

## Duplication and Loss of Chromosome 21 in Two Children With Down Syndrome and Acute Leukemia

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Acute leukemia in Down syndrome (DS) is often associated with additional changes in the number or structure of chromosome 21. We present two DS patients whose leukemic karyotypes were associated with changes in chromosome 21 ploidy. Patient 1 developed acute lymphocytic leukemia (type L1); disomy for chromosome 21 was evident in all blast cells examined. Loss of the paternal chromosome in the leukemic clone produced maternal uniparental disomy with isodisomy over a 25-cM interval. The second patient had acute monoblastic leukemia (type M5) with tetrasomy 21 in all leukemic cells. DNA polymorphism analysis showed duplicate paternal chromosomes in the constitutional genotype. The maternal chromosome was subsequently duplicated in the leukemic clone. The distinct inheritance patterns of chromosome 21 in the blast cells of these patients would appear to indicate that leukemogenesis occurred by different genetic mechanisms in each individual.

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**KEY WORDS:** Down syndrome, acute lymphocytic leukemia, acute myelogenous leukemia, monoblast, uniparental disomy, tetrasomy, ploidy

### INTRODUCTION

Although the molecular bases for acute leukemias of childhood have been established for several types of chromosomal rearrangements, it is unknown why children with constitutional trisomy 21 (Down syndrome;

DS) are predisposed to develop hematologic malignancies. Compared to controls, children with DS develop acute leukemia (AL) earlier and have an approximately 15-fold increased risk of AL [Robison et al., 1984; Miller, 1970]. Acute myeloid leukemia (AML) predominates in the newborn period [Fong and Brodeur, 1987; Rosner and Lee, 1972]; however, there is also an increased risk of acute lymphocytic leukemia (ALL) during the first decade of life [Kalwinsky et al., 1990]. AML in these patients is often of the M7 type (megakaryocytic leukemia) [Watson et al., 1993].

Trisomy 21 is also among the most frequent numerical abnormalities in the leukemic blasts of non-Down syndrome patients with ALL and AML [Rowley, 1981; Watson et al., 1993]. Granulocytic and monocytic lineages predominate in non-DS patients with trisomy 21 as the sole or primary abnormality in the leukemic clone [Dewald et al., 1990; Sakurai and Swansbury, 1982]. The gain or loss of one or more chromosomes in ALL and AML, when present in DS, is similar to that found in the cells of patients with AL but without trisomy 21, and is often related to the type of leukemia [Rowley, 1981].

Newborn DS patients frequently present with a transient leukemoid reaction (TLR), which is characterized by neutrophilia resembling chronic myelogenous leukemia [Fong and Brodeur, 1987; Hecht et al., 1986; McCoy and Epstein, 1987; Rowley, 1981]. Moreover, the higher prevalence of TLR in phenotypically normal individuals with trisomy 21 mosaicism suggests that the abnormal karyotype is causally related to TLR [Ferster et al., 1986; Sikand et al., 1980; Rogers et al., 1983; Brodeur et al., 1980]. The leukocytosis and the abnormal karyotype usually disappear without treatment [Heaton et al., 1981], suggesting that the trisomic cells may temporarily gain a proliferative advantage over normal cells. TLR has been suggested to represent the first stage of the disease, with individuals carrying a second mutation being at increased risk for developing AL [Scholl et al., 1982].

Disruption due to translocation of chromosome 21, involving either one of two distinct genes, *AML1* or *ERG*, results in acute myelogenous leukemia [Miyoshi et al., 1991; Shimizu et al., 1993; Nucifora et al., 1993].

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The most common translocation, t(8;21)(q22;q22), creates a novel fusion transcript, *ETO-AML1*, from genes whose sequences are related to those involved in cell cycle control and pattern development on chromosomes 8 and 21, respectively [Erickson et al., 1992; Nisson et al., 1992]. *AML1* is also interrupted in therapy-related myelodysplastic syndrome [Nucifora et al., 1993] as well as in chronic myelogenous leukemia in blast crisis due to a recurrent translocation, t(3;21)(q26.2;q22). A chimeric gene is formed in the t(16;21)(p11;q22) rearrangement [Shimizu et al., 1993], consisting of the *ETS*-related DNA-binding domain of the transcriptional regulator, *ERG* [Reddy and Rao, 1991; Siddique et al., 1993], and of *TLS/FUS*, a gene encoding RNA-binding protein that is similar to the *EWS* gene involved in Ewing sarcoma [Ichikawa et al., 1994]. The predicted fusion products have been suggested to act by affecting multiple differentiation pathways in hematopoietic stem cells, leading to a heterogeneous clinical and histopathological presentation of leukemia [Karp and Broder, 1994].

In addition to changes in the numerical and structural constitution of chromosome 21, X chromosome aneuploidy occurs more often in the lymphoblasts of DS patients than in individuals with ALL where trisomy 21 is present [Pui et al., 1993; Mitelman et al., 1990]. Isochromosome of 9q may also be more frequent in DS patients with ALL [Kalwinsky et al., 1990]. The distribution of secondary structural and numeric abnormalities present in non-DS patients with trisomy 21 and leukemia differs significantly from the DS/AL population [Raimondi et al., 1992; Ankathil et al., 1992; Furuya et al., 1992]. Apart from the t(8;21) translocation found in 40% of patients with AML subtype M2 (and to a lesser extent t(16;21) and t(3;21)), the inconsistent representation of chromosomes other than 21 in different cells of the leukemia clone suggests the possibility that some of these abnormalities may arise after the initial leukemogenic event.

We present two Down syndrome patients who developed AL with alterations in their constitutional karyotype that resulted in consistent numerical changes in chromosome 21. Patient 1 demonstrated loss of a single copy of chromosome 21 and ALL. Acute monoblastic leukemia was diagnosed in patient 2, with tetrasomy 21 in blast cells. Chromosome 21 loss is relatively uncommon in DS patients with AL, whereas gain of this chromosome is one of the most frequent abnormalities in leukemia in these children [Kalwinsky et al., 1990]. In order to understand how changes in chromosome 21 ploidy might be related to the development of leukemia in these two individuals, we have deduced the parental origins of the chromosomes involved in these events using DNA polymorphisms.

## MATERIALS AND METHODS

### Cytogenetic Studies

Bone marrow specimens in sodium heparin were cultured in parallel in 1) RPMI supplemented with 20% fetal bovine serum, 2) Chang medium D, and 3) medium 199 supplemented with 5% fetal bovine serum. The cultures were harvested as direct preparations and GTG-

banded. A blood specimen from patient 2 was similarly processed, except that cultures were incubated for 24, 48, or 96 hr both in the presence and absence of phytohemagglutinin (PHA).

After destaining, fluorescence in situ hybridization (FISH) was performed with a chromosome 21 painting probe according to the manufacturer's instructions (Oncor, Gaithersburg, MD).

### DNA Polymorphism Analysis

This study was approved by the Clinical Investigation Committee of the Pennsylvania State University College of Medicine. After obtaining informed consent, 5-cc samples of aspirated bone marrow (BM) were acquired during routine BM examination performed at time of diagnosis and on subsequent therapeutic marrow examinations of patient 2. At the conclusion of treatment (and/or upon remission), either a blood sample or skin biopsy was obtained to establish the patient's constitutional genotype; 20–30-cc aliquots of blood were also obtained from both parents of the patients. Genomic DNA was isolated from blood and blasts by purification over a Ficoll-Hypaque gradient (Pharmacia, Uppsala, Sweden) followed by a nonorganic extraction procedure [Buffone, 1985; Miller et al., 1988]. Polymorphic short-sequence-repeat (SSR) markers were used to distinguish the parental origin of chromosomes 21 in the patient's lymphocytes or fibroblasts, blast, and remission bone marrow cells [Pangalos et al., 1994; Weber and May, 1989].

DNA from patient 1 was extracted from formalin-fixed, paraffin-embedded BM sections (10  $\mu$ m). Genomic templates suitable for polymerase chain reaction (PCR) amplification were produced by preextension PCR (PEP) using random 15-mers as described by Zhang et al. [1992] and Li et al. [1990].

The polymorphic chromosomal 21 loci used in the present study were D21S369, D21S215, D21S258, D21S120, D21S13, D21S172, D21S406, D21S11, D21S145, D21S214, D21S222, D21S260, D21S217, D21S265, D21S210, D21S269, D21S263, D21S1280, D21S216, D21S262, GART, IFNAR, D21S65, D21S167, D21S266, D21S59, D21S270, D21S1238, D21S259, D21S265, D21S168, HMG14A, D21S257, D21S264, D21S268, D21S49, *PFKL*, and D21S171. For information concerning the oligonucleotides used per marker, their locations on the physical and/or genetic map, and respective PCR reaction conditions, refer to the Genome DataBase (Johns Hopkins University), Avramopoulos et al. [1993], and McInnis et al. [1993].

The parental origin of the allele associated with the nondisjoined chromosome was determined according to Antonarakis et al. [1990] and Pangalos et al. [1994]. The origin of the duplicated chromosomes (in the trisomy 21 and in the leukemic cells) was determined by internal comparison of allele intensities for each locus at different markers along the chromosome. Copy number determination of nonpolymorphic or uninformative genetic loci was performed by densitometric analysis of multiple autoradiographic exposures of gels. The parental origin of the additional 21 chromosome in the tetrasomy 21 clone of patient 2 was confirmed at non-

polymorphic genetic loci by measuring changes in the relative ratio of the integrated densities of alleles at informative loci in the constitutional (fibroblasts or post-treatment) vs. the blast cell line.

PCR amplification of 2- $\mu$ l aliquots of PEP reaction products from patient 1 at selected, informative genetic loci was carried out as described above, except that the number of cycles was optimized for each locus tested. Thirty cycles of amplification were determined to be appropriate for producing detectable products at D21S172 and D21S214.

An *Eco*RI polymorphism at the *AML1* locus [Birn et al., 1993], an *Msp*I polymorphism at the *ETS2* locus [Creau-Goldberg et al., 1987], and a *Bam*HI fragment that spans the breakpoint cluster region of the *ERG* gene were examined by Southern hybridization analysis of restriction-digested genomic DNA from monoblasts, remission bone marrow, and fibroblasts of patient 2, and from lymphocytes of her parents. The *AML1* probe was obtained from Dr. Guiseppina Nucifora (GN201) [Birn et al., 1993]. A synthetic probe derived from the coding domain of the *ETS2* gene was generated by PCR-amplification of a 309-bp genomic segment (positions 1891–2200; GenBank locus J041021) [Watson et al., 1988]. The *ERG* cDNA probe was produced by random-primed reverse transcription of total RNA followed by PCR (positions 775–885) [Rao et al., 1987], and was designed to detect sequences flanking the intron containing all of the known translocation breakpoints. The number of genomic copies of *AML1* or *ETS2* sequences at diagnosis and remission were estimated by comparison of band intensities after rehybridization with a non-polymorphic, chromosome 13-specific probe, H2-26 (D13S28) [Tantravahi et al., 1989]. The chromosome 21 locations of *AML1*, *ERG*, and *ETS2* with respect to the other polymorphic markers studied in this patient are presented in Table III.

## RESULTS

### Clinical Diagnosis

**Patient 1.** This 3-year-old Amish girl with Down syndrome presented with a WBC of 6,200 with 24% blasts, hemoglobin 2.9 gm/dl, hematocrit 8.8%, and a platelet count of 6,200/mm<sup>3</sup>. Differential on peripheral blood included 24% blasts. Physical exam documented presence of Down syndrome, bruising, petechiae, mild hepatomegaly, and left lower lobe pneumonia. Bone marrow aspirate morphology was 98% L1 lymphoblasts. Immunohistochemical staining of blasts showed granular PAS positivity in the cytoplasm; Sudan black, peroxidase, and combined esterase stains were negative. Flow cytometry showed a standard I2 (HLA-DR), B4 (CD-19), and CALLA positive immunophenotype, consistent with acute lymphocytic leukemia of childhood. Spinal fluid cell count and cytology were normal. Treatment was initiated on Children's Cancer Group Protocol 1881 [Trigg et al., 1994], Regimen B, with vincristine, prednisone, L-asparaginase, and intrathecal methotrexate. Day 14 bone marrow showed early response to therapy, and day 28 bone marrow demonstrated remission. Therapy was completed without ma-

ior complications in 26 months, and she remains in remission 2 years off-therapy.

**Patient 2.** This 2 $\frac{1}{2}$ -year-old Caucasian girl with Down syndrome presented with fever, pharyngitis, diffuse erythematous maculopapular rash, hepatosplenomegaly, cervical adenopathy, and the following blood counts: white blood cell count, 11,400/mm<sup>3</sup>; hemoglobin, 8.4 gm/dl; hematocrit, 25.9%; platelet count, 24,000/mm<sup>3</sup>; and a differential including 9 blasts, 3 promyelocytes, 2 myelocytes, 2 metamyelocytes, 8 bands, 19 neutrophils, 47 lymphocytes, 2 monocytes, and 2 basophils. Initial bone marrow aspirate and biopsy documented a general dysmyelopoiesis and a small (22%) undifferentiated population of 12–15  $\mu$  diameter blasts of inconclusive morphology. Flow cytometry demonstrated a small blast population of 20% of total nucleated cells, and equivocal phenotyping: 12 (HLA-DR) 74%, B4 (CD-19) 0%, MY 7 (CD-13) 11%, MY 9 (CD-33) 19%, CD34-progenitor cells 47%, and T1 (CD-5) 4%. The patient was observed without treatment, and over 1 week, peripheral blood blast count rose to 20% with a repeat bone marrow test showing a predominant undifferentiated blast population with similar flow cytometry results. Diagnosis of acute monoblastic leukemia was made, based on strongly positive Napthol-AS-D immunohistochemical staining. Spinal fluid cell count and cytology were normal. Treatment was initiated on Children's Cancer Group Protocol 2891 [Woods et al., 1994] with daunomycin, Ara-C, etoposide, 6-thioguanine, and dexamethasone. A day 7 bone marrow specimen showed early response to therapy, as did the patient's clinical signs and symptoms. A day 43 bone marrow specimen showed no evidence of disease in a 95% cellular specimen. Chemotherapy treatment was completed in 9 months, and the patient remains in remission 8 months after cessation of therapy.

### Cytogenetics

The only consistent karyotypic abnormality present in a direct analysis of BM from patient 1 was the loss of a single copy of chromosome 21 in 4 cells (Table I). Disomy 21 was confirmed in a total of 10 metaphase cells. A heterochromatic marker chromosome larger than chromosome 21 was present in 3 of 4 cells karyotyped. Fluorescence in situ hybridization (FISH) with a chromosome 21 painting probe did not highlight the marker chromosome (results not shown). Three cells also displayed monosomy X, and one of the monosomy X cells showed trisomy 18.

The chromosome 21 karyotype of an unstimulated BM sample from patient 2 showed tetrasomy 21 in the 9 cells that were analyzed (Table I). Eight unstimulated peripheral blood lymphocytes exhibited predominantly the same karyotype. Trisomy and tetrasomy 21 cells were equally represented in PHA-stimulated peripheral blood. Karyotypes of marrow cells harvested 7, 14, and 35 days postchemotherapy revealed virtual complete repopulation with 47,XX,+21 cells (results not shown). Skin fibroblasts were 47,XX,+21. Aneuploidy of chromosomes X, 4, 5, 6, 9, 11, 18, and 19, isochromosome 17q, and an unidentified marker chromosome were inconsistently present in different metaphase cells from this patient.

TABLE I. Leukemia Karyotypes of DS Patients  
(A) Direct Analyses of Bone Marrow

Patient	Pretreatment	Posttreatment
1	47,XX, +mar 1 [1 cell]/46,X,-X, +18 [1 cell]/46,X,-X, +mar 1 [3 cells]	47,XX, +21 [20 cells] <sup>a</sup>
2	50,XX, +11, +19, -20, +21, +21, +mar 1 [1 cell]/48,XX, +21, +21 [3 cells]/48,X,-X, +11, +21, +21 [1 cell]/48,X,-X, -4, +5, +9, +21, +21 [1 cell]/47,-X,-X, +i(17q), +21, +21 [1 cell]/48,XX,+X, -6, +i(17q), -18, +21, +21 [1 cell]	47,XX,+21 [8 cells]/50,XX, +1, +1, -2, +7, +8, +9, +11, -12, -13, -15, +19, +21 [1 cell]/66,XX, +1, +1, +2, +2, +3 +3, +4, +4, +5, -6, +7, +8, +9, +10, +11, +11, +12, -13, -14, -14, +16, +17, +17, +19, -20, +21, +21, +21, +22 [1 cell] <sup>b</sup>

<sup>a</sup> At remission.<sup>b</sup> Five weeks postchemotherapy.

(B) Short-Term Cultures of Peripheral Blood, Patient 2

Culture conditions	47,XX, +21	48,XX, +21, +21
Pretreatment		
Unstimulated, 24 hr	0	9
Unstimulated, 48 hr	0	9
PHA-stimulated, 48 hr	15	2
PHA-stimulated, 96 hr	32	0
Five weeks postchemotherapy		
Unstimulated	20	0

### Parental Origin of Chromosome 21 in the Leukemic Clone

**Hypoploidy of chromosome 21 in patient 1.** This DS patient was originally diagnosed in 1991 with ALL, and a limited quantity of archival bone marrow was available for genotype studies of the blast clone. The constitutional genotype of this child demonstrated maternal meiosis I nondisjunction, based on the inheritance of heterozygous maternal alleles at D21S172 (which is approximately 5 cM from the most centromeric genetic marker) and D21S214 (Table II). Sev-

eral other proximal markers displayed genotypes consistent with the inheritance of both maternal alleles (D21S265, D21S269, D21S262, and D21S210). Recombination in maternal meiosis occurred between D21S167 and D21S259, and all genetic loci distal to D21S167 were reduced to homozygosity (D21S266, D21S268, D21S171, and *PFKL*; see Table II).

The analysis of DNA isolated from formalin-fixed, paraffin-embedded sections revealed the loss of paternal alleles at D21S172 (Fig. 1a) and D21S214 (Table II), producing maternal uniparental disomy of chromosome 21

TABLE II. Genotypes of Chromosome 21 Polymorphisms in Patient 1\*

Genetic locus	Proband bone marrow at diagnosis	Proband lymphocytes: at remission	Origin of constitutional nondisjunction		Origin of leukemogenic event	Reduced (R) or nonreduced (NR)
			Paternal	Maternal		
D21S120		122	12	12		NR
D21S13E		111	11	11		
D21S172	12	123	33	12	M1	NR
D21S214	12	123	33	12	M	NR
D21S210		112 <sup>a</sup>	11	12		NR
D21S265		223 <sup>a</sup>	12	23		NR
D21S269		112 <sup>a</sup>	11	12		NR
D21S262		123				NR
D21S270		111	11			
D21S167		112 <sup>a</sup>	11	12		NR
D21S267		223 <sup>a</sup>	13	22	M	
HMG14A		113	13	11		
D21S259		333	23	13		R
D21S268		144 <sup>a</sup>	12	34	M	R
D21S266		113 <sup>a</sup>	23	12	M	R
<i>PFKL</i>		112 <sup>a</sup>	23	12	M	R
D21S171		133 <sup>a</sup>	12	34	M	R

\*P, paternal segregation error; M, maternal segregation error; M1, maternal segregation error, probably during meiosis I; NR, maternal genotype heterozygous, proband heterozygous for maternal alleles; R, maternal genotype heterozygous, proband homozygous for maternal alleles.

<sup>a</sup>Based on a comparison of the relative intensities of alleles [Pangalos et al., 1994].

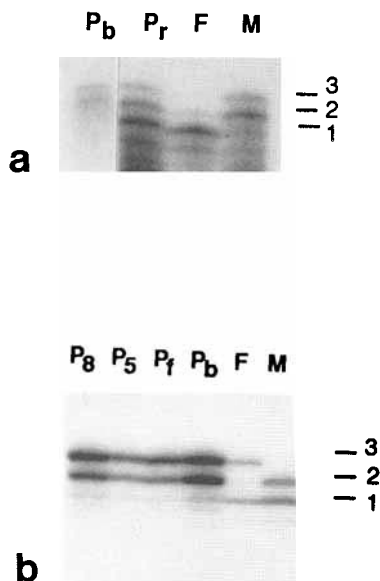


Fig. 1. Representative molecular genetic analyses of chromosome 21 SSR loci. Genotypic analysis of (a) locus D21S172 of patient 1 and (b) locus D21S216 of patient 2. Symbols designate proband bone marrow, Blast, P<sub>b</sub>; bone marrow, remission, P<sub>r</sub>; bone marrow, 8 weeks posttreatment, P<sub>8</sub>; bone marrow, 5 weeks posttreatment, P<sub>5</sub>; fibroblast, P<sub>f</sub>. Maternal (M) and paternal (F) genotypes were determined by conventional PCR amplification techniques. The lymphoblast genotype of patient 1 was determined by amplification of the SSR polymorphism after preextension PCR [Zhang et al., 1992] of archival bone marrow with random primers (different exposures of the same gel were combined due to the low yield of amplification products from the archival sample). Alleles are numbered according to Pangalos et al.

in the lymphoblast clone. This result also indicates that the disomy 21 karyotype was representative of the entire population of leukemic cells. We suggest that the loss of the paternal chromosome in this patient may have unmasked either a recessive mutant oncogene or a mutant tumor suppressor gene in the isodisomy domain of this patient (Fig. 2a; see Discussion). Since she displays heterozygous maternal inheritance centromeric to and including D21S262, it follows that the gene(s) predisposing to the development of ALL in this patient would be unlikely to reside in this proximal 21q interval.

**Hyperploidy of chromosome 21 in patient 2.** Acute monoblastic leukemia in patient 2 arose in a cell line with a predominantly tetrasomy 21 karyotype (Tables I and II). Analysis of genotypes from remission bone marrow and fibroblasts showed the ratio of intensities of the band corresponding to the paternally-inherited allele to be twice that of the corresponding maternal allele at the following genetic loci: D21S120, D21S11, D21S145, D21S217, D21S265, D21S1280, D21S65, D21S216, D21S49, and *PFKL* (Table III; Fig. 1b). Identical paternal alleles were inherited at heterozygous paternal markers spanning most of the q arm, indicating that the constitutional nondisjunction event involved two identical paternal chromosomes. Either a postzygotic segregation error involving the paternal chromosome, or else meiosis II nondisjunction (in the absence of recombination during the preceding meiosis I), could have occurred in this patient. The outcomes of both types of events are currently indistinguishable; however, a mitotic event may be more likely [Antonarakis et al., 1993].

Comparison of the parental alleles in the remission marrow with those of the leukemia clone revealed that the fourth chromosome 21 in the leukemia clone was maternally derived. Densitometric analysis showed the intensity of the maternal allele to be uniformly increased in the leukemic cell line relative to the paternal allele at multiple genetic loci along 21q (e.g., D21S11, D21S222, D21S270, and D21S216; Fig. 1b, Table III). These results suggest that the monoblast cell line consisted of equal numbers of paternal and maternal copies of chromosome 21 (Fig. 2b). This genotype may have arisen via a mitotic segregation error of the maternally-derived chromosome in the progenitor of the monoblast cell line.

Southern hybridization analysis indicated that the *AML1*, *ERG*, and *ETS2* genes were neither rearranged nor deleted in the leukemic cells of patient 2. An *AML1* probe detected homozygous 3.0-kb *EcoRI* alleles in monoblast, 8-weeks posttreatment fibroblast genomic DNA (Table III). A 7.3-kb *BamHI* fragment spanning intron 5 of the *ERG* gene, which has been shown to be consistently disrupted in other AML patients [Shimuzu et al., 1993], was not rearranged in this patient (results not shown). Similarly, a polymorphic probe derived from the *ETS2* locus detected homozygous 1.2-kb *MspI* alleles (Table III). Densitometric analysis of both *AML1* and *ETS2* in genomic DNA were consistent with karyotypic determinations of tetrasomy and trisomy 21, re-

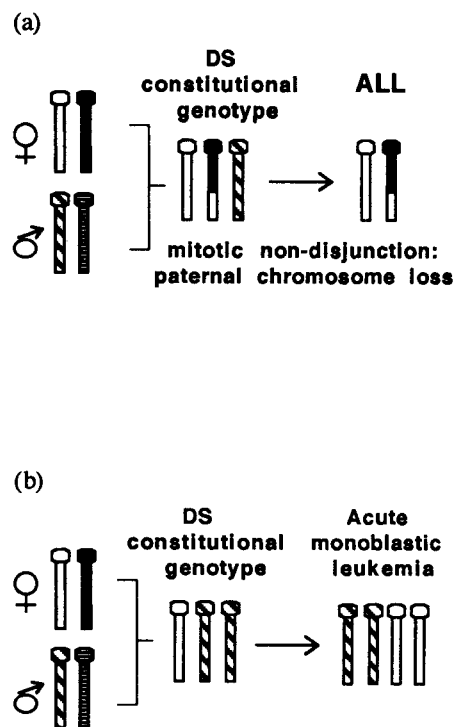


Fig. 2. a: Development of maternal uniparental disomy 21 in the ALL clone of patient 1. b: Evolution of tetrasomy 21 karyotype in acute monoblastic leukemia of patient 2.

TABLE III. Genotypes of Chromosome 21 SSR Polymorphisms in Patient 2\*

Genetic locus	Proband fibroblasts	Proband bone marrow: 8 weeks posttreatment	Proband bone marrow: 5 weeks posttreatment	Proband bone marrow: 1 week posttreatment	Proband bone marrow: pretreatment	Paternal	Maternal	Origin of constitutional nondisjunction event	Origin of leukemogenic event	Reduced or nonreduced
D21S369	222	222				12	22			
D21S215	111		111	111	1111	11	11			R
D21S258	222	222			2222	12	23		M	R
D21S120	122	122			1122	23	11	P		R
D21S13	111	111		111	1111	12	12		M	R
D21S172	122	122		122	1122	23	12	P	M	R
D21S406	122	122			1122	12	11		M	R
D21S11	223	223, I (3:2) = 0.6			2233, I (3:2) = 1.0	12	34	P	M	R
D21S145	334	334			3344	13	24	P	M	R
D21S214	223	223		223	2233	12	23	P	M	R
D21S222	112, I (2:1) = 0.9				1122, I (2:1) = 1.7	13	12		M	R
D21S260	111	111			1111	11	12	P	M	R
D21S217	223	223			2233	12	13		M	R
D21S265	112	112			1122	13	23	P	M	R
D21S269	111	111		111	1111	11	11		M	
D21S263	111	111			1111	12	11		M	
D21S1280	223	223			2233	24	13	P	M	R
D21S216	112	112, I (2:1) = 0.7			1122, I (2:1) = 1.1	13	23	P	M	R
GART	112	112			112 <sup>a</sup>	11	12		M	
IFNAR	122	111			1111	11	11	P	M	R
D21S65	122	122			1122	23	14		M	
AML1	111	111			1111	11	11		M	
D21S167	222	222		222	2222	12	22		M	
D21S266	111	111			1111	12	11		M	
D21S59	111	111			1111	11	11		M	
D21S270	223, I (3:2) = 0.8				2233, I (3:2) = 1.1	12	23		M	R
ERG <sup>b</sup>	111	111			1111	11	11	P	M	R
D21S1238	122	122			1122	12	11		M	
D21S259	112	112			112 <sup>a</sup>	13	12		M	
ETS2	111	111			1111	11	11		M	
D21S257	111	111		111	1111	11	11		M	
D21S264	122	122		122	122 <sup>a</sup>	12	12		M	R
D21S49	223	223			2233	23	13	P	M	R
PFKL	112	112			1122	12	23	P	M	R

\*Genetic markers and genes are ordered from centromere to telomere. M, missegregation of maternal chromosome; P, missegregation of paternal chromosome; R, reduced; duplicate nondisjoined chromosomes; NR, nonreduced; nondisjoined chromosomes derived from distinct homologs at this locus; I (X:Y), ratio of background-corrected intensities; allele X/allele Y.

<sup>a</sup>Due to allele stuttering, it is not possible to distinguish between 1122 and 1112.

<sup>b</sup>Southern hybridization analysis of the *ERG* gene was carried out with a nonpolymorphic probe that detected a 7.0-kb *Bam*HI fragment.

spectively, in the pretreatment and 8-weeks posttreatment cell lines. In the absence of a point mutation or another subtle rearrangement at either of these loci, these results would appear to exclude the common translocation mechanism by which rearrangements at *AML1* or *ERG* lead to AML.

## DISCUSSION

The contrasting findings of uniparental inheritance of chromosome 21 in the lymphoblasts of patient 1 and biparental inheritance in patient 2 suggest the possibility that different genetic mechanisms may be responsible for the development of this condition in each of these DS patients. We propose the following mechanism to explain leukemogenesis in patient 1. Trisomy 21 has been suggested to represent the first of two events that result in leukemia in patients with DS, the second being a somatic event [Scholl et al., 1982]. Increased susceptibility to leukemia might be a consequence of nondisjunction of identical chromosomal alleles at a homozygous, recessive mutant oncogene or tumor-suppressor locus in patient 1. Assuming that the mutant allele is rare in the population, the paternally-derived chromosome 21 allele would be presumed to carry a normal allele at this locus. Loss of this allele may have unmasked homozygous, mutant alleles at a locus telomeric to D21S262, in a manner analogous to the mechanism responsible for expression of mutant tumor-suppressor genes [Cavenee et al., 1985]. Loss of the paternal chromosome and the resultant maternal isodisomy in this patient is the only one of three possible chromosome missegregation events capable of producing a homozygous chromosome 21 genotype in blasts from this patient. While this mechanism may not apply to all patients with DS and ALL, the loss of heterozygosity documented in patient 1 would appear to merit further examination in other individuals.

The homozygous, maternally-derived chromosomal domain in lymphoblasts from patient 1 would appear to be exclusive of the locus responsible for TLR, which has been mapped to proximal 21q [Abe et al., 1989]. *AML1*, *ETS2*, and *ERG* each fall within the isodisomic interval in this patient (Table III). Since this patient was ascertained retrospectively, it was not possible to evaluate the sequences of these candidates in the leukemia clone.

The presence of both paternally- and maternally-derived chromosome 21 alleles in the monoblast clone of patient 2 would not be consistent with "unopposed" expression of a mutant tumor-associated gene on this chromosome. This genotype has also been reported in other DS patients with AL and tetrasomy 21 [Onondera et al., 1992]. Tetrasomy 21 has been proposed to result in a dose-dependent overexpression of genes that stimulate growth [Onondera et al., 1992; Cicchetti et al., 1992] or of genes that sequester transcriptional regulators of genes encoding growth-inhibitors [Oliner et al., 1992; Vogelstein and Kinzler, 1992].

Although a significant proportion of individuals with DS may be predisposed to develop leukemia, a relatively small number of these individuals manifest the disease. Additional somatic events or mutations in other genes may be necessary for leukemogenesis [Knudson, 1988]. Both of the patients presented here

displayed secondary cytogenetic abnormalities that are commonly found in other DS patients with leukemia (-X) and in non-DS patients with trisomy 21 in their leukemic clones (1: +18; 2: +X,+8,-20). However, differences in the distribution of secondary chromosomal abnormalities in DS vs. non-DS patients with +21 have not been of prognostic significance [Watson et al., 1993].

The identification of other DS patients with ALL with homozygous domains that overlap the isodisomic interval found in patient 1 will be needed to delineate loci that predispose these individuals to develop leukemia. By contrast, the chromosome 21 genotype of patient 2 would not contribute to localization of a gene predisposing DS patients to develop acute monoblastic leukemia. Previous studies have not identified a single homozygous locus common to DS patients with acute leukemia [Lorber et al., 1992]. Our results would appear to suggest that both the type of leukemia and the probable genetic mechanism of its evolution will be important in guiding genetic linkage studies aimed at defining some of the genes associated with acute leukemia in these patients.

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