Congenital Aplastic Anemia Caused by Mutations in the SBDS Gene: A Rare Presentation of Shwachman-Diamond Syndrome

Taco W. Kuijpers, MD, PhD*; Eline Nannenberg, MD‡; Marielle Alders, MSc†; Robbert Bredius, MD, PhD§; and Raoul C. M. Hennekam, MD, PhD*‡

ABSTRACT. Clinical Findings. Aplastic anemia was diagnosed at birth for a first child from healthy nonconsanguineous parents. The girl had hypoglycemia, which normalized within 2 months. Cow milk allergy was suspected initially, because of skin lesions and diarrhea, followed by severe growth retardation. Clinical and radiologic symptoms gradually became typical for Shwachman-Diamond syndrome. Two common mutations in the SBDS gene (183-184TA→CT [K62X] and IVS2(258)+2T→C [C84fs]) were found.

Results. Bone marrow transplantation from a matched unrelated donor was unsuccessful. The genetic information from the deceased patient enabled us to perform prenatal molecular studies during the subsequent pregnancy, successfully predicting a nonaffected child.

Conclusions. This report describes for the first time the hematologic abnormalities of congenital aplastic anemia and prolonged neonatal hypoglycemia as the presenting symptoms of Shwachman-Diamond syndrome. The finding of common mutations in the presence of these symptoms at birth suggests the lack of a clear phenotype-genotype relationship in this syndrome.

Abbreviations. SDS, Shwachman-Diamond syndrome; CHH, cartilage/hair hypoplasia; DKC, dyskeratosis congenita; G-CSF, granulocyte colony-stimulating factor; BM, bone marrow; BMT, bone marrow transplantation; PCR, polymerase chain reaction.

Shwachman-Diamond syndrome (SDS) (Online Mendelian Inheritance in Man no. 260400) is a rare autosomal recessive disorder that usually manifests itself in infancy or early childhood. The disease is extremely heterogeneous, showing a wide variety of abnormalities and symptoms. It is characterized mainly by exocrine pancreatic insufficiency, short stature, and bone marrow (BM) dysfunction. Several studies have shown that, with advancing age, 40% to 60% of patients exhibit pancreatic insufficiency. Elevated liver enzyme levels and hepatomegaly have been observed in the first years of life, with subsequent improvement without complications (similar to the pancreatic insufficiency). Intermittent neutropenia is the most common hematologic finding in SDS. Hematologic manifestations other than neutropenia include anemia, increased fetal hemoglobin levels, thrombocytopenia, and aplastic anemia. As with other constitutional BM failure syndromes, there is a tendency toward malignant myeloid transformation. Recombinant human granulocyte colony-stimulating factor (G-CSF) has been used for some SDS subjects with severe neutropenia but is not recommended because of the risk of acute myeloid leukemia, although the exact prevalence of the disease and its induction by G-CSF are difficult to establish.

Growth retardation is a typical manifestation. Weight and length are deficient at birth and remain below normal with time. Some patients with SDS present with short stature only, rather than malnutrition or malabsorption, which suggests an inherent growth problem. A broad spectrum of skeletal abnormalities, including metaphyseal dysostosis and epiphyseal dysplasia, has been found to be associated with this syndrome. Additional clinical features include immune dysfunction, liver disease, renal tubular defects, insulin-dependent diabetes mellitus, and psychomotor retardation.

No unifying pathogenic mechanism has yet been shown to be responsible for SDS, although the genetic basis of this rare disease was recently described. Indirect lines of evidence indicate that the orthologs may function in RNA metabolism. YLR022c has been clustered with genes encoding RNA-processing enzymes. Restriction digestion or sequencing of polymerase chain reaction (PCR) products from affected individuals showed ~75% of alleles associated with SDS were the result of gene conversion, which was confined to a short segment with a maximal size of 240 base pairs. Approximately 90% of affected individuals carry at least 1 converted allele, and 60% carry 2 converted alleles. Alleles from affected individuals without conversion mutations had other changes in the coding region of SBDS, which led to frameshift and missense changes.

We present an unusual case of congenital aplastic anemia combined with transient hypoglycemia during early infancy with a diagnosis of SDS, which was confirmed by the identification of 2 common mutations in the SBDS gene.

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**METHODS**

**Hematologic Studies**

Morphologic analyses demonstrated hypocellular BM in smears, which was confirmed with BM biopsies. No excess collagen or signs of fibrosis, disturbed BM stroma development, or disorganized hematopoiesis was observed. Absolute numbers of progenitor B cells (CD19⁺, CD10⁻, CD24⁻), T cells (CD2⁺, CD3⁺, CD4⁺, CD8⁺), natural killer cells (CD3⁻, CD16⁺, CD56⁺), and myeloid cells (CD15⁺, CD14⁺, CD16⁺, CD65⁺) were determined with standard fluorescence-activated cell-sorting procedures. Colony-forming units of the erythroid and granulocyte/macrophage progenitors were determined in 10- to 14-day semisolid cultures and compared with normal age-matched values.

**Histochemical Analyses**

Histochemical and immunophenotypic analyses of the liver and muscle were conducted with standard staining procedures, with a streptavidin-biotin complex method for paraffin-embedded sections and a 3-step, indirect, immunoperoxidase method, with 3-amino-9-ethylcarbazole as a substrate, for frozen sections. Electron microscopy was performed with tissue samples simultaneously, with Karnovsky embedding.

**Molecular Studies**

Genomic DNA from peripheral mononuclear cells and fibroblasts from the patient were extracted with standard methods. The SDBS gene was amplified in separate PCRs with primer sets identical to those described by Boocock et al, with essentially the same genomic PCR conditions as described. Direct sequencing was performed with an ABI PRISM Big Dye Terminator v1.1 cycle sequencing kit (Applied Biosystems, Foster City, CA), and PCR products were separated in 1% SeaKem (FMC BioProducts, Rockland, ME) gels, purified with a Qiagen gel extraction kit (Qiagen, Hilden, Germany), and sequenced automatically (ABI3100 sequencer; Applied Biosystems).

**CLINICAL REPORT**

The proband was the first child of a nonconsanguineous couple with no family history of congenital abnormalities. The 32-year-old white mother and the 31-year-old father were both healthy. At the 39th week of gestation, contractions started spontaneously. During the delivery fetal distress developed, prompting a cesarean section. A pale girl with a birth weight of 2790 g (10th percentile), length of 38 cm (<3rd percentile), and occipital-frontal circumference of 35 cm (10th percentile) was born; she experienced respiratory failure, which necessitated artificial respiration. The patient had low hemoglobin levels and hypoglycemia (Table 1), which were immediately corrected. Chest radiographs did not show any defects (in particular, no abnormal ribs, vertebrae, or humeri).

No firm diagnosis was made at that time. Kleihauer tests to determine fetomaternal blood loss yielded negative results. The girl was weaned from the ventilator but, for unknown reasons, exhibited periods of tachypnea for ~8 weeks. Ultrasonographic evaluations of the head yielded normal results. Echocardiography showed nonclosure of the foramen ovale, with moderate left-to-right shunting. The pulmonary artery pressure was 35 mm Hg. Microbiologic cultures all yielded negative results. Serologic tests for congenital infections yielded negative results, as did PCR tests for cytomegalovirus, parvovirus B19, herpes simplex virus, and Epstein-Barr virus. The patient experienced persistent periods of unexplained hypoglycemia, with transient lactate acidemia (peak: 5.4 mmol/L). She was extensively evaluated for metabolic disorders, in particular mitochondrial disorders, but no clues were found in a liver biopsy, muscle biopsy, and mitochondrial DNA analysis. The patient was treated with decreases.

### TABLE 1. Hematologic and Immunologic Blood Values of the Propositus

<table>
<thead>
<tr>
<th></th>
<th>Birth</th>
<th>2 wk</th>
<th>12 mo*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hematologic findings</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb, mmol/L</td>
<td>2.8t</td>
<td>4.2†</td>
<td>5.5t</td>
</tr>
<tr>
<td>Hct, L/L</td>
<td>0.23</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Reticulocytes (10⁶ cells/mL)</td>
<td>2.6</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>MCV, fl</td>
<td>106</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>Platelets (10⁶ cells/mL)</td>
<td>75</td>
<td>21</td>
<td>50</td>
</tr>
<tr>
<td>Leukocytes (10⁶ cells/mL)</td>
<td>1.8</td>
<td>4.2</td>
<td>4.4</td>
</tr>
<tr>
<td>Metamyelocytes, %</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Bands formed, %</td>
<td>8</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>5</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>87</td>
<td>80</td>
<td>75</td>
</tr>
<tr>
<td><strong>Chemical findings</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilirubin, μmol/L</td>
<td>63</td>
<td>166</td>
<td>6</td>
</tr>
<tr>
<td>ALAT, IU/L</td>
<td>110</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>ASAT, IU/L</td>
<td>94</td>
<td>40</td>
<td></td>
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<tr>
<td>LDH, IU/L</td>
<td>414</td>
<td>576</td>
<td>0.7</td>
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<tr>
<td>Creatinine, μmol/L</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>0.9</td>
<td>2.2</td>
<td>4.4</td>
</tr>
<tr>
<td><strong>Immunologic findings</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes (10⁶ cells/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3⁺ T cells (10⁶ cells/mL)</td>
<td>3.4</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>CD3⁺/CD4⁺ Th cells (10⁶ cells/mL)</td>
<td>2.5</td>
<td>2.1</td>
<td></td>
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<tr>
<td>CD3⁺/CD8⁺ Tc cells (10⁶ cells/mL)</td>
<td>0.6</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>CD19⁺ B cells (10⁶ cells/mL)</td>
<td>0.2†</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>CD16/CD56⁺ NK cells (10⁶ cells/mL)</td>
<td>0.03†</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

Hb indicates hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; LDH, lactate dehydrogenase; Th, T helper; NK, natural killer; Tc, cytotoxic cells.

* With erythropoietin (400 E/kg) and G-CSF (30 μg/kg, 3 times per wk, subcutaneously).
† Outside the normal range of values for lymphocyte counts.
ing amounts of diazoxide until glucose levels were completely normalized.

Fanconi anemia was considered because of the aplastic anemia (Fig 1) but was excluded because of normal mitomycin C test results. Neonatal lupus was excluded because of the absence of autoantibodies in the mother and the newborn. Thrombopoietin levels were strongly increased (786 kU/L; normal: <40 kU/L); the absence of mutations in the MPL gene made severe congenital amegakaryocytic thrombocytopenia unlikely. Moreover, plasma levels of folic acid and vitamin B12 were normal; ferritin levels increased within the first 3 months, from 24 to 890 μg/L, after 4 erythrocyte transfusions. Chromosomal analysis indicated a normal female pattern (46, XX).

At the age of 4 months, the patient was discharged with tube feeding and erythropoietin treatment (400 U/kg, administered subcutaneously, twice weekly). She was thought to have a cow milk allergy because of persistent diarrhea and small, itchy, eczematous, skin lesions on the limbs, chest, and back. Growth failure became more evident during follow-up monitoring. Insulin-like growth factor-1, growth hormone, and thyroid hormone tests produced normal results. Metaphyseal dysplasia was noted radiologically (Fig 2), which led to the suspicion of SDS. Although stools seemed well formed and of normal consistency, fat malabsorption was detected (78%), and elastase in fecal samples was repeatedly determined to be undetectable (<15 μg/g).

Pancreatic enzymes were administered orally, but substitution was without effect on the patient’s decreased growth rate during follow-up monitoring. The patient experienced recurrent pneumonia and diarrhea, recovering rapidly with rehydration and antibiotic treatment. Subcutaneous administration of G-CSF was initiated with a dose of 10 μg/kg; with time, the dose was increased to 30 μg/kg, to maintain the patient’s absolute neutrophil count above 500 to 600 cells per μL. At the age of 14 months, the patient was admitted for allogeneic BM transplantation (BMT) with BM cells from a matched unrelated donor. Before admission, there were no signs of infections or hemorrhagic diathesis.

Examination at admission showed a pale dystrophic girl, with weight of 6980 g (<3 SD) and height of 68 cm (<3 SD). The tonsils were present, and the lymph nodes were not enlarged. There was hepatomegaly but no enlargement of the spleen. The patient had no clinical jaundice. No proof of a recent (viral) infection was obtained. The BM was hypocellular,
without much erythropoiesis. The myeloid lineage was hypoplastic; megakaryocytes were not detected. Clonal derangements and myelodysplasia were excluded on the basis of morphologic, immunophenotyping, and cytogenetic studies (eg, monosomy 7 or i(7q)).

Immunologic studies indicated no defects. Lymphocyte proliferation tests yielded largely normal results with activation with mitogens or combined CD3/CD28 receptor signaling (data not shown). A normal-sized thymus was observed with computed tomography; no lung abnormalities or scarring was observed. Echocardiography showed normal anatomic dimensions and good ventricular contractions, with normal fractional shortening for age. Liver and kidney functions were normal at that time. Microbiologic cultures and PCR tests yielded negative results for several viruses (ie, all herpes viruses, human immunodeficiency virus, and parvovirus B19) in nose washings, feces, urine, and/or blood.

The patient received $2.5 \times 10^8$ nucleated cells per kg. Graft-versus-host disease prophylaxis consisted of methotrexate and cyclosporine A. Posttransplantation supportive care consisted of total gastrointestinal decontamination in a strict protective (sterile) environment. Hematologic engraftment failed. On the 30th day after BMT, circulatory insufficiency became evident during *Escherichia coli* sepsis. A second infusion of donor BM was performed on the 47th day. Thereafter, the patient experienced clinical gastroenteritis and a systemic adenovirus infection that worsened her condition, with rapidly increasing viral DNA concentrations. She died on the 69th day, as a result of multiple-organ failure.

Sequence analysis showed that the patient was compound heterozygous for 2 common mutations in exon 2 of the *SBDS* gene (Fig 3). These mutations were previously identified among SDS patients. Analysis of *SBDS* genomic sequences in each of the parents confirmed the presence of the genetic changes in the heterozygous condition, ie, 183-184TA→CT, resulting in a stop codon (K62X), in the mother and IVS2(258)+2T→C, leading to a C84 frameshift splicing event, in the father.

Recently, the mother became pregnant again and asked for prenatal diagnosis. A chorionic villus biopsy showed that the fetus was a carrier of the IVS2(258)+2T→C (C84fs) mutation only, and a healthy boy was born.

**DISCUSSION**

Several short stature-skeletal dysplasias can be associated with varying degrees of hematologic deficiency or immunodeficiency. BM failure syndromes almost always occur in childhood until adulthood, with symptoms related to the predominant cytopenia, and often culminate in multilineage failures with time. Congenital BM failures have been characterized primarily as single-lineage failures, as seen in Kostmann syndrome or other variants of severe chronic neutropenia, congenital amegakaryocytic thrombocytopenia or thrombocytopenia with absent radii syndrome, or Diamond-Blackfan anemia.

The presentation of SDS for our patient was highly unusual. The *SBDS* gene product is presumed to function in RNA processing. This function and its clinical implications are reminiscent of those of the *RMRP* gene, which, when defective, causes cartilage/hair hypoplasia (CHH) and impairs hematologic and immunologic functions. However, the growth failure in SDS is proportional and differs from the disproportional growth in CHH. Moreover, the exocrine pancreas insufficiency is most typical for SDS, whereas Hirschsprung disease may coincide with CHH. Dyskeratosis congenita (DKC) also is
caused by a defect in RNA processing.\textsuperscript{21,22} The defective protein, dyskerin, in X-linked DKC is associated not only with H/ACA small nucleolar RNA but also with human telomerase RNA, which was found to be defective in autosomal DKC.\textsuperscript{23} Telomerase adds simple sequence repeats to chromosome ends by using an internal region of its RNA as a template. Interestingly, shortened telomeres have been demonstrated in the leukocytes of patients with SDS,\textsuperscript{20,24} although it remains to be shown whether this results from defective telomerase activity or hyperploilation.

Overt BM failure at birth has never been observed in SDS, although aplastic anemia, with transfusion dependence, has been noted at older ages during childhood and adolescence.\textsuperscript{5,6} In contrast to our case, the symptoms of pancreatic insufficiency are suggested to be most prominent in SDS at the time of diagnosis and especially during infancy, although they resolve with time for \textgreater{}50\% of patients.\textsuperscript{1–3} In our case, the diagnosis of SDS was finally established at the age of 6 months, because of the combined hematologic abnormalities, growth failure with metaphyseal dysplasia, mild hepatitis, and fat malabsorption, with undetectable elastase and chymotrypsin in the feces. The BM failure reacted marginally to the addition of growth factors such as erythropoietin and G-CSF.

Although BMT or hematopoietic stem cell transplantation has been attempted for treatment of the hematologic disturbances of SDS, few survivors have been reported to date.\textsuperscript{25–28} Poor outcomes are often related to excessive cardiac and other organ toxicity resulting from the conditioning regimen before transplantation and a stromal cell defect that is part of the syndrome.

CONCLUSIONS

The female patient presented here exhibited congenital aplastic anemia, which was diagnosed after 6 months as SDS. The early onset of the hematologic symptoms is unusual for SDS. Transient diabetes mellitus in early infancy was described for a single case of SDS,\textsuperscript{29} but our patient experienced transient hypoglycemia instead. In addition, the patient showed extreme proportional dwarfism once the metaphyseal dysplasia became apparent. Taken together with the findings of Boocock et al.,\textsuperscript{8} the common mutations in the present case and our analysis of 10 additional SDS cases (T.W. Kuipers, M. Alders, and R.C.M. Hennekam, unpublished observations) suggest that a clear genotype-phenotype relationship may not exist in SDS. This would be in agreement with the lack of concordance in hematologic findings among affected siblings and the wide variability within families.\textsuperscript{3}

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REFERENCES

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