, and 3 patients died of refractory disease without receiving allogeneic HSCT. In the MDS group (n = 3), 2 patients were alive and categorized as in CR after receiving allogeneic HSCT and 1 patient died of graft-versus-host disease and fungal infection after receiving allogeneic HSCT. In total, after a median follow-up period of 28 months (range: 8-63 months), 10 patients are alive and categorized as in CR and 8 patients are dead. The median survival time was 15 months (range: 6-62 months).

### Cytogenetic and molecular analyses

All 18 patients in the present study series were successfully assessed using MC analysis. G-banded karyotyping was performed using standard techniques. Whenever possible, 20 metaphases were analyzed. Chromosomal abnormalities are described according to the International System for Human Cytogenetic Nomenclature (14). Fluorescence in situ hybridization (FISH) analysis was performed if requested by the clinician.

RT-PCR was performed on 15 AML patients to detect fusion gene transcripts, including *RUNX1-RUNX1T1*, *PML-RARA*, *CBFB-MYH11*, *BCR-ABL*, and *MLL-AF4*. Notably, 7 AML patients were analyzed using multiplex RT-PCR (HemaVision; Bio-Rad Laboratories, Hercules, CA, USA), which can detect 28 different leukemia-associated translocations. *c-KIT* mutation analysis was performed on 2 patients with the *RUNX1-RUNX1T1* fusion transcript. *FLT3* was analyzed in 14 AML patients to detect the *FLT3-ITD* and *FLT3-D835* mutations. The *NPM1* mutation was analyzed in 5 patients with AML.

### Array CGH and SNP microarray analysis

Genomic DNA (gDNA) was isolated from frozen BM (n = 17) or PB samples (n = 1) using the QiagenDNeasy Blood & Tissue Kit (P/N 69504; Qiagen Inc., Valencia, CA, USA) and processed according to the manufacturer's protocol for the Agilent SurePrint G3 Cancer CGH+SNP 4 × 180K microarray (P/N G4869A; Agilent Technologies, Santa Clara, CA, USA). All samples were processed according to the instructions for Agilent's oligonucleotide array-based CGH for gDNA analysis and the enzymatic labeling of blood, cells, or tissues (high-throughput option; ver-

Table 1. Patient characteristics

	No.
Total	18
Sex	
Male	7
Female	11
Age (yr)	
Median (range)	11 (0.5-17)
Diagnosis	
AML	15
AML with recurrent genetic abnormalities	9
AML with t(8;21)(q22;q22); <i>RUNX1-RUNX1T1</i>	2
AML with inv(16)(p13;q22); <i>CBFB-MYH11</i>	1
AML with t(15;17)(q22;q12); <i>PML-RARA</i>	1
AML with 11q23 ( <i>MLL</i> ) abnormalities	3
AML with t(6;9)(p23;q34); <i>DEK-NUP214</i>	2
AML with MRC	2
AML, not otherwise categorized	4
AML with maturation	1
Acute myelomonocytic leukemia	1
Acute megakaryoblastic leukemia	1
Acute basophilic leukemia	1 (2)*
MDS	3
RAEB-2	1
RCMD	1
Unclassifiable	1

\*Patient 6 (AML with 11q23) abnormalities was diagnosed as having morphologically acute basophilic leukemia. AML, acute myeloid leukemia; AML with MRC, acute myeloid leukemia with myelodysplasia-related changes; MDS, myelodysplastic syndrome; RAEB, refractory anemia with excess blasts; RCMD, refractory cytopenia with multilineage dysplasia.

Table 2. Treatment outcomes and genetic aberrations detected using MC and CGH+SNP microarray analyses

Pa-tient	Age (yr)	Sex	WHO classification	Genetic aberrations				Treatment outcomes				
				Karyotype (MC)	Molecular aberration	CNA (size, Mb) (CGH microarray)	LOH (size, Mb) (SNP microarray)	HSCT	Relapse Status	OS (mo)	Cause of death	
1	7	M	AML with t(8;21)(q22;q22)	45,X,-Y,t(8;21)(q22;q22)	<i>RUNX1-RUNX1T1</i> <i>C-KIT<sup>mut</sup></i>	L: Yp11.32-q12 (59.2)	No	Yes	No	Alive	25	-
2	5	M	AML with t(8;21)(q22;q22)	45,X,-Y,t(8;21)(q22;q22)	<i>RUNX1-RUNX1T1</i> <i>C-KIT<sup>mut</sup></i>	L: 1q42.13-q42.2 (2.2) L: Yp11.31-q12 (56.7)	No	Yes	Yes	Dead	45	DOD
3	15	F	AML with inv(16)(p13;q22)	46,XX,inv(16)(p13.1q22),dic(22)(p11.2)	<i>CBFB-MYH11</i>	G: 22q11.1-q13.33 (33.9)	No	Yes	No	Alive	30	-
4	16	F	AML with t(15;17)(q22;q12)	46,XX,t(15;17)(q22;q21)	<i>PML-RARA</i>	No	15q22.2-q23 (6.5) Xq11.1-q21.1 (17.1)	No	No	Alive	8	-
5	17	M	AML with 11q23 abnormalities	46,XY,t(9;11)(p22;q23)	<i>FLT3-ITD</i>	No	No	Yes	Yes	Dead	12	DOD
6	2	F	AML with 11q23 abnormalities (acute basophilic leukemia)	45,XX,t(2;5;17;6)(p21;q35;q21;p21)t(9;11;10)(q22;q23;p12.3),der(12)t(12;18)(p11.2;q11.2),-18	<i>MLL-AF10</i>	L: 12p13.33-p12.3 (17.9) L: 18p11.32-p11.21 (15.2)	18q12.1-q12.2 (5.7) Xp22.2-p22.11 (10.2) Xq22.2-q23 (11.8)	Yes	No	Alive	19	-
7	15	M	AML with 11q23 abnormalities	46,XY,t(6;11)(q27;q23)	<i>MLL-AF6</i>	No	No	Yes	No	Alive	8	-
8	12	F	AML with t(6;9)(p23;q34)	47,XX,t(6;9)(p23;q34),+add(21)(q21)	<i>DEK-NUP214</i>	G: 13q21.32-q34 (46.9)	5q14.1-q14.3 (5.4)	Yes	No	Alive	16	-
9	14	F	AML with t(6;9)(p23;q34)	46,XX,t(6;9)(p23;q34)	<i>DEK-NUP214</i> <i>FLT3-ITD</i>	No	13q12.2-qter (81.1)	No	Yes	Dead	13	DOD
10	15	F	AML with MRC	46,XX	<i>FLT3-ITD</i>	No	7q31.1-q31.2 (5.8)	No	Yes	Dead	6	DOD
11	9	M	AML with MRC	46,XY,del(2)(q33),del(9)(q13q32)	No	L: 2q32.3-q37.2 (40.2) L: 9q21.11-q31.1 (37.2)	No	Yes	Yes	Dead	27	DOD
12	8	F	AML with maturation	47,XX,+6	No	G: 6p25.3-q27 (170.7)	6p11.1-q27 (166.6)	Yes	Yes	Alive	18	-
13	0.5	F	Acute myelomonocytic leukemia	46,XX,t(8;9)(p11.2;q34)	No	No	No	No	No	Alive	12	-
14	1	F	Acute megakaryoblastic leukemia	46,XX	No	G: 8p23.3-q24.3 (146.0) G: 19p13.3-q13.43 (58.7)	1p33-p32.3 (6.0)	Yes	No	Dead	11	TRM
15	14	F	Acute basophilic leukemia	46,XX	No	No	1p33-p32.3 (6.0) 9p24.3-13.2 (32.5)	No	Yes	Dead	13	DOD
16	10	M	MDS, RCMD	46,XY	ND	No	No	Yes	No	Alive	62	-
17	10	F	MDS, RAEB	46,XX	ND	No	No	Yes	No	Alive	36	-
18	15	M	MDS, unclassifiable	48,XX,+8,inv(9)(p12q13),+21	ND	G: 8p23.3-q24.3 (146.1) G: 21p11.2-q22.3 (37.3)	No	Yes	No	Dead	8	TRM

MC, metaphase cytogenetics; CGH, comparative genomic hybridization; SNP, single-nucleotide polymorphism; CNA, copy number alteration; LOH, loss of heterozygosity; ND, not done; G, gain; L, loss; HSCT, hematopoietic stem cell transplantation; OS, overall survival; DOD, died of disease; TRM, treatment-related mortality.

sion 6.3; P/N G4410-90010).

gDNA was digested using the *AluI* and *RsaI* restriction endonucleases, which enable the identification of SNPs located within the enzymes' recognition sites. Experimental samples were labeled with cyanine 5-conjugated dUTP and hybridized to the SurePrint G3 Cancer CGH+SNP 4 × 180K microarrays in the presence of Cot-1-DNA (Invitrogen, Carlsbad CA, USA) for 24 hr at 65°C. A reference HapMap sample with a known genotype (European male; NA 12891\_v1) was labeled with cyanine 3-conjugated dUTP and hybridized to the same microarrays. The microarray slides were scanned at 3-μm resolution using an Agilent microarray scanner, and images were extracted using Agilent Feature Extraction software (v10.10) that was integrated in Agilent CytoGenomics 2.0.6.0. Data were analyzed using Agilent CytoGenomics 2.0.6.0 with an Aberration Detection Method-2 (ADM-2) algorithm (threshold 8.0). Results were analyzed according to genome build Hg19.

### Statistical analysis

The Kaplan-Meier method was used to calculate survival outcomes. Outcomes were compared using the log-rank test. In this study,  $P < 0.05$  is considered statistically significant. All statistical analyses were performed using SPSS (Statistical Package for the Social Science, version 18.0; IBM, Somers, NY, USA).

### Ethics statement

The study was approved by the institutional review board of Asan Medical Center (2011-0570). Informed consent was waived by the board.

## RESULTS

### Conventional cytogenetics

In the AML group ( $n = 15$ ), MC analysis revealed normal karyotypes in 3 patients and chromosomal aberrations in the other 12 patients, including balanced translocation (11 translocations in 10 patients) and/or chromosomal CNAs such as numerical chromosomal abnormalities and/or the duplication or deletion of chromosomal segments (3 gains and 6 losses in 7 patients). In the MDS group ( $n = 3$ ), MC analysis revealed normal karyotypes in 2 patients and balanced translocation and two chromosomal additions was noted in the other 1 patient. In total, MC analysis revealed CNAs in 8 patients. These results are summarized in Table 2 and Table 3.

### Molecular genetic abnormalities

RT-PCR analysis demonstrated *RUNX1-RUNX1T1* with *c-KIT* mutation ( $n = 1$ ), wild-type *c-KIT* ( $n = 1$ ), *CBFB-MYH11* ( $n = 1$ ), *PML-RARA* ( $n = 1$ ), *MLL-AF10* ( $n = 1$ ), *MLL-AF6* ( $n = 1$ ), and *DEK-NUP214* ( $n = 2$ ), which is consistent with conventional cytogenetic analysis. Fourteen patients with AML were ana-

**Table 3.** Comparison of cytogenetic defects detected using MC and CGH+SNP microarray analyses

	MC (patients)	CGH+SNP microarray (patients)
Copy number alteration	11 ( $n = 8$ )	14 ( $n = 9$ )
Gain	5 ( $n = 4$ )	7 ( $n = 5$ )
Loss	6 ( $n = 4$ )	7 ( $n = 4$ )
Loss of heterozygosity	NA	12 ( $n = 8$ )
Balanced rearrangement	11 ( $n = 10$ )	NA

MC, metaphase cytogenetics; CGH, comparative genomic hybridization; SNP, single-nucleotide polymorphism; NA, not applicable.

lyzed for the *FLT3* mutation, and 3 patients demonstrated the *FLT3*-ITD mutation. None of these 3 patients demonstrated the *NPM1* mutation. One of these 3 patients with the *FLT3* mutation demonstrated a normal karyotype, whereas 2 patients demonstrated  $t(9;11)$  and  $t(6;9)$ , respectively. These results are shown in Table 2.

### CNA identification

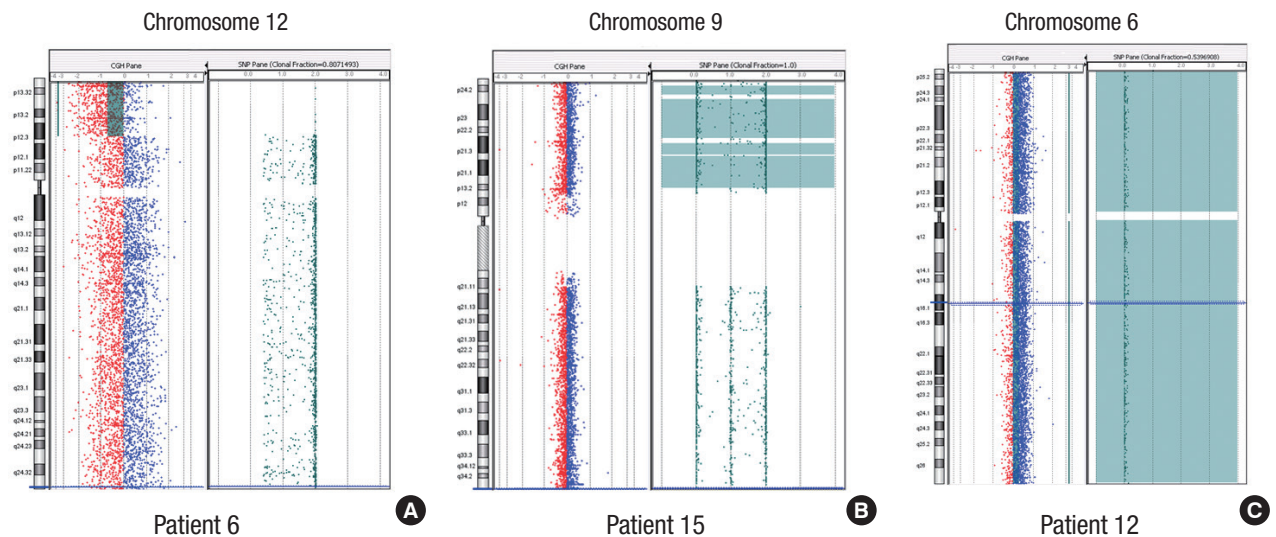
The CGH panel (Agilent SurePrint G3 Cancer CGH+SNP 4 × 180K microarrays) detected 14 CNAs (7 gains and 7 losses) in 9 of 18 patients (50%), while conventional cytogenetics detected 11 CNAs (5 gains and 6 losses) in 8 of 18 patients (44%; Table 3). All genomic imbalances detected using MC analysis were recognized by CGH, whereas 2 gains and 1 loss detected by CGH were undetected by MC analysis. In addition, an accurate gain region that was erroneously detected by MC was corrected using CGH (patient 8). Moreover, CGH more accurately detected breakpoints. In patient 6, CGH detected an accurate breakpoint in derivative chromosome 12, including the loss of 12p13.33-p12.3 (Fig. 1A). In 11 patients with balanced translocations, CGH did not detect any alterations in the breakpoints of the translocated chromosome. Recurrent segmental gain or loss was not identified.

The cryptic CNAs detected using CGH include the following. In patient 2 with 45,X,-Y,t(8;21)(q22;q22), CGH detected additional small deletions in 1q42.13-q42.2 (2.24 Mb). In patient 8, who demonstrated 47,XX,t(6;9)(p23;q34),+add(21)(q21) according to MC analysis, CGH revealed that the addition of 21q21 was actually the gain of 13q21.32-q34. In patient 14, initial BM examination revealed acute myelofibrosis without evidence of hematological malignancy. Using the samples collected during the initial BM examination, MC analysis demonstrated a normal karyotype whereas CGH detected trisomy 8 and 19. Subsequent BM examination performed 2 months later using MC revealed acute megakaryoblastic leukemia with 54,XX,+2,+6,+7,+8,+8,+19,+19,+22. Table 2 summarizes all of the CNAs detected using MC and CGH.

### LOH identification

Using Agilent SurePrint G3 Cancer CGH+SNP 4 × 180K microarrays, LOHs > 5 Mb were detected in 12 regions in 8 of 18 pa-





**Fig. 1.** Genetic aberrations detected using CGH+SNP microarray analysis. (A) Copy loss at 12p13.33-p12.3 in patient 6. (B) Large terminal LOH in 9p in patient 15. (C) Homozygous acquisition of chromosome 6 in patient 12. CGH, comparative genomic hybridization; SNP, single-nucleotide polymorphism; LOH, loss of heterozygosity.

tients (44%), and LOHs > 10 Mb were detected in 6 regions in 5 of 18 patients (28%). Of 12 LOHs, 9 LOHs were involved in interstitial segments, 2 in terminal regions, and 1 in whole chromosomes. Thus, LOHs > 10 Mb that involved terminal regions or whole chromosome were detected in 3 regions in 3 of 18 patients (17%; Table 2). Of 12 LOHs, 1 LOH was a copy-gain LOH involving trisomy 6 and the others were CN-LOHs. Three of 8 patients demonstrated > 1 LOH. LOH affected the chromosomal arms of 1p, 5q, 9p, 7q, 13q, 6,15q, 18q, Xp, and Xq. Recurrent LOH was also observed at 1p33-p32 ( $n = 2$ ).

Notably, of 3 patients with the *FLT3*-ITD mutation (patients 5, 9, and 10), patient 9 demonstrated LOH 13q-harboring *FLT3*, which resulted in a high allelic ratio of 1.847; however, patient 5 did not demonstrate LOH and patient 10 demonstrated a small LOH at 7q. Patient 9 achieved delayed CR after 3 cycles of induction chemotherapy and experienced early relapse. This patient subsequently died of refractory disease.

Among patients with acute basophilic leukemia (patients 6 and 15), only patient 6 demonstrated LOH at Xq22, and survived in CR after receiving allogeneic HSCT. Patient 15 demonstrated 2 LOHs involving 1p33-p32 (described above as recurrent LOH) and 9pter-p13, which is a telomeric defect > 30 Mb (Fig. 1B). Notably, patient 15 demonstrated the aberrant expression of lymphoid antigens (CD10 and CD19) at diagnosis. Patient 15 demonstrated early relapse during consolidation chemotherapy and subsequently died of refractory disease.

MC analysis determined trisomy 6 in patient 12, which resulted from LOH and concomitant isochromosome formation according to SNP microarray analysis (Fig. 1C). Patient 12 relapsed 8 months after receiving allogeneic HSCT and received reinduction chemotherapy followed by a second round of allogeneic HSCT.

#### Comparison of CGH+SNP microarray and MC analyses

Table 3 compares MC and CGH+SNP microarray analysis for the detection of cytogenetic defects. MC detected 11 CNAs in 8 patients, while CGH+SNP microarray revealed 14 CNAs in 9 patients as well as 12 LOHs in 8 patients. Of 5 patients with normal karyotypes (patients 10, 14, 15, 16, and 17), CGH+SNP microarray revealed LOH in 2 patients (patients 10 and 15) and LOH and genomic gain in 1 patient (patient 14), revealing that 3 patients actually had genetic abnormalities. Of 13 patients with abnormal MC, CGH+SNP microarray detected additional, previously cryptic CNAs ( $n = 2$ ) and LOHs ( $n = 5$ ) in 6 patients. In total, 9 patients demonstrated additional aberrations, including CNAs ( $n = 3$ ) and/or LOHs ( $n = 8$ ).

#### Clinical impact of lesions identified using CGH+SNP microarray

Of 5 patients with normal karyotypes determined using MC analysis, 2 patients without hidden genetic abnormalities survived after receiving allogeneic HSCT. Contrarily, 3 patients with LOH and/or CNA detected using CGH+SNP microarray died of refractory disease (patients 10 and 15) or pulmonary hemorrhage following allogeneic HSCT (patient 14). All 3 patients with LOH > 10 Mb involving the terminal region or whole chromosome demonstrated relapse (patient 12) or disease progression (patients 9 and 15; Table 2). Analyzing the impacts of LOH on the survival outcome in 15 patients with AML, 3 patients with terminal LOH > 10 Mb showed significantly inferior relapse-free survival (RFS) rate ( $P = 0.041$ ). Overall survival did not differ between patients with or without terminal LOH > 10 Mb (Fig. 2).

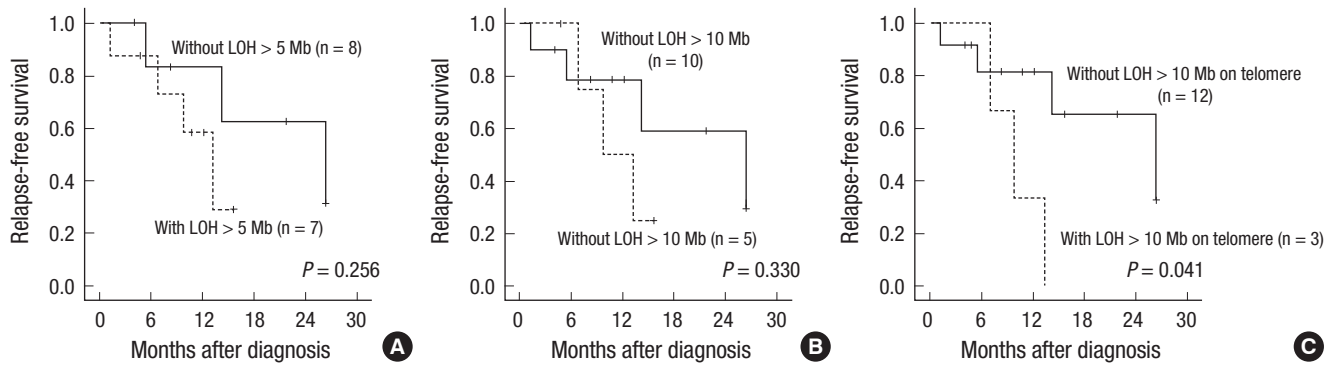


Fig. 2. LOH and relapse-free survival in patients with acute myeloid leukemia. LOH, loss of heterozygosity.

## DISCUSSION

In this study, genetic aberrations were analyzed in children with AML or MDS using high-resolution genome-wide CGH+SNP microarray. Our results show that CGH+SNP microarray not only confirms the CNAs detected by MC analysis but also simultaneously identifies cryptic CNAs and CN-LOHs. CGH+SNP microarray detected more CNAs than MC analysis (14 CNAs in 9 patients vs. 11 CNAs in 8 patients) and revealed LOHs that were not detected by conventional cytogenetic analysis in 8 of 18 patients (44%). Notably, 3 of 5 patients with normal karyotypes demonstrated cryptic genetic aberrations, which underscores the genetically heterogeneous nature of cytogenetically normal AML. Moreover, CGH+SNP microarray facilitated the detection of additional previously cryptic abnormalities in patients with abnormal karyotypes and determined the exact sites of chromosomal abnormalities, which were erroneously detected by MC analysis.

Previous studies show that SNP microarray and oligonucleotide CGH can more sensitively detect chromosomal abnormalities than MC analysis in AML and MDS patients (15-17). Cytogenetically normal AML (17, 18), as well as specific AML subtypes such as AML with complex karyotypes (19), core-binding factor AML (20), acute promyelocytic leukemia (21), and secondary AML (15), reportedly demonstrate hidden genetic alterations that can be detected using array CGH and/or SNP microarray. In our present study, patients within the same WHO classification, as well as those with normal karyotypes, demonstrated individually different, additional cryptic abnormalities according to CGH+SNP microarray. These results imply that clinical heterogeneity attributes to genetic aberrations in patients with similar MC results, especially patients with normal karyotypes.

The clinical implications of CGH and SNP microarray analyses have been evaluated in previous studies, which report the potential prognostic impact of previously cryptic CNAs and CN-LOHs (15, 16). However, because prognostic values were evaluated in various patient populations by diverse studies, the clinical

applications of such results are still limited. In normal-karyotype MDS, patients with cryptic genomic imbalances demonstrate inferior survival (16). Similarly, patients with SNP microarray lesions, including acquired somatic CN-LOH, exhibit a poorer overall survival and event-free survival than patients with primary AML and normal MC (15). It is notable that among 5 of our patients with normal MC, 3 patients with cryptic genetic aberrations did not survive while 2 patients without cryptic aberrations remain alive and relapse-free. Moreover, all 3 of our patients with large, terminal LOHs demonstrated significantly inferior RFS. These observations may imply the prognostic significance of cryptic genetic aberrations. However, it was difficult to evaluate the significance of the prognostic impact of hidden CNAs and CN-LOHs in our study population as it was small and heterogeneous. Thus, prognostic implication of cryptic genetic aberrations should be investigated in a larger homogeneous population.

In our present study series, LOHs > 5 Mb were detected at 12 regions in 8 of 18 patients (45%), and LOHs > 10 Mb were detected in 6 regions in 5 of 18 cases (28%). This study, however, did not include matched DNA controls because of its retrospective design, and LOH frequency was most likely overestimated. When more stringent LOH size and location criteria were applied, LOHs > 10 Mb involving the terminal regions or whole chromosome were detected in 17% of patients. This is consistent with previous studies reporting that de novo AML is characterized by a low burden of genomic alterations in comparison with pediatric acute lymphoblastic leukemia (ALL) (22).

The detection of recurrent somatic LOH has contributed to the identification of novel mutations associated with myeloid oncogenesis, such as *JAK2* in MPD (23) and *TET2* (24) and *FZH2* (25) in AML. In addition, recurrent somatic LOH indicates a mechanism by which leukemic cells can increase the dose of the mutated gene (15). The individual cases in this study may suggest a mechanism whereby genetic mutations in known oncogenes or suppressors can increase, contributing to leukemogenesis and phenotypic variations in same subtypes.

Patient 9 demonstrated LOH 13q containing *FLT3*, which re-

sulted in a high allelic *FLT3*-ITD ratio. Previous studies report that LOH 13q is associated with homozygous *FLT3*-ITD mutation; thus, the presence of LOH 13q may be linked to clinical outcomes in patients with *FLT3*-ITD mutation (26).

Patient 12 also demonstrates an important revelation regarding leukemogenesis. Trisomy 6 has been reported as a nonrandom primary numerical anomaly in myeloid disorders that abrogates the usual favorable prognosis associated with *RUNX1/RUNX1T1* in AML patients (27, 28). As putative candidate genes, *IRF4* and *DEK* in 6p25.3 and 6p22.3, respectively, may be associated with myeloid leukemogenesis and prognosis in AML with trisomy 6. The role of trisomy 6 in leukemogenesis and its impact on prognosis can be hypothetically attributed to increased oncogenes in chromosome 6, which is caused by LOH and concomitant isochromosome formation. LOH in chromosome 6 in AML patients with trisomy 6 has not been described in other studies. This implies that leukemogenesis in other trisomy karyotypes could be explained by the imprinting effects of certain genes due to acquired trisomy-caused LOH.

This study included 2 patients with acute basophilic leukemia (patients 6 and 15). CGH+SNP microarray revealed different genetic profiles that may underlie differences in phenotypes and genetic aberrations. Patient 6 demonstrated *MLL* rearrangement and LOH Xq22.1, which harbors *BEX1*. Interestingly, *BEX1* expression levels in *MLL*-rearranged AML cells are higher than in wild-type-*MLL* AML cells, and *BEX1* could play a role in AML leukemogenesis and *MLL* rearrangements (29). In addition, CGH demonstrated the loss of *ETV6*-harboring 12p13.3 in patient 6. The loss of *ETV6* is reportedly associated with acute basophilic leukemia (30). Patient 15 demonstrated LOH 9p, which harbors *JAK2*, *CDKN2A*, and *PAX5*. *CDKN2A* reportedly influences the risk of developing ALL (31). *PAX5* is also reportedly concurrently deleted in *CDKN2A* deletion in B-lineage ALL (32). Segmental LOH 9p could explain this concurrent deletion. It is notable that patient 9 demonstrated the aberrant expression of lymphoid antigens. This biphenotypic feature may be associated with LOH 9p and the harboring of tumor suppressors, which reportedly impact the risk of developing ALL.

In summary, our present findings show that CGH+SNP microarray is superior to conventional cytogenetic analysis for detecting genetic aberrations (other than balanced translocations) in childhood AML and MDS. CGH+SNP microarray simultaneously detects previously cryptic CNAs and LOHs, thereby decreasing the reported proportion of patients with a normal karyotype. Moreover, segmental LOHs harboring oncogenes or tumor suppressors associated with leukemogenesis may implicate pathogenic and prognostic significance. In order to refine the prognostic significance and delineate the pathogenic role of hidden genetic aberrations in childhood AML and MDS, a prospective study on a larger pediatric population with matched DNA samples is needed.

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## DISCLOSURE

The authors have no relevant financial relationships to disclose or conflicts of interest to report.

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