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Ph-Positive CML in Blastic Phase with Monosomy 7 in a Down Syndrome Patient

Monitoring by Interphase Cytogenetics and Demonstration of Maternal Allelic Loss

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ABSTRACT: *We report a case of Ph-positive chronic myelocytic leukemia in blastic phase in an 11-year-old boy with Down syndrome. Monosomy 7 was the only additional chromosomal anomaly in the blastic clone. Fluorescence in situ hybridization analysis on interphase nuclei with a centromeric probe specific to chromosome 7 proved to be efficient in disease monitoring, and showed, together with the results of chromosome analysis on metaphases, that B-lymphocytes at the origin of an EBV-established line were not part of the leukemic clone. The study of DNA polymorphisms showed that the origin of the constitutional trisomy 21 was a maternal anaphase I nondisjunction, that the chromosome 7 lost in the blastic marrow clone was the maternal one, and led us to postulate that the mother's chromosomes are prone to impairment of normal disjunction. The study of allelic losses of chromosome 7 loci proved to be a further possibility for disease monitoring.* © *Elsevier Science Inc., 1997*

INTRODUCTION

Monosomy 7 is a frequently acquired clonal abnormality in acute myeloid leukemias (AML), and in preleukemic myelodysplastic syndromes (MDS), both in adults and in children. It is associated with typical clinical course, prognostic implications, and hematologic features, so that it is possible to describe a specific myeloproliferative disorder associated with this chromosome anomaly [1, 2], and some authors termed this disorder "monosomy 7 syndrome" (M7S) [3, 4]. The existence of this M7S was generally accepted in the classifications of childhood MDS [5]. Monosomy 7 is also found in the blastic phase (BP) of chronic myelocytic leukemia (CML), where it is an additional change to the Philadelphia chromosome (Ph) in 4.4% of the cases (personal observation on 137 cases, unpublished); a similar incidence was reported by Rowley and Testa [6].

Here we report on a Down syndrome subject with CML in BP and monosomy 7. The cytogenetic and molecular data of this case raised a number of points worthy of discussion.

Case Report

B. C., male, born in 1983, was the first son of unrelated parents (mother's age 23; father's age 24) and was diagnosed to be affected with Down syndrome at birth. The clinical picture was typical and a chromosome analysis confirmed the presence of trisomy 21.

In October 1994 the finding of leukocytosis (WBC 36 × 10^6/ml) and increased LDH (2,600 mU/ml) led to an examination of the bone marrow, which was hypercellular with atypical myelocytes and metamyelocytes, undifferentiated blasts, dyserythropoiesis, and dysmegakaryocytopoiesis. This finding, together with the results of chromosomal and molecular analysis, led to a diagnosis of Ph-positive CML. Hydroxyurea therapy was started, but a marrow control in January 1995 revealed a relevant increase of blasts (50% of the cells), and, together with immunologic and

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cytochemical data, indicated a progression to BP. A course of intensive chemotherapy was administered with only partial results (30 to 40% blasts in the bone marrow after the first cycle, 5% after the second). In the following months the disease progressed with short periods of partial remission, a fungal bronchopneumonia was diagnosed in December 1995, and the boy died in the same month because of massive lung hemorrhage.

In 1990 the mother had a second pregnancy, prenatal diagnosis was performed through amniocentesis, and the result indicated a karyotype 47,XY,+21. The pregnancy was then terminated. In 1995 a third pregnancy, monitored by prenatal diagnosis, led to the birth of a healthy male.

CYTOGENETIC STUDIES

Chromosome analyses were performed on bone marrow (BM) direct preparations and 24 hour to 48 hour cultures, and on peripheral blood (PB) unstimulated cultures with standard techniques. Two acquired anomalies were found in BM and PB in addition to the constitutional trisomy 21: a Ph chromosome by standard translocation t(9;22), and a monosomy 7 in addition to the Ph, both present throughout the entire course of the disease. The results are shown in Table 1.

Fluorescence in situ hybridization (FISH) analysis was made at the onset of the disease (October 1994), and after the polychemotherapy of the BP had started and a partial remission had been obtained (February 1995). Probe pZ7.6B (a gift of Professor M. Rocchi, Bari), which hybridizes specifically to centromeric alphoid sequences of chromosome 7 at convenient stringency conditions, was used according to standard procedures on nuclei from bone marrow samples and from a lymphoblastoid line established with EBV in June 1995: the karyotype of this cell line was consistently 47,XY,+21 (66 mitoses analyzed). Appropriate controls were added to the two FISH experiments (at onset and remission), and all the results are listed in Table 2.

MOLECULAR STUDIES

The origin of the extra chromosome 21 was investigated comparing polymorphisms of the patient and of his parents. Both RFLP on Southern experiments and STR by PCR and electrophoresis were analyzed, according to standard methods. The results with the interpretation of the origin for each marker are listed in Table 3. Allele distribution at loci D21S258 and D21S11 indicated a maternal I nondisjunction.

We studied some loci on chromosome 7 by STR polymorphism analysis to obtain evidence of allelic loss in the bone marrow, and to identify if the maternal or paternal chromosome 7 had been lost. The analysis was repeated four times, at the onset of the disease, at the beginning of the BP, at partial remission, and at relapse, when highly different proportions of monosomic 7 cells were present in the marrow (Tables 1 and 2). The same study was made on WBC of the patient at remission and at relapse, and on his parents' WBC. Informative polymorphisms are shown in Figure 1. For each of the three loci one allele was clearly less intense or not evident in the BM of onset (lane 6) and of BP (lane 5), but not at remission (lane 4: BM; lane 2: PB). The allelic loss was again evident both in the BM and PB obtained at relapse (data not shown). The allele absent or of lower intensity was consistently the maternal one.

DISCUSSION

The CML of the patient here reported was monitored by repeated routine chromosome analyses, but information

Table 1 Results of chromosome analyses

Date	Material	No. mitoses		
		+21	+21,Ph	+21,Ph,−7
10/17/94	BM	—	1	3
11/16/94	PB	—	3	12
1/11/95	BM	—	—	13
2/1/95	BM	41	—	1
3/7/95	BM	39	—	1
4/4/95	BM	1	—	27
6/21/95	BM	—	—	12
6/21/95	PB	—	—	15

Abbreviations: BM, bone marrow; PB, peripheral blood unstimulated cultures.

Table 2 Results of FISH analysis with chromosome 7 centromeric probe on nuclei from BM samples, EBV-established lymphoblastoid line (LL), and controls

Material	No. nuclei	No. spots (%)			
		0	1	2	3
Control 1	460	2	16(3%)	440(95%)	2
Control 2	453	1	11(2%)	440(97%)	1
BM onset	581	38(6%)	443(76%)	100(17%)	—
Control 3	650	27(4%)	71(11%)	551(85%)	1
BM remission	1277	47(4%)	314(25%)	908(71%)	8
LL	536	17(3%)	42(8%)	463(86%)	14

Table 3 Results of polymorphism analysis of the listed chromosome 21 loci

Locus	Proband	Mother	Father	Error type
D21S215	122	12	12	NI
D21S258	124	24	13	M I
D21S120	122	12	22	M I/P I, II
M21(S13) TaqI	++−	+−	+−	NI
D21S192	122	22	12	M I, II/P I
1H(S52) BglII	+++	++	++	NI
D21S11	124	12	34	M I
36B(S11) TaqI	−−+	+−	+−	NI
D21S213	112	11	12	M I, II/P I
H8(S17) BglII	++−	+−	+−	NI
102(S25) HindIII	+−−	−−	++	M I, II
D21S212	112	11	12	M I, II/P I

Abbreviations: NI, not informative; M I, II, P I, II, nondisjunction at maternal or paternal meiosis I or II.

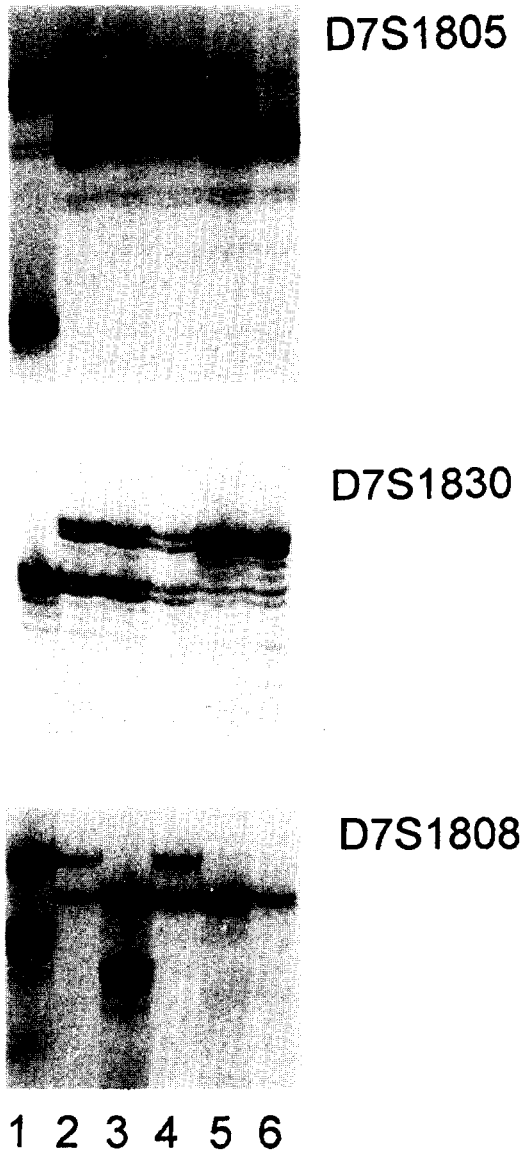

Figure 1 Results of chromosome 7 STR analysis of the loci indicated. Lane 1: Mother's WBC; lane 2: proband's WBC at remission; lane 3: father's WBC; lane 4–6: proband's BM at remission, BP, and CML onset, respectively.

more relevant for the clinical management was obtained when FISH analysis of nuclei was added. The relevance of interphase analyses in monosomy 7 associated with AML and MDS has been already stressed in the literature [7–9]. In our case, where monosomy 7 is additional to the Ph, it is noteworthy that we demonstrated it at the beginning of the disease, with a considerable advance (three months) to a clinically overt BP: at the onset of CML, when only 4 mitoses were seen at chromosome analysis, and 3 of them with monosomy 7 (Table 1), 76% of an informative number of BM nuclei (581) were demonstrated to be monosomic (Table 2). In fact the −7 clone led then to the BP. When partial remission was obtained, and 1 cell out of 42 showed a Ph and −7 at chromosome analysis (Table 1),

Table 4 Comparison among chromosome 7 allelic loss, chromosome analysis results, and FISH on nuclei

Date (disease phase)	Material	Allelic loss	Metaphases −7	Nuclei −7
10/17/94 (CML onset)	BM	+	3/4	76%
1/11/95 (BP)	BM	+	13/13	NE
2/1/95 (remission)	BM	−	1/42	25%[a]
6/21/95 (relapse)	BM	+	12/12	NE
	PB	+	15/15	NE

Abbreviations: BM, bone marrow; PB, peripheral blood unstimulated cultures; NE, not examined.

[a]Note that the control results in this experiment showed 11% nuclei with one signal (Table 2): the proportion of −7 BM nuclei should be considered lower accordingly.

FISH data revealed that a significant proportion of BM cells belonged to the −7 clone (Table 2), which was predictive of the relapse that in fact soon took place. Thus interphase monitoring of the blastic −7 clone proved to be of prognostic relevance.

B-lymphocytes are thought to be part of the leukemic clone in Ph-positive CML [10], whereas conflicting evidence is available on the involvement of B-lymphocytes in −7 AML/MDS [11–13]. The results of chromosome analysis and FISH nuclei score made on the EBV-established line of our patient indicate that the B-lymphocytes that gave origin to this line did not share a Ph chromosome, nor monosomy 7 with the −7 blastic marrow clone.

The origin of the constitutional trisomy 21 of our patient was maternal anaphase I nondisjunction (Table 3), as in 73.11% of the cases [14]. The chromosome 7 lost in the blastic marrow clone was the maternal one (Fig. 1). Available data concerning the parental origin of chromosome 7 loss in 21 children with AML/MDS showed the loss of the maternal 7 in 11, of the paternal in 10 [15, 16]. Our case is the first one in which the origin of chromosome 7 loss is investigated in CML, where it should be considered a secondary event. If the chromosome 7 is lost as consequence of mitotic nondisjunction, and if we take into account the young age of the mother (23 years) and her subsequent pregnancy with a trisomic 21 fetus, we may postulate that the mother's chromosomes are prone to impairment of normal disjunction. The existence of a constitutional predisposition to nondisjunction is supported by the known risk of recurrence of aneuploidies after trisomy 21, by double aneuploidies, by some mitotic mutants described in the literature with multiple somatic, and even clonal, aneuploidies [17]. A similar etiology might account also for multiple aneuploidies not infrequently found in leukemic cells and not associated with any structural abnormalities.

The evidence of allelic loss of chromosome 7 obtained in our patient is compared with cytogenetic and FISH data in Table 4. Allelic loss was evident at CML onset, and at

BP, but not during remission, and again demonstrable at relapse both on BM and PB. Thus, allelic loss correlated well with cytogenetic data, and proved to be a further possibility for disease monitoring.

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