

## Case Report

# Molecular Characterization of a Portuguese Patient with Shwachman-Diamond Syndrome

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## INTRODUCTION

Shwachman-Diamond syndrome (SDS) a rare autosomal recessive disorder described first time 1964 (1), is characterized by the association of exocrine pancreatic and bone marrow dysfunction. Other systemic findings (skeletal, liver and psychomotor) or problems secondary to bone marrow dysfunction may also be detected (1–4). Intermittent or persistent neutropenia is the most common hematologic finding, but anemia and thrombocytopenia are present in approximately 40% of the patients (1–4).

In 2002, fine mapping identified the locus for SDS in band 7q11. More recently Boocock et al. (5) identified 18 positional candidate genes in this locus and examined eight of them for occurrence SDS-associated changes. They found alterations only in a previously uncharacterised gene. This gene, designated *SBDS* (Shwachman-Bodian-Diamond syndrome), is composed of five exons spanning 7.9Kb. The authors also described a pseudogene (*SBDSP*) with 97% homology to *SBDS* (5).

Only two reports have described mutations in the *SBDS* gene. The original article described 14 different mutations found in 158 SDS families, mostly of European ancestry. The majority of *SBDS* mutations were found to occur within a 240-bp region around exon 2 and resulted from gene conversion as the result of recombination with the pseudogene (5). In the other report, mutations were identified in four patients; two recurring mutations and three novel changes were found (6).

We describe a Portuguese patient with SDS found to be compound heterozygous for two previously described mutations (183–184TA>CT + 201A>G and 258+2T>C).

## CASE REPORT

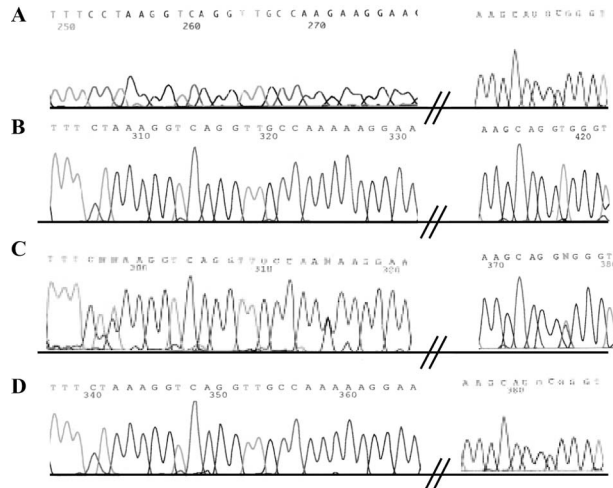
A 3-year-old boy presented with failure to thrive, diarrhea, developmental delay and neutropenia. His perinatal history was uneventful. During his third month of life the child was evaluated because of failure to thrive, anaemia and hypotonia. A diagnosis of cytomegalovirus infection was established. During his first 3 years intermittent neutropenia was documented, with values ranging from normal to severe ( $200 \times 10^9/L$ ). Initially increased values of aspartate and alanine aminotransferases later normalized, as did other liver function test values, including a normal serum bilirubin,  $\gamma$ -glutamyl transferase and alkaline phosphatase. Clotting studies were normal.

At the time of his referral, height and weight below the fifth percentile was noted with no dysmorphic features. Laboratory investigations included hemoglobin of 10.5 g/dL and a white blood cell count of  $4700/mm^3$  with a neutrophil count of  $1645/mm^3$  and a platelet count of  $216000/mm^3$ . Liver function tests were normal, as was the chloride sweat test. A 72-hour fecal fat collection revealed an abnormally increased fat loss. The fecal elastase level was 15.9  $\mu g/g$  (normal  $>200 \mu g/g$ ), consistent with pancreatic failure. The hemoglobin F level was elevated for his age (2.76%) and defective neutrophil mobility was observed (0.3 mm; normal range, 1.23–1.77). Bone marrow aspirate with direct metaphase karyotype study was normal. The skeletal survey revealed a metaphyseal chondrodysplasia of the left hip and rib flaring.

Normal growth rate was re-established at the age of 4 years after institution of pancreatic enzyme supplementation, and his height and weight attained the 25th percentiles.

Molecular analysis of the *SBDS* gene, after parental consent, was performed by direct sequencing of exon 2, using the primers SBDSEX2F: 5' CTGAGGTTACAGT-GACCCGAGA and SBDSEX2R: 5' CTTTCCTCCAGAAAACAGCCT. Results revealed the presence of

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**FIG. 1.** Direct sequence chromatograms of exon 2 of *SBDS* and *SBDSP*. Normal sequence of exon 2 of *SBDSP* (A) and *SBDS* (B). Patient sequence revealing the presence of two mutations and one polymorphism in *SBDS* (C). Patient sequence of one of the bands obtained by polymerase chain reaction single-strand conformational analysis, revealing the presence of the 258+2T>C mutation and absence of 183–184TA>CT mutation and absence of 201A>G polymorphism (D).

two previously described mutations, namely 183–184TA>CT and 258+2T>C (Fig. 1). The nucleotides of the polymorphisms 129–71G and 201A were found to be A and G, respectively. To determine whether the two mutations were allelic, polymerase chain reaction single-strand conformational analysis was performed. After electrophoresis on 8% acrylamide gels, the separated bands were excised, purified and re-amplified for subsequent sequencing in both directions, using the same forward and reverse primers in independent sequencing reactions. The 183–184TA>CT mutation and 201A>G polymorphism were found to be present in one of the alleles and the 258+2T>C mutation and the 129–71G>A polymorphism were present in the other. Direct sequencing of the pseudogene copy was also performed, using the same *SBDS* forward primer (SBDSEX2F) and the specific *SBDSP* primer SBDSPEX2R: 5'CGGACGTTGCAGTGAGCC (Fig. 1).

## DISCUSSION

A wide spectrum of phenotypic abnormalities has been described among patients with SDS (2–4). In the child reported here, the diagnosis of SDS was made on the basis of pancreatic dysfunction and hematological abnormalities (intermittent neutropenia and anaemia) found in

virtually all SDS patients, but also in light of an inappropriate height and weight, skeletal abnormalities and reduction of neutrophil chemotaxis.

SDS is a rare and heterogeneous disorder, which raises difficulties in its diagnosis in some patients. The presence of a hot spot region in exon 2 of the *SBDS* gene was an important finding in terms of facilitating diagnosis. In fact, direct sequencing of this exon has enabled the detection of mutations, in at least one of the alleles, in approximately 89% of SDS patients (5). These mutations are the result of conversion events between *SBDSP* and *SBDS* (5).

Before recognizing the involvement of *SBDS*, genotype-phenotype correlations could only be inferred on the basis of clinical manifestations (4). Concordance between affected siblings, presumed to have the same genotype, was found only in terms of pancreatic disease expression, providing very little evidence of genotype-phenotype correlation. The identification of the *SBDS* gene and mutations related with SDS will likely improve our understanding of the observed spectrum of disease phenotypes. Only one previous report (7) tentatively correlated the *SBDS* mutations with the nature, frequency and age-related changes of the radiographic skeletal abnormalities. The authors found no correlation between the genotype and the skeletal phenotype.

Detailed descriptions of the clinical profiles of SDS patients with the corresponding genotypes, as in the present report, could be important in establishing a more global genotype-phenotype correlation or provide steps towards the identification of other factors involved in the disease manifestation.

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