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Molecular anatomy of chromosome 7q deletions in myeloid neoplasms: Evidence for multiple critical loci

(instability/unbalanced translocation/monosomy/leukemia)

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ABSTRACT **Complete or partial deletions of the long arm of chromosome 7 (7q- and -7) are nonrandom abnormalities seen in primary and therapy-induced myelodysplasia (MDS) and acute myelogenous leukemia (AML). Monosomy 7, occurring as the sole cytogenetic anomaly in a small but significant number of cases, may denote a dominant mechanism involving critical tumor suppressor gene(s). We have determined the extent of allele loss in cytogenetically prescreened MDS and AML patients for microsatellite markers from chromosome 7q22 and 7q31. Whereas >80% of these cases revealed allele loss for the entire region, a rare case of the 7q- chromosome showed allele loss for only the proximal 7q31.1 loci flanked by the markers *D7S486* and *D7S2456*, and a case of monosomy 7 revealed allele loss for loci at both 7q31 and 7q22 with retention of sequences between these sets of loci. Furthermore, a case of AML with no cytogenetic anomaly of chromosome 7 revealed a submicroscopic allelic imbalance for a third distal locus, *D7S677*. These findings suggest the presence of three distinct critical loci that may contribute alone or in combination to the evolution of MDS and AML. The data also provide molecular evidence for unbalanced translocation with noncontiguous deletions, as an alternate mechanism underlying monosomy 7.**

Complete and interstitial losses of chromosome 7 (-7, 7q-) are nonrandom anomalies seen in *de novo* and therapy-induced myeloid neoplasms as well as leukemias arising from occupational or environmental exposure to mutagens. Thus far, there have been no molecular investigations on monosomy 7 in adult myeloid neoplasms, although this anomaly is more common than the interstitial deletions of the long arm (7q-). The importance of monosomy 7 is evident from the small but significant number of adult acute myelogenous leukemia (AML) cases in which it occurs as the sole anomaly (1). Childhood monosomy 7 seen secondary to Fanconi's anemia, juvenile chronic myelogenous leukemia, Down's syndrome as well as familial monosomy 7 may share genetic alterations associated with adult myeloid neoplasms (2). The poor prognosis associated with chromosome 7 anomalies in adult myelodysplasia (MDS) and AML (3, 4) underscores the need for molecular analysis.

Interstitial deletions of 7q, seen in a variety of solid tumors with distinct segments of loss at bands 7q11, 7q22, and 7q31 that are apparently unique to specific neoplasms, have raised the possibility of a number of tumor suppressor genes in the long arm of chromosome 7 (5). In the past 2 years, delineation of the critical regions of loss in 7q has been the focus of several investigations on solid tumors as well as hematopoietic neoplasms (6–16). Fluorescence *in situ* hybridization analyses have delineated a critical region of loss at 7q22 in myeloid malignancies (15, 16). Other studies have raised the possibility of unbalanced translocations of fragments of chromosome 7 being located on marker chromosomes; patients who were perceived to be monosomic by centromere-specific probes showed strong hybridizations to marker chromosomes with chromosome 7-painting probes (17). A correlation between survival and deletion limits in 77 cases of myeloid neoplasms, with the 7q- chromosome characterized by high resolution banding, suggests that loss of 7q31 loci may be implicated in refractoriness to therapy (18).

Although chromosomal deletions are indicative of loss of function, direct evidence for the tumor suppressor activity is provided by reversion of tumorigenicity in nude mice by micro cell hybrids containing human chromosome 7q31 (19). Furthermore, introduction of 7q loci restores cellular senescence-like behavior to immortalized human fibroblasts (20, 21).

The physical mapping and sequencing efforts on chromosome 7 have yielded yeast artificial chromosome (YAC) and bacterial artificial chromosome contigs with a large number of polymorphic markers and sequence-tagged sites that are linked physically (22). Availability of these reagents in the public domain facilitates rapid and accurate definition of the interstitial deletions. Interesting to note is the invariant allele loss for three distinct microsatellite markers (*D7S523*, *D7S486*, and *D7S522*), which lie within a <3-Mb interval on 7q31, in malignancies of the breast, ovary, prostate, lung, and colon (6, 8, 14, 23–26). However, loss of heterozygosity analysis on a large panel of breast tumors (n = 637) by the European consortium has revealed a 12% discrepancy between laboratories in the interpretation of allele loss, casting doubts on the accuracy of the molecular delineations (10).

Hematopoietic neoplasms provide a unique advantage to study allele loss in that the samples can be prescreened by conventional cytogenetics. We have developed a highly sensitive assay to determine loss of heterozygosity in fractions of peripheral blood or bone marrow enriched in leukemic cells (27). To delineate the critical region of loss in myeloid malignancies and to ascertain whether monosomy 7 represents a distinct genotypic entity with loss of an entire chromosome 7, >20 polymorphic markers from the commonly deleted 7q22–31 interval were used to screen paired myeloid leukemic and normal cells from patients prescreened to be 7q- or -7; additionally, a panel of myeloid leukemia patients with poor

Abbreviations: AML, acute myelogenous leukemia; MDS, myelodysplasia; contigs, contiguous deletions; RA, refractory anemia; YAC, yeast artificial chromosome.

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prognosis and no cytogenetic anomalies of chromosome 7 were searched for submicroscopic deletions. Whereas most patients showed allele loss for all of the loci tested, three cases delineated distinct regions of loss at 7q31 and 7q22. The findings are consistent with a model in which the putative tumor suppressor genes on 7q may act singly or cooperate with one another.

MATERIALS AND METHODS

Cases and Cytogenetics. Routine cytogenetic analyses were done on all of the MDS and AML patients seen at the Department of Hematology, University of Texas M. D. Anderson Cancer Center, as detailed elsewhere (28). Patients 1–22 had numerous cytogenetic anomalies in addition to the 7q- or -7 abnormalities. Data presented on patient 23 (Fig. 4) are from a 62-year-old female patient with a history of MDS progressing to AML. A bone marrow specimen was obtained at this time (February 1991), when her peripheral leukocyte count was ≈4,500, with 20% blasts. For cytogenetics, the marrow cells were cultured for 24 h without mitogen, and standard Giemsa-banded preparations were made. Twenty-two of 31 metaphases examined (71%) constituted a clone with the karyotype: 46 X,-X, t(5;18;17)(q31;p11;q11). The mononuclear blast population isolated from fixed portions of marrow specimens collected at this time was used for dinucleotide polymorphism analyses.

Dinucleotide Polymorphism Analyses. All of the primer sequences and amplification conditions were obtained from the Genome Database (http://www.gdb.org). The polymorphic dinucleotide markers spanning the 7q22–31.1 interval, used in the present study, are depicted in Fig. 1. PCR was performed as described (27, 29). Additional enrichment of leukemic blasts was obtained from the Ficoll buoyant fraction by short term culturing of cells in RPMI 1640 medium containing 30% fetal bovine serum. Con A-stimulated T cells were grown in short term culture from the Ficoll buoyant fraction whenever the sample size permitted. In some instances, the Ficoll buoyant fraction was a mixture of diploid lymphoid cells and abnormal leukemic blasts whereas the pelleted granulocytic fraction was derived from the malignant clone. In such cases, the amplification products from triplicate PCR of the Ficoll buoyant and pelleted fractions were quantitated on a phosphorimager, and the ratio between the alleles was calculated (27). The presence of a single allele in the peripheral blood or bone marrow samples enriched in granulocytes or blasts in conjunction with the presence of two alleles in the short term cultured T cells or lymphoid enriched fractions indicated loss of heterozygosity.

YAC Contigs. The chromosome 7 physical maps can be found at http://www.nhgri.nih.gov/DIR/GTB/CHR7. Additional World-Wide Web Sites on chromosome 7 mapping data are as reported by Bouffard *et al.* (22).

Fragile Sites. The fragile site designation was obtained from the Genome Database.

RESULTS

Characterization of Allele Loss in Patients with 7q- Chromosome. Seven cases of myeloid leukemia with 7q- chromosome were investigated to determine the extent of allele loss within the critical 7q22–7q31 region. As shown in Fig. 2 *A* and *B*, patients 1–6 have interstitial deletions that span the entire 7q22–31 loci. In contrast, patient 7 shows retention of all of the 7q31.1–31.2 polymorphic loci telomeric to *D7S687* and centromeric to *D7S525.* Thus, the uncharacteristically small interstitial deletion in this AML patient spans the proximal 7q31.1 segment flanked by *D7S486* and *D7S2456*. Fig. 2*C* depicts the alleles detected in short term cultured T cells (lane 1) and leukemic blasts (lane 2) of this patient. Unambiguous

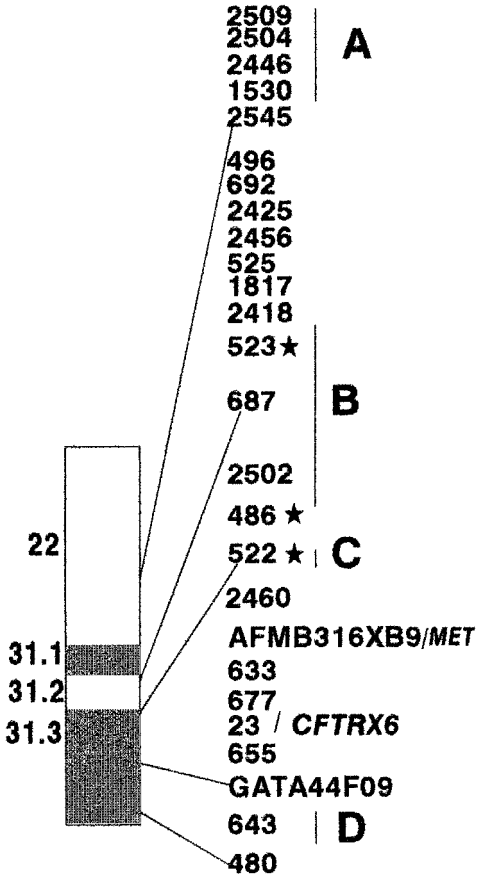

FIG. 1. Polymorphic markers from 7q22–31.3. The prefix *D7S* is omitted. The distances between the loci are not to scale. Order of loci is based on the physical linkage in YAC contigs (22). Consistent region of allele loss as delineated by other studies correspond to *A*(15,16), *B*(6,8,14), *C*(23–26), and *D*(7). *CFTRX6*, polymorphism in exon VI of the *CFTR* gene; *MET*, *C-MET* protooncogene; ∗, loci identified to show invariant allele loss.

allele losses are identified for *D7S687* and *D7S525*, whereas the amplification pattern is identical between the T cells and leukemic blasts (lanes 1 and 2) for all of the flanking markers. The critical region of loss as defined by this patient includes the *D7S523 and D7S687* loci, identified to be from a region deleted in prostate, ovarian, and breast tumors (Fig. 1 and the references therein).

Characterization of Allele Loss in Monosomy 7 Patients. Fifteen cases of preleukemic MDS and AML identified to be monosomic for chromosome 7 were examined for loss of heterozygosity of the 24 polymorphic markers. As shown in Fig. 3*A*, patients 8–20 showed allele loss for all of the 13 loci in the 7q31.1–7q31.2 region. Patients 21 and 22 appear to retain both alleles of all these loci due to unidentified translocations of chromosome 7 that may be retained on the marker chromosomes. Further centromeric walk (Fig. 3*B*) revealed that patients 21 and 22 retained both alleles of all of the loci. It was surprising that, patient 20, who was a case of AML, retained a segment of the 7q22-q31.1 region containing *D7S1817*, *D7S525*, *D7S2456*, *D7S2425*, *D7S692*, and *D7S496*. The telomeric end of this segment resides between the microsatellite markers *D7S1817* and *D7S2418* (Fig. 3*B*).

Fig. 3*B* also shows the status of allele loss in the monosomy 7 patients within the critical 7q22 region flanked by *D7S2545* and *D7S2509*. Patients 8–18 show allele loss for all of the markers within this interval, whereas patient 19 retains the 7q22 markers. Of interest is patient 20, who retains both alleles of *D7S2545* but has lost the proximal loci *D7S2446*, *D7S2504*,

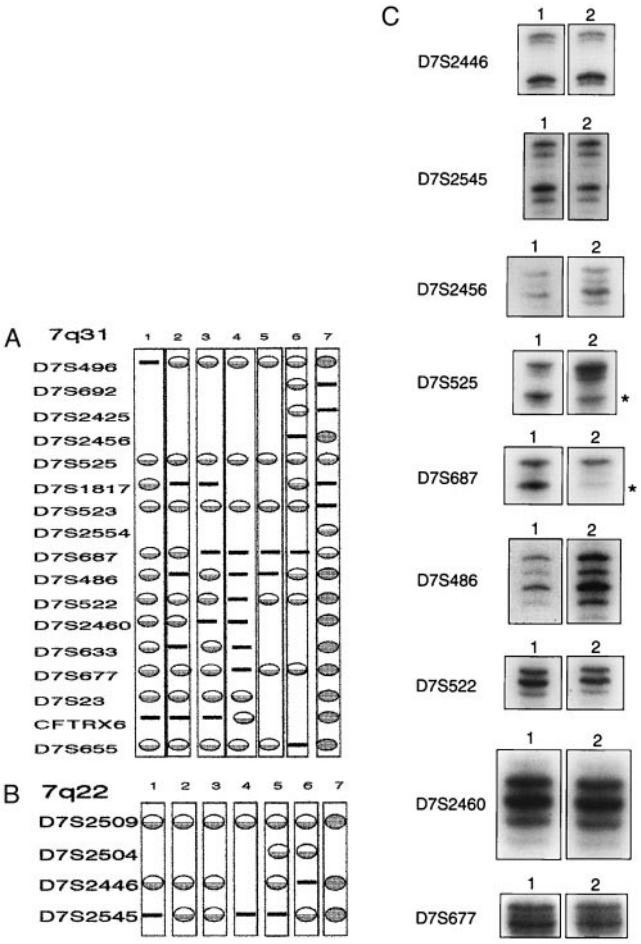

FIG. 2. (*A* and *B*) Allele loss in AML and MDS patients with 7q- chromosome. Loss of heterozygosity was determined as described in *Materials and Methods*. Half-filled circle, loss of heterozygosity; filled circle, retention of both alleles; lines, noninformative. (*C*) Patient 7 shows an uncharacteristically small interstitial deletion. Representative autoradiograph revealing allele loss for the *D7S687* and *D7S525* loci. Lanes: 1, short term cultured T cells; and 2, Leukemic blasts. *, deleted alleles.

and *D7S2509*, suggesting that the telomeric boundary of the critical 7q22 locus resides centromeric to *D7S2545*.

Fig. 3*C* depicts the products of PCR amplification in patient 20, whose chromosomal loss is apparently noncontiguous. In contrast to the bi-allelic amplification pattern for a population enriched in T cells (lane 1), loss of the *D7S486*, *D7S523*, and *D7S2418* loci is seen in the short term cultured leukemic blasts (lane 2). The chromosomal break between *D7S2418* and *D7S1817* is evident by the loss of the smaller allele for the former and presence of two alleles of the latter in the malignant clone (lane 2). The physical linkage between *D7S2418* and *D7S1817* is estimated to be <1.1 Mb because they are contained within the YAC yWSS4526 (22). Fig. 3*C* also shows that both alleles of *D7S2456* and *D7S2545* are present in the leukemic blasts. Previous studies that delineated distal limits of the critical 7q22 locus have demonstrated that the sequences from the YAC yWSS3710 spanning *D7S2545* were retained on a 7q- chromosome (15). The chromosomal break in patient 20 appears to reside within a neighboring YAC yWSS642 with an insert size of 600 kbp. *D7S1530*, for which the leukemic blasts show loss of heterozygosity, and *D7S2545,* with both alleles retained in this patient (Fig. 3*C*), have been localized to this YAC.

Submicroscopic Deletion of D7S677 in a Case of AML in Evolution. To delineate further the critical regions of loss, a panel of MDS and AML patients, who were cytogenetically diploid for chromosome 7, were screened for a subset of 7q22–7q31 markers. A single case of AML in evolution (patient 23) showed an apparent allelic imbalance for *D7S677* as revealed by the decreased amplification of the smaller allele. In contrast, there were no readily detectable differences between the two alleles of *D7S522* and *D7S687* in this patient (Fig. 4). A restricted amount of material and lack of informativeness for several other markers hindered an extensive delineation of the critical region flanking the *D7S677* locus. Nonetheless, retention of both alleles of *D7S522* and *D7S687* pinpoints the significance of a critical region in the band 7q31.3.

Results presented in Figs. 2–4 have allowed us to delineate three distinct critical regions in myeloid neoplasms. First, patient 7 showed a critical locus of loss spanning the proximal 7q31 region, which is flanked by *D7S486* and *D7S2456*. Second, direct evidence for allele loss at distinct 7q22 and 7q31 loci is provided by patient 20, who appears to retain the segment of chromosome 7q between *D7S1817* and *D7S2545* on a marker chromosome. Finally, a submicroscopic allelic imbalance for *D7S677* in patient 23 implies a third critical region.

DISCUSSION

Recent progress in the physical mapping and sequencing of human chromosome 7 has resulted in the availability of physically linked contigs with a high density of polymorphic markers (22). In this study, we used these reagents to delineate a subset of the critical segments of deletions in preleukemic MDS and AML. Loss of heterozygosity analysis in myeloid disorders as described in this report are highly reliable and sensitive because of the comparison with diploid T cell populations. Furthermore, absence of replication errors, which are frequent in solid tumors, facilitates unambiguous interpretation of allele loss in myeloid neoplasms (refs. 30 and 31 and J.F. and L.N., unpublished results). This precision is demonstrated by the results obtained for patients 7 and 20 (Figs. 2*C* and 3*C*), in whom the interstitial deletion limits in 7q31 and 22 could be mapped to <2 Mb.

Delineation of the Critical Regions of Loss in 7q31.1. The uncharacteristically small deletion seen in patient 7, whose distal break is between *D7S687* and *D7S486,* may provide important clues in delineating a novel locus. The physical distance between *D7S486* and *D7S687* is estimated to be <1.5 Mb (22). The *D7S687* locus is shown to be the centromeric limit of the consistent region of loss in a study of 73 cases of breast tumors that showed invariant allele loss for *D7S486* (8). This region of loss resides within the *D7S523-D7S486* interval identified to be the site of allelic imbalance in prostate cancer (14). The losses in patients 7 and 20 also include the *D7S523* locus, which appears to be deleted consistently in advanced ovarian tumors (6). All of these findings may be pinpointing the same critical region; the studies on prostate and ovarian malignancies did not screen for loss of *D7S687*. Taken together, the *D7S687* and *D7S486* loci appear to delineate a critical segment of loss with an estimated distance of <1.5 Mb, which is part of a well characterized YAC contig (22).

The submicroscopic allelic imbalance of the *D7S677* locus in patient 23 may indicate a tumor suppressor locus or trisomy of a small segment whose centromeric end resides between *D7S522* and *D7S677*. The *c-MET* protooncogene, which is mutated constitutionally in familial papillary renal carcinomas exhibiting trisomy for chromosome 7, resides within the *D7S522-D7S677* interval (32). Furthermore, *D7S677* is present in a nonchimeric CEPH YAC 212H1 (with an insert size of 480 kbp), whose telomeric end spans intron 12 of the *CFTR* gene (H.L. and L.N., unpublished results). An increased frequency of leukemia and melanoma has been observed among the first degree relatives of patients with cystic fibrosis, although the

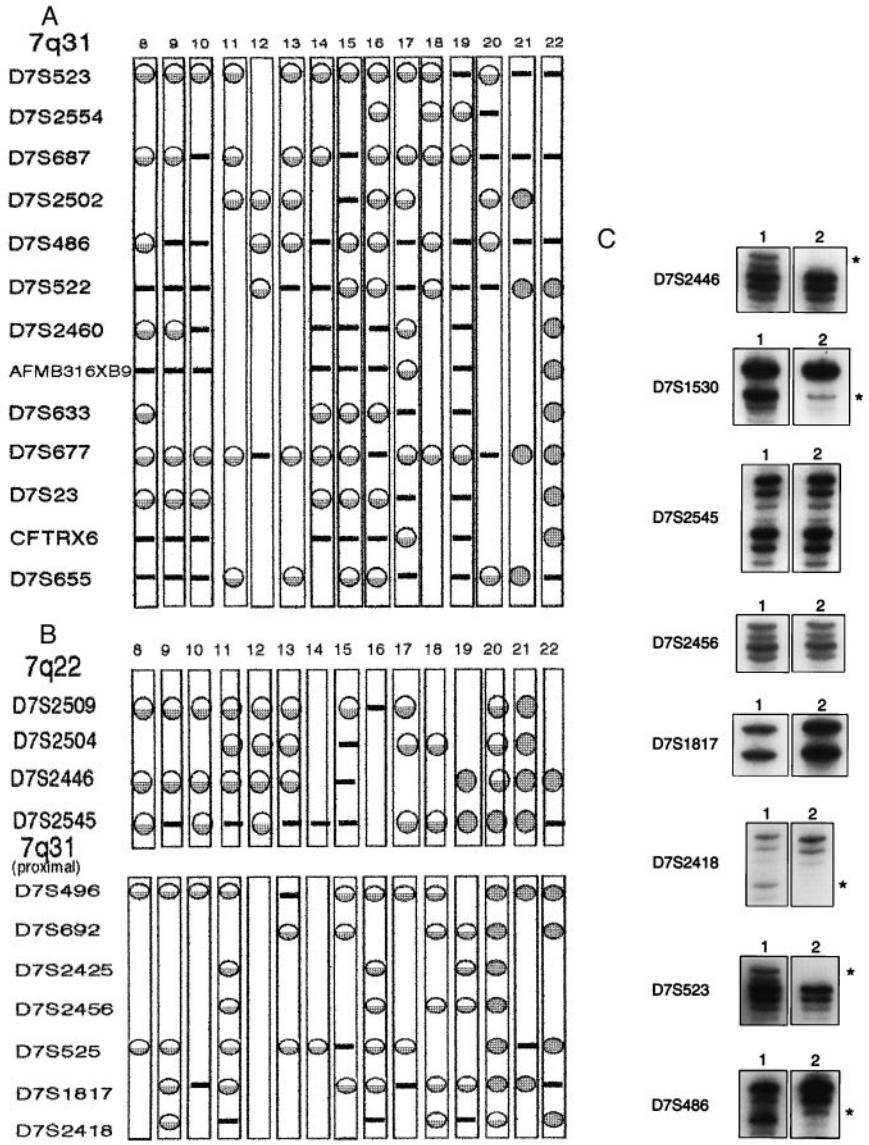

FIG. 3. (*A* and *B*) Allele loss in AML and MDS patients with monosomy 7. Loss of heterozygosity was determined as described in *Materials and Methods*. Half-filled circle, loss of heterozygosity; filled circle, retention of both alleles; lines, noninformative. Patients 21 and 22 show apparent retention of all of the 7q31 loci. Patient 20 shows retention of markers centromeric of *D7S2418* and shows a break within the *D7S2545-D7S2446* interval. (*C*) Representative autoradiograph revealing allele loss for *D7S486, D7S523, D7S2418, D7S1530*, and *D7S2446* loci. Lanes: 1, short term cultured Ficoll buoyant fraction enriched for T cells by Con A stimulation; and 2, short term cultured leukemic blasts. ∗, deleted alleles.

molecular mechanism behind this association is unknown (33). Characterization of additional polymorphic markers and identification of cases similar to patient 23 will allow better understanding of the heterogeneity of molecular lesions associated with anomalies of the proximal 7q31.3 region.

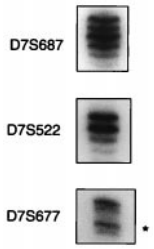

FIG. 4. Allelic imbalance of the *D7S677* locus. Bone marrow samples from patient 23, who was cytogenetically diploid for chromosome 7, were analyzed as described in *Materials and Methods*. ∗, decreased amplification of the smaller *D7S677* allele.

Delineation of the Critical Region of Loss at 7q22. Recent reports have delineated this locus to be flanked by *D7S2545* (on the telomeric side) and *D7S2446* (on the centromeric side); sequences from YACs containing these loci hybridized to the 7q- chromosome (15, 16). The availability of a large number of highly informative markers allowed further narrowing of the telomeric limit of this locus between *D7S1530* and *D7S2545* (Fig. 3*C*). The interstitial deletions in six of the seven 7q- patients and 19 of the 22 monosomy 7 cases include the proximal 7q22 locus; isolation of the candidate tumor suppressor gene will elucidate whether both the 7q31 and 7q22 loci contribute to leukemogenesis in a subset of these patients.

Instability of Chromosome 7q Sequences. Fluorescence *in situ* hybridization studies with chromosome 7-specific probes revealed that a subset of patients identified to be monosomic gave a single signal with the centromeric probe but retained chromosome 7 sequences on marker chromosomes (as detected by painting probes), raising the possibility of chromosomal instability (17). Patient 20, with the multiple noncontiguous deletions, not only appears to localize the critical

regions of instability but also provides a unique opportunity to characterize the sequences between *D7S1530* and *D7S2545* as well as the break between *D7S2418* and *D7S1817*. Of interest, the noncontiguous deletion appears to be unique to chromosome 7 loci in this patient because chromosome 5 sequences (for which he was monosomic) appear to show a contiguous loss (J.F. and L.N., unpublished results).

Future studies will determine whether the *D7S1530-D7S2545* interval is the site of the aphidicolin-induced rare fragile site Fra 7F at 7q22 (34). Nonetheless, thus far there have been three other reports of chromosomal breaks (two cases of therapy-induced myeloid leukemia and a single case of chronic myelogenous leukemia in blast crisis) within the *D7S2509-D7S2545* interval (15, 16). In an analogous scenario, the loss of heterozygosity patterns in ovarian tumors implies that noncontiguous deletions of 7q31 loci may be frequent (7). Indeed, one of the critical segments identified by Edelson *et al.* (7) resides between *D7S687* and *D7S2502*, a locus that may be the site of the distal break in patient 7 in the present study (Fig. 2).

A preliminary report on allele loss for chromosome 7 markers suggested that mitotic recombination in a pluripotent stem cell, around the commonly deleted 7q31 loci, might account for the observed high degree of homozygosity in patients with 7q- chromosome (35). Results presented in the present study (Figs. 2 *A* and *B* and 3 *A–C*) do not reveal an unexpectedly high incidence of homozygosity in the 22 cases. In addition, analysis of nonhematopoietic samples from patient 19 (Fig. 3*A*) demonstrated that he is constitutively uninformative for the loci between *D7S633* and *D7S486*.

Localization of the regulatory subunit IIB of protein kinase A as well as *DRA* (which is down-regulated in colonic adenoma) to the 7q22–31 locus raises the possibility of these being candidate tumor suppressor genes (22). However, both of these genes map to the island of retention in patient 20, namely the *D7S525–D7S2545* interval. Similarly, retention of both alleles of *D7S486* in patient 7 excludes a suppressor role for all of the genes telomeric of this locus in a subset of AML and MDS patients.

In summary, we have provided direct evidence for three distinct segments of chromosomal loss in the bands 7q22–31 in patients with myeloid neoplasms. Availability of these loci as molecularly cloned DNA with accurate physical linkage information coupled with the ready accessibility of the DNA sequence information for some of these segments in the public domain (22) provides powerful reagents for the isolation of the candidate tumor suppressor genes.

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