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Isochromosome (7)(q10) in Shwachman Syndrome Without MDS/AML and Role of Chromosome 7 Anomalies in Myeloproliferative Disorders

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ABSTRACT: *Shwachman syndrome (SS) is an autosomal recessive disorder in which bone marrow dysfunction is observed, with development of myelodysplastic syndromes (MDS) and acute myeloid leukemias (AML) in up to one third of the cases. Inconclusive data are available as to increased chromosome breakage in SS, while chromosome 7 anomalies, and often an isochromosome (7)(q10), are frequent in cases with MDS/AML. We report on the consistent presence of an i(7)(q10) in the bone marrow and blood lymphocytes in one of two sisters affected with SS without any clinical or cytological signs of MDS/AML. Thus, this patient was either a case of constitutional mosaicism for the i(7)(q10), or this had to be acquired in a nondysplastic and non-neoplastic marrow clone. DNA polymorphism analysis demonstrated the paternal origin of the i(7q). We postulate that the SS mutation acts as a mutator gene, and causes karyotype instability; abnormal clones would thus arise in the marrow, and chromosome 7 anomalies, i(7q) in particular, will in turn lead to MDS/AML. If this interpretation is correct, it would be also an indication to consider chromosome 7 anomalies in general, out of SS, as primary changes in MDS/AML pathogenesis.*

INTRODUCTION

Shwachman syndrome (SS) is a rare autosomal recessive disease characterized by exocrine pancreatic insufficiency, short stature, skeletal abnormalities with metaphyseal dysostosis of the long bones, bone marrow dysfunction with peripheral blood cytopenias, and a variety of other less common findings [1]. A tendency to develop myelodysplastic syndromes (MDS) and acute myeloblastic leukemias (AML) is documented in up to one third of the cases [2].

Cytogenetic results in SS have been reported mostly in cases who developed a myeloproliferative disorder. Dror et al. [3] reviewed 16 SS cases with chromosome analyses performed to study the acquired anomalies of the cells involved in AML or MDS, and postulated that the presence of an isochromosome for the long arm of a number 7, i(7)(q10) may be a fairly specific marker of the myeloid malignant transformation in SS.

We report here two sisters with SS, together with the cytogenetic and molecular data obtained in one of them, which demonstrated the constitutional presence of an i(7)(q10), in the absence of any sign of MDS or AML.

CASE REPORTS

Case 1

A female (A. L.), born by caesarean section in 1981 at full-term of a pregnancy complicated by gestosis, was the first child of healthy, unrelated parents. Her father's and mother's heights were 161.4 and 151.2 cm, respectively. Her birth weight was 2,350 g. She suffered from recurrent pneumonia, and at the age of 1 year, her weight was 3,750 g (<3rd percentile) and her length was 60 cm (<3rd percentile). When she was 3-years-old, exocrine pancreatic insufficiency was diagnosed because of increased excretion of nitrogen, lactic acid, and lipids; trypsin levels and lipase activity after a stimulation test were low. Anemia

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was present with 8 g/dL Hb, and thrombocytopenia with 40,000 platelets/mm^3. The marrow was severely hypoplastic.

She died of intracranial hemorrhage in the course of pneumonia in 1985.

Case 2

M. L., female born in 1982, sister of A. L., was born by caesarean section at full-term of an uncomplicated pregnancy. Her birth weight was 3,000 g. Her neonatal period was complicated by anorexia, diarrhea, and frequent vomiting. The patient was evaluated at the age of 2 months for her undernourished state: her length was 50 cm (<3rd percentile), her weight was 3,100 g (<3rd percentile), and her head circumference 36 cm (<10th percentile).

At the age of 3 months, exocrine pancreatic insufficiency was diagnosed on the basis of increased excretion of nitrogen, lactic acid, and lipids. A secretion stimulation test showed low levels of trypsin and lipase activities. Sweat chloride tests were repeatedly negative. Liver function tests were normal. A diagnosis of SS was made, and therapy started with pancreatic enzymes, low-fat diet, and fat-soluble vitamins. Laboratory data revealed a hemoglobin of 11.5 g/dL, white blood cell count ranging from 3,000 to 5,200/mm^3 (neutrophils 400–500/mm^3), and platelets 250,000/mm^3. Neutrophil chemotaxis, phagocytosis, and intracellular killing were consistently normal. In spite of persistent neutropenia, the girl did not suffer from recurrent infections. Cellular and humoral immune tests were within normal limits. Re-examined at the age of 18 months, she showed radiological findings of metaphyseal dysostosis; her stature was 55 cm (−7.2 SD).

At 10.2 years of age, her stature was 102 cm (−6 SD), bone age was 6 years, and an extensive endocrinological evaluation was performed. A blunted growth hormone (GH) response to pharmacological stimuli indicated a complete GH deficiency (peaks of 4.2 and 1.88 ng/mL after arginine and l-dopa administration, respectively). Serum GH peak values (29 ng/mL) following GH-releasing hormone injection showed that the deficiency was of hypothalamic origin. No associated hormonal deficiencies were observed.

Nuclear magnetic resonance imaging of the hypothalamus and pituitary regions showed normal findings.

The patient was then treated with rh-GH as described by Marseglia et al. [4], with good results during the first 2 years of treatment. At 14.5 years of age, she was 123.3 cm (−6.7 SD) and fit into the second stage of pubertal development.

From the age of 9 years, leukopenia worsened gradually (average values <1,500 WBC/mm^3), as did anemia (average Hb <9 g/dL), and thrombocytopenia (platelets < 40,000/mm^3). No specific symptoms followed a gradual impairment of neutrophil chemotaxis, phagocytosis, and intracellular killing.

In 1997, the patient's conditions deteriorated due to recurrent infections, frequent hemorrhages, and severe liver insufficiency, when the WBC dropped to <1,000/mm^3, Hb was consistently <7 g/dL, and platelets <5,000/mm^3. Bone marrow was almost aplastic, but without any clinical or cytological evidence of dysplasia, and she died in January 1998.

MATERIALS AND METHODS

Cytogenetic Studies

Repeated PHA-stimulated blood lymphocyte and skin fibroblast cultures, as well as bone marrow direct preparations and cultures, were performed by routine techniques on case 2, while in case 1 and their parents, only blood cultures were made. From case 2, an EBV-stimulated lymphoid cell line was established in December 1997 and analyzed. QFQ- and GTG-banding techniques were used for chromosome analyses.

Fluorescence in situ hybridization (FISH) with a library for painting of chromosome 7 (Cambio, UK) was performed on the bone marrow sampled on December 2, 1997, of case 2, to rule out the possibility that the abnormal chromosome originated from a more complex rearrangement, including material from chromosomes other than 7.

Molecular Studies

Molecular studies were performed to gather information on the parental origin of the anomaly. We extracted DNA with routine techniques from the cells of the lymphoid line and of bone marrow sampled on December 2, 1997 of case 2, and from her parents' blood samples. The DNA recovered from a blood sample obtained in December 1997, when the patient was severely leukopenic, was not suitable for molecular analysis. Genotyping of STRPs (Research Genetics) was performed by use of standard procedures. The PCR amplifications were performed in 8-μl reaction mixtures containing 20 ng genomic DNA; 330 nM of each primer, 200 μM of dCTP, dGTP, and dTTP; 25 μM of dATP, 1 μCi (α^{35}S) dATP, 50 mM KCl; 10 mM TRIS pH 9; 1.5 mM $MgCl_2$; 0.1% Triton X-100; 0.01% gelatin and 0.2 U of *Taq* polymerase. The PCR conditions consisted of an initial denaturation step followed by 30 cycles of 94°C for 40 seconds, 57°C for 40 seconds, 72°C for 40 seconds, and a final extension at 72°C for 5 minutes, using a PTC-100 thermal cycler (MJ Research). Four microliters of each PCR product were resolved by electrophoresis on denaturing (7 M urea) 6% polyacrylamide gels for 3–5 hours at 50 W. Gels were fixed in 10% methanol, 10% acetic acid, and dried and exposed to x-ray film at room temperature.

RESULTS

Cytogenetic Studies

Chromosome analyses on blood cultures of case 1 (110 cells scored) and of both parents (50 cells each) showed normal karyotypes.

In Case 2, a mosaicism was detected: the majority of the cells from PHA-stimulated blood cultures repeatedly showed a normal karyotype and a minority an isochromosome of the long arm of chromosome 7 (i(7)(q10), Table 1). Skin fibroblasts from two different biopsies consistently

showed a normal karyotype, whereas analyses on marrow material repeatedly showed only cells with the i(7)(q10), both from direct preparations and 24–48-hour cultures (Table 1). The painting with the chromosome 7 library confirmed that the abnormal chromosome did not include material from chromosomes other than 7. The lymphoblastoid cell line was cytogenetically normal (Table 1).

The results of a search for increased spontaneous chromosome breakage in both cases 1 and 2 have been reported [5].

Molecular Studies

Informative results were obtained at the following loci: D7S2514, D7S507, D7S2506, and D7S1830 located on the short arm of chromosome 7, and D7S796 and D7S495 located in 7q (Fig. 1). Five more loci, located on chromosomes other than 7, were tested to confirm paternity. All short arm loci demonstrated the loss of paternal alleles in the bone marrow sample, whereas both maternal and paternal alleles were present with equal intensity in the lymphoid line. Long arm loci showed both maternal and paternal alleles; however, paternal ones were consistently of higher intensity in marrow cells, and also, although at a variable degree, in the lymphoid cell line.

DISCUSSION

Cytogenetic data on SS patients are scarce, and may be summarized as follows. The only case in the literature with a constitutional chromosome anomaly is one with a de novo reciprocal t(6;12) reported by Masuno et al. [6], who postulated a candidate region for SS locus around the breakpoints of this translocation [7]; however no conclusion was reached by linkage analysis [8].

Inconclusive data are available as to possible chromosome instability [5, 9–11]; however, these reports deal specifically with chromosome breakage and give no detail on karyotypes and possible other abnormalities.

Most cytogenetic reports on SS concern acquired anomalies in cases with AML or MDS. Dror et al. [3] reviewed 16 such cases, and pointed out that chromosome 7 was involved in 11 of them, and that the anomaly was an isochromosome for the long arm, i(7)(q10), in 4. They concluded that the i(7)(q10) may be a fairly specific marker of myeloid malignant transformation in SS. This conclusion is particularly intriguing independently from SS, if we take into account the overall high frequency of monosomy 7 in MDS and AML, and the discussion whether it should be regarded as a primary or secondary event in their pathogenesis [12]. Again, monosomy 7 is specifically associated with MDS and AML developing in patients with different forms of congenital neutropenia, SS included, under therapy with recombinant human granulocyte colony-stimulating factor (rhG-CSF) [13]. Thus, the understanding of the meaning of chromosome 7 anomalies in MDS/AML of SS may shed light on their role in MDS/AML pathogenesis in general. An isochromosome (7)(q10), in particular, was recurrently reported as an acquired anomaly in different tumors, and also in MDS/AML [14, 15]. Among lymphoid malignancies, an i(7)(q10) was recently associated in particular with peripheral T-cell lymphoma [16, 17], where it may be regarded as a primary change, and in natural killer cell lymphoma [18].

In our case 2, we found the i(7)(q10) in the marrow and in PHA-stimulated lymphocytes, in the absence of any MDS-AML development, possibly indicating that Dror et al.'s conclusion [3] may be incorrect, at least if the i(7)(q10) is considered as an anomaly acquired in a dysplastic or neoplastic clone.

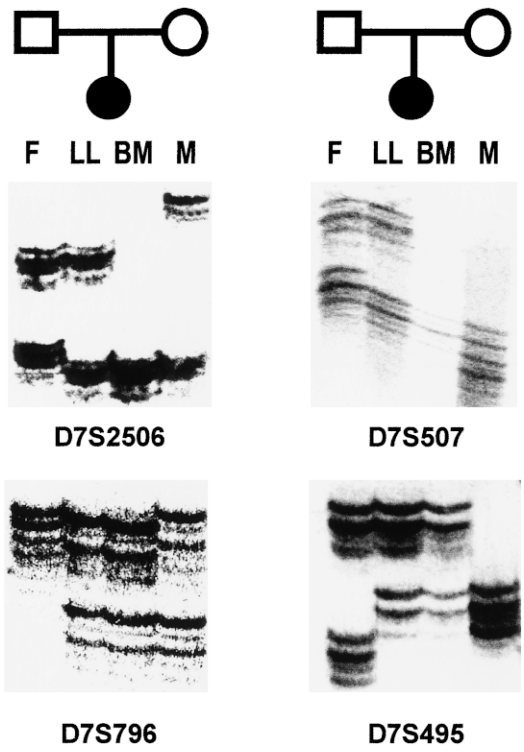
Two alternative interpretations of our data are possible:

Table 1 Results of chromosome analyses in case 2

Date	Material	Karyotype
02/02/88	PB	46,XX,i(7)(q10)[3]/46,XX[30]
02/03/88	SF	46,XX[110]
05/22/90	PB	46,XX,i(7)(q10)[1]/46,XX[29]
05/12/92	PB	46,XX,i(7)(q10)[7]/46,XX[22]
05/13/92	BM	46,XX,i(7)(q10)[12]
06/30/92	BM	46,XX,i(7)(q10)[17]
11/13/92	PB	46,XX,i(7)(q10)[6]/46,XX[20]
01/15/93	SF	46,XX[80]
04/02/93	PB	46,XX,i(7)(q10)[3]/46,XX[36]
12/02/97	BM	46,XX,i(7)(q10)[30]
12/02/97	LL	46,XX[26]

Abbreviations: PB, peripheral blood PHA-stimulated cultures; SF, fibroblast cultures from skin biopsies; BM, bone marrow direct preparations and 24–48-hour cultures; LL, EBV-established lymphoid cell line.

Figure 1 Pattern of four chromosome 7 polymorphic loci, two mapped on the short arm (upper row), two on the long arm (lower row), on DNA from case 2 and her parents. F, father; LL, lymphoid cell line; BM, bone marrow; M, mother.



a constitutional tissue-confined mosaicism, or an acquired anomaly in a non-neoplastic clone. The first one is supported by the consistent presence of i(7)(q10) in marrow and PHA-stimulated lymphocytes (Table 1). No dysmorphic sign indicating the presence of the i(7)(q10) and independent from SS was noticed. The i(7)(q10) is so frequent in SS that it is certainly specifically associated with it; however, as we observed, it may precede dysplastic or neoplastic transformation of marrow cells. This does not exclude its importance in evolution toward MDS and AML, but in our patient this evolution was in fact never observed over a period of 9 years. Chromosome 7 anomalies, in general, and i(7)(q10), in particular, in SS should be regarded as the first mutation in a possible multistep carcinogenesis and, according to the interpretation of a tissue-confined mosaicism, this would be the second example of a predisposing mosaicism, after trisomy 8 [19].

Alternatively, chromosome 7 anomalies in SS may be considered as acquired in a nondysplastic and non-neoplastic marrow clone. Its relevance to MDS/AML development might be the same, and the only difference would be the timing (from embryogenesis to adult life) and the cell type in which they arise.

In any case, some type of karyotype instability, leading to chromosome 7 anomalies, and more frequently to i(7)(q10), seems to be specific to SS and part of the pleiotropic effect of the SS mutation. The possible increased chromosome breakage reported in SS [5, 9–11] might represent another consequence of this instability. We postulate that the SS gene acts as a mutator gene, causing karyotype instability. Thus, cytogenetically abnormal clones arise in the marrow, and when chromosome 7 is involved MDS/AML, are likely to develop. In a comparison with MDS and AML in general, this may be an argument for considering chromosome 7 anomalies as a primary event in their pathogenesis.

Nowell stressed the importance of mutator genes in hematopoietic malignancies [20], and, in myeloproliferative disorders, Laneauville et al. suggested that the product itseslf of *BCR/ABL* rearrangement in chronic myelocytic leukemia is able to induce further structural and numerical chromosome anomalies [21]. Similarly, SS might be another example of a mutator phenotype leading the MDS/AML.

The analysis of chromosome 7 STRPs showed a paternal origin of the i(7)(q10) (Fig. 1). Abnormal phenotypes observed as a consequence of maternal uniparental disomy 7, as in Silver-Russell syndrome [22], and the evidence for imprinted genes on the long arm of chromosome 7 [23] warrant the investigation on the parental origin of chromosome 7 anomalies in further cases of SS and/or MDS/AML to evaluate possible imprinting relevance.

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