

SNP array–based karyotyping: differences and similarities between aplastic anemia and hypocellular myelodysplastic syndromes

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In aplastic anemia (AA), contraction of the stem cell pool may result in oligoclonality, while in myelodysplastic syndromes (MDS) a single hematopoietic clone often characterized by chromosomal aberrations expands and outcompetes normal stem cells. We analyzed patients with AA (N = 93) and hypocellular MDS (hMDS, N = 24) using single nucleotide polymorphism arrays (SNP-A) complementing routine cytogenetics. We hypothesized that clinically important cryptic clonal aberrations may exist in

some patients with BM failure. Combined metaphase and SNP-A karyotyping improved detection of chromosomal lesions: 19% and 54% of AA and hMDS cases harbored clonal abnormalities including copy-neutral loss of heterozygosity (UPD, 7%). Remarkably, lesions involving the HLA locus suggestive of clonal immune escape were found in 3 of 93 patients with AA. In hMDS, additional clonal lesions were detected in 5 (36%) of 14 patients with normal/noninformative routine cytogenetics. In a subset of

AA patients studied at presentation, persistent chromosomal genomic lesions were found in 10 of 33, suggesting that the initial diagnosis may have been hMDS. Similarly, using SNP-A, earlier clonal evolution was found in 4 of 7 AA patients followed serially. In sum, our results indicate that SNP-A identify cryptic clonal genomic aberrations in AA and hMDS leading to improved distinction of these disease entities. (*Blood*. 2011;117(25): 6876-6884)

Introduction

Approximately 10% of patients with myelodysplastic syndromes (MDS) present with a hypocellular BM and distinction of these patients from those with aplastic anemia (AA) is often a diagnostic challenge.¹⁻⁴ The morphologic diagnosis of MDS relies on the presence of dysplastic features and detection of clonal chromosomal abnormalities. In particular, detection of recurrent genomic lesions supports the diagnosis of a neoplastic clonal process. However, low cellularity of the aspirates in AA and hypocellular MDS (hMDS) often hampers precise morphologic assessment and leads to unsuccessful cytogenetic testing, resulting in misdiagnosis.

Around 50% of patients with MDS, including hypocellular cases, show a normal karyotype by metaphase cytogenetics (MC), making the distinction from AA more difficult. Similarly, MDS can also develop as a late clonal complication of AA; the evolution rate of clonal chromosomal defects may be as high as 20% in 10 years, but the risk factors for evolution have not been identified.⁵⁻⁸ Karyotype abnormalities encountered in this setting often include loss of chromosomes 6 and 7, and trisomy 8.⁵ Identification of clonal progression is an important diagnostic task, as the prognosis of patients with AA-derived MDS is less favorable and treatment choices differ, in particular when high-risk chromosomal abnormalities are involved.

Many investigators believe that the presence of chromosomal abnormalities is not compatible with the diagnosis of AA.^{9,10} However, some diagnostic guidelines do not preclude a diagnosis of AA even if abnormal cytogenetics is present in otherwise

hypocellular marrow.¹¹ In some instances, hematopoiesis may be oligoclonal because of depletion of stem cell reserves. A clinical correlate for this phenomenon may be detection of nonrecurrent or transient clonal chromosomal abnormalities.^{6,7,10} In contrast, in true MDS, clonal markers result from the malignant expansion of an abnormal hematopoietic clone characterized by chromosomal defects.

Recently developed array-based DNA technologies allow for a very precise assessment of unbalanced genomic lesions and may overcome the inherent limitations of metaphase karyotyping in terms of its low resolution. Whole-genome array-based analysis of single nucleotide polymorphisms (SNP) reaches a much higher level of genomic resolution; precise mapping of data can be generated as a result of the dense distribution of SNP probes throughout the genome.¹² SNP arrays (SNP-A) have the additional technical advantage of detecting copy-neutral loss of heterozygosity.¹³⁻¹⁵ Such lesions are characterized by duplication of one parental allele with the concomitant loss of the other, and can be a result of mitotic recombination.¹⁶⁻¹⁸ Previously, we applied SNP-A technology in the study of MDS and acute myeloid leukemia (AML)¹⁹⁻²¹ and found new or additional chromosomal abnormalities in patients with normal MC, and in those with known aberrations. Most relevant to this study is the fact that SNP-A karyotyping does not require induction of cell division and thus can be performed on interphase cells, a feature that may be important in

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Table 1. Patient general characteristics

Patient group	N	Sex	Median age, y (range)	Presentation*	Post-IS†	Response to IS	SCT (median days from diagnosis to SCT)
AA	93	52F/41M	42 (5-80)	33	75‡	63% (47/75)	4 (100)
hMDS	24	8F/16M	60 (16-83)	23	3	33% (1/3)	5 (174)

IS indicates immunosuppressive therapy; SCT, stem cell transplant; F, female; M, male; AA, aplastic anemia; SNP-A, single nucleotide polymorphism array; and hMDS, hypocellular myelodysplastic syndrome.

*Patients with sample at presentation.

†Patients with sample post-IS (52 samples available for SNP-A analysis).

‡Eleven patients had samples pre- and post-IS.

hypocellular specimens with expected poor growth in vitro, often encountered in hMDS or AA.

Based on the advantages of SNP-A karyotyping, we theorized that application of this technique to the analysis of AA and hMDS will improve diagnostic precision and thereby enable a better distinction of MDS from AA through detection of cryptic clonal abnormalities typical of MDS. In AA, the identification of transient clonal defects may be indicative of stem cell pool contraction and extrinsic selective pressure. We studied patients with AA and hMDS using SNP-A as a karyotyping tool. This is the first comprehensive application of high-resolution SNP-A to identify and characterize submicroscopic chromosomal aberrations in diseases with profoundly hypocellular BM.

Methods

Patients and controls

BM aspirates and peripheral blood samples were collected from 2002 to 2010 from patients with AA (N = 93) with median follow-up of 1437 days (17-7216 days, including follow-up for patients diagnosed before 2002) and hMDS (N = 24) with median follow-up of 924 days (37-2495 days) diagnosed on clinical grounds using routine diagnostic tools (Tables 1 and 2). Median follow-up was 1308 days for AA patients diagnosed after 2002. Informed consent for sample collection was obtained in accordance with protocols approved by the Institutional Review Board of Cleveland Clinic (Cleveland, OH). Patients with AA were diagnosed according to the International Study of Aplastic Anemia and Agranulocytosis.²² For the initial diagnosis of AA, only patients lacking dysplasia in the myeloid and megakaryocytic series were included and only patients with normal or noninformative (no growth) cytogenetics were classified as having AA.⁵ Furthermore, diagnostic evaluation required exclusion of T- and B-cell lymphoproliferative disorders by flow cytometry and TCR rearrangement studies when indicated. In selected patients treated with immunosuppression (antithymocyte globulin [ATG]), samples were collected before treatment and in various intervals after treatment.

A diagnosis of hMDS was made based on the presence of dysplastic features and the overall clinical presentation including the presence of cytopenias, the presence of blasts (< 5% in the BM or < 2% in peripheral blood) or/and chromosomal abnormalities by metaphase cytogenetics, and a decreased cellularity of the marrow of < 20%. When indicated based on clinical suspicion, immunohistochemical staining for CD34 was performed to rule out/in collections of immature cells.

Pathomorphologic evaluation of the marrow was performed by a hematopathologist not associated with the study in a blinded fashion

because the study was conceived after the time of specimen review. All controversial cases were a subject of consensus pathology conference per standard operating procedure. To reflect this, some diagnostic definitions recognize the presence of small percentage of abnormal cytogenetics in AA cases.^{11,23}

Cytogenetics

Cytogenetic analysis was performed on fresh aspirates according to the standard metaphase karyotyping protocol: G-banding of chromosomes was performed using trypsin and Giemsa. Karyotypes were analyzed according to the ISCN guidelines.²⁴

DNA extraction

DNA was extracted using the ArchivePure DNA extraction kit (5 Prime). For the analysis of germline karyotypes, CD3⁺ lymphocytes were isolated using magnetic beads (Miltenyi Biotec) from mononuclear cells obtained by density gradient centrifugation.

SNP-A preparation

Affymetrix Human Mapping 250K Array and Genome-Wide Human SNP Array 6.0 were used for karyotyping. Total BM DNA was uniformly used for SNP-A. Use of purified progenitor cells would likely result in a higher diagnostic yield but would be impractical in a routine clinical setting; additionally, in the context of AA separation is often unsuccessful because of hypocellularity.

Biostatistical evaluation of SNP-A data

Overall, for outcome and other analyses only somatic chromosomal lesions were used. Briefly, signal intensity was analyzed and SNP calls determined using Gene Chip Genotyping Analysis Software Version 4.0 (GTYPE). Copy number was investigated using a Hidden Markov Model and Copy Number Analyzer for Affymetrix GeneChip Mapping 500K arrays (CNAG Version 3).²⁵ Results of Affymetrix 6.0 arrays were analyzed using Genotyping Console software (Affymetrix). Segmental loss of heterozygosity was identified by a statistical assessment of the likelihood that consecutive SNP loci would exhibit heterozygosity given the corresponding allelic frequency of a particular SNP in the normal population (CNAG). For assay validation, 5 samples were repeated on the same array type, while 48 samples were run on both the 250K and 6.0 arrays.

Copy number variants and nonclonal areas of germline-encoded copy-neutral loss of heterozygosity were excluded using a stringent algorithm.²¹ Copy number variants found in 117 internal 259 Hapmap²⁶ and 627 Framingham Heart Study controls were excluded. Lesions that were seen by MC and confirmed by SNP-A analysis were not further validated.

Table 2. Patients with clonal disease (AA) and progression to sAML (MDS)

Patient group	N	Median age, y (range)	Clonal evolution of AA	Progression to sAML	Median time from diagnosis to clonal evolution, d
AA	14	57 (28-75)	15% (14/93)	NA	1145 (427-2671)
hMDS	8	61 (44-72)	NA	33% (8/24)	234 (28-631)

AA indicates aplastic anemia; MDS, myelodysplastic syndrome; hMDS, hypocellular MDS; sAML, secondary acute myeloid leukemia; and NA, not applicable.

For other lesions, germline analysis was used for confirmation (DNA from corresponding CD3⁺ lymphocytes or serial samples showing emergence or disappearance of a chromosomal defect; N = 46). Well-characterized MDS-associated lesions—such as, for example, UPD7q, del(7q), monosomy 7, or trisomy 21—did not require confirmation.

In controls, small stretches of homozygosity were frequently found; using a cutoff value of 4 Mb and > 100 homozygous calls, most of these areas were excluded from further analysis in controls and patients. In our control cohort, copy-neutral loss of heterozygosity was detected in around 8% of samples. The germline or somatic derivation of this type of lesion could be interpolated from the size and location of these areas; in the control cohort, the average size was 8.7 Mb and most were interstitial. Thus, any interstitial area of copy-neutral loss of heterozygosity < 25 Mb (95% confidence interval [CI]) was excluded from the analysis of clonal lesions, as they most likely represent germline events. In contrast, large and/or telomeric defects do not occur in nonclonal control DNA and therefore were considered true somatic abnormalities. The remaining regions of copy-neutral loss of heterozygosity were confirmed by analysis of germline DNA. Furthermore, lesions that were identified preimmunosuppression and that disappeared postimmunosuppression were also considered somatic.

Results

Patient characteristics

We analyzed a large cohort of patients with AA (N = 93) and hMDS (N = 24) using SNP-A to detect the presence of cryptic chromosomal aberrations (Table 1). A total of 33 patients with AA were examined before immunosuppression, 11 of whom were studied serially thereafter. Another 52 AA patients were analyzed postimmunosuppression. We also analyzed samples of 9 of 14 AA patients who, in the course of their disease, evolved to MDS or AML (Table 2). There were 3 patients who had unsuccessful cytogenetics because of no growth. Of the 24 hMDS patients studied, 10 received supportive care only, 7 were treated with either 5-azacitidine, decitabine, or lenalidomide, 3 were treated with horse ATG (hATG) and 4 received more than one of these drugs. Evolution to secondary acute myeloid leukemia (sAML) was observed in 8/24 patients. The median age was 42 and 60 years for patients with AA and hMDS, respectively (Tables 1,2). Overall, 4 AA and 5 hMDS patients underwent BM transplantation.

Comparison of metaphase cytogenetics and SNP-A karyotyping

The methodology and principles of SNP-A–based karyotyping have been described in detail in earlier reports.^{20,21,27} In general, SNP-A can be used for genotyping and determination of genomic copy number, and thereby cytogenetic analysis of unbalanced chromosomal defects as well as copy-neutral loss of heterozygosity. An exemplary ideogram obtained from a patient is shown in Figure 1A. The presence of small defects (both copy-neutral loss of heterozygosity as well as copy number changes) detected using SNP-A has to be interpreted carefully as they may represent nonpathologic germline variants. In general, large recurrent lesions such as monosomy 7 or those identified by both cytogenetics and SNP-A did not require further verification. All other abnormalities were verified to be somatic by comparison to sorted nonclonal CD3⁺ cells, (Figure 1B) or by following an algorithm for identifying somatic aberrations as explained in “Biostatistical evaluation of SNP-A data.”²¹ The size, frequency, and distribution of small interstitial regions of copy-neutral loss of heterozygosity detected in patients were similar to those seen in controls (not shown). While true germline DNA (eg, skin biopsies) was not available,

T cells were polyclonal in all cases (see diagnostic criteria for AA), and therefore were used for paired testing when appropriate. In addition, lesions which occurred/disappeared in the course of the disease on serial examinations were also considered somatic (N = 5). Using this approach, we have identified new, previously cryptic lesions undetectable by MC in both AA and hMDS as well as we confirmed known defects.

In AA patients tested at presentation (per definition all with normal or noninformative cytogenetics), defects were identified by SNP-A in 10 (30%) of 33. At different time points postimmunosuppression, an abnormal karyotype was seen in 13% (7 of 52) of patients by MC while SNP-A revealed clonal defects in 19% of patients (10 of 52, $P = .59$). Overall, when we combined MC and SNP-A analysis, we improved the detection yield of abnormal karyotypes to 19% (18 of 93), consistent with the presence of clonal hematopoiesis (Figure 2A). In the hMDS cohort, abnormal chromosomes were detected by MC in 42% (10 of 24). When routine MC was combined with SNP-A analysis, an abnormal karyotype was detected in 54% of patients (13 of 24, $P = .56$) compared with metaphase cytogenetics alone. Of note is that noninformative MC results were resolved in 3 of 3 AA and 3 of 3 MDS patients. In addition, 4 hMDS patients who presented with normal MC showed clonal abnormalities by SNP-A, a finding consistent with the originally assigned diagnosis. When histomorphology and flow cytometry were compared between AA patients with and without clonal lesions, no differences were detected (see supplemental Table 1, available on *Blood* Web site; see the Supplemental Methods link at the top of the online article).

In addition, we analyzed our AA cohort based on their age (< 18 vs > 19 years). When SNP-A were applied, clonal lesions were found in 2 of 15 in the younger than 18-year-old group compared with 16 of 78 in the older than 19-year-old group ($P = .72$). No one in the younger group evolved to MDS/AML while 14 of 78 in the older group had clonal evolution and the median follow-up was 2377 days and 1335 days, respectively.

Analysis of chromosomal abnormalities detected by SNP-A analysis in AA and hMDS

In AA patients who were studied preimmunosuppression, we found microdeletions in 3 patients, monosomy 7 in 1 patient and microduplications in 2 patients (Figure 2B). In patient 38, we detected 2 microdeletions (12q21.1 and 6p22.1) before immunosuppression. These lesions disappeared after treatment, signifying their transient somatic nature. In patients evaluated by SNP-A karyotyping at later time points in the clinical course, monosomy 7 was found in 7 patients. In addition, 2 patients showed microduplications (1 patient had microduplications in addition to monosomy 7) and 1 patient showed a microdeletion in addition to monosomy 7. 5 of these patients showed a normal karyogram by routine cytogenetic examination and 1 was noninformative at presentation because of no growth. Detection of clonal abnormalities was consistent with diagnosis of secondary MDS.

In hMDS, a total of 13 patients showed chromosomal abnormalities by SNP-A analysis (Figure 2B). We found nonrecurrent microdeletions in 9 patients, 2 of whom had concomitant microduplications. Another 4 patients also had nonrecurrent microduplications. In patient 122, the initial diagnosis of hMDS was based on histomorphology while metaphase cytogenetics did not show any clonal abnormalities. In the subsequent course, monosomy 7 was detected 2 months earlier by SNP-A compared with metaphase cytogenetics, indicating that initial diagnosis was correct (see Figure 4).

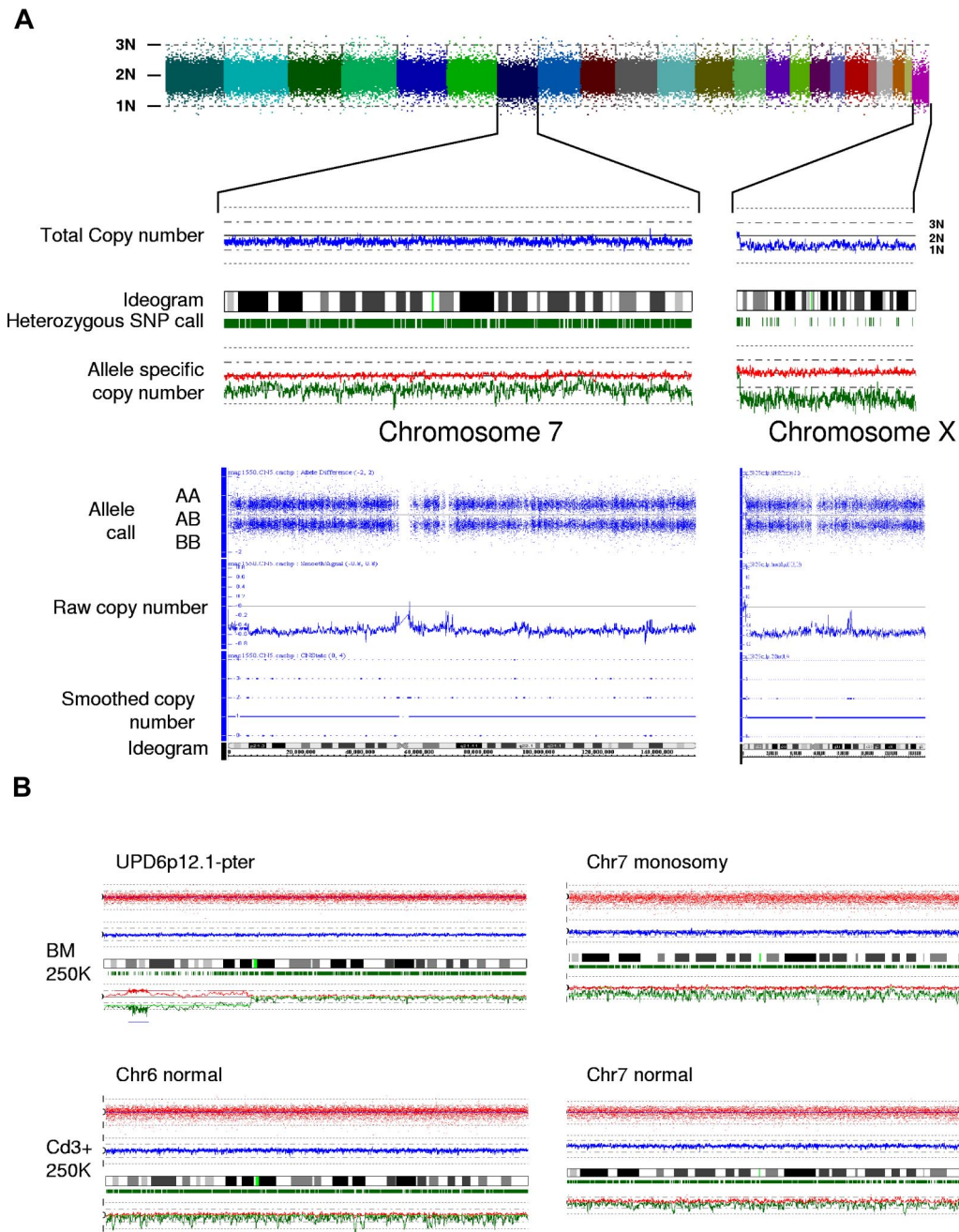


Figure 1. Detection of somatic genomic loss in BM failure syndromes using SNP-A analysis. (A) At the top, a whole-genome view is shown for patient 57. Individual dots represent the raw signal intensity of a specific SNP, which indicates the copy number at that locus. In general, the copy number is around 2N. Each chromosome is represented by a different color, from chromosome 1 on the left to the X chromosome on the right. Loss of chromosome 7, as well as the “physiologic” loss of the X chromosome in this male patient can be seen. At a higher level of resolution, the total and allele-specific copy numbers can be investigated for individual chromosomes. In the middle, Affymetrix 250K array karyograms for chromosomes 7 and X are displayed. The blue line represents smoothed total copy number, while the green ticks below the ideogram represent heterozygous calls. Although both chromosomes 7 and X show a reduced copy number, indicating loss of the entire chromosome, the presence of a large number of heterozygous calls along the length of chromosome 7 is consistent with the clonal nature of this lesion, compared with the X chromosome which has almost no heterozygous calls. Those that remain most likely reflect technical artifacts. At the bottom, karyograms from Affymetrix 6.0 array analysis of the same patient are shown. Raw and smoothed copy number tracks, as well as allele calls (blue dots), are shown. A reduction in the total copy number, as well as loss of heterozygous calls, are seen for both chromosomes, indicative of deletion. (B) Large clonal lesions were detectable in patients with AA by SNP-A. The results are shown for an exemplary patient (no. 96). On the short arm of chromosome 6, loss of heterozygous calls (heterozygous SNP call and allele-specific copy number tracks) with a normal diploid copy number marked a region of copy-neutral loss of heterozygosity in the somatic (BM) but not germline (CD3⁺) configuration. Similarly, clonal monosomy 7 was identified in this patient.

One advantage of SNP-A is the ability to detect copy-neutral loss of heterozygosity not visible by MC. In AA, clonal regions of copy-neutral loss of heterozygosity were identified in a total of 7 patients (17q11.1qter/58 Mb, 6p21.1q15/51 Mb, 6p22.1/ < 10 Mb, 1p36.13-p34.2/25.6 Mb, 6p12.1/55 Mb, 11q14.1qter/

56 Mb, 22q11.23qter/27.5 Mb, 3q12.2qter/97 Mb; Figure 2B) but we did not find regions of copy-neutral loss of heterozygosity in our hMDS cohort. Using an earlier proposed stringent algorithm combining genomic location and size criteria for the identification of acquired copy-neutral loss of heterozygosity²¹

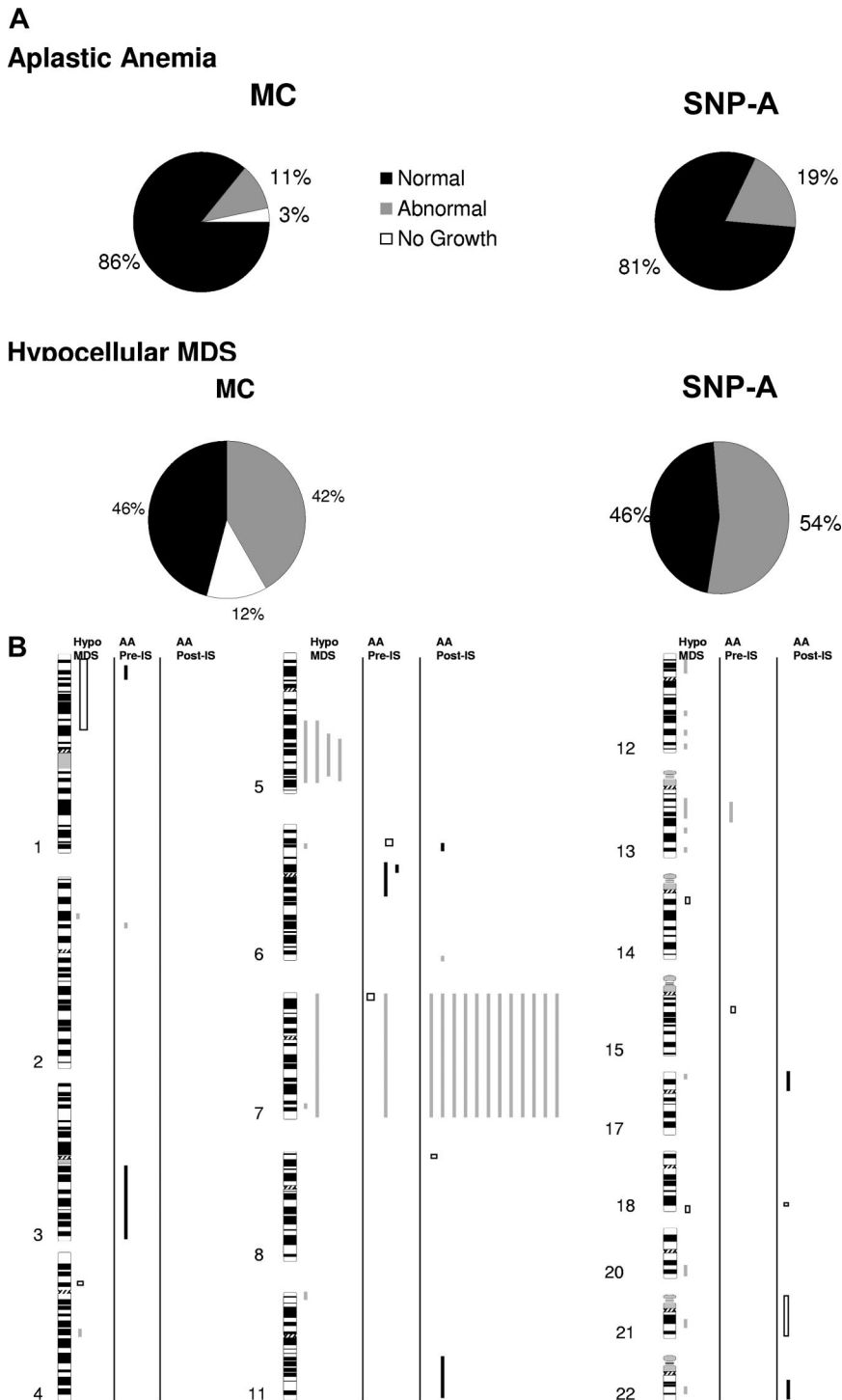


Figure 2. Frequency and genomic distribution of lesions detected by SNP-A. (A) Metaphase karyotyping identified lesions in a subset of patients with AA (top left); however, 3% of the patients had noninformative MC because of failure of growth. When MC and SNP-A karyotyping were combined, the detection rate for chromosomal lesions was increased (top right). In addition, the noninformative cases were resolved. For hMDS, when MC and SNP-A karyotyping were combined, the detection rate for was increased from 42% to 54% (bottom). (B) Genomic distribution of lesions detected by SNP-A in the analyses of hMDS and AA. □ and illustrate genomic gains and losses, respectively. ■ depicts regions of segmental UPD.

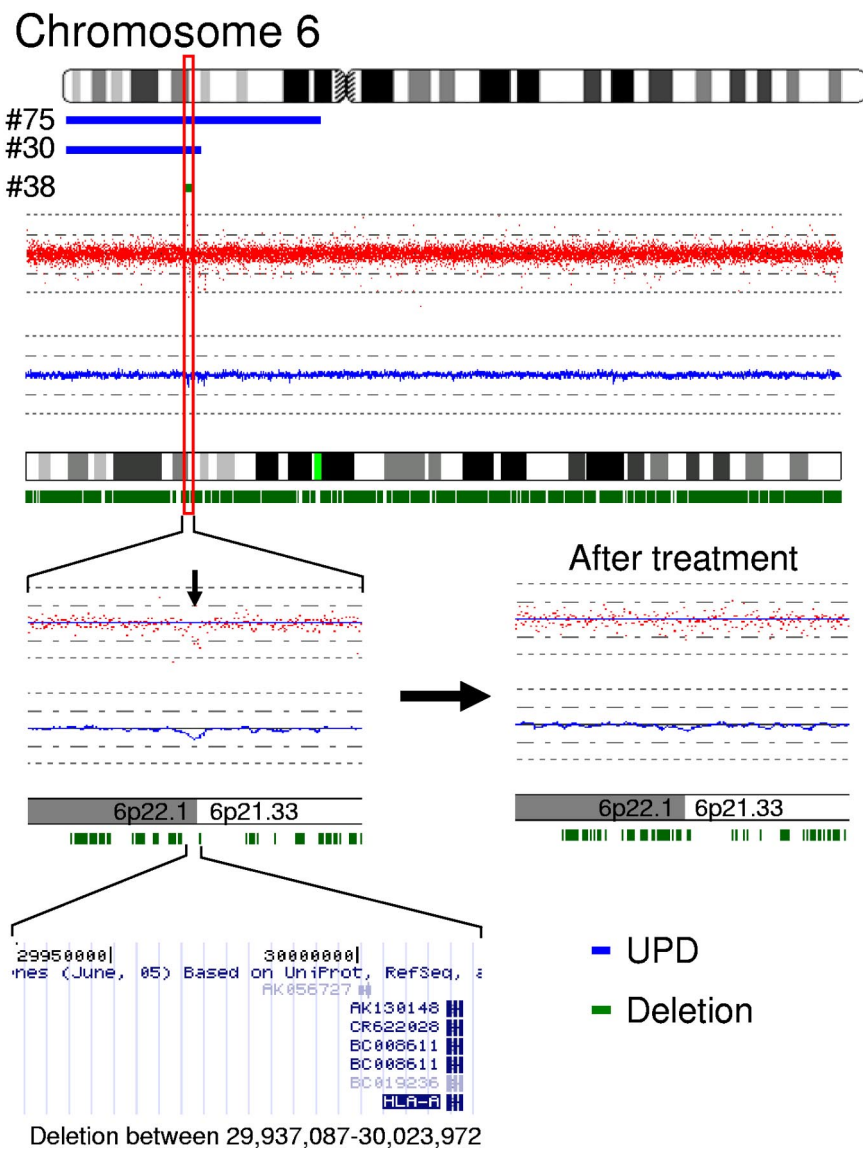
as well as analysis of CD3⁺ cells, we verified the somatic nature of these lesions. Of note, regions of copy-neutral loss of heterozygosity were detected in 5 patients with normal metaphase cytogenetics.

Clinical correlations

When lesions detected by SNP-A were compared between patient groups, clonal chromosomal aberrations are more prevalent in patients with hMDS than in those with AA (19% vs 54%, $P = .0007$). More significantly, chromosomal lesions were less

frequently seen at presentation than in patients with a longer disease history. Within the 7 cases with copy-neutral loss of heterozygosity (in 1 patient 2 regions of copy-neutral loss of heterozygosity were found), we identified a shared region on 6p22.1p21.33 in 2 patients. In an additional patient, a microdeletion was detected which defined the boundaries of a commonly affected area on chromosome 6 (no. 38, Figure 3). This region involved the HLA-A locus. The corresponding microdeletion was of somatic origin as determined in serial testing. It is noteworthy that 2 other patients displayed smaller, germline-encoded runs of

Figure 3. SNP-A identifies genomic regions with potential pathogenic significance in AA. We identified 2 overlapping regions of copy-neutral loss of heterozygosity (blue bars) on the short arm of chromosome 6 in patients with AA; a microdeletion at 6p22.1 (86 KB, green bar) in a third patient defined a minimally affected region (top). This region contained the *HLA-A* locus. The patient with the microdeletion (no. 48) was treated with immunosuppression. After immunosuppression, the lesion disappeared, confirming the somatic nature of the lesion.



copy-neutral heterozygosity involving this locus (not shown). Loss of heterozygosity in this area would lead to clonally restricted change in the HLA type as affected patients were heterozygous for the HLA-A locus.

Of the 10 AA patients seen at presentation with SNP-A clonal defects, 7 were evaluable for response after immunosuppression; 3 (43%) of 7 of these patients responded. In contrast, patients with a normal SNP-A karyotype before therapy achieved a response rate of 56% (14 of 25; not statistically significant). Of note, the overall response in our unselected AA cohort was 63% (all patients who received ATG/cyclosporine A, 47/75), consistent with the results of previously published US trials (60%-70%).²⁸⁻³⁰ In our hMDS cohort, 3 patients were treated with ATG and 1 showed hematologic response, consistent with studies of ATG in MDS.

Clonal evolution and detection of genomic lesions by SNP-A

Among the entire AA cohort, 15% of patients (14 of 93) had malignant evolution (median time to evolution of 1145 days, Table 2). Of these transformed patients, 9 had evaluable samples, and we focused on a longitudinal analysis of these patients. Monosomy 7, a

common recurrent chromosomal defect in the context of AA disease progression, was detected in 7 of 9 transformed patients. Of these 7 patients, monosomy 7 was detected earlier in 4 patients using SNP-A. One patient was studied at presentation (no. 46, no growth by MC); 2 had normal cytogenetics by metaphase cytogenetics (no. 66 and no. 2) and in 1 the routine examination was noninformative because of no growth of cells during culture (no. 46; Figure 4).

In our hMDS cohort, 8 (33%) of 24 evolved to AML (median time to evolution = 234 days, Table 2). One patient (no. 122) had monosomy 7 at presentation by SNP-A, while his karyotype by MC only showed a constitutional duplication of the Y chromosome. When survival was compared between hMDS patients to patients who had AA-derived MDS (from the time of AA diagnosis), primary hMDS was associated with shorter survival (841 vs 2133 days, *P* = .01) and time-to-disease progression (234 vs 407 days, *P* = .0003; Table 3). When we compared survival of primary hMDS to patients with AA-derived MDS from the time of their evolution, there was no statistically significant difference in survival (841 vs 1360 days, *P* = .9).

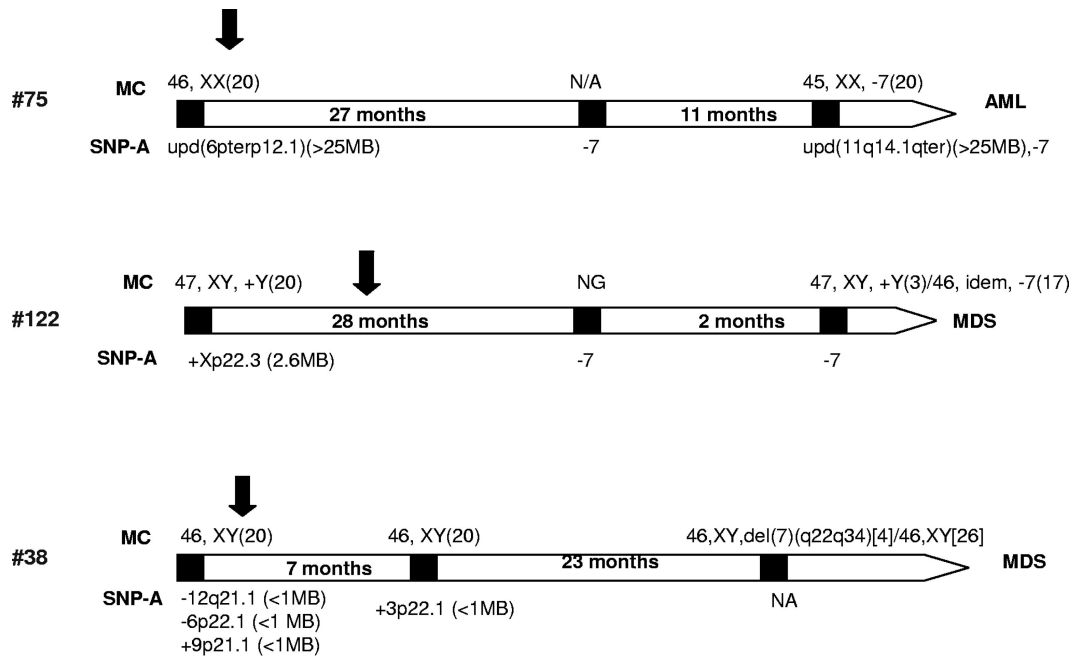


Figure 4. Behavior of SNP-A characterized lesions through the clinical course. Using SNP-A–based karyotyping, clonal monosomy 7 was identified earlier in some patients in our cohort (nos. 122, 75, 38). In addition, SNP-A analysis identified clonal lesions in a patient (no. 38) before immunosuppression that disappeared posttreatment. Number 75 had normal cytogenetics by MC at presentation but SNP-A analysis revealed a uniparental disomy (UPD). Black squares indicate clinical time points where karyotyping was performed using SNP-A and/or metaphase cytogenetics. The metaphase karyotype is given above and the SNP-A-based karyotype below the bars representing the clinical course. NG indicates no growth of the metaphase culture; and NA, result not available. Black arrows indicate when immunosuppression was initiated. The time given in months indicates the length of time between karyotype timepoints.

Discussion

In this study, we adopted SNP-A as a karyotyping tool to facilitate the discovery of new chromosomal defects in AA. While SNP-A achieve excellent resolution and allow for detection of copy-neutral loss of heterozygosity, they have relatively low sensitivity (around 25%).²⁰ However, this potential shortcoming may in fact help to avoid the detection of minor clones lacking clinical relevance. For example, clonal lesions affecting < 2 of 20 metaphases by routine cytogenetics are considered of unclear clinical significance. SNP-A karyotyping will not replace metaphase cytogenetics; the greatest clinical impact is achieved when both techniques are applied together or in targeted fashion in problematic cases.³¹ Of utmost importance for this project was that SNP-A karyotyping can be

performed on nondividing cells, which can be of great advantage in hypocellular and poorly growing AA BM.

Our results demonstrate that SNP-A can be applied in AA to enhance and/or complement metaphase cytogenetics-based detection of clonal chromosomal defects, which could support the diagnosis of MDS. It can also be used in diagnostically challenging cases and thus aid the distinction of AA from hMDS. Aside from improving diagnostic accuracy, early detection of clonal lesions using SNP-A may allow for better monitoring of the clinical course, identifying patients who may evolve from AA to MDS or even leukemia earlier. For instance, we detected the presence of monosomy 7 earlier during the clinical course in 4 (44%) of 9 of AA patients who eventually had malignant evolution, a finding that under clinical circumstances would likely modify the management of these patients. This efficient detection of monosomy 7 may be

Table 3. Comparison of primary hMDS and AA-derived MDS

Clinical characteristics	Primary hMDS, N = 24	Secondary MDS due to AA, N = 10
Age (y)	56 (16-83)	59 (28-75)
Karyotyping by MC	11	5
Normal	10	5
Abnormal	3	0
No growth		
IPSS*		
Low risk (IPSS: low to int – 1)	2	4
High risk (IPSS: Int – 2 and higher)	19	6
Median survival (d)	841 (37-2495)	1360 (797-3185)†, P = .9 2133 (797-3185)‡, P = .009
Time to progression (range)	234 (28-631)	407 (167-1683), P = .0003

MDS indicates myelodysplastic syndrome; hMDS, hypocellular MDS; AA, aplastic anemia; IPSS, International Prognostic Scoring System; and MC, metaphase cytogenetics.

*In 3 patients, there was no growth in MC hence IPSS not available; †from diagnosis of MDS; and ‡from diagnosis of AA.

because of the fact that postmitotic cells were derived from the aberrant clone, and the fraction of dividing progenitors with this lesion amenable to metaphase karyotyping was low. That the detection of monosomy 7 reflects clonal evolution can be deduced from the absence of this defect on preceding evaluations, or subsequent progression to morphologically manifest MDS and AML. Additional, previously cryptic chromosomal lesions were found more frequently in patients with hMDS, supporting the notion that the initial morphologic diagnosis was correct in most instances. Of note, a diagnostic golden standard does not exist.

Principally, there are 2 possible explanations for the detection of clonal defects in some patients with AA. First, clonal defects may indicate pseudoclonality because of depletion of the stem cell reserve and clonal defects represent intrinsically nonpathogenic defects which tend to disappear with restoration of normal hematopoietic function. Such a form of clonality could be because of recruitment of a defective stem cell that operates as the sole supplier of blood cell progeny at any given time and recruitment of such a stem cell is random. In such a scenario, a chromosomal abnormality serves as a marker of oligoclonality rather than a pathogenic lesion. Consistent with this theory, several lesions which were detected at presentation were of a transient nature. It is also noteworthy that some investigators do not routinely exclude the diagnosis of AA in patients with abnormal cytogenetics, as these patients may still respond to immunosuppression and the lesions may constitute a marker of clonality rather than marker of dysplasia.¹⁰ A suitable example of such a clonal lesion is trisomy 8, which has been associated with responsiveness to ATG and cyclosporine therapy.^{5,32,33}

However, certain defects, in particular those with a known poor prognostic value, are clearly signatures of a malignant clonal process if detected. In this case, our results suggest that MDS, rather than AA, may be the correct diagnosis in those patients who had normal MC but heretofore cryptic lesions visualized by SNP-A. This conclusion is supported by our preliminary observation that patients with clonal lesions seem to have a lower response rate to immunosuppression, suggesting that some clonal chromosomal defects could constitute a marker of future clonal evolution. Whether chromosomal aberrations represent negative predictive factors for response as suggested by these initial results has to be confirmed in longer prospective studies. Along these lines, detection of monosomy 7 by additional FISH study in AA with a normal karyotype was associated with a shorter interval to MDS diagnosis. Alternatively, hMDS may have been the proper initial diagnosis in these patients.³⁴

While early detection of poor clonal markers (ie, monosomy 7) in controversial cases may imply more aggressive management, identification of chromosomal abnormalities in hMDS may also allow for better prognostic assessment as exemplified by MDS patients with noninformative metaphase cytogenetics. In these cases, the assignment of an IPSS score may be possible when SNP-A karyotyping is performed.

As shown in previous studies, copy-neutral loss of heterozygosity is a common clonal lesion in hematologic malignancies and in this setting may help distinguish AA from MDS. Because copy-neutral loss of heterozygosity cannot be detected by other cytogenetic techniques and if present as a sole unrecognized defect, it establishes the diagnosis of clonal disease. In past studies, copy-neutral loss of heterozygosity indicated the presence of homozygous mutations, as for *JAK2*, *CBL*, *EZH2*, *TET2*, and many others.^{19,35-37}

Identification of recurrent defects may allow for the mapping of genes that are involved in the pathogenesis of clonal evolution and in the phenotype of the aberrant clone. Conversely, extrinsic pressure may drive clonal immune escape. Interestingly, we have shown a shared defect of chromosome 6 harboring the HLA-A gene cluster. One could speculate that clones with deletion of one of the HLA alleles represent escape mutants selected through immune pressure. In such a scenario, a mutant clone would lack a restrictive HLA presentation allele. After immunosuppression, such selective advantage would not be present and the HLA-deficient clone would be “diluted” out by normal clones. That patients who displayed loss of heterozygosity involving the HLA-A locus have a similar clinical phenotype, with pancytopenia and aplasia, supports this notion. Analogous mechanisms have been recently described for relapsed AML in the context of GVL disease after allogeneic partially mismatched BMT.³⁸

In summary, our results demonstrate that SNP-A karyotyping in AA and related BM failure syndromes can complement metaphase cytogenetics and lead to the identification of clonal chromosomal lesions consistent with either highly clonal hematopoiesis or malignant evolution. In some instances, SNP-A findings may have clinical relevance with regard to the clinical management. Long-term follow-up studies will show whether SNP-A will facilitate identification of AA at risk for clonal evolution.

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Authorship

Contribution: M.G.A. collected, analyzed, and interpreted data, performed statistical analysis, and wrote the manuscript; M.W. performed research, collected, analyzed, and interpreted data, and wrote the manuscript; H.M. performed research and collected data; M.S. collected data; R.V.T. collected data; M.K. provided vital patient samples; C.L.O. performed research, collected, analyzed and interpreted data, and wrote the manuscript; M.A.S. provided vital samples and edited the manuscript; and J.P.M. designed research, analyzed, and interpreted data, and wrote the manuscript.

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