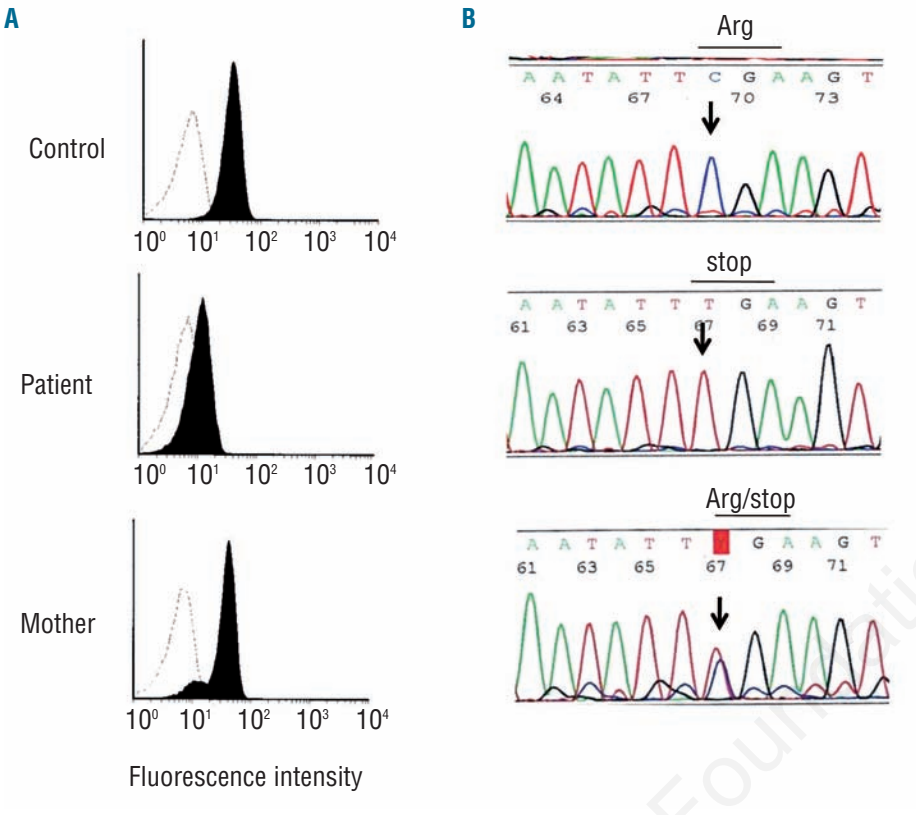

Figure 1. (A) XIAP expression in lymphocytes. Flow cytometric detection of XIAP in lymphocytes from the patient, his mother and the control using anti-XIAP antibodies (clone 48, BD Biosciences, San Jose, CA, USA). Filled histograms represent XIAP staining, while open histograms represent the isotype control antibody. (B) Sequence analysis of XIAP gene. Electropherograms of exon 1 of the *XIAP* gene from the patient, his mother and the control. Arrows indicate the substitution of cytosine to thymine at nucleotide 840. The patient possessed a nonsense mutation, R238X. A heterozygous mutation was detected in the mother.

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Mutations of the Shwachman-Bodian-Diamond syndrome gene in patients presenting with refractory cytopenia – do we have to screen?

Children diagnosed with acquired hypocellular myelodysplastic syndrome (MDS), such as refractory cytopenia (RC), share clinical features with patients suffering from inherited bone marrow failure (IBMF). The Shwachman-Diamond syndrome (SDS; OMIM #260400) is an autosomal recessive disorder associated with bone marrow failure, pancreatic exocrine insufficiency, short stature and liver abnormalities. Other symptoms, such as eczematous lesions, oral disease, cognitive/behavioral problems, immune dysfunction or urinary tract anomalies, may occur.[1] In addition, SDS predisposes to the development of leukemia.[1] Mutations in the Shwachman-Bodian-Diamond Syndrome gene (*SBDS*) are found in approximately 90% of SDS patients.[2] Studies in yeast suggest an important role of the SBDS protein in RNA metabolism.[1]

In children with suspected RC or IBMF, meticulous clinical examination is important because RC and IBMF cannot be distinguished by hematologic or morphological features alone. Underlying congenital disorders may be missed specifically in cases of hypocellular RC and normal karyotype. Our group recently reported in this journal 2 patients with germline mutations of the human Telomerase RNA Component (*TERC*) gene among 80 children with hypocellular RC.[3] Here we hypothesized

that some children presenting with hypocellular MDS might have constitutional SDS identifiable only by mutational analysis.

One hundred and twenty patients with RC, enrolled in the prospective study 98 of the European Working Group of Myelodysplastic Syndromes in Childhood (EWOG-MDS), were screened for SBDS mutations. Patients included were diagnosed with primary hypocellular RC between 1 July 1998 and 31 December 2006 after central reference review of bone marrow biopsies. The diagnosis followed guidelines later adopted by the World Health Organization.[4] Children with an abnormal clonal karyotype or myelofibrosis were excluded from the analysis. Fanconi anemia had been ruled out in all cases by mitomycin C / diepoxybutane sensitivity testing. Patients' age ranged from 0.2 to 19.0 years (median 10.3 years). Three patients had short stature, 2 showed bone abnormalities. Kidney or urinary tract anomalies were present in 4 patients, and cognitive or behavioral problems were noted in 3 children. Further symptoms indicative of SDS were not reported in our cohort. Mutational analysis of the *SBDS* gene was performed through genomic DNA sequencing using peripheral blood or bone marrow, as described elsewhere.[5-8]

We detected one male patient who carried a heterozygous c.258+2T>C mutation. This change is predicted to disrupt the donor splice site of intron 2 and is therefore regarded pathogenic if present in homozygous or compound-heterozygous form.[2] The alteration was present in leukocytes and fibroblasts, demonstrating germline origin. The boy was diagnosed with RC at the age of 14 years. He initially presented with transfusion-dependent pancytopenia. The patient had no clinical history of pancreatic exocrine failure, skeletal abnormalities or other SDS symptoms. Both parents were healthy. Allogeneic hematopoietic stem cell transplantation (HSCT) was performed six months after diagnosis from an 1 HLA-mismatched donor, using a reduced intensity regimen with fludarabin and thiotepa. The boy developed acute and chronic GvHD of the skin. Severe adverse events were not observed and the boy remains in stable condition more than three years after HSCT without symptoms typical of SDS. It is likely that the heterozygous c.258+2 T>C lesion encountered here represents the background allele frequency in the general population, which is estimated at 1/110.[1]

Another patient was heterozygous for an SBDS sequence alteration not previously described. c.127G>T is a single nucleotide variation in exon 1 predicted to result in an amino acid exchange (Val43Leu). The c.127G>T is located at the -2 position of the exon splice site and corresponds to the sequence of the highly homologous *SBDS* pseudogene. We reasoned that the proximity to the splice site might affect RNA splicing. We tested this possibility by reverse-transcriptase polymerase chain reaction but found regular transcripts (*data not shown*). It remains unclear whether the c.127G>T renders the SBDS protein non-functional or represents a polymorphism.

Other SBDS sequence variations detected include c.635 T>C, a known benign variation[8] (8/120 children), c.651 C>T, a synonymous nucleotide change (6/120 children) and c.201 A>G, also a synonymous nucleotide change (9/120 children). We did not identify any patient with a homozygous or compound-heterozygous SBDS lesion in our cohort. Calado *et al.* reported that a small fraction of patients with aplastic anemia were heterozygous for the c.258+2 T>C mutation and that these cases had shortened telomeres.[9] We have not measured telomere length in our study. Whether heterozygosity for the c.258+2 T>C predisposes to RC, as suggested for aplastic anemia,[9] remains speculative.

In summary, we analyzed 120 children with primary hypoplastic RC for *SBDS* gene mutations and found one patient with a heterozygous mutation previously reported as pathogenic, one patient with a heterozygous non-synonymous missense mutation not described in the literature, and several patients with silent or benign sequence variants. These patients were clinically indistinguishable from other RC patients. We conclude that among 120 children given the diagnosis of RC, there was no SDS patient misdiagnosed as RC. When performing HSCT for RC, the failure to recognize underlying SDS is a potential pitfall in the light of increased regimen-related toxicity in SDS patients.[10] However, our data do not indicate that this might be an important clinical issue. We, therefore, do not recommend mutational SBDS screening in RC patients who lack clinical features suggestive of SDS.

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Key words: myelodysplastic syndrome, childhood, bone marrow failure.

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