ORIGINAL ARTICLE

Microarray-Based Genomic Analysis Identifies Germline and Somatic Copy Number Variants and Loss of Heterozygosity in Acute Myeloid Leukaemia

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ABSTRACT

Introduction: Insights into molecular karyotyping using comparative genomic hybridization (CGH) and single nucleotide polymorphism (SNP) arrays enable the identification of copy number variations (CNVs) at a higher resolution and facilitate the detection of copy neutral loss of heterozygosity (CN-LOH) otherwise undetectable by conventional cytogenetics. The applicability of a customised CGH+SNP 180K DNA microarray in the diagnostic evaluation of Acute Myeloid Leukaemia (AML) in comparison with conventional karyotyping was assessed in this study. **Methods:** Paired tumour and germline post induction (remission sample obtained from the same patient after induction) DNA were used to delineate germline variants in 41 AML samples and compared with the karyotype findings. **Results:** After comparing the tumour versus germline DNA, a total of 55 imbalances (n 5-10 MB = 21, n 10-20 MB = 8 and n >20 MB = 26) were identified. Gains were most common in chromosome 4 (26.7%) whereas losses were most frequent in chromosome 7 (28.6%) and X (25.0%). CN-LOH was mostly seen in chromosome 4 (75.0%). Comparison between array CGH+SNP and karyotyping revealed 20 cases were in excellent agreement and 13 cases did not concord whereas in 15 cases finding could not be confirmed as no karyotypes available. **Conclusion:** The use of a combined array CGH+SNP in this study enabled the detection of somatic and germline CNVs and CN-LOHs in AML. Array CGH+SNP accurately determined chromosomal breakpoints compared to conventional cytogenetics in relation to presence of CNVs and CN-LOHs.

Keywords: Acute myeloid leukaemia, Copy number variants, Loss of heterozygosity, Microarray

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INTRODUCTION

Acute myeloid leukaemia (AML) is a heterogeneous malignant haematopoietic disorder that is characterized by an increase in immature myeloid cells. It is a result of an arrest of normal cell differentiation in the bone marrow. AML is also known as a disease marked by heterogeneity in diagnosis, classification, response to therapy and survival. Recent insights into the genomic landscapes of AML have led to tremendous advancement in understanding the molecular pathogenesis of this disease. Currently, the state-of-the-art in the diagnosis of AML relies on the integration of clinicopathological findings which include morphologic assessment, immunophenotypic analysis, and genetic studies.

The advent of molecular cytogenetics using comparative genomic hybridization (CGH) and a similar microarray analysis of single nucleotide polymorphism (SNP) has permitted comprehensive genome-wide assessments at resolutions higher than conventional cytogenetics. Molecular karyotyping enables the elucidation of genetic alterations that may have a significant role in the pathogenesis of AML and could lead to better stratification of diagnosis and prognosis. In terms of resolution, molecular karyotyping allows the detection of genomic lesions of ~ 400 kb in size, thus surpassing conventional cytogenetics (3-5 Mb) (1). Microarray platforms utilises customised probes that are designed down to a single exon resolution permitting detection of submicroscopic genetic lesions including microduplications and microdeletions that may be clinically relevant. Moreover, all DNA, tumour and non-tumour is represented and so there is no selection or bias. Besides copy number variations (CNVs), SNP – array facilitates the detection of uniparental disomy (UPD),

undetectable by conventional cytogenetics which are unable to detect copy neutral loss of heterozygosity (CN-LOH) (2-6).

Therefore, molecular karyotyping using a combination of CGH+SNP DNA microarray can complement conventional cytogenetics not only in the diagnosis but also in the classification and prognostication of AML. In addition, the discovery of cryptic chromosomal aberrations and novel disease related genomic regions is possible through the utilisation of CGH+SNP DNA microarray in a clinical setting. The present investigation identifies somatically-acquired genetic aberrations in AML by using CGH+SNP DNA microarray and compares the findings with karyotyping.

MATERIALS AND METHODS

Subject Recruitment and Selection

This cross-sectional study was performed on AML subjects (n=41) diagnosed and treated at Haematology Department, Hospital Ampang and Medical Department, Hospital Sultanah Aminah, Johor Baru over a period of 14 months (July 2013 till September 2014). Only cases with paired remission samples were included to facilitate determination of somatic aberrations. All cases were diagnosed by incorporating clinical and laboratory information which included morphologic assessment, flow cytometry immunophenotyping, conventional cytogenetics and molecular studies based on WHO Classification (2008) (7). Subjects of this study include 18 males (aged between 21 and 77 years with median of 54) and 23 females (aged between 14 and 71 years with median of 52). All samples were collected upon patient's informed consent during this study period. The Medical Research Ethics Committee of the Ministry of Health Malaysia approved this study (NMRR-12-844-12051) alongside the Research Ethics Committee Universiti Putra Malaysia (FPSK [EX14] P037).

DNA Extraction

DNA was extracted from bone marrow aspirates and peripheral blood samples using QIAamp® DNA Mini and Blood Mini Kit. Agilent Male and Female reference DNA supplied with the SureTag Complete DNA Labelling Kit were used as reference. The Agilent reference DNA used in this study were well characterised CNVs and CN-LOHs derived single individual normal male and female individuals of Caucasian ethnicity. Sample quantity and quality were assessed by UV-VIS Spectrophotometry (NanoDrop ND-1000), gel electrophoresis (2200 TapeStation) and Fluorometer (Qubit 2.0).

Custom Agilent SurePrint G3 CGH+SNP 180K Design

All samples were processed using custom Agilent SurePrint G3 CGH+SNP 180K (Agilent Technologies, Santa Clara, USA). This custom chip was designed in November 2013 based on Cancer Cytogenomics Microarray Consortium (CCMC) design and Human Genome 19 (Hg19), distributed by University of California, Santa Cruz (UCSC) genome browser (this build is derived from Genome Reference Consortium 37 (GRCh37) prepared by Genome Reference Consortium.

This platform allowed simultaneous detection of CNVs and CN-LOHs. Resolution of the SNP array ranged between 5 to 10 Mb. Additional custom probes for 47 genes were added to array with eleven genes covering every exon (TP53, DNMT3A, TET2, ASXL1, MLL, IKZF1, PAX5, EZH2, FLT3, NOTCH1 and ATM). The coverage of probes for the exon resolution gene is between 5-7 probes/exon. The other 36 genes are as follow: CEBPA, FHIT, ETV6, CBFB, NF1, MYC, ET52, RUNX1, RB1, CDKN2A, CCND1, PDGFRA, PDGRFB, FGFR1,ABL1, BCOR, BCORL1, BRAF,CBL, CCND3, EZH2, FAM5C, FBXW7, GATA1, HNRNPK, ID3, IDH1, IDH2, JAK2, KDM6A, KIT, KRAS, MPL, NPM1, NRAS, PHF6, PTPN1, RAD21, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, U2AF1, WT1, and ZRSR1.

Array CGH+SNP experiment was carried out based on the protocol provided by the manufacturer. Genomic DNA was subjected to restriction digestion using AluI and RsaI restriction enzymes which overlap with known SNP sites. Completeness of digestion was assessed before sample labelling by using DNA 1000 assay kit on Agilent 2100 Bioanalyzer. Array CGH required a two colour labelling protocol where the sample was labelled with Cy5 and gender matched Agilent's Human Reference DNA Male or Female was labelled with Cy3. Random primers and the exo-Klenow fragment were used to differentially label gDNA with fluorescent-labelled nucleotides. Nanodrop 2000 UV-VIS was used to analyse DNA yield, specific activity and degree of labelling before the sample and matched reference DNA were combined, followed by hybridization in the presence of Cot-1 DNA (Agilent Technologies, Santa Clara, USA) at 67°C for 24 hours. By utilizing Agilent microarray scanner, the slides were scanned at 3µm resolution followed by image processing via Agilent Feature Extraction Software version 12.0.0.7.

Array CGH+SNP Data Analysis

Analysis was integrated with Cytogenomics software version 2.9.2.4. Mosaic analysis method was selected for all sample analysis with an Aberration Detection Method-2 (ADM-2) algorithm, threshold set at 6.0, Fuzzy Zero switched off and GC correction setting was switched on. Result analysis was based on genome build Hg19.

We used Agilent reference DNA (male and female) derived from Caucasian population in this study as these reference DNAs are genotyped and well characterized by the manufacturer. To reduce the bias in the comparison of CNVs and CN-LOHs in Caucasian versus Malaysian genome, we have utilised a customised Malaysian normal recurrent CNVs customised genome track

created by Clinical Haematology Referral Laboratory, Hospital Ampang. This customised genome track are well characterised based on gender and ethnicity of Malaysians (10).

Paired Tumour and Germline DNA Analyses

Paired tumour and germline (post induction remission sample obtained from the same patient) DNA were used to delineate germline variants. Tumour and matched germline DNA aberrations and LOH intervals reports generated by Cytogenomics software version 2.9.2.4 were compared for all cases. Matching aberrations present in tumour and matched germline DNA were considered germline and changes present only in tumour were classified as tumour related changes.

In this study, apart from using SNP probes to detect the presence of CN-LOH, we used these probes to confirm the CGH probe findings in detection of chromosomal gains and deletion (CNVs). Hence, we focussed on aberrations above 5 Mb to match the resolution limit of SNP probes with the array CGH probes. Aberrations were then grouped according to sizes: less than one Mb, one to five Mb, five to ten Mb, 10 - 20 Mb, and larger than 20 Mb (8).

Exclusion of Germline Lesions for International System for Human Cytogenetic Nomenclature (ISCN), 2013

Karyotypes were retrieved (if available) and compared with array CGH+SNP findings. Data before and after exclusion of germline CNVs and SNPs were generated for each case to illustrate somatic nature of these lesions. Germline CNVs and SNPs were suppressed manually in the Cytogenomics software version 2.9.2.4 before generating ISCN, 2013 nomenclature for all the samples (9). This was to ensure the final data only represents the tumour related aberrations present in each sample.

Statistical Analyses

Classification of type of aberrations, grouping of aberrations based on sizes, location and coordinates were performed using Statistical Package of Social Science (SPSS) version 20 (SPSS Inc., Chicago, IL, USA).

RESULTS

Paired tumour and germline DNA analysis

After comparing tumour DNA versus germline DNA, a total of 55 imbalances (n 5-10 Mb = 21, n 10-20 Mb = 8 and n >20 Mb = 26) were identified. Regions with aberrations above 20 Mb were mostly losses (n=9 regions) whereas regions with smaller aberrations of 5-10 Mb were CN-LOH (n=9). Twenty eight regions with losses, 15 regions with gains and CN-LOH in 12 regions were detected. The most commonly gained chromosome was 4 (26.7%) whereas losses were frequent in chromosome 7 (28.6%) and X (25.0%). CN-LOH was mostly seen in chromosome 4 (75.0%). In chromosome 4, gains were seen in the p and q arms whereas in X chromosomes, losses of p and q arms were identified as the recurrent aberration in our cases series as shown in Table I

Germline Only Genomic Aberrations

Apart from identifying tumour specific genomic regions, we identified 23 genomic imbalances which were only seen in germline DNA (n 5-10 Mb = 14, n 10-20 Mb =3 and n >20 Mb = 6). Of these about 87 % were CN-LOH (n 5-10 Mb = 11, n 10-20 Mb = 3 and n >20 Mb = 6). However, there was no recurrent aberration seen in the germline samples. We have compared the germline CNVs identified in this study with a customised Malaysian normal recurrent CNVs and CN-LOH track established by Clinical Haematology Referral Laboratory Hospital Ampang which was established using 48 healthy and normal Malaysian adults (10). List of chromosomes, cytobands, coordinates and types of aberrations are summarized in Table II.

Exclusion of Germline Lesions in the CGH+SNP Analysis

Summary of the CGH+SNP results before and after exclusion of germline lesions based on International System for Human Cytogenetic Nomenclature (ISCN),

Table I: Recurrent genomic aberrations detected in tumour

Chromosome	Cytoband	Size (Mb)	Start	Stop	Aberration Type
4	p16.3 - p11	49.492586	12,440	49,505,025	Gain
4	p16.3 - p11	49.523083	12,440	49,535,522	Gain
4	q11 - q35.2	137.79361	52,672,433	190,466,045	Gain
4	q11 - q35.2	138.20208	52,672,433	190,874,516	Gain
X	p22.33 - p11.1	55.551544	2,695,374	58,246,917	Loss
X	p22.33 - p11.1	55.84845	2,695,374	58,543,823	Loss
X	p22.33 - p11.1	55.84845	2,695,374	58,543,823	Loss
X	q11.1 - q28	93.1679	61,934,507	155,102,406	Loss
X	q11.1 - q28	93.1679	61,934,507	155,102,406	Loss
X	q11.1 - q28	93.320806	61,781,601	155,102,406	Loss

Table II: Germline only genomic aberrations with cytoband, size, and coordinate

Chromosome	Cytoband	Size (Mb)	Start	Stop	Aberration Type
1	p31.3 - p31.1	6.019609	68,886,499	74,906,107	CN-LOH
1	q32.1 - q42.2	27.140594	205,441,074	232,581,667	CN-LOH
3	p14.2 - p13	11.357126	62,820,908	74,178,033	CN-LOH
3	q26.31 - q27.2	9.1492	175,384,006	184,533,205	CN-LOH
4	q21.1 - q32.1	81.924655	78,345,009	160,269,663	CN-LOH
4	q22.1 - q22.3	6.663701	91,297,445	97,961,145	CN-LOH
5	p15.2 - p14.3	10.975143	9,992,043	20,967,185	CN-LOH
6	q22.32 - q23.3	10.210033	126,895,368	137,105,400	CN-LOH
10	q22.3 - q24.33	27.418842	78,194,812	105,613,653	CN-LOH
10	q11.21 - q11.23	5.035224	45,904,882	50,940,105	Loss
11	q22.3	5.000256	103,948,375	108,948,630	CN-LOH
12	q21.31 - q21.33	5.306067	84,392,856	89,698,922	CN-LOH
12	q24.11 - q24.33	20.063904	109,937,907	130,001,810	CN-LOH
13	q14.3 - q21.2	5.448647	54,411,259	59,859,905	CN-LOH
14	q12 - q22.2	25.950688	29,504,400	55,455,087	CN-LOH
14	q21.2 - q22.1	6.106469	45,046,941	51,153,409	CN-LOH
15	q15.1 - q21.1	6.166393	41,069,619	47,236,011	CN-LOH
16	q21	5.277076	60,292,273	65,569,348	Gain
16	q23.3 - q24.3	5.055068	83,847,630	88,902,697	Loss
17	p13.1 - p11.2	7.496026	9,865,311	17,361,336	CN-LOH
17	q22 - q23.2	6.364245	53,010,381	59,374,625	CN-LOH
17	q22 - q24.1	6.655919	57,263,393	63,919,311	CN-LOH
18	q21.31 - q23	22.989711	54,989,859	77,979,569	CN-LOH
21	q11.2 - q22.2	26.932082	14,458,104	41,390,185	Gain
21	q21.3 - q22.12	7.403755	30,255,817	37,659,571	Gain
21	q22.2 - q22.3	6.6613	41,429,018	48,090,317	Loss

2013 as listed in Table III (9).

Comparison of CGH+SNP Analysis and Karyotyping

CGH+SNP results were compared with the conventional karyotyping results when available. Out of 41 cases, karyotyping results were normal in 17 cases, 9 cases showed structural aberrations, 7 cases had no analysable chromosome spreads and in 8 cases, karyotypes were not available. Excellent agreements between the karyotype and CGH+SNP results were observed in 20 cases, with CGH+SNP providing more precise breakpoint definition. In 13 cases karyotypes were not in agreement with CGH+SNP results; in 4 cases with normal karyotype, regions of CN-LOH were detected (S05,S09,S24,S26) and in another 3 cases array CGH+SNP detected aberrations which was missed by conventional karyotyping (S03,S31,S36). As expected, translocations were not detected by CGH+SNP in 6 cases. The array findings could not be confirmed in 15 cases (7 cases with no analysable chromosome spread by karyotyping and 8 cases with no results available). Table IV summarises CGH+SNP findings and subjects with abnormal karyotype.

DISCUSSION

Within 49% of the cases, the results of karyotype and CGH+SNP analysis were observed to be in concordance. Moreover, CGH+SNP analysis provided accurate breakpoint size and involved genes within chromosomal analysis. In 32% of the cases, however, karyotype findings did not match results of CGH+SNP analysis. Out of these cases, three cases (S03,S31,S36), displayed erroneous karyotypes due to failure to map the exact location of abnormalities and one of the cases showcased a complex karyotype. Additionally, four cases with normal-karyotype revealed CN-LOH regions available through CGH+SNP analysis, which would not be detectable using conventional cytogenetics methods. As expected, translocations seen in 6 cases were not detected by CGH+SNP analysis. CGH+SNP analysis also discovered chromosomal aberrations in five out of seven cases whereby analysable spreads were not available through conventional karyotyping means.

We have included cases with no karyotypes in this study as to reveal the benefits of array CGH+SNP in cases where no karyotypes are available due to technical and sampling reasons. Significant limitations are faced

Table III: CGH+SNP results before and after exclusion of germline lesions based on ISCN (2013)

Subject ID	Before Germline Exclusion	After Germline Exclusion
S01	arr Xp22.33(63,303-2,348,740)x1,Xq11.1q28(61,781,601-155,232,877)x1,Yp11.32p11.2(13,303-10,084,438) x1,Yq11.1q12(13,135,703-59,335,883)x1,2p23.3(25,456,917-25,537,174)x2,2p12p11.2(76,134,547-84,198,744) x2,2p11.2(89,185,302-89,319,978)x3,4q12q13.1(56,670,475-65,652,347)x2,4q27(121,348,002-121,533,345)x1,4q3 4.2q35.1(177,489,679-183,315,577)x2,6q15q16.1(91,670,945-97,101,702)x2,7p12.1p11.2(51,568,969-54,972,575) x2,8p23.1(7,239,491-7,779,266)x1,9q34.3(139,393,369-139,418,283)x2,10q11.22(46,938,469-47,717,349)x1,1 0q26.13(124,347,870-124,351,275)x2,14q32.33(106,331,956-107,216,227)x3,14q32.33(106,469,365-106,513,022)x3, 15q11.1q13.1(20,399,030-28,377,123)x2,16p13.11(16,364,101-16,772,356)x1,16p11.2p11.1(31,735,156-35,257,727) x2,16q21(60,107,797-65,919,653)x2,16q24.3(89,809,755-90,088,436)x2,17p13.1(7,572,822-7,590,998)x2,18p11.3 1p11.23(5,607,798-7,759,400)x2	arr(1-22)x2,(XY)x1
S02	arr Xp22.33p22.32(91,932-5,441,039)x2,2p23.3(25,457,245-25,497,999)x2,2p11.2(89,141,608-89,319,978) x4,8p22(15,952,011-16,021,744)x3,8p11.22(39,258,894-39,381,514)x10,8q23.3(116,493,571-117,094,928)x1,9p13. 1p11.2(38,812,449-46,953,126)x2,14q32.33(106,331,956-106,943,374)x3,14q32.33(106,469,365-106,513,022)x3,1 5q11.1q11.2(20,399,030-22,586,951)x4,17q11.2(29,546,107-29,562,744)x3,17q21.33(48,262,645-48,275,584)x2,2 2q11.22(23,056,562-23,244,636)x9	arr(1-22,X)x2
S03	arr Xp22.33(63,303-1,945,721)x2,Xp11.23(48,525,493-48,658,937)x3,2p23.3(25,456,917-25,537,174) x2,2p16.3(49,231,108-52,519,423)x2,2p11.2(89,141,608-89,319,978)x5,2p11.2p11.1(89,538,815-92,184,540) x2,4q13.2(70,108,664-70,223,408)x1,4q28.3(138,123,923-138,261,712)x1,4q34.3q35.1(177,998,382-183,337,339) x2,7p12.1p11.2(51,376,150-54,742,296)x2,8p22(15,952,011-16,021,744)x4,8p11.22(39,258,894-39,381,514)x10,8 q11.21q11.23(50,806,102-53,436,068)x2 hmz,8q24.3(145,532,050-145,897,770)x2,9p11.2(44,318,661-45,036,533) x3,9q34.3(139,390,541-139,418,283)x2,11p12(38,035,794-42,125,623)x2,14q21.2q21.3(45,979,670-48,518,396)x2, 14q32.33(106,037,979-106,239,525)x3,14q32.33(106,331,956-106,561,182)x3,14q32.33(106,375,298-106,449,894) x3,14q32.33(106,469,365-106,513,022)x3,14q32.33(106,566,837-106,943,374)x3,15q14(34,695,166-34,841,446) x1,16p11.2(32,015,694-34,363,720)x2,17p13.1(7,572,822-7,590,998)x2,17q21.33(48,263,136-48,275,584)x2,1 7q25.3(79,047,509-79,975,300)x2,22q11.21(19,707,321-19,716,812)x2,22q11.22(23,056,562-23,244,636)x10	arr 7p22.3p11.2(58,764-56,935,608)x3,7q 11.21q36.3(63,374,309-158,943,158)x1
S04	arr 1q21.1(144,950,149-145,081,881)x2,2p11.2(89,141,608-89,319,978)x3,5p13.3(29,327,285-29,562,431) x4,8p22(15,952,011-16,021,744)x4,8p11.22(39,258,894-39,381,514)x10,9p13.1p11.2(39,058,058-47,212,321)x2,1 4q32.33(106,331,956-106,544,481)x3,14q32.33(106,469,365-106,513,022)x3,14q32.33(106,636,701-106,943,374)x3,15 q11.1q11.2(20,055,137-22,698,581)x3,22q11.22(23,056,562-23,244,636)x9	arr(1-22,X)x2
S05	arr Xp22.33(63,303-2,865,335)x2,Xp11.4(38,577,856-40,329,863)x1,2p23.3(25,456,917-25,537,174)x2,2p 12p11.2(77,020,973-83,975,111)x2,2p11.2(89,141,608-89,319,978)x4,4q12q13.2(58,661,978-66,853,487) x2,8p22(15,952,011-16,021,744)x3,8p11.22(39,258,894-39,381,514)x10,8q23.2q23.3(111,730,471-114,131,155)x2, 9p13.1p11.2(38,459,475-47,212,321)x2,9q34.3(139,389,744-139,418,283)x2,10q26.13(124,314,524-124,395,668)x2 ,13q14.3q21.33(53,755,412-71,903,342)x2,14q11.2(19,376,762-20,421,677)x3,14q32.33(106,331,956-106,552,084) x3,14q32.33(106,469,365-106,497,204)x3,14q32.33(106,636,701-106,943,374)x4,16q21(60,292,273-65,569,348) x2,17p13.1(7,572,822-7,590,998)x2,17q22q24.1(57,455,620-63,176,847)x2 hmz,19p13.3(502,228-1,656,386)x2,2 2q11.1(16,114,195-16,434,984)x3,22q11.22(23,056,562-23,244,636)x10	arr 17q22q24.1(57,455,620-63,176,847) x2 hmz
S06	arr Xp22.33(63,303-2,656,392)x2,Xq27.2(140,673,376-140,738,473)x1,2p23.3(25,456,917-25,537,174) x2,2p11.2(89,141,608-89,319,978)x4,4p14(38,774,784-40,746,231)x2,4q12q13.2(58,415,025-67,199,889) x2,5q32(149,433,880-149,516,292)x2,7q11.23(73,396,886-73,481,111)x2,8p22(15,952,011-16,021,744)x4, 8p11.22(39,258,894-39,381,514)x10,8q24.3(145,737,350-145,743,935)x2,9p22.1p21.3(18,847,288-22,472,338)x2 hm z,9p22.1p21.3(19,600,306-22,347,561)x1,9p13.2(36,838,638-37,015,082)x2,9p13.1p11.2(39,167,334-45,783,387)x2 ,9q34.3(139,390,541-139,438,547)x2,10q26.13(124,291,833-124,396,755)x2,14q11.2(19,572,252-20,421,677)x2,1 4q11.2(22,571,278-23,421,023)x2,14q13.1q13.3(34,857,622-36,600,754)x2,14q32.33(105,052,722-106,243,254)x2, 14q32.33(106,331,956-106,526,266)x3,14q32.33(106,469,365-106,513,022)x3,14q32.33(106,636,701-106,943,374) x4,15q11.2(22,000,090-22,698,581)x2,16p13.3(2,103,321-2,138,687)x2,16p11.2(32,573,808-34,384,670) x2,16q21(60,211,425-65,545,445)x2,17p11.2p11.1(21,546,572-22,203,007)x3,17q21.33(48,263,136-48,275,584)x2,2 2q11.21(19,707,321-19,721,668)x2,22q11.22(23,056,562-23,244,636)x10	arr(1-22,X)x2
S07	arr 1p36.13(16,833,957-17,241,809)x2,2p25.3(63,431-386,519)x3,2p23.3(25,457,245-25,537,174) x2,2p11.2(89,141,608-89,319,978)x4,2p11.2p11.1(89,538,815-92,273,822)x2,5p14.3p14.1(19,877,286-24,646,268) x2 hmz,8p23.1(7,239,491-7,779,266)x3,8p22(15,952,011-16,021,744)x4,8p11.22(39,258,894-39,381,514)x10,9p 13.1p11.2(39,058,058-47,212,321)x2,9q34.3(139,390,541-139,440,753)x2,11p12(36,736,289-39,968,503)x2 hmz ,12p11.1(33,465,932-33,822,244)x3,14q11.2(19,376,762-20,421,677)x3,14q32.33(106,331,956-106,552,084)x3,1 4q32.33(106,469,365-106,513,022)x3,14q32.33(106,636,701-106,943,374)x4,15q11.1q11.2(20,399,030-22,297,110) x3,16p11.2(32,015,694-34,427,859)x2,17q21.33(48,263,136-48,275,584)x2,22q11.21(18,661,724-18,889,098)x1,2 2q11.22(23,056,562-23,244,636)x10,22q11.23(25,672,585-25,892,401)x1	arr(1-22)x2,(XY)x1
S08	arr Xp22.33(145,768-2,591,200)x2,2p23.3(25,458,523-25,537,174)x2,2p11.2(89,141,608-89,319,978) x4,4p16.3p11(12,440-49,505,025)x3,4q11q35.2(52,672,433-190,466,045)x3,5q23.1(121,034,626-121,315,329) x1,8p23.3p11.1(194,625-43,727,674)x3,8p23.1(7,303,187-7,779,266)x3,8p22(15,952,011-16,021,744)x3,8p11.2 2p11.1(39,189,481-43,520,355)x2 hmz,8p11.22(39,258,894-39,381,514)x3,8q11.1q24.3(46,924,418-146,293,435)x3,8q1 1.1q11.21(47,763,671-51,191,546)x2 hmz,9p13.1p11.2(39,167,334-47,212,321)x3,9q12q21.11(65,632,517-71,016,040) x3,9q34.3(139,390,541-139,418,283)x3,14q11.2(19,434,575-20,143,830)x3,14q32.33(106,331,956-106,943,374)x3 ,14q32.33(106,469,365-106,497,204)x3,16p11.2(32,015,694-34,427,859)x2,17q21.33(48,264,131-48,276,293)x2,2 1q21.3(29,011,058-29,281,955)x1,22q11.22(23,056,562-23,244,636)x10	arr 4p16.3p11(12,440-49,505,025)x3,4q 11q35.2(52,672,433-190,466,045)x3,8p 23.3p11.1(194,625-43,727,674)x3,8q11. 1q24.3(46,924,418-146,293,435)x3
S09	arr Xp22.33(145,768-888,218)x2,Xp11.3(43,599,360-44,036,619)x3,2p23.3(25,457,245-25,537,174) x2,2p11.2(89,141,608-89,319,978)x4,2p11.2p11.1(89,538,815-92,273,822)x2,3p12.1p11.1(87,074,534-90,064,515) x2 hmz,3q11.2(93,989,454-94,298,117)x2 hmz,7p14.1(38,295,198-38,382,567)x2,7q11.23(73,449,704-73,481,111) x2,8p22(15,952,011-16,021,744)x4,8p11.22(39,258,894-39,381,514)x10,8q11.21q11.23(50,060,960-53,880,247)x2 hmz,9p11.2(44,318,661-45,036,533)x3,9q34.3(139,390,541-139,418,283)x3,10q26.13(124,347,870-124,351,275)x5 ,12q13.2q14.1(56,069,231-61,972,806)x2 hmz,13q21.1(57,760,470-57,913,415)x1,14q11.2(19,572,252-20,421,677) x3,14q11.2(22,436,040-23,011,311)x2,14q32.33(106,331,956-106,552,084)x3,14q32.33(106,469,365-106,497,204)x3 ,14q32.33(106,636,701-106,943,374)x3,15q11.1q11.2(20,481,702-22,698,581)x3,17q21.33(48,263,136-48,277,213) x2,19p13.3(1,206,486-1,656,386)x2,21q11.2q21.1(15,580,827-18,422,045)x2 hmz,22q11.1(16,054,691-16,434,984) x3,22q11.21(18,661,724-18,889,098)x1,22q11.21(19,707,321-19,721,668)x3,22q11.22(23,056,562-23,244,636)x10,2 2q12.1(28,193,727-28,196,474)x3	arr 12q13.2q14.1(56,069,231-61,972,806) x2 hmz
S10	arr Xp22.33(145,768-2,390,502)x1,Yp11.32p11.2(95,768-10,045,809)x1,Yq11.223q11.23(24,108,372-27,617,606) x1,1q31.1(189,331,252-189,535,626)x1,1q43(237,633,579-240,599,264)x2,2p23.3(25,458,440-25,537,174) x2,2p11.2(89,185,302-89,319,978)x4,8p23.1(7,239,491-7,779,266)x1,8p11.22(39,258,894-39,381,514)x1, 9q34.3(139,390,541-139,438,547)x2,10q11.22(46,938,469-47,691,285)x1,10q26.13(124,347,870-124,351,275)x3 ,14q32.33(106,331,956-107,216,227)x3,14q32.33(106,469,365-106,513,022)x3,16q21(60,292,273-65,919,653) x2,17p13.1(7,572,822-7,590,998)x2,17q21.33(48,263,136-48,275,584)x2,21p11.2(10,743,033-10,838,151)x2	arr(1-22)x2,(XY)x1

(table continues)

Table III: CGH+SNP results before and after exclusion of germline lesions based on ISCN (2013) (*Continued*)

Subject ID	Before Germline Exclusion	After Germline Exclusion
S11	arr Xp11.22p11.21(50,325,888-57,999,932)x2 hmz,Xq11.2q12(63,663,978-67,645,007)x2 hmz,1p36.32(2,822,585-5,271,086)x2,1p36.23p36.22(8,180,442-10,490,014)x2,1p36.13(16,833,957-17,091,284) x3,1q43(237,728,098-240,076,771)x2,2p23.3(25,457,245-25,537,174)x2,2p11.2(89,141,608-89,319,978)x4,2 p11.2p11.1(89,342,480-92,035,200)x2,4p14(38,960,195-40,869,416)x2,7p12.1p11.2(51,670,577-55,357,325) x2,8p22(15,952,011-16,021,744)x4,8q24.22(132,311,479-136,004,054)x2 hmz,9q34.3(139,390,541-139,418,283)x2,1 2q24.11q24.13(110,641,965-112,939,841)x2,14q11.2(19,376,762-20,421,677)x3,14q32.33(106,331,956-106,526,266) x3,14q32.33(106,416,316-106,449,894)x3,14q32.33(106,469,365-106,497,204)x3,14q32.33(106,636,701-106,943,374) x4,16q21(59,804,745-65,569,348)x2,17q21.33(48,261,448-48,276,293)x2,22q11.1(16,054,691-16,434,984)x3,2 2q11.22(23,056,562-23,244,636)x10	arr(1-22,X)x2
S12	arr Xp22.33(145,768-2,656,392)x1,Yp11.32p11.2(95,768-10,084,438)x1,2p23.3(25,457,245-25,536,922)x2,2p1 2p11.2(82,457,898-85,503,996)x2 hmz,2p11.2(89,185,302-89,319,978)x3,5p13.1p11(41,623,186-46,105,865)x2 hmz,5q11.1q11.2(49,623,539-52,549,193)x2 hmz,7p12.2(50,344,456-50,344,689)x6,8p23.1(7,239,491-7,779,266) x2,8p11.22(39,258,894-39,381,514)x1,9p11.2(44,318,661-47,212,321)x2,9q34.3(139,390,541-139,440,753)x2,1 0q11.22(46,938,469-47,691,285)x2,11q22.3(103,709,577-107,118,116)x2 hmz,14q11.2(19,728,641-20,421,677)x3, 14q32.33(105,955,083-106,243,254)x2,14q32.33(106,331,956-107,211,941)x3,14q32.33(106,469,365-106,513,022) x3,14q32.33(106,636,701-106,803,307)x3,15q11.1q13.2(20,012,645-30,870,463)x2,16p12.2(22,563,832-22,739,647) x2,16p11.2p11.1(33,798,324-35,257,727)x2,16q21(60,292,273-65,569,348)x2,17q21.33(48,262,645-48,273,578)x2,1 7q25.3(79,047,509-79,979,742)x2,18p11.21(14,285,197-15,362,169)x2,20q11.21(30,946,056-31,021,801)x2,21p11. 2p11.1(9,412,632-11,151,933)x2,22q11.21(18,661,724-18,830,629)x1	arr(1-22)x2,(XY)x1
S13	arr Yq11.1q11.21(13,250,971-13,637,320)x1,1p21.3(96,737,383-99,657,787)x2 hmz,1q1 2q21.1(142,552,678-143,467,738)x3,2p23.3(25,462,140-25,537,174)x2,2p11.2(89,185,302-89,319,978) x3,2q21.2(132,649,661-132,917,197)x3,4p16.3(12,440-71,611)x3,4p16.3(1,785,020-1,807,211) x2,4p11(49,215,538-49,535,522)x3,4q35.2(190,465,986-190,678,708)x2,5q32(149,441,077-149,513,209) x2,7p22.2(2,949,734-2,985,479)x2,7q11.23(73,456,979-73,480,302)x2,8p23.1(7,239,491-7,779,266)x1, 8q24.3(145,736,666-145,742,554)x3,9p13.1p12(40,297,447-41,241,382)x2,9p12p11.2(43,464,701-43,910,995)x2 ,9q34.3(139,390,541-139,438,547)x3,10q11.22(46,938,469-47,691,285)x2,10q26.13(124,325,486-124,358,140) x3,11q13.4(71,752,020-74,336,144)x2,11q22.3(108,093,454-108,094,056)x2,12p11.21(31,108,073-32,538,445) x2,13q11(19,024,748-19,420,378)x3,14q11.2(19,159,168-20,143,830)x2,14q21.3q22.1(49,789,136-51,189,256)x2,14q 32.32q32.33(103,430,083-104,837,294)x2,14q32.33(106,110,414-106,239,525)x3,14q32.33(106,331,956-107,216,227) x3,14q32.33(106,469,365-106,513,022)x3,14q32.33(106,535,379-106,566,896)x1,14q32.33(106,636,701-106,803,307) x3,15q11.1q11.2(20,149,475-22,698,581)x3,15q14(34,695,166-34,841,446)x1,16p13.3(2,088,438-2,138,687) x2,16p11.2p11.1(31,986,127-34,777,244)x2,17p13.1(8,045,240-8,055,686)x2,17q11.2(29,557,005-29,562,744) x2,17q25.3(79,929,994-79,975,493)x2,18p11.21(14,196,900-15,325,187)x3,19p13.3(1,613,331-1,619,870) x2,19p13.3(4,361,166-4,364,108)x3,19q13.2(42,387,965-42,799,912)x3,19q13.2(42,415,076-42,780,391)x1,21p11. 2p11.1(9,412,632-11,176,309)x3,21q11.2q22.2(14,458,104-41,390,185)x3,21q11.2q21.1(15,336,412-18,485,980)x3, 21q21.1(20,967,012-21,288,932)x3,21q21.2q21.3(24,101,544-27,229,275)x3,21q21.3q22.12(30,255,817-37,659,571) x3,21q22.13q22.2(38,344,540-41,347,582)x3,21q22.2q22.3(41,429,018-48,090,317)x1,21q22. 2q22.3(41,464,836-47,376,507)x2 hmz,21q22.3(44,518,722-46,115,982)x1,21q22.3(47,737,355-47,863,878)x1,2 2q11.21(18,551,722-18,830,629)x1,22q12.1(28,193,727-28,197,407)x3	arr(1-22)x2,(XY)x1
S14	arr Xp22.33(63,303-2,656,392)x1,Yp11.32p11.31(13,303-2,606,392)x1,Yq11.1q11.21(13,250,971-13,872,561) x1,1p35.1(32,739,700-32,741,965)x3,1q12q21.1(142,552,678-145,311,102)x2,2p23.3(25,461,919-25,537,174) x3,2p11.2(89,185,302-89,319,978)x3,4p16.3p11(12,440-49,535,522)x3,4q11q35.2(52,672,433-190,874,516) x3,4q31.3(151,211,578-155,355,055)x3,5q32(149,433,880-149,514,558)x3,6q25.3(156,504,597-157,095,208) x1,7p22.2(2,949,734-2,985,479)x2,7p12.2(50,444,439-50,459,342)x3,7q11.23(73,432,241-73,483,107) x3,8p23.1(7,239,491-7,779,266)x1,8q24.3(145,736,666-145,741,874)x3,9p11.2(44,418,648-47,212,321)x3, 9q34.3(139,390,541-139,440,753)x3,10q26.13(124,325,486-124,381,604)x3,11p12(36,476,372-41,019,615)x2 hm z,11q13.4(71,715,018-71,725,437)x3,11q22.3(108,093,454-108,094,386)x3,14q32.2(98,129,865-99,360,391)x1,1 4q32.33(106,113,380-106,239,525)x3,14q32.33(106,331,956-107,216,227)x3,14q32.33(106,469,365-106,513,022) x3,14q32.33(106,636,701-106,803,307)x3,16p13.3(2,100,439-2,138,687)x3,16p13.11(15,476,833-16,311,070)x2 ,16p12.2(22,563,832-22,780,312)x3,16p11.2p11.1(31,986,127-35,257,727)x2,17p13.1(7,573,861-8,055,686)x2 ,17q12q21.2(37,811,339-38,513,367)x2,17q21.33(48,256,223-48,278,913)x3,17q25.3(79,941,446-79,975,300) x3,19p13.3(1,217,820-1,830,585)x2,19p13.3(4,361,166-4,364,108)x3,19q13.2(42,386,884-42,799,912)x3,1 9q13.32(45,855,524-45,873,671)x3,20q11.21(30,921,700-31,019,558)x2,22q11.21(18,551,722-18,830,629)x1,2 2q11.21(19,707,321-19,716,812)x3,22q11.22q11.23(23,244,577-23,632,565)x3,22q12.1(28,193,391-28,197,407)x3,2 2q12.3(36,680,285-36,722,637)x3,22q13.1(40,807,285-40,832,480)x3	arr 4p16.3p11(12,440-49,535,522)x3,4q1 1q35.2(52,672,433-190,874,516)x3
S15	arr 2p23.3(25,459,981-25,537,174)x2,2p11.2(89,185,302-89,319,978)x3,5p15.33(459,407-900,380)x3, 7q11.21(64,701,173-65,092,262)x3,8p23.1(7,239,491-7,779,266)x1,8p11.22(39,258,894-39,381,514)x1, 9q34.3(139,390,541-139,418,283)x2,10q11.22(46,938,469-47,691,285)x1,14q32.33(106,331,956-107,216,227)x3,1 4q32.33(106,469,365-106,513,022)x3,14q32.33(106,803,248-107,216,227)x3,15q11.2(22,297,051-22,586,951)x1,1 7q21.33(48,261,783-48,273,578)x2,21p11.2(10,710,871-10,838,151)x3,22q11.21(18,661,724-18,830,629)x1	arr(1-22)x2,(XY)x1
S16	arr Xp22.33(63,303-2,656,392)x1,Yp11.32p11.31(13,303-2,606,392)x1,1p36.32(3,258,786-4,533,747) x2,1p34.1(45,797,437-45,806,268)x2,1q23.1(156,833,328-156,846,353)x2,2p23.3(25,458,440-25,537,174) x3,2p23.2(29,177,012-29,629,281)x2,2p12p11.2(77,525,189-84,089,013)x2,2p11.2(89,185,302-89,319,978) x2,2p11.2(89,342,480-90,248,715)x1,2q13(110,862,477-110,964,737)x2,2q22.1(139,543,888-141,930,930) x2,3p21.31(48,712,000-48,727,183)x2,3p14.2p14.1(63,611,060-66,842,564)x2 hmz,4p16.3(12,440-56,887) x3,4p16.3(1,801,459-1,810,070)x2,4q12q13.2(58,415,025-67,472,979)x2,5q32(149,433,311-149,516,292)x3, 6p12.3p12.2(48,440,605-52,055,434)x2,6q16.1(95,456,808-95,662,059)x1,7p22.2(2,949,734-2,987,287)x2,7 p12.3p11.2(48,356,663-55,273,357)x2,7q11.23(73,442,449-73,481,111)x2,7q11.23(75,169,284-75,190,709) x2,8p23.2(3,047,146-5,302,666)x2,8p23.1(7,220,322-7,779,266)x1,8p11.23(38,270,907-38,287,231)x2, 8p11.22(39,258,894-39,381,514)x0,8q24.3(145,737,350-145,742,554)x2,9p13.2p11.2(36,839,052-47,212,321)x2, 9q12q21.11(65,632,517-70,901,873)x2,9q34.3(139,390,541-139,438,547)x3,10q11.21(43,600,689-43,620,437)x2, 10q11.22(46,938,469-47,672,307)x1,10q23.1(82,668,806-85,284,029)x2,10q24.32(104,154,391-104,162,707)x3,1 0q26.13(124,325,486-124,377,004)x3,11q13.4(71,715,018-71,747,017)x2,11q22.3(108,158,472-108,160,532)x1,1 2q13.13(53,554,538-53,566,453)x2,12q24.31(121,426,649-121,451,456)x2,14q32.33(105,052,722-106,269,317)x2 ,14q32.33(106,331,956-107,216,227)x3,14q32.33(106,469,365-106,561,182)x3,15q11.2(22,297,051-22,648,497) x1,15q26.1(91,428,387-91,447,053)x2,16p13.3(2,105,434-2,138,073)x3,16p13.13(10,989,225-11,017,773)x2 ,16p13.11(15,808,279-16,311,070)x2,16p13.11(16,364,101-16,772,356)x1,16p12.3(18,608,635-18,779,974) x1,16p12.2(22,563,832-22,758,443)x2,16p11.2(32,207,321-32,624,637)x2,16p11.2(33,304,631-33,773,163) x1,16q24.3(89,805,670-90,025,225)x2,17p13.1(7,572,822-8,055,686)x3,17q21.33(48,263,136-48,275,584)x3 ,17q25.3(79,941,446-79,975,300)x2,19p13.3(1,219,360-1,656,386)x2,19p13.11(17,935,889-18,896,635)x2,1 9q13.2(40,737,857-40,748,500)x2,19q13.2(42,386,884-42,799,051)x2,19q13.32(45,837,564-45,873,671)x2,1 9q13.33(48,228,949-48,260,496)x2,20q11.21(30,901,343-31,021,801)x2,21q22.3(47,731,338-47,930,431)x2,2 2q11.21(19,168,291-19,851,138)x2,22q11.23(23,574,219-23,657,804)x2,22q12.3(36,680,285-36,716,968)x2	arr(1-22)x2,(XY)x1

(table continues)

Table III: CGH+SNP results before and after exclusion of germline lesions based on ISCN (2013) (*Continued*)

Subject ID	Before Germline Exclusion	After Germline Exclusion
S17	arr Xp22.33(63,303-2,656,392)x1,Yp11.32p11.31(13,303-2,606,392)x1,1p31.1p21.2(70,314,219-102,056,219)x1,1p31.1p21.1(70,422,901-102,223,286)x2 hmz,1p21.1p13.3(103,253,503-108,146,890)x2,1q25.3(183,195,938-183,209,457)x2,2p23.3(25,461,919-25,536,922)x2,2p16.3(49,373,865-51,986,860)x2,2p11.2(89,185,302-89,319,978)x2,2q22.1(139,586,429-141,819,727)x2,3p26.3(1,601,269-2,562,334)x2,4q12q13.2(57,723,271-67,472,979)x2,4q24(106,067,203-106,068,168)x5,5q11.1q11.2(50,206,819-52,354,843)x2,5q13.2(68,849,594-70,636,824)x1,5q32(149,433,311-149,514,558)x2,6p12.3(48,440,605-51,649,477)x2,7p12.3p11.2(48,315,444-55,273,357)x3,7p12.2(50,344,456-50,344,689)x3,7q11.23(73,432,241-73,481,035)x2,8p23.1(7,220,322-7,779,266)x1,9p13.2(36,838,638-37,033,992)x2,9q34.3(139,390,541-139,438,547)x2,10q11.22(46,938,469-47,672,307)x1,10q24.32(104,154,391-104,162,707)x2,10q26.13(123,883,701-124,987,771)x2,11q21(95,889,755-95,961,627)x2,12q24.11q24.13(110,609,255-112,519,684)x2,14q32.33(104,708,728-106,251,883)x2,14q32.33(106,331,956-107,216,227)x3,14q32.33(106,469,365-106,513,022)x3,14q32.33(106,636,701-106,803,307)x3,15q11.2(22,297,051-22,648,497)x1,15q14(34,695,166-34,841,446)x2,16p13.3(2,088,438-2,138,687)x2,17p13.1(7,573,861-10,021,521)x2,17q21.33(48,263,136-48,275,584)x3,20q11.21(30,956,726-31,021,801)x2	arr 1p31.1p21.2(70,314,219-102,056,219)x1
S18	arr Xp22.33(63,303-2,656,392)x1,Xp11.23(48,525,493-48,891,441)x1,Yp11.32p11.31(13,303-2,606,392)x1,1p36.13(18,957,565-19,072,669)x2,1p36.13(19,969,814-19,984,527)x2,1p35.1(32,739,700-32,751,421)x2,1q21.2(148,609,572-149,224,043)x1,1q23.1(156,833,328-156,846,353)x2,2p23.3(25,457,245-25,537,174)x3,2p23.2(29,402,944-29,606,628)x2,2p11.2(89,185,302-89,319,978)x3,2p11.2(89,342,480-90,248,715)x1,2q13(113,860,604-114,046,312)x2,4p16.3(1,801,459-1,809,469)x2,4q24(106,067,203-106,068,228)x4,5q32(149,433,880-149,516,292)x2,7p12.2(50,332,185-50,459,474)x3,7p12.2(50,427,655-50,436,941)x1,7q11.23(73,442,449-73,481,111)x2,7q11.23(75,165,519-75,228,544)x2,7q36.1(148,504,728-148,515,262)x2,8p23.1(7,239,491-7,779,266)x1,8p11.22(39,258,894-39,381,514)x0,8q24.3(145,737,350-145,741,403)x2,9p13.2(36,839,459-37,034,645)x2,9q34.3(139,390,541-139,438,547)x3,10q11.21(43,571,616-43,623,741)x2,10q11.22(46,938,469-47,691,285)x1,10q24.32(104,154,391-104,162,707)x3,10q26.13(124,291,833-124,396,755)x2,11q13.4(71,715,797-71,747,017)x2,11q22.3(108,093,454-108,094,351)x2,12q24.31(121,426,649-121,439,580)x2,14q32.33(106,037,979-106,263,147)x2,14q32.33(106,331,956-107,216,227)x3,14q32.33(106,469,365-106,513,022)x3,14q32.33(106,531,557-106,561,182)x3,14q32.33(106,636,701-106,803,307)x3,14q32.33(106,906,901-107,216,227)x3,15q26.1(91,428,387-91,439,009)x2,16p13.3(2,105,434-2,138,687)x2,16q13q21(56,973,009-58,789,627)x2,16q24.3(89,388,113-89,799,712)x2,17p13.1(7,572,822-8,055,686)x2,17q12(36,861,875-36,880,910)x2,17q12(37,863,329-37,883,968)x2,17q21.33(48,263,136-48,275,584)x3,19p13.3(1,610,115-1,656,386)x2,19p13.3(1,830,527-4,349,889)x2,19p13.11(17,935,889-17,958,844)x2,19p13.11(18,848,025-18,892,515)x2,19q13.2(42,350,228-42,799,051)x2,19q13.32(45,837,564-45,873,671)x2,20q11.21(30,946,056-31,021,678)x2,21q22.12(36,245,802-36,295,397)x1,22q11.1(16,153,099-16,417,067)x1,22q11.21(18,661,724-18,830,629)x2,22q11.21(19,707,321-19,721,668)x2,22q12.1(28,194,834-28,197,407)x2	arr(1-22)x2,(XY)x1
S19	arr 1q21.2(149,041,013-149,202,620)x1,2p11.2(89,185,302-89,319,978)x3,2q14.2(121,886,806-122,336,492)x3,3p26.3(243,433-403,431)x2,8p23.1(7,239,491-7,779,266)x1,8p11.1(43,128,013-43,520,355)x2 hmz,8q11.1q11.21(46,940,022-51,547,523)x2 hmz,9p13.1(39,092,761-40,719,201)x2,9p12p11.2(43,315,599-43,874,898)x1,10q11.22(46,938,469-47,691,285)x1,10q26.13(124,347,870-124,351,275)x4,12p13.31p12.1(9,924,796-22,235,083)x1,14q11.2(19,255,744-20,421,677)x3,14q11.2(22,277,272-22,952,279)x2,14q32.33(106,331,956-107,216,227)x3,14q32.33(106,469,365-106,497,204)x3,14q32.33(106,469,365-106,561,182)x3,14q32.33(106,636,701-106,803,307)x10,14q32.33(106,850,403-107,211,941)x3,15q11.2(22,318,597-22,648,497)x1,17q21.31(44,485,771-44,762,618)x3,22q11.21(18,661,724-18,830,629)x1	arr 12p13.31p12.1(9,924,796-22,235,083)x1
S20	arr Xq27.2q27.3(141,690,336-145,591,175)x2 hmz,2p11.2(89,141,608-89,319,978)x4,2p11.2(89,538,815-90,003,752)x3,4q22.1q24(90,114,138-101,127,081)x2 hmz,6q15(89,272,006-91,954,911)x2 hmz,8p22(15,952,011-16,021,744)x4,8p11.22(39,258,894-39,381,514)x10,9p13.1p12(40,654,070-43,429,530)x2,14q11.2(19,255,744-20,421,677)x3,14q32.33(106,331,956-106,552,084)x3,14q32.33(106,357,649-106,392,806)x3,14q32.33(106,469,365-106,513,022)x3,14q32.33(106,636,701-106,943,374)x4,15q11.1q11.2(20,399,030-22,698,581)x3,22q11.21(18,661,724-18,830,629)x1,22q11.22(23,056,562-23,244,636)x10	arr 4q22.1q24(90,114,138-101,127,081)x2 hmz
S21	arr Xp22.33(63,303-1,945,721)x2,Xp11.23(48,525,493-48,658,937)x3,2p23.3(25,456,917-25,537,174)x2,2p16.3(49,231,108-52,519,423)x2,2p11.2(89,141,608-89,319,978)x5,2p11.2p11.1(89,538,815-92,184,540)x2,4q13.2(70,108,664-70,223,408)x1,4q28.3(138,123,923-138,261,712)x1,4q34.3q35.1(177,998,382-183,337,339)x2,7p12.1p11.2(51,376,150-54,742,296)x2,8p22(15,952,011-16,021,744)x4,8p11.22(39,258,894-39,381,514)x10,8q11.21q11.23(50,806,102-53,436,068)x2 hmz,8q24.3(145,532,050-145,897,770)x2,9p11.2(44,318,661-45,036,533)x3,9q34.3(139,390,541-139,418,283)x2,11p12(38,035,794-42,125,623)x2,14q21.2q21.3(45,979,670-48,518,396)x2,14q32.33(106,037,979-106,239,525)x3,14q32.33(106,331,956-106,561,182)x3,14q32.33(106,375,298-106,449,894)x3,14q32.33(106,469,365-106,513,022)x3,14q32.33(106,566,837-106,943,374)x3,15q14(34,695,166-34,841,446)x1,16p11.2(32,015,694-34,363,720)x2,17p13.1(7,572,822-7,590,998)x2,17q21.33(48,263,136-48,275,584)x2,17q25.3(79,047,509-79,975,300)x2,22q11.21(19,707,321-19,716,812)x2,22q11.22(23,056,562-23,244,636)x10	arr(1-22,X)x2
S22	arr Xp22.33(63,303-2,656,392)x1,Yp11.32p11.31(13,303-2,606,392)x1,2p23.3(25,456,917-25,537,174)x2,2p11.2(89,185,302-89,319,978)x3,6p22.1(29,675,515-30,098,664)x1,8p23.1(7,239,491-7,779,266)x1,8p11.22(39,258,894-39,381,514)x1,9q34.3(139,390,541-139,438,547)x2,10q11.22(46,938,469-47,691,285)x1,14q32.33(106,113,380-106,239,525)x3,14q32.33(106,331,956-107,216,227)x3,14q32.33(106,469,365-106,513,022)x3,14q32.33(106,636,701-106,803,307)x3,17q21.33(48,262,645-48,273,777)x2,22q11.21(18,661,724-18,830,629)x1	arr(1-22)x2,(XY)x1
S23	arr Xp22.33p11.1(2,695,374-58,246,917)x1,Xp11.23(48,013,124-48,896,732)x1,Xq11.1q28(61,934,507-155,102,406)x1,Xq11.2(63,409,439-63,429,474)x1,Xq26.1(129,199,147-129,244,885)x1,1q43(237,728,098-240,076,771)x2,2p23.3(25,457,245-25,537,174)x2,2p11.2(89,141,608-89,319,978)x4,2p11.2p11.1(89,538,815-92,184,540)x2,2q32.1q32.2(188,471,271-191,169,948)x2 hmz,8p23.1(7,239,491-7,779,266)x1,8p22(15,952,011-16,021,744)x4,8p11.22(39,258,894-39,381,514)x10,9p13.1p11.2(39,167,334-47,212,321)x2,9q13q21.11(66,151,746-71,046,091)x2,9q34.3(139,390,541-139,418,283)x2,12q12(41,634,082-43,750,710)x1,14q11.2(19,376,762-20,421,677)x3,14q32.33(106,037,979-106,243,254)x2,14q32.33(106,331,956-106,552,084)x3,14q32.33(106,469,365-106,513,022)x3,14q32.33(106,566,837-107,124,579)x2,16p11.2(32,015,694-34,384,670)x2,17q21.33(48,261,783-48,275,584)x2,22q11.22(23,056,562-23,244,636)x10	arr Xp22.33p11.1(2,695,374-58,246,917)x1,Xq11.1q28(61,934,507-155,102,406)x1
S24	arr 2p11.2(89,185,302-89,319,978)x4,4q12q13.1(58,728,642-64,671,240)x2 hmz,4q21.23q22.2(86,243,959-94,736,376)x2 hmz,4q23q25(100,500,130-108,394,042)x2 hmz,4q25(108,624,907-113,483,843)x2 hmz,4q26(116,914,182-120,612,158)x2 hmz,4q28.1q28.2(124,165,289-129,503,678)x2 hmz,4q31.21q31.3(144,333,710-152,861,685)x2 hmz,4q32.1q33(157,578,308-170,984,825)x2 hmz,4q34.1q35.2(172,152,981-190,449,761)x2 hmz,8p23.1(7,239,491-7,779,266)x1,8p11.22(39,258,894-39,381,514)x1,9p23(11,952,522-12,163,289)x1,9p13.1p11.2(38,459,475-47,212,321)x2,10q11.22(46,938,469-47,691,285)x1,11p12(37,610,467-40,278,016)x2 hmz,14q32.33(106,331,956-107,216,227)x3,14q32.33(106,469,365-106,561,182)x3,16p11.2p11.1(32,550,921-35,257,727)x2,17q21.33(48,263,792-48,274,043)x2	arr 4q12q13.1(58,728,642-64,671,240)x2 hmz,4q21.23q22.2(86,243,959-94,736,376)x2 hmz,4q23q25(100,500,130-108,394,042)x2 hmz,4q28.1q28.2(124,165,289-129,503,678)x2 hmz,4q31.21q31.3(144,333,710-152,861,685)x2 hmz,4q32.1q33(157,578,308-170,984,825)x2 hmz,4q34.1q35.2(172,152,981-190,449,761)x2 hmz

(table continues)

Table III: CGH+SNP results before and after exclusion of germline lesions based on ISCN (2013) (*Continued*)

Subject ID	Before Germline Exclusion	After Germline Exclusion
S25	arr 2p23.3(25,456,917-25,537,174)x2,2p11.2(89,141,608-89,319,978)x4,7p14.1(38,295,198-38,378,991) x1,8p22(15,952,011-16,021,744)x4,8p11.22(39,258,894-39,381,514)x10,9p21.3p11.2(20,574,205-47,212,321)x2,9q2 1.2q21.32(80,617,300-85,947,216)x2,11q23.3q25(119,172,038-134,934,196)x2,14q11.2(19,709,805-20,421,677)x3, 14q11.2(22,358,442-23,019,786)x1,14q32.33(106,331,956-106,552,084)x3,14q32.33(106,375,298-106,392,806)x3,1 4q32.33(106,416,316-106,465,578)x3,14q32.33(106,469,365-106,513,022)x3,14q32.33(106,555,946-106,943,374)x2 ,15q15.1(40,494,506-42,062,587)x2,16q21(62,851,908-65,712,545)x2 hmz,17q21.33(48,264,359-48,274,043)x2,17q2 2q24.1(57,263,393-63,919,311)x2 hmz,22q11.22(23,056,562-23,244,636)x10	arr 9q21.2q21.32(80,617,300-85,947,216) x3
S26	arr 2p11.2(89,185,302-89,319,978)x4,7q21.12q21.13(87,148,265-90,826,257)x2 hmz,8p23.2(2,351,976-2,794,488) x3,8p23.1(7,239,491-7,779,266)x1,8p11.22(39,258,894-39,381,514)x0,9q34.3(139,393,586-139,438,408)x2,1 0q11.22(46,938,469-47,691,285)x1,10q21.1(57,630,800-60,333,847)x2 hmz,10q26.13(124,347,870-124,351,275) x4,10q26.3(135,252,867-135,386,958)x3,12q21.31q21.33(84,392,856-89,698,922)x2 hmz,14q1 2q22.2(29,504,400-55,455,087)x2 hmz,14q32.33(106,331,956-107,216,227)x3,14q32.33(106,469,365-106,513,022)x3,1 4q32.33(106,636,701-106,803,307)x3,14q32.33(106,850,403-107,216,227)x3,16p11.2p11.1(32,015,694-35,257,727)x2, 18q21.2q21.31(48,917,970-54,128,364)x2 hmz,22q11.21(18,661,724-18,830,629)x1	arr 18q21. 2q21.31(48,917,970-54,128,364)x2 hmz
S27	arr Xp22.33(145,768-2,778,548)x2,2p23.3(25,456,917-25,537,174)x2,2p11.2(89,141,608-89,319,978) x5,8p23.1(7,220,322-7,779,266)x3,8p22(15,952,011-16,021,744)x4,8p11.22(39,258,894-39,381,514) x10,9p23(10,273,068-13,331,466)x2 hmz,9q34.3(139,390,541-139,438,371)x2,11q22.3(108,127,062-108,173,473)x2,1 1q23.3(118,338,407-118,354,958)x4,14q21.2q22.1(45,046,941-51,153,409)x2 hmz,14q32.33(106,331,956-106,552,084) x3,14q32.33(106,375,298-106,392,806)x3,14q32.33(106,416,316-106,449,894)x3,14q32.33(106,469,365-106,513,022) x3,14q32.33(106,555,946-106,943,374)x3,15q11.1q11.2(20,399,030-22,558,756)x3,17p13.1(7,572,822-7,590,998)x3,1 7q21.33(48,264,131-48,274,043)x2,22q11.22(23,056,562-23,244,636)x10	arr(1-22,X)x2
S28	arr Xp22.33(63,303-2,865,335)x2,Xq21.31(90,058,366-91,519,765)x1,1p36.32(2,335,096-4,901,389)x2 ,1q21.2(149,041,013-149,202,620)x1,2p23.3(25,456,917-25,537,174)x2,2p12(78,658,294-78,833,712) x1,2p11.2(89,141,608-89,319,978)x4,6p22.2p21.33(26,311,321-30,467,695)x2 hmz,7p14.1(38,295,198-38,369,498) x1,8p22(15,952,011-16,021,744)x4,8p11.22(39,258,894-39,381,514)x10,10q23.31(89,644,976-91,626,668)x2,1 4q11.2(19,434,575-20,143,830)x2,14q11.2(22,358,442-23,019,786)x1,14q32.33(106,037,979-106,232,567)x3,1 4q32.33(106,331,956-106,552,084)x3,14q32.33(106,357,649-106,465,578)x3,14q32.33(106,469,365-106,513,022) x3,14q32.33(106,636,701-106,943,374)x4,15q11.2(22,318,597-22,586,951)x1,16p11.2(33,339,542-34,363,720)x2,1 7q21.33(48,261,783-48,275,584)x2,22q11.21(19,707,321-19,825,562)x2,22q11.22(23,056,562-23,244,636)x10	arr(1-22,X)x2
S29	arr Xp22.33(63,303-2,656,392)x1,Yp11.32p11.31(13,303-2,606,392)x1,2p23.3(25,456,917-25,537,174) x2,2p11.2(89,185,302-89,319,978)x3,6p22.1(29,675,515-30,098,664)x1,8p23.1(7,239,491-7,779,266)x1, 8p11.22(39,258,894-39,381,514)x1,9q34.3(139,390,541-139,438,547)x2,10q11.22(46,938,469-47,691,285)x1,1 4q32.33(106,113,380-106,239,525)x3,14q32.33(106,331,956-107,216,227)x3,14q32.33(106,469,365-106,513,022)x3,1 4q32.33(106,636,701-106,803,307)x3,17q21.33(48,262,645-48,273,777)x2,22q11.21(18,661,724-18,830,629)x1	arr(1-22)x2,(XY)x1
S30	arr Yq11.21q11.223(13,675,923-22,550,312)x1,Yq11.223(23,021,108-23,114,008)x1,1p36.33p36.22(746,608-9,349,080) x2,2p23.3(26,692,246-26,801,287)x4,2p11.2(89,185,302-89,319,978)x4,2q21.1(130,907,973-131,144,665)x3,3p26. 3p11.1(68,644-90,489,421)x1,3p26.3p11.1(107,994-90,064,515)x2 hmz,3q11.1q29(93,538,467-197,840,323)x1,3q11. 2q29(93,989,454-197,802,470)x2 hmz,3q13.13q21.2(111,119,902-125,370,235)x1,3q22.1(129,739,996-130,003,876)x1, 5q14.3q35.3(85,701,372-180,696,806)x1,5q14.3q35.3(85,770,686-180,614,665)x2 hmz,5q32(149,427,775-149,545,238) x1,7p22.3p12.1(65,558-53,303,719)x1,7p22.3p21.3(90,167-8,717,542)x1,7p22.3p21.3(891,338-8,865,997)x2 hmz,7p21. 3p21.1(10,306,955-16,652,639)x2 hmz,7p21.3p21.1(10,649,696-16,535,901)x1,7p14.3p12.3(33,883,154-47,103,211)x2 hmz,7p14.3p12.3(34,145,878-47,097,701)x1,7p12.3p12.1(48,050,043-53,303,719)x1,7p12.2(50,444,542-50,459,342)x2, 7q21.11q36.3(85,631,245-159,030,335)x2 hmz,7q21.11q36.3(85,765,132-159,075,079)x1,8p23.1(7,239,491-7,779,266) x1,8p11.22(39,258,894-39,381,514)x1,9p23(10,721,924-12,405,079)x1,9p23(12,424,341-13,234,954) x3,9p22.3(15,870,462-16,343,955)x1,9p22.3p21.3(16,375,027-21,584,042)x3,9p22.3p22.2(16,401,595-17,869,257)x3 ,9p21.3p21.2(22,801,570-26,833,141)x2 hmz,9p21.3p21.2(22,822,144-26,851,028)x1,9p21.2(26,886,731-27,718,516) x3,9p21.1(28,606,661-28,932,450)x1,9p21.1(28,957,785-29,066,933)x4,9p21.1(29,085,216-29,544,123) x1,9p21.1(29,879,482-31,923,974)x3,9p21.1(31,341,152-31,923,974)x3,9p13.3(33,957,741-34,203,001)x4,9p13. 3p13.1(34,556,560-40,719,201)x1,9p13.3p13.2(35,113,110-36,620,362)x1,9p13.1(38,403,205-40,322,594)x1,9p1 2p11.2(43,315,599-43,841,603)x1,10q11.22(46,938,469-47,691,285)x1,10q26.13(124,347,870-124,358,140)x3,11p1 5.5p15.4(205,172-4,609,561)x2,12p13.33(1,176,383-2,387,033)x3,12p13.33p13.31(1,176,383-8,291,515)x3,12p13.3 3p13.31(2,704,800-8,048,500)x4,12p13.2p12.1(10,832,372-21,312,063)x1,12p13.2p12.1(10,847,527-21,320,686)x2 hm z,12p12.1(21,356,582-22,482,505)x3,12p12.1p11.23(25,889,551-26,713,340)x1,13q11q12.3(19,149,060-30,342,672) x3,13q11q12.13(19,227,564-26,035,359)x3,13q12.13q12.3(26,379,864-30,322,265)x3,13q12. 3q31.2(29,720,371-88,051,460)x2 hmz,13q12.3q31.1(30,404,904-87,111,281)x1,14q11.2(19,663,407-20,421,677)x1 ,14q32.33(106,331,956-107,216,227)x3,14q32.33(106,469,365-106,561,182)x3,14q32.33(106,803,248-107,211,941) x3,16p13.11(15,496,393-16,289,746)x3,19p13.3(715,326-1,207,182)x3,22q11.21(18,661,724-18,830,629)x1,2 2q11.21(21,536,794-21,704,972)x1,22q11.23(25,644,713-25,892,401)x3	arr Yq11.2 1q11.223(13,675,923-22,550,312)x1,3p 26.3p11.1(68,644-90,489,421)x1,3q11. 1q29(93,538,467-197,840,323)x1,3q13 .13q21.2(111,119,902-125,370,235)x1, 5q14.3q35.3(85,701,372-180,696,806) x1,7p22.3p12.1(65,558-53,303,719) x1,7p22.3p21.3(90,167-8,717,542)x1,7p2 1.3p21.1(10,649,696-16,535,901)x1,7p14 .3p12.3(34,145,878-47,097,701)x1,7p12. 3p12.1(48,050,043-53,303,719)x1,7q21.1 1q36.3(85,765,132-159,075,079)x1,9p22 .3p21.3(16,375,027-21,584,042)x3,9p13. 3p13.1(34,556,560-40,719,201)x1,12p13 .33p13.31(1,176,383-8,291,515)x3,12p1 3.33p13.31(2,704,800-8,048,500)x4,13q 11q12.3(19,149,060-30,342,672)x3,13q1 1q12.13(19,227,564-26,035,359)x3,13q12 .3q31.1(30,404,904-87,111,281)x1
S31	arr Xp22.33(63,303-2,865,335)x2,2p23.3(25,457,245-25,537,174)x3,2p11.2(89,141,608-89,319,978) x3,4p16.3(23,036-56,887)x3,4q24(106,067,203-106,068,168)x3,6q21(110,294,224-113,665,595)x2 hmz, 7p12.2(50,344,227-50,469,882)x3,7q11.23(73,449,704-73,481,035)x3,7q22.1q36.3(100,780,826-158,923,949)x1,7 q22.1q36.3(100,985,743-159,030,335)x2 hmz,7q33(137,560,258-137,593,146)x1,7q36.1(148,504,728-148,514,004) x1,8p22(15,952,011-16,021,744)x4,9p13.2(36,839,459-37,034,645)x3,9p13.1p11.2(38,459,475-47,212,321)x2, 9q34.3(139,390,098-139,438,547)x3,10q26.3(135,242,075-135,386,958)x3,11p15.5p11.12(192,358-51,538,651)x3, 11q11q25(54,909,688-134,934,196)x3,11q23.3(118,342,216-118,353,292)x3,14q11.2(19,794,577-20,338,802)x3,1 4q32.33(106,037,979-106,269,317)x3,14q32.33(106,331,956-106,943,374)x3,14q32.33(106,469,365-106,513,022) x3,15q25.3(86,809,339-86,980,779)x3,17p13.3p13.1(44,684-7,572,867)x1,17p13.3p13.1(72,083-7,940,754)x2 hmz,17p13.3(1,959,507-1,961,561)x3,17p12p11.2(13,230,329-20,927,835)x2 hmz,17p12p11.2(13,236,413-22,128,401) x1,17q11.1(25,458,873-25,569,094)x2 hmz,17q21.33(48,263,136-48,275,584)x3,19q13.41(53,052,678-53,433,028)x3,2 0q11.21(30,946,056-31,021,801)x2,22q11.22(23,056,562-23,244,636)x10	arr 7q22. 1q36.3(100,780,826-158,923,949)x1,11 p15.5p11.12(192,358-51,538,651)x3,11 q11q25(54,909,688-134,934,196)x3,17 p13.3p13.1(44,684-7,572,867)x1,17p1 2p11.2(13,236,413-22,128,401)x1
S32	arr Xq28(154,830,427-154,929,279)x3,2p11.2(89,141,608-89,319,978)x3,2p11.2p11.1(89,538,815-92,273,822) x2,8p22(15,952,011-16,021,744)x4,8p11.22(39,258,894-39,381,514)x10,9p13.1p11.2(39,167,334-47,212,321)x2, 14q11.2(19,376,762-20,143,830)x3,14q11.2(22,358,442-23,019,786)x2,14q32.33(106,331,956-106,943,374)x3,1 4q32.33(106,469,365-106,513,022)x3,14q32.33(106,555,946-106,712,967)x3,15q11.1q11.2(20,399,030-22,698,581)x3, 22q11.22(23,056,562-23,244,636)x10	arr(1-22,X)x2
S33	arr 2p25.3(10,702-664,362)x2,2p23.3(25,459,981-25,537,174)x2,2p11.2(89,141,608-89,319,978)x5 ,2p11.2p11.1(89,402,898-92,184,540)x2,4p16.3(12,440-56,887)x3,4p14(38,999,534-40,815,250)x2, 6q16.3(103,488,284-103,652,304)x1,8p22(15,952,011-16,021,744)x5,8p11.22(39,258,894-39,381,514) x10,9p13.1(38,694,064-38,710,922)x2 hmz,9p13.1p11.2(39,115,086-46,361,897)x2,9q21.1 1q31.1(71,046,033-103,362,618)x1,9q21.11q31.1(71,046,583-103,571,020)x2 hmz,9q34.3(139,390,541-139,438,408) x2,10q26.13(124,347,870-124,351,275)x1,14q11.2(19,376,762-20,421,677)x3,14q32.33(105,955,083-106,243,254)x2,1 4q32.33(106,331,956-106,552,084)x3,14q32.33(106,357,649-106,392,806)x3,14q32.33(106,416,316-106,449,894)x3,1 4q32.33(106,469,365-106,513,022)x3,14q32.33(106,636,701-106,943,374)x5,14q32.33(107,230,639-107,287,505)x3,18 q12.1q12.2(30,069,445-33,901,772)x2 hmz,22q11.22(23,056,562-23,244,636)x10	arr 9q21.1 1q31.1(71,046,033-103,362,618)x1

(table continues)

Table III: CGH+SNP results before and after exclusion of germline lesions based on ISCN (2013) (*Continued*)

Subject ID	Before Germline Exclusion	After Germline Exclusion
S34	arr ,1q12q21.1(142,552,678-144,931,798)x2,2p23.3(25,472,637-25,498,457)x2,2p11.2(89,185,302-89,301,214) x2,4p16.3(1,757,061-1,807,316)x2,4q13.1q13.2(61,127,613-70,298,721)x2 hmz,5q35.1(170,735,089-170,741,580) x2,7p12.2(50,344,456-50,344,689)x4,8p23.1(7,220,322-7,779,266)x1,9p13.1p11.2(39,008,321-47,212,321)x2,9q 12q21.11(65,632,517-70,403,378)x2,9q34.3(139,390,541-139,490,407)x2,10q11.22(46,938,469-47,691,285)x2,1 0q26.13(124,347,870-124,351,275)x1,14q11.2(21,575,069-23,731,373)x2,14q32.33(106,331,956-107,216,227)x3,1 4q32.33(106,469,365-106,561,182)x3,14q32.33(106,636,701-106,803,307)x3,14q32.33(107,001,431-107,203,073) x3,15q11.2(22,318,597-22,586,951)x1,16p11.2(33,304,631-33,773,163)x1,17q11.2(28,670,800-29,880,441) x1,19p13.3(1,191,934-1,232,206)x2,19q13.2q13.31(43,261,778-43,783,380)x2,21p11.2p11.1(9,647,088-11,151,933)x2, 22q11.21(18,661,724-18,830,629)x1,22q12.1(28,193,727-28,196,474)x2	arr 4q13.1q13.2(61,127,613-70,298,721) x2 hmz
S35	arr 1p36.13(16,833,957-17,051,239)x2,1p36.12(20,909,782-21,066,243)x3,2p23.3(25,457,245-25,537,174) x2,2p11.2(89,185,302-89,319,978)x3,4q24(106,067,148-106,068,168)x4,5q13.2(68,849,594-70,636,824) x1,7p12.2(50,344,456-50,344,689)x5,8p23.1(7,239,491-7,779,266)x1,9q34.3(139,390,541-139,418,283)x2,1 0q11.22(46,938,469-47,691,285)x1,10q22.2(76,788,224-76,790,091)x1,10q26.13(124,347,870-124,351,275)x5,1 4q32.33(106,331,956-107,216,227)x3,14q32.33(106,469,365-106,561,182)x3,15q11.1q11.2(20,481,702-21,941,153)x2, 15q11.2(22,297,051-22,669,111)x1,17q21.33(48,264,359-48,275,584)x2	arr(1-22)x2,(XY)x1
S36	arr 1q21.1(144,942,423-145,311,102)x3,2p11.2(89,141,608-89,319,978)x6,2q21.1q21.2(130,711,448-133,076,697)x2 ,5q14.3q34(83,516,470-162,772,648)x2 hmz,5q14.3q34(83,525,460-162,507,695)x1,7p12.2(50,344,456-50,344,689) x6,8p22(15,952,011-16,021,744)x6,8p11.22(39,258,894-39,381,514)x10,9p13.1p11.2(39,254,329-47,212,321)x2,1 4q11.2(19,376,762-20,421,677)x6,14q32.33(106,331,956-106,561,182)x3,14q32.33(106,469,365-106,513,022)x3,15q11 .1q11.2(20,399,030-22,698,581)x3,22q11.1(16,054,691-16,434,984)x3,22q11.22(23,056,562-23,244,636)x10	arr 5q14.3q34(83,525,460-162,507,695)x1
S37	arr 1p36.13(16,833,957-17,241,809)x3,1q21.2(149,041,013-149,202,620)x0,1q21.2(149,223,984-149,775,936) x1,2p23.3(25,458,440-25,537,174)x2,2p21(42,430,345-45,770,687)x2 hmz,2p11.2(89,141,608-89,319,978) x4,8p22(15,952,011-16,021,744)x4,8p11.22(39,258,894-39,381,514)x10,9p11.2(44,224,278-47,212,321)x2, 9q34.3(139,390,541-139,440,753)x2,14q11.2(19,376,762-20,421,677)x3,14q32.33(105,955,083-106,243,254)x2,1 4q32.33(106,331,956-106,552,084)x3,14q32.33(106,367,922-106,392,806)x3,14q32.33(106,416,316-106,465,578) x3,14q32.33(106,469,365-106,513,022)x3,14q32.33(106,555,946-106,943,374)x3,16p13.3(2,105,434-2,138,687)x2,1 7q21.33(48,263,136-48,278,913)x2,22q11.1(16,054,691-16,197,064)x3,22q11.22(23,056,562-23,244,636)x10	arr(1-22,X)x2
S38	arr 1q21.1(144,930,656-145,079,845)x2,2p11.2(89,141,608-89,319,978)x4,5q22.1q22.3(111,447,639-114,710,474) x2 hmz,6p22.2p22.1(25,739,644-29,503,782)x2 hmz,6p11.2(57,205,883-58,055,076)x3,8p22(15,952,011-16,021,744) x4,8p11.22(39,258,894-39,381,514)x10,9p12p11.2(43,540,034-47,212,321)x2,14q11.2(19,255,744-20,290,684)x3,1 4q32.33(106,331,956-106,552,084)x3,14q32.33(106,354,395-106,392,806)x3,14q32.33(106,469,365-106,513,022)x3, 14q32.33(106,636,701-106,943,374)x4,15q11.1q11.2(20,399,030-22,698,581)x3,16p11.2(32,207,321-33,798,368)x2,2 2q11.22(23,056,562-23,244,636)x10	arr(1-22,X)x2
S39	arr Xp22.33p11.1(2,695,374-58,543,823)x1,Xp22.2p21.3(10,715,887-25,472,209)x1,Xp11.23(48,665,599-48,880,703) x1,Xq11.1q28(61,781,601-155,102,406)x1,Xq24(118,767,374-118,832,973)x1,Xq26.1(129,199,147-129,244,885) x1,2p23.3(25,456,917-25,537,174)x3,2p23.2(29,419,621-29,606,628)x2,2p16.3p16.2(49,570,941-53,212,136) x2,2p11.2(89,141,608-89,319,978)x4,2q22.1(138,227,855-141,735,908)x2,2q32.3q33.1(196,064,399-199,776,769) x2 hmz,4q24(106,066,996-106,180,973)x2,5q23.1(120,179,961-120,274,616)x1,6p25.3(283,968-381,382)x1 ,7p22.1p21.3(6,916,324-7,335,136)x3,7q11.23(73,449,704-73,481,111)x2,7q21.11(78,781,210-84,891,396) x2,8p22(15,952,011-16,032,825)x4,8p11.22(39,258,894-39,381,514)x10,8q24.3(145,737,350-145,741,403) x2,9p13.2(36,839,459-37,034,645)x2,9p13.1p11.2(39,008,321-47,212,321)x2,9q34.3(139,390,541-139,438,547)x3 ,10p12.2(24,270,288-24,580,655)x1,14q11.2(19,255,744-20,143,830)x3,14q32.33(106,037,979-106,239,525)x3,1 4q32.33(106,331,956-106,544,481)x3,14q32.33(106,354,395-106,392,806)x3,14q32.33(106,469,365-106,513,022) x3,14q32.33(106,636,701-106,943,374)x2,16p11.2(32,207,321-33,773,163)x1,17p13.1(7,572,822-7,590,998) x3,17q12(36,859,669-36,884,594)x2,17q21.33(48,263,136-48,275,584)x3,19p13.3(1,206,486-1,663,934)x2,1 9p13.11(17,935,889-17,966,794)x2,19q13.2q13.32(42,350,228-45,873,671)x2,20q11.21(30,956,726-31,021,801)x2 ,20q13.32(57,414,967-57,953,455)x2,22q11.21(19,707,321-19,716,812)x3,22q11.22(23,056,562-23,244,636)x3,2 2q11.22(23,087,094-23,144,851)x3,22q11.22(23,152,416-23,201,852)x3,22q12.3(36,680,285-36,707,231)x2	arr Xp22.33p11.1(2,695,374-58,543,823) x1,Xp22.2p21.3(10,715,887-25,472,209) x1,Xq11.1q28(61,781,601-155,102,406)x1
S40	arr Xp22.33(63,303-2,656,392)x3,Xp11.23(48,542,216-48,547,818)x3,1p35.1(32,739,700-32,751,421) x2,1p34.1(45,797,437-45,806,268)x2,1q22(155,154,933-155,212,375)x2,1q23.1(156,833,328-156,846,353) x3,1q43(237,668,915-240,419,384)x2,2p23.3(25,456,917-25,537,174)x3,2p23.2(29,416,368-29,551,250) x2,2p11.2(89,141,608-89,319,978)x4,2p11.2(89,342,480-90,248,715)x1,2q14.3(127,807,997-127,828,379) x2,3p21.31(48,712,000-48,723,181)x2,3q29(194,826,801-195,789,500)x2,3q29(196,730,908-196,755,703) x3,4p16.3(12,440-45,941)x3,4p16.3(1,802,707-1,809,469)x2,4p14p13(38,830,948-41,269,183) x2,4q24(106,067,203-106,068,228)x3,5q32(149,433,880-149,516,292)x2,7p12.2p11.2(50,305,021-55,273,357) x3,7p12.2(50,427,655-50,436,941)x1,7q11.23(73,449,704-73,481,111)x3,7q11.23(75,176,243-75,189,138) x2,8p22(15,952,011-16,043,322)x4,8p11.22(39,258,894-39,381,514)x10,8q24.3(145,737,350-145,743,935) x2,9p13.3(35,074,930-35,088,472)x2,9p13.2(36,839,459-37,034,645)x3,9q22.32(98,201,651-98,513,438)x2, 9q34.3(139,390,541-139,438,547)x3,10q23.1(82,691,740-85,600,474)x2,10q24.32(104,154,391-104,161,803)x3 ,11q13.3(70,253,461-70,280,132)x2,11q13.4(71,715,018-71,729,992)x3,11q22.3(108,093,454-108,094,351)x2,1 2q13.3(57,853,745-57,864,423)x2,12q24.31(121,426,649-121,438,978)x2,14q11.2(19,794,577-20,421,677)x3,1 4q32.33(106,037,979-106,239,525)x3,14q32.33(106,331,956-106,555,991)x3,14q32.33(106,469,365-106,513,022) x3,14q32.33(106,561,123-106,943,374)x3,15q11.1q12(20,012,645-26,332,772)x2,15q26.1(91,428,387-91,437,286) x2,16p13.3(2,105,434-2,138,687)x3,16p13.13(10,989,225-11,017,773)x2,16p13.11(15,802,738-16,225,792) x2,16p11.2(32,015,694-34,427,859)x2,16q21(60,292,273-65,919,653)x2,16q24.3(89,322,147-89,357,532)x2 ,16q24.3(89,811,420-90,030,199)x2,17p13.1(7,572,822-7,590,998)x3,17q12(36,859,669-36,884,187)x2,17 q12q21.2(37,850,874-38,513,367)x2,17q21.33(48,263,136-48,275,584)x3,17q25.3(79,832,664-79,975,300) x2,18q21.2(50,181,426-51,053,152)x2,19p13.3(1,219,360-1,653,000)x3,19p13.3(4,360,346-4,366,948)x2,1 9p13.11(17,935,889-17,958,844)x3,19q13.2q13.32(42,386,884-45,873,671)x2,19q13.33(48,228,949-48,260,496)x2 ,20q11.21(30,946,056-31,021,801)x2,20q13.32(57,407,840-57,495,925)x2,22q11.1q11.21(17,593,629-18,889,098) x2,22q11.21(19,707,321-19,721,668)x3,22q11.22(23,056,562-23,244,636)x3,22q11.22(23,087,094-23,144,851) x3,22q11.22(23,152,416-23,201,852)x3,22q12.1(28,192,866-28,197,407)x2,22q12.3(36,680,285-36,707,231)x2,2 2q13.1(40,802,894-40,820,240)x2	arr(1-22,X)x2
S41	arr Xp22.33p11.1(2,695,374-58,543,823)x1,Xp11.23(48,074,226-48,896,732)x1,Xq11.1q28(61,934,507-155,102,406) x1,2p23.3(25,456,917-25,537,174)x2,2p11.2(89,141,608-89,319,978)x4,7q11.23(76,095,829-76,593,996) x1,8p22(15,952,011-16,021,744)x4,8p11.22(39,258,894-39,381,514)x10,9q34.3(139,390,541-139,418,283)x2,1 4q32.33(106,331,956-106,850,462)x3,14q32.33(106,469,365-106,497,204)x3,15q11.1q11.2(20,399,030-22,698,581) x3,16p11.2(32,573,808-34,363,720)x2,17q21.33(48,263,136-48,274,043)x2,22q11.21(19,707,321-19,716,812)x2,2 2q11.22(23,056,562-23,244,636)x10,22q13.32(48,655,121-48,771,433)x1	arr Xp22.33p11.1(2,695,374-58,543,823) x1,Xq11.1q28(61,934,507-155,102,406)x1

Table IV: CGH+SNP findings and subjects with abnormal karyotype

ID	After Germline Exclusion	Karyotype
S08	arr 4p16.3p11(12,440-49,505,025)x3,4q11q35.2(52,672,433-190,466,045)x3,8p23.3p11.1(194,625-43,727,674)x3,8q11.1q24.3(46,924,418-146,293,435)x3	48, XX, +4, +8 [13]
S17	arr 1p31.1p21.2(70,314,219-102,056,219)x1	46, XY, t(11;20)(p15;p11.2) [26]
S23	arr Xp22.33p11.1(2,695,374-58,246,917)x1,Xq11.1q28(61,934,507-155,102,406)x1	45, X, t(8;21)(q22;q22), der(4) dup(4q?) [12].
S30	arr Yq11.21q11.223(13,675,923-22,550,312)x1,3p26.3p11.1(68,644-90,489,421)x1,3q11.1q29(93,538,467-197,840,323)x1,3q13.13q21.2(111,119,902-125,370,235)x1,5q14.3q35.3(85,701,372-180,696,806)x1,7p22.3p12.1(65,558-53,303,719)x1,7p22.3p21.3(90,167-8,717,542)x1,7p21.3p21.1(10,649,696-16,535,901)x1,7p14.3p12.3(34,145,878-47,097,701)x1,7p12.3p12.1(48,050,043-53,303,719)x1,7q21.11q36.3(85,765,132-159,075,079)x1,9p22.3p21.3(16,375,027-21,584,042)x3,9p13.3p13.1(34,556,560-40,719,201)x1,12p13.33p13.31(1,176,383-8,291,515)x3,12p13.33p13.31(2,704,800-8,048,500)x4,13q11q12.3(19,149,060-30,342,672)x3,13q11q12.13(19,227,564-26,035,359)x3,13q12.3q31.1(30,404,904-87,111,281)x1	42,XY,-3,del(5)(q12q33),-7,der(12)t(3;12)(p14;p12),-13,-14,der(19)t(19;?)(q12.3;?)[7]/43,idem,+mar[10]/44,idem,+mar,+mar[8]
S31	arr 7q22.1q36.3(100,780,826-158,923,949)x1,11p15.5p11.12(192,358-51,538,651)x3,11q11q25(54,909,688-134,934,196)x3,17p13.3p13.1(44,684-7,572,867)x1,17p12p11.2(13,236,413-22,128,401)x1	46, XX, t(11;17)(q13;p11.2), der(21)add(21)(q22) [22]
S33	arr 9q21.11q31.1(71,046,033-103,362,618)x1	46, XX, t(8;21)(q22;q22), del(9)(q22) [8]
S36	arr 5q14.3q34(83,525,460-162,507,695)x1	46,XX, del(5)(p13) [18] / 46, XX [4]
S37	arr(1-22,X)x2	46,XX, t(13;15;17)(q21;q24;q21) [15]
S39	arr Xp22.33p11.1(2,695,374-58,543,823)x1,Xp22.2p21.3(10,715,887-25,472,209)x1,Xq11.1q28(61,781,601-155,102,406)x1	45,X,-X, t(8;21)(q22;q22) [30]

with the conventional karyotyping method which are dependent on cell growth, mitotic index of the dividing cells and analysable chromosome spread but array CGH+SNP are not dependant on any of these factors as the starting material for the array CGH+SNP is DNA. There is no necessity to have the karyotypes for all cases as the array CGH+SNP findings complements each other as the SNP probes in the designs aids in the confirmation of CGH probes in this study except for the identification of translocation which are not possible using our array design.

Upon comparison with other studies of the AML genome using array CGH and SNP platforms. (Table V), our team found that only several aberrations were recurrent (7.3%) and they displayed a low frequency (<5.5%). Low burden of CNVs were also observed in our cases, supported by previous reports (11, 12) where they have reported similar findings in AML where lower frequencies of CNVs are seen compared to other subtypes of leukaemia. These findings, however, are distinct from reports by Mehrotra et al (2014) and Jun et al (2014) where they discovered over two-fold of CNVs in their studies. Studies by Mehrotra et al and Jun et al lacked systematic use of germline DNA and so there could be over estimation of somatic CNVs (13,14). Based on Table III of this study, we have clearly shown that the use of germline DNA is one of the critical procedure in the assessment of an AML genome to distinctly determine somatic CNVs from the germline CNVs. Matched germline in this study revealed that most of the CNVs seen may not have been directly related to the pathogenesis of AML. The systemic application of matched tumour and germline samples equipped our study with unequivocal delineation of somatic genetic alteration including CNVs and CN-LOHs, which led to the exclusion of CNVs and CN-LOHs from our analysis which in turn generated lower numbers of genomic aberrations in our study.

As opposed to Mehrotra et al (2014), who included microdeletion in kilo base pairs of unknown significance, we focussed our analysis on somatic genomic alterations above 5 Mb in size based on our SNP probe resolution (13) as SNP probes were utilized to confirm the CGH probes findings. We discovered 419 somatic CNVs below 5 Mb in this study which was confirmed by a subsequent study with larger cohort of patient samples with similar disease condition (15)

Walter and his team reported 201 CNVs (35 kb to 200 Mb), in which about 88 CNVs were below 5 Mb. Their sample size was 86 AML cases compared to 41 AML cases in this study. They performed paired analysis of

Table V: Comparison of findings from various studies with this study

	Akagi et al (2009) [14]	**Mehrotra et al (2014)** [12]	**Walter et al (2009)** [10]	**Jun et al (2011)** [13]	**This Study**
Number of patients	38	48	86	133	41
Array type	250K SNP	Custom 44K oligo-nucleotide genomic array	SNP 6.0	SNP 6.0	Custom CGH+SNP 180K
Cases	30 AML-NK, 8 MDS	AML	60 de novo AML	133 AML-NK	41 AML
Use of Germline	0	0	60	0	41
CNVs	19 cases had one or more CNVs	170 total chromo-somal aberrations (including mi-crodeletion)	201 CNVs	Total 113 SNP lesions	55 CNVs
Gains	Deletion and dupli-cation in 9 patients	71	201 CNVs	23	15
Loss		99		55	28
CN-LOH	12 patients	-	8 regions of UPD in 7 of 86 samples	35	12
Normal karyotype by CGH and/or SNP	19 patients	21	43	90	22
One or more genomic aberrations by CGH and/ or SNP	18 patients	27	38	43	19

tumour and germline DNA and validated their findings using a high resolution custom NimbleGen CGH 12X135 array including CNVs of smaller than one Mb (11). Our study utilised lower resolution of array CGH+SNP and so the number of CNVs seen were fewer.

Losses were more frequently seen in chromosome 7 where CGH+SNP revealed heterogeneous breakpoints in three cases. In case S30, segmental deletion of p arm that included five breakpoints: 7p12.3 - p12.1, 7p21.3 - p21.1, 7p22.3 - p21.3, 7p14.3 - p12.3, 7p22.3 - p12.1 spanning between 5.3 Mb to 53.3 Mb, and loss of entire q arm of chromosome 7. In case S31, segmental deletion of 7q22.1-q36.3 (100,780,826-158,923,949) was seen together with structural aberrations involving other chromosomes. In case S03, loss of 7q arm were observed as the sole chromosomal aberration. Other studies have reported that complete or interstitial deletion of chromosome 7 (-7,7q-) are non-random abnormalities observed in de novo AML and myeloid neoplasms. Region of losses identified within this study in the three cases encompasses 7q22 and 7q36, the region harbouring one or more tumour-suppressor genes (TSGs) inactivated in AML. Besides that, our findings are in agreement with other studies which reported that at least three distinct loci are recurrently deleted 7q22, 7q31.1 and 7q31.3 by allelotyping studies in AML (16, 17).

Two cases (S23, S41) had Monosomy X as the sole abnormality and one case (S39) had a Monosomy X with t(8;21)(q22;q22) . Only sporadic reports on Monosomy X as a sole recurring chromosomal abnormality were available; with none specific for AML. Monosomy X as a sole recurring abnormality was reported in other haematological malignancies including myelodysplastic syndrome and acute lymphoblastic leukaemia (18, 19). A study on cytogenetic profile in Iranian AML patients reported that Monosomy X was seen in two patients but no details if it was a sole recurrent abnormality or concurrent with other chromosomal aberrations (20).

Trisomy 4 was the most frequent and recurrent chromosomal gain detected in this study. Trisomy 4 was the only abnormality detected in case S14 whereas in case S08, which coexisted with Trisomy 8. As the only chromosomal aberration detected in S14, trisomy 4 in AML is uncommon and the consequences are also unknown (21,22). Two probable candidate genes were identified: PDGFRA, KIT. Some studies on Trisomy 4 have focussed on KIT mutations to study its role in leukopoiesis. Their findings support that there is an association between trisomy 4 and overexpression of KIT in leukemic cells (23, 24). This could have potentially led to deregulated expression and proliferation capacity of the cells. Rearrangement of PDGFRA was associated with AML in other studies (25). We did not perform mutational analysis on KIT and PDGFRA and so we are unable to assess the role of these genes in the leukopoiesis of AML.

We also found interesting regions of UPD/CN-LOH in two cases occurring in q arm of chromosome 4. In case S24, seven segmental stretches of CN-LOH were

seen ranging from 5.3 Mb to 18.3 Mb (58,728,642-190,449,761) in chromosome 4q. These findings appear to be unique to our study as we did not find any report with similar region of CN-LOH except in one study by Raghavan et al (2008) who found CN-LOH of chromosome 4q(87,522,823-term) in a relapsed AML sample (26). Although their findings were within the same 4q arm, it was detected in a relapsed AML sample and the pathogenic consequences of these aberrations in AML are still unknown.

We also elucidated stretches of CN-LOH in three different cases which were not reported elsewhere: patient S09 had CN-LOH in chromosome 12 q13.2 - q14.1 (56,069,231-61,972,806); patient S05 had CN-LOH in chromosome 17q22 - q24.1 (57,455,620-63,176,847); patient S26 had CN-LOH in chromosome 18q21.2 - q21.31 (48,917,970-54,128,364). These regions are novel findings which could contribute in AML pathogenesis as the changes are only seen in the tumour DNA. All the four cases with CN-LOHs in this study were cytogenetically normal. It was reported that CN-LOHs are common in myeloid malignancies, even in cases with AML-NK. CN-LOHs have also been implicated with microsatellite instability in AML-NK. Besides that, CN-LOHs may also have homozygous somatic mutation where initially it was a heterozygous mutation duplicated through UPD that inactivates or totally knocks out a tumour suppressor gene such as TP53 in UPD17. Secondly, a double dose of mutated gene produced which leads to increased proliferation as oncogenic mutations can result through CN-LOHs (3, 27). Since these stretches of CN-LOHs were found only in a few cases, assignment of prognostic significance will require systematic assessment of CGH+SNP in larger series of AML cases with investigation into the underlying mechanism which caused CN-LOH.

With the use of germline DNA in this study, somatic lesions were successfully delineated from inherited sequence variants. The use of germline DNA avoided overestimation of genomic aberrations. Our findings do not agree with the opinion of Akagi et al (2009) where they stated that CN-LOH occurs only in the leukaemia samples but not in the corresponding germline DNA. In our study, only 22% of somatic lesions where CN-LOHs whereas in the germline DNA about 87% were CN-LOHs (16).

This further supported our claim that it is of utmost importance to utilise corresponding germline DNA in each AML case as large number of CNVs and CN-LOHs were present as inherited sequence variants and are unique in each individual subject or patient. It is interesting to note that our CNVs and CN-LOHs that seen in the patients are not reported in the normal recurrent CNVs and CN-LOHs customised genome track established by Clinical Haematology Referral Laboratory Hospital Ampang which are established using normal healthy Malaysian adults (10). These could be due to either our CNVs are truly unique and seen among our case series or the germline CNVs and CN-LOHs could be associated in the pathogenesis of stage preceding leukaemic transformation of these patients or could be associated with clonal expansion even when patients have attained CR (28). However, our hypothesis requires another level of in depth study on a larger cohort with application of whole genome sequencing on the germline DNA and tumour DNA. Germline DNA should be obtained from a different source from the same patient such as buccal swab or tissue instead of the patient's remission samples in order to accurately ascertain germline and somatic aberrations in future studies. In this study, we have limitations in terms of obtaining other sources of germline DNA.

CONCLUSION

In this study, we first demonstrated the advantages of array CGH+SNP compared to conventional karyotyping method which are limited in terms of resolutions, identification of cryptic genomic aberrations and other technical and sampling issues. Apart from that, we have distinctly exhibited the benefits of using germline DNAs to segregate somatic aberrations in this study. Array CGH+SNP holds great potential in the clinical setting as the results are ready in quicker turn around time compared to conventional method and aids efficient patient management. Even with the rapid development of other sequencing technologies, array CGH+SNP technology is still relevant in the diagnosis, prognostication and stratification of patients based on their genomic aberrations.

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REFERENCES

1. Wisniewska J, Bi W, Shaw C, Stankiewicz P, Kang SHL, Pursley AM, et al. Combined array CGH plus SNP genome analyses in a single assay for optimised clinical testing. European Journal of Human Genetics 2014; 22: 79-87.
2. Heinrichs S, Li C, Look AT. SNP array analysis in hematologic malignancies: avoiding false

discoveries. Blood. 2010; 115(21): 4157-61.
3. O'Keefe C, McDevitt M, Maciejewski JP. Copy neutral loss of heterozygosity: a novel chromosomal lesion in myeloid malignancies. Blood. 2010; 115(14): 2731-39.
4. Vermeesch JR, Brady PD, Sanlaville D, Kok K, Hastings RJ. Genome-wide arrays: quality criteria and platforms to be used in routine diagnostics. Hum Mutat. 2012; 33(6): 906-15.
5. Maciejewski JP, Tiu V, O'Keefe C. Application of array-based whole genome scanning technologies as a cytogenetic tool in haematological malignancies. British J of Haem. 2009; 146: 479-88.
6. Heinrichs S, Look AT. Identification of structural aberrations in cancer by SNP array analysis. Genome Biology. 2007; 8(7): 219.
7. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukaemia: rationale and important changes. Blood. 2009; 114: 937-51.
8. Bajaj R, Xu F, Xiang B, Wilcox K, DiAdamo AJ, Kumar R, et al. Evidence-based genomic diagnosis characterized chromosomal and cryptic imbalances in 30 elderly patients with myelodysplastic syndrome and acute myeloid leukaemia. Molecular Cytogenetics 2011; 4: 3.
9. Shaffer LG, McGowan-Jordan J, Schmid M. An International System for Human Cytogenetic Nomenclature. ISCN 2013; S Karger, Basel.
10. Angeli Ambayya, Andrew Octavian Sasmita, Carey Wee Kai Li, Dan Belluoccio, Chang Kian Meng, Jameela Sathar, Qian-Yun Zhang, Subramanian Yegappan. Population Characterisation of Copy Number Variants in Blood in Malaysians. (Submitted for MSH Annual Scientific Meeting (19-21th April 2018), Malaysia.
11. Walter MJ, Payton JE, Ries RE, Shannon WD, Deshmukh H, Zhao Y, et al. Acquired copy number alterations in adult acute myeloid leukaemia genomes. PNAS. 2009; 106(31): 12950-5.
12. Kьhn MW, Radtke I, Bullinger L, Goorha S, Cheng J, Edelmann J, et al. High-resolution genomic profiling of adult and paediatric core-binding factor acute myeloid leukaemia reveals new recurrent genomic alterations. Blood. 2012; 119(10): e67-75.
13. Mehrotra M, Luthra M, Ravandi F, Sargent RL, Abraham R, Mishra BM, et al. Identification of clinically important chromosomal aberrations in acute myeloid leukaemia by array-based comparative genomic hybridization. Leukaemia & Lymphoma. 2014; 55(11): 2538-48.
14. Jun HY, Jungwon H, Hee-Jin K, Sun-Hee K, Hyeoung-Joon K, Yeo-Kyeoung K, et al. Adverse prognostic impact of abnormal lesions detected by genome-wide single nucleotide polymorphism array-based karyotyping analysis in acute myeloid leukaemia with normal karyotype. J of Clin Oncol. 2011; 29(35): 4702-8.
15. Ambayya A, Chang KM, Lim SM, Yegappan S. Identification of germline and somatic aberrations in acute myeloid leukemia genome by using customised DNA microarray. In: Proceedings of XXIXth International Symposium on Technological Innovations in Laboratory Hematology. Milano, Italy: International Journal of Laboratory Hematology; 2016.
16. Akagi T, Ogawa S, Dugas M, Kawamata N, Yamamoto G, Nannya Y, et al. Frequent genomic abnormalities in Acute Myeloid Leukaemia/ Myelodysplastic syndrome with normal karyotype. Haematologica. 2009; 94: 213-23.
17. Abdelrazik HN, Farawila HM, Sherif MA, Al Ansary M. Molecular characterization of chromosome 7 in acute myeloid leukaemia and myelodysplastic patients. African Journal of Health Sciences. 2006; 13(3-4): 33-42.
18. Bueno JL, Watson A, Dainton MG, Hughes DM, Killick S, Treleaven JG, et al. Monosomy X as the Sole Cytogenetic Abnormality in Acute Lymphoblastic Leukaemia: A Report of Two New Patients. Leukaemia & Lymphoma 1999; 32(3-4): 381-4.
19. Abruzzese E, Rao PN, Slatkoff M, Cruz J, Powell BL, Jackle B, et al. Monosomy X as a recurring sole cytogenetic abnormality associated with myelodysplastic diseases. Cancer Genet Cytogenet. 1997; 93(2): 140-6.
20. Toogeh GH, Keyhani AHNM. Cytogenetics findings in acute myeloid leukaemia. Acta Medica Iranica. 2013; 41(4): 227-32.
21. Gupta V, Minden MD, Yi QL, Brandwein J, Chun K. Prognostic significance of trisomy 4 as the sole cytogenetic abnormality in acute myeloid leukaemia. Leuk Res. 2003; 27(11): 983-91.
22. Bains A, Lu G, Yao H, Luthra R, Medeiros LJ, Sargent RL. Molecular and clinicopathologic characterization of AML with isolated Trisomy 4. AJCP. 2012; 137: 387-94.
23. Schnittger S, Kohl TM, Haferlach T, Kern W, Hiddemann W, Spiekermann K, et al. KIT-D816 mutations in AML1-ETO-positive AML are associated with impaired event-free and overall survival. Blood. 2006; 107(5): 1791-9.
24. Ferrari S, Grande A, Zucchini P, Manfredini R, Tagliafico E, Rossi E, et al. Overexpression of c-kit in a leukaemic cell population carrying a trisomy 4 and its relationship with the proliferative capacity. Leuk Lymphoma. 1993; 9(6): 495-501.
25. Bain BJ. Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB or FGFR1. Haematologica. 2010; 95(5): 696–98.
26. Raghavan M, Smith LL, Lillington DM, Chaplin T, Kakkas I, Molloy G, et al. Segmental uniparental

disomy is a commonly acquired genetic event in relapsed acute myeloid leukaemia. Blood. 2008; 112(3): 814-21.
27. Makeshima H, Maciejewski JP. Pathogenesis and consequences of uniparental disomy in cancer. Clin Cancer Res. 2011; 17: 3913-23.
28. Takahashi K, Wang F, Kantarjian H, Doss D, Khanna K, Thompson E, et al. Preleukaemic clonal haemopoiesis and risk of therapy-related myeloid neoplasms: a case-control study. Lancet Oncol. 2017; 18(1): 100-111.