Helsinki Medical Imaging Center and
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SHWACHMAN-DIAMOND SYNDROME

Clinical, Genetic and Radiological Study

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ACADEMIC DISSERTATION

To be presented,
with the permission of the Medical Faculty
of the University of Helsinki,
for public examination in the Niilo Hallman Auditorium,
Hospital for Children and Adolescents, Helsinki,
on October 10, 2008, at 12 noon.

Helsinki 2008
C'est le temps que tu as perdu pour ta rose qui fait ta rose si importante

Antoine de Saint-Exupéry

To

Tomi, Elias, Erkka, and Erno
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This thesis is based on the following publications which are referred to in the text by Roman numerals:


LIST OF ABBREVIATIONS

For clarity, those abbreviations used only in the tables, clarified in their footnotes, have not been included in the list below.

AML  Acute myeloid leukemia
BMC  Bone mineral content
BMD  Bone mineral density
CT   Computed tomography
DXA  Dual-energy X-ray absorptiometry
LTM  Lean tissue mass
LV   Left ventricle
MRCP Magnetic resonance cholangiopancreatography
MRI  Magnetic resonance imaging
OFC  Occipitofrontal circumference
pSBDS Shwachman-Bodian-Diamond pseudogene
SBDS Shwachman-Bodian-Diamond gene
SBDS Shwachman-Bodian-Diamond protein
SD   Standard deviation
SDS  Shwachman-Diamond syndrome
S-25-OHD Serum concentration of 25-OH-vitamin D
T1   Longitudinal relaxation
T2   Transverse relaxation
TE   Time to echo
TR   Time of repetition
1. ABSTRACT

Background: Shwachman-Diamond syndrome (SDS) is a rare autosomal recessive disorder in which the cardinal symptoms arise from exocrine pancreatic insufficiency and bone marrow dysfunction. Previous studies have suggested increased risk of fatal complications among Finnish SDS infants. The genetic defect responsible for the disease was recently identified; the SBDS gene is located at chromosome 7q11 and encodes a protein of unknown function. The discovery of the SBDS gene has opened new insights into the pathogenesis of this multiorgan disease.

Objective: This study aimed to assess phenotypic and genotypic features of Finnish patients with SDS.

Methods: Seventeen Finnish patients with a clinical diagnosis of SDS were included in the study cohort. Extensive clinical, biochemical, and imaging assessments were performed to elucidate the phenotypic features, and the findings were correlated with the SBDS genotype. Imaging studies included abdominal magnetic resonance imaging (MRI), brain MRI, cardiac echocardiography including tissue Doppler examination, and cardiac MRI. The skeletal phenotype was assessed by dual-energy X-ray absorptiometry (DXA) and bone histomorphometry.

Results: Twelve patients had mutations in the SBDS gene. In MRI, a characteristic pattern of fat-replaced pancreas with occasional enhancement of scattered parenchymal foci and of pancreatic duct was noted in the SBDS mutation-positive patients while the mutation-negative patients did not have pancreatic fat accumulation. The patients with SBDS mutations had significantly reduced bone mineral density associated with low-energy peripheral fractures and vertebral compression fractures. Bone histomorphometry confirmed low-turnover osteoporosis. The patients with SBDS mutations had learning difficulties and smaller head size and brain volume than control subjects. Corpus callosum, cerebellar vermis, and posterior fossa structures were significantly smaller in SDS patients than in controls. Patients with SDS did not have evidence of clinical heart disease or myocardial fibrosis. However, subtle diastolic changes in the right ventricle and exercise-induced changes in the left ventricle contractile reserve were observed.
Conclusions: This study expanded the phenotypic features of SDS to include primary low-turnover osteoporosis and structural alterations in the brain. Pancreatic MRI showed characteristic changes in the SBDS mutation-positive patients while these were absent in the mutation-negative patients, suggesting that MRI can be used to differentiate patients harboring SBDS mutations from those without mutations. No evidence for clinical cardiac manifestations was found, but imaging studies revealed slightly altered myocardial function that may have clinical implications. These findings confirm the pleiotropic nature of SDS and underscore the importance of careful multidisciplinary follow-up of the affected individuals.
2. INTRODUCTION

Shwachman-Diamond syndrome (SDS, MIM # 260400), also known as congenital lipomatosis of the pancreas, Shwachman syndrome or Shwachman-Bodian-Diamond syndrome, is an autosomal recessive disorder that was recognized as a clinical entity in the early 60’s. Harry Shwachman, Louis Diamond, Frank Oski, and Kon-Taik Khaw, from Harvard Medical School, Boston, reported a new syndrome consisting of pancreatic insufficiency and neutropenia (Shwachman et al. 1963, Shwachman et al. 1964). They concluded that “patients with unexplained neutropenia should be evaluated for pancreatic function, and patients with the diagnosis of cystic fibrosis in the presence of normal sweat electrolytes and lack of respiratory disease should be re-evaluated. This disease appears to have a much better prognosis than cystic fibrosis, and the two conditions should be carefully separated” (Shwachman et al. 1964).

The clinical features of SDS include involvement of multiple organ systems that are not directly linked to each other. Exocrine pancreatic insufficiency is a characteristic gastrointestinal manifestation; in the developed countries, SDS is the second most common cause of pancreatic insufficiency in children after cystic fibrosis. The hematological features link SDS to inherited bone marrow failure syndromes; it is thought to be the third most common inherited blood dyscrasia after Fanconi anemia and Diamond-Blackfan anemia. The bone involvement with metaphyseal chondrodysplasia associates SDS with skeletal dysplasias, a heterogeneous group of diseases with abnormalities in the skeletal development.

The recent discovery of disease-causing mutations in the SBDS gene has lead to growing understanding of the genetic and molecular basis of SDS. Moreover, genotypic characterization of an increasing number of patients has enabled more precise phenotypic definition of this variable disorder. As stated in 1964 by Shwachman et al. (Shwachman et al. 1964): “With identification of more patients with this clinical entity, it is hoped that the complexities of etiology and interrelationship of the various aspects of the syndrome will be resolved.”
3. REVIEW OF THE LITERATURE

3.1. Shwachman-Diamond syndrome

3.1.1. History

In 1964, Shwachman et al. described six American children, three of whom were initially thought to suffer from atypical cystic fibrosis (Shwachman et al. 1964). The children presented with failure to thrive, pancreatic insufficiency, neutropenia, growth retardation, absence of pulmonary disease and normal sweat electrolytes, elevated fetal hemoglobin levels, and mild galactosuria. Three of these patients were siblings, suggesting inherited cause for the disorder. In the same year, Martin Bodian, Wilfried Sheldon, and Reginald Lightwood from The Hospital for Sick Children, London, reported two patients and reviewed the literature of 18 autopsy-proven cases of “congenital lipomatosis of the exocrine pancreas”, thus identifying the characteristic pancreatic histopathological feature of this newly recognized syndrome (Bodian, Sheldon & Lightwood 1964).

After these first cases, several small and large cohort studies from different centers around the world further elucidated the clinical characteristics of this rare disorder (Table 1) which was first named after Shwachman. In 1980, an extensive study of phenotypic and histopathological features of British patients with SDS was published (Aggett et al. 1980). The increased prevalence of leukemia in SDS was soon noted (Woods et al. 1981). Savilahti’s group reported fatal myocardial involvement among Finnish children with SDS (Savilahti & Rapola 1984), a constant defect in neutrophil locomotion (Ruutu et al. 1984), and aberrant phagocyte function (Repo, Savilahti & Leirisalo-Repo 1987). In 1996, a wide spectrum of phenotypic abnormalities among Canadian patients with SDS was described but exocrine pancreatic dysfunction was an invariable abnormality (Mack et al. 1996). The same year, the risk of leukemic transformation in SDS patients was shown to be considerably higher than previously thought and clonal aberrations to be frequent (Smith et al. 1996). Immunologic dysfunction associated with SDS was also reported (Mäki et al. 1978, Aggett et al. 1980, Dror et al. 2001). In 1999, the report of a large international cohort of 88 SDS patients suggested autosomal recessive mode of inheritance and described varied phenotypic features as well as clinical presentation changing with age (Ginzberg et al. 1999). Further evidence for recessive inheritance was provided by segregation analysis of the families in this large international cohort (Ginzberg et al. 2000). Linkage and haplotype analyses of the families and affected indi-
### TABLE 1. Large cohort studies on Shwachman-Diamond syndrome.

<table>
<thead>
<tr>
<th>PATIENTS</th>
<th>M/F</th>
<th>AGE RANGE AT STUDY</th>
<th>TYPE OF STUDY</th>
<th>MAIN FINDINGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shwachman et al. 1964</td>
<td>6 American</td>
<td>3/3</td>
<td>1-10 y</td>
<td>Clinical study</td>
</tr>
<tr>
<td>Aggett et al. 1980</td>
<td>21 British</td>
<td>10/11</td>
<td>0.9-29 y</td>
<td>Clinical and retrospective study</td>
</tr>
<tr>
<td>Savilahti &amp; Rapola 1984</td>
<td>16 Finnish</td>
<td>10/6</td>
<td>5-20 y</td>
<td>Clinical, 17-year follow-up</td>
</tr>
<tr>
<td>Berrocal et al. 1995</td>
<td>6 Spanish</td>
<td>2/4</td>
<td>0.9-10 y</td>
<td>Clinical and radiological study</td>
</tr>
<tr>
<td>Mack et al. 1996</td>
<td>25 Canadian</td>
<td>17/8</td>
<td>0.5-29.5 y</td>
<td>Clinical</td>
</tr>
<tr>
<td>Smith et al. 1996</td>
<td>21 English</td>
<td>12/9</td>
<td>0.6-43 y</td>
<td>Clinical study</td>
</tr>
<tr>
<td>Cipolli et al. 1999</td>
<td>13 Italian</td>
<td>8/5</td>
<td>0.5-16 y</td>
<td>Clinical, 15-year follow-up</td>
</tr>
<tr>
<td>Ginzberg et al. 1999</td>
<td>88 international cohort</td>
<td>55/33</td>
<td>0.2-31.9 y</td>
<td>Questionnaire</td>
</tr>
<tr>
<td>Dror et al. 1999, Dror et al. 2001</td>
<td>13 Canadian</td>
<td>7/6</td>
<td>1-18 y</td>
<td>Clinical</td>
</tr>
<tr>
<td>Mäkitie et al. 2004</td>
<td>15 Canadian (SBDS +)</td>
<td>8/7</td>
<td>0.9-19.9 y</td>
<td>Longitudinal radiological study</td>
</tr>
<tr>
<td>Kuijpers et al. 2005</td>
<td>22 Dutch (15 SBDS +)</td>
<td>13/9</td>
<td>&lt;1-35 y</td>
<td>Hematological</td>
</tr>
<tr>
<td>Kawakami et al. 2005</td>
<td>9 Japanese (7 SBDS +)</td>
<td>6/3</td>
<td>0.5-30 y</td>
<td>Clinical</td>
</tr>
</tbody>
</table>

*Includes 2 patients reported by Bodian, †includes 8 patients reported by Aggett, ‡includes 6 patients reported by Mack, ††all patients also reported by Ginzberg. SBDS+, verified mutations in the SBDS gene.
viduals with SDS identified a single gene locus at the centromeric region of chromosome 7 (Goobie et al. 2001). In 2003, the gene involved in SDS was discovered (Boocock et al. 2003). Thereafter, several disease-causing mutations have been identified in this Shwachman-Bodian-Diamond gene, \textit{SBDS} (Nicolis et al. 2005, Costa & Santos, 2008).

Skeletal involvement with metaphyseal dysplasia and delayed bone age affecting a subset of patients with SDS was recognized in several early reports (Burke et al. 1967, Fellman, Kozlowski & Senger 1972, McLennan & Steinbach 1974, Aggett et al. 1980, Mack et al. 1996, Ginzberg et al. 1999). A radiographic study on Canadian \textit{SBDS} mutation-verified patients in 2004 was able to show that skeletal changes are, in fact, present in all patients with SDS and \textit{SBDS} mutations, but their severity and localization varies with age (Mäkitie et al. 2004). Lack of genotype-phenotype correlation was demonstrated in the skeletal (Mäkitie et al. 2004), hematological (Kuijpers et al. 2005), and clinical features in SDS (Kawakami et al. 2005).

Current research is focusing on the definition of the full clinical spectrum of SDS and on the deeper understanding of its etiopathogenesis as well as the molecular and the biological aberrations behind this disease. The elucidation of the underlying impairment in biological pathways, in turn, could allow for development of molecular therapeutic applications in the future.

3.1.2. Genetics and molecular basis of Shwachman-Diamond syndrome

Shwachman-Bodian-Diamond gene, \textit{SBDS}, that lies in the long arm of chromosome 7 at the cytogenetic position 7q11 (Boocock et al. 2003) is composed of 5 exons and has a 1.6 kb mRNA transcript. The gene resides in a block of genomic sequence that is locally duplicated resulting in two versions of the \textit{SBDS}: the functional gene and its non-functional pseudogene that is 97% identical with the functional gene. The genetic material from the pseudogene, \textit{pSBDS}, contains errors that, when introduced into the \textit{SBDS} gene, result in the disruption of the gene’s instructions to make a protein, SBDS. Gene conversion mutations resulting from an exchange of genetic material between the \textit{SBDS} gene and the nearby pseudogene at meiosis have been found in 89% of individuals with SDS, with 60% having two converted alleles (Boocock et al. 2003). Two gene conversion mutations predominate. A splice-site mutation 258+2T>C (hypomorphic allele) changes a single DNA nucleotide in intron 2. The other common mutation, 183-184TA>CT (null allele), changes two nucleotides in the \textit{SBDS} gene and introduces a premature stop signal in the instructions for making the SBDS protein. While heterozygosity for the hypomorphic and null alleles and ho-
mozygosity for the hypomorphic allele are the most common mutations in patients with SDS, no patients have been found homozygous for the null allele. Homozygous 183-184TA>CT mutations and, consequently, complete absence of the SBDS protein product are thus believed to result in early embryo lethality which is also seen in the animal model (Zhang et al. 2006). The mouse ortholog for SBDS has been shown to express ubiquitously in the majority of embryonic and adult mouse tissues, but increased expression is found in rapidly proliferating cells (Zhang et al. 2006).

The SBDS gene encodes a protein of 250 amino acids, SBDS. SBDS belongs to a highly conserved protein family and its wide occurrence in all archaea and plants indicates a fundamental role in cellular biology (Shammas et al. 2005). The exact function of SBDS protein, however, is to be defined. SBDS localizes within the nucleus and the cytoplasm; the protein shuttles in and out of the nucleus during cell cycles (Austin, Leary & Shimamura 2005). SBDS-deficient cells have been demonstrated to undergo accelerated apoptosis (Dror et al. 2002, Rujkijyanont et al. 2008). Defects in SBDS protein are associated with mitotic spindle destabilization and genomic instability in bone marrow cells of the patients with SDS (Austin et al. 2008). Recent studies have revealed underlying impairment of ribosomal biogenesis in SDS, linking this disorder to other diseases with ribosomal dysfunction such as Diamond-Blackfan anemia, dyskeratosis congenita, and cartilage-hair hypoplasia (Ganapathi et al. 2007, Menne et al. 2007, Ganapathi & Shimamura 2008).

Almost 90% of the patients who meet the current diagnostic criteria for SDS have mutations in SBDS (Boocock et al. 2003). Hitherto, more than 50 mutations in the gene have been identified (Boocock et al. 2003, Nakashima et al. 2004, Nicolis et al. 2005, Costa & Santos 2008). Most of these mutation-positive patients (up to 60%) are compound heterozygotes of the two common conversion mutations (Boocock et al. 2003). Others carry a common mutation on one chromosome and either a rare mutation on the second chromosome or no identified mutations. However, in a subset of patients with a clinical phenotype of SDS, no mutations can be found, even after extensive laboratory testing; the absence of identified mutations in SBDS has been reported in 11% (Boocock et al. 2003) to 18% (Woloszynek et al. 2004) of the patients with a clinical diagnosis of SDS.

3.1.3. Epidemiology

To date, approximately 500 cases of SDS have been described in the literature. The estimated incidence is 1/50.00-1/75.000, and the carrier frequency is estimated to be 1/110 (Goobie et al. 2001). Although the median age at diagnosis is 1 year, the age of diagnosis has ranged from a newborn baby to a middle-aged adult. The early studies by Shwachman et al. (1964)
and Aggett et al. (1980) demonstrated equal numbers of both sexes among affected individuals. Despite autosomal mode of inheritance, more recent studies have shown male predominance with male-female ratio from 1.7:1 (Ginzberg et al. 1999) to 1.5:1 (Alter 2007). Patients with SDS have a high risk of developing hematologic malignancy; age-dependent cumulative probability of leukemia is estimated to be over 70%. The projected median survival age is 35 years. To date, there are no reports of solid tumours in patients with SDS (Alter 2007).

3.1.4. Clinical presentation

SDS is a clinical diagnosis and the current diagnostic criteria include the demonstration of both exocrine pancreatic insufficiency and bone marrow dysfunction (Table 2). The verification of SBDS mutations confirms a clinical diagnosis. However, a negative SBDS mutation result does not exclude SDS (Shimamura 2006). Classically, patients with SDS present in early infancy with failure to thrive, steatorrhea with voluminous fatty diarrhea, hematological disturbances, recurrent infections, and short stature. However, the condition is highly variable and several other organs may also be affected (Table 3). Furthermore, clinical features vary over time making the diagnosis at a later age more challenging.

**TABLE 2. Current diagnostic criteria of Shwachman-Diamond syndrome:**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Exocrine pancreatic dysfunction (at least one of the following):</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Abnormal quantitative pancreatic stimulation test</td>
</tr>
<tr>
<td></td>
<td>b. Serum cationic trypsinogen below the normal range</td>
</tr>
<tr>
<td></td>
<td>c. Serum pancreatic isoamylase under 12U/L (after 3 years of age)*</td>
</tr>
<tr>
<td></td>
<td>d. Abnormal 72-hour fecal fat analysis plus normal intestinal biopsy</td>
</tr>
<tr>
<td></td>
<td>e. Abnormal fecal elastase</td>
</tr>
<tr>
<td><strong>II. Hematological abnormalities (at least one of the following):</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1. Chronic single lineage or multilineage cytopenia:</td>
</tr>
<tr>
<td></td>
<td>a. Neutropenia; repeated count &lt;1500 X 10⁶/l</td>
</tr>
<tr>
<td></td>
<td>b. Hemoglobin concentration &lt; 2 SD</td>
</tr>
<tr>
<td></td>
<td>c. Thrombocytopenia &lt;150 X 10⁹/l</td>
</tr>
<tr>
<td></td>
<td>2. Myelodysplastic syndrome</td>
</tr>
<tr>
<td><strong>Supportive clinical features for diagnosis:</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Skeletal dysplasia</td>
</tr>
<tr>
<td></td>
<td>Short stature</td>
</tr>
<tr>
<td></td>
<td>Liver abnormalities</td>
</tr>
<tr>
<td></td>
<td>Recurrent infections</td>
</tr>
</tbody>
</table>

*(Ip et al. 2002)*
TABLE 3. Clinical spectrum of Shwachman-Diamond syndrome.

<table>
<thead>
<tr>
<th>ORGAN</th>
<th>ABNORMALITY</th>
<th>FREQUENCY</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal system:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low serumtrypsinogen</td>
<td>76-98%</td>
<td>Mack 1996, Ginberg 1999, Cipolli 1999</td>
</tr>
<tr>
<td></td>
<td>Abnormal pancreatic stimulation test</td>
<td>100%</td>
<td>Aggett 1980, Mack 1996, Ginberg 1999</td>
</tr>
<tr>
<td></td>
<td>Clinical improvement of pancreatic function</td>
<td>45-60%</td>
<td>Mack 1996, Cipolli 1999</td>
</tr>
<tr>
<td></td>
<td>Steatosis, perportal inflammation and fibrosis in histopathology</td>
<td>common-100%</td>
<td>Bodian 1964, Aggett 1980, Mack 1996</td>
</tr>
<tr>
<td>Intestine</td>
<td>Hirschsprung disease, ulcerative colitis, small intestine villus atrophy</td>
<td>rare</td>
<td>Burke 1967, Mack 1996, Ginberg 1999</td>
</tr>
<tr>
<td>Hematology:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- intermittent</td>
<td>66%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- persistent</td>
<td>33%</td>
<td></td>
</tr>
<tr>
<td>Bone marrow</td>
<td>Bone marrow hypoplasia</td>
<td>36-100%</td>
<td>Aggett 1980, Smith 1996, Dror 1999</td>
</tr>
<tr>
<td></td>
<td>Cyto genetic aberrations</td>
<td>common</td>
<td>Smith 2002, Dror 2002, Mellink 2004</td>
</tr>
<tr>
<td></td>
<td>Aplastic anemia</td>
<td>occasional</td>
<td>Kuipers 2004, Dror 2005</td>
</tr>
<tr>
<td></td>
<td>ALL, juvenile myelomonocytic leukemia</td>
<td>rare</td>
<td>Smith 1996, Cipolli 1999</td>
</tr>
<tr>
<td>Immune system:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impaired neutrophil chemotaxis</td>
<td></td>
<td>100%</td>
<td>Aggett 1980, Ruutu 1984, Dror 2001</td>
</tr>
<tr>
<td>T cell abnormalities</td>
<td></td>
<td>common</td>
<td>Dror 1999, Dror 2001</td>
</tr>
<tr>
<td>Growth:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------------</td>
<td>--------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>Delayed bone age</td>
<td>100%</td>
<td>Aggett 1980, Mäkitie 2004</td>
</tr>
<tr>
<td></td>
<td>Oral soft tissue disorders, periodontitis</td>
<td>common</td>
<td>Ho 2008</td>
</tr>
<tr>
<td>Skin:</td>
<td>Skin scaling, ichthyosis in infancy</td>
<td>30-62%</td>
<td>Aggett 1980, Savilahti 1984</td>
</tr>
<tr>
<td></td>
<td>Learning difficulties</td>
<td>common</td>
<td>Ginzberg 1999, Cipolli 1999</td>
</tr>
<tr>
<td></td>
<td>Central pontine myelinolysis, necrotizing pontine leukencephalopathy</td>
<td>3 patients</td>
<td>Steinsapir 1985, Mah 1987, Anders 1993</td>
</tr>
<tr>
<td></td>
<td>Delayed myelination in MRI</td>
<td>1 patient</td>
<td>Kamoda 2005</td>
</tr>
<tr>
<td></td>
<td>Agenesis of corpus callosum in CT</td>
<td>1 patient</td>
<td>Todovic-Guid 2006</td>
</tr>
<tr>
<td></td>
<td>Abnormal posterior sensitivity during EEG</td>
<td>6 of 13 patients</td>
<td>Aggett 1980</td>
</tr>
<tr>
<td>Heart:</td>
<td>Myocardial fibrosis</td>
<td>rare</td>
<td>Sacrez 1969, Nezelof 1979, Savilahti 1984</td>
</tr>
<tr>
<td></td>
<td>Cardiomegaly</td>
<td>1 patient</td>
<td>Graham 1980</td>
</tr>
<tr>
<td></td>
<td>Increased cardiotoxicity in stem cell transplantation</td>
<td>3 patients</td>
<td>Tsai 1990, Fleiz 2002, Dror 2005, Donadieu 2005</td>
</tr>
<tr>
<td>Kidney:</td>
<td>Variable glycosuria</td>
<td>unknown</td>
<td>Shwachman 1964, Aggett 1980</td>
</tr>
<tr>
<td></td>
<td>Ureterocele, duplex kidney</td>
<td>2 patients</td>
<td>Mack 1996</td>
</tr>
<tr>
<td>Endocrine and reproductive system:</td>
<td>Delayed puberty</td>
<td>30%</td>
<td>Aggett 1980</td>
</tr>
<tr>
<td></td>
<td>Growth hormone deficiency</td>
<td>1 patient</td>
<td>Kornfeld 1995</td>
</tr>
<tr>
<td></td>
<td>Hypogonadotropic hypogonadism</td>
<td>1 patient</td>
<td>Raj 2003</td>
</tr>
<tr>
<td></td>
<td>Hypothyroidism</td>
<td>1 patient</td>
<td>Shimamura 2006</td>
</tr>
<tr>
<td></td>
<td>Testicular fibrosis</td>
<td>1 patient</td>
<td>Graham 1980</td>
</tr>
<tr>
<td>Eyes:</td>
<td>Retinitis pigmentosa, coloboma, strabismus, keratitis</td>
<td>rare</td>
<td>Aggett 1984, Ginzberg 1999</td>
</tr>
</tbody>
</table>
**Pancreatic dysfunction**

Exocrine pancreatic insufficiency is one of the diagnostic hallmarks and an invariable feature of SDS (Shwachman et al. 1964, Mack et al. 1996). Histological specimens of the pancreas have revealed extensive fatty replacement of pancreatic acini with preserved islets of Langerhans and ductal architecture (Bodian, Sheldon & Lightwood 1964, Aggett et al. 1980). Pancreatic dysfunction is usually diagnosed within the first six months of life and in 90% of the patients during the first year (Mack et al. 1996). Ductular electrolyte and fluid secretion has been shown to remain normal, but the secretion of proteolytic enzymes is severely decreased leading to steatorrhea (Hill et al. 1982, Mack et al. 1996). The exocrine function of the pancreas ameliorates with increasing age in some patients. In cross-sectional and longitudinal data nearly 50% of the patients showed improved fat absorption and near normal fecal fat balance by the age of 4 years. Despite the relief in subjective symptoms, all patients had a persistent deficit of enzyme secretion in quantitative studies on pancreatic function (Mack et al. 1996).

Fecal elastase 1 test has been used to rule out pancreatic insufficiency in SDS. An abnormal test result, however, should be interpreted with caution because malabsorption, due to other reasons, may give erroneous results (Beharry et al. 2002). Serum trypsinogen and isoamylase levels have been shown to be more useful markers for pancreatic dysfunction in SDS (Ip et al. 2002). In patients under 3 years of age, low trypsinogen level reliably indicates exocrine pancreatic dysfunction. In this age group, isoamylase measurement is less useful due to overlapping with the values for healthy young children. In a significant number of patients, serum trypsinogen values normalize after the first years of life but isoamylase level remains constantly low. Therefore, serum isoamylase measurement provides a useful parameter to assess pancreatic function in patients over 3 years (Ip et al. 2002).

Endocrine function of the pancreas is generally unaffected in SDS. However, both children and adults with type 1 and type 2 diabetes mellitus have occasionally been reported (Shmerling et al. 1969, Aggett et al. 1980, Mack et al. 1996, Ginzberg et al. 1999, Filippi et al. 2002, Mäkitie et al. 2004, Kawakami et al. 2005, Kawashima et al. 2006, Rosendahl et al. 2006), even a rare form of neonatal-onset diabetes mellitus has been described (Kamoda et al., 2005).

**Hematological features**

Hematological abnormalities are the diagnostic cornerstones for SDS. The most frequent abnormality is neutropenia that occurs in 88-100% of the patients (Aggett et al. 1980, Mack et al. 1996, Ginzberg et al. 1999). Neutropenia can be either persistent or intermittent, with fluctuation from normal to severely low cell count levels. In about two thirds of the patients, neutropenia is intermittent, in the remaining patients it persists. Normochromic anemia with low reticulocyte count is encountered in 80%, and thrombocytopenia
occurs in 24-88% of the patients with SDS (Dror & Freedman 2002, Dror 2005). Cytopenias affecting all three cell lines are seen 10-65% of the patients, occasionally resulting in severe bone marrow aplasia (Kuijpers et al. 2004, Dror 2005).

Bone marrow features in SDS are variable; bone marrow is usually hypocellular with increased fat deposition (Aggett et al. 1980, Dror & Freedman 1999), but it may occasionally be hypercellular or normocellular (Smith et al. 1996, Ginzberg et al. 1999); the bone marrow cell content does not necessarily correlate with the peripheral cell counts. Accelerated angiogenesis with abnormal tortuous microvessels in bone marrow has been observed in SDS (Leung et al. 2006).

Patients with SDS are at risk of clonal bone marrow abnormalities and malignant transformation, particularly of acute myeloid leukemia (Aggett et al. 1980, Mack et al. 1996, Smith et al. 1996, Dror et al. 2005). Clonal changes - most often monosomy 7, 7q deletion, isochromosome (7q), trisomy 8, and 20q deletion - may fluctuate and sometimes even become undetectable over time (Dror et al. 2002, Smith et al. 2002, Kuijpers et al. 2005). Isochromosome (7q) abnormalities, commonly seen in SDS but an uncommon finding in other bone marrow failure syndromes, and 20q deletion do not seem to associate with malignant bone marrow transformation in SDS (Mellink et al. 2004). Current data suggest that trisomy 8 and chromosome 7 abnormalities other than those involving isochromosome (7q) may contribute to leukemic progression in patients with SDS (Dror et al. 2002, Smith et al. 2002). Males are excessively overrepresented among SDS patients with leukemic transformation; 92% of the reported SDS patients with leukemia are males (Dror et al. 2002). SDS-related leukemia has a poor prognosis. Patients with SDS usually fail to respond to chemotherapy and appear to have pronounced susceptibility to complications and organ toxicity in conjunction with stem cell transplantation; a recent review of 36 reported patients revealed that 83% of the patients had succumbed from therapy-related complications (Dror 2005).

**Infections and immunologic abnormalities**

Patients with SDS are prone to recurrent infections of viral and bacterial origin, and the risk of fatal overwhelming sepsis, particularly at early age, is increased (Grinspan & Pikora 2005). This susceptibility to infections is thought to result mainly from neutropenia, but serious infections may occur even in patients with protective neutrophil levels. While Aggett reported that a large number of patients had indeed suffered from recurrent infections at early age, he noticed that the severity and the frequency of infections did not correlate with the degree of neutropenia. However, the risk of both infections and neutropenia tended to diminish over time (Aggett et al. 1980).
The most common reported infections in SDS are pneumonia (33%), recurrent otitis media (29%), and skin infections or skin abscesses (15%). Viral infections such as parvovirus, measles, and cytomegalovirus have also been observed in SDS. Paradoxically, fungal infections, hallmark infections of severe neutropenia, have been reported in SDS only in conjunction with stem cell transplantation. (Grinspan & Pikora 2005).

Several immunologic abnormalities have been found in SDS. Defective neutrophil migration and chemotaxis are seen in most patients (Aggett et al. 1979, Ruutu et al. 1984, Dror et al. 2001, Stepanovic et al. 2004), as well as aberrations in phagocyte function (Repo, Savilahti & Leirisalo-Repo 1987). Disturbances in both B and T cell functions have been reported (Hudson & Aldor 1970, Doe 1973, Mäki et al. 1978, Aggett et al. 1980, Dror et al. 2001). The observed T cell abnormalities include low percentage of total circulating T lymphocytes, CD3+CD4+ cell subpopulations, an inversed CD4:CD8 ratio, and low proliferative response. Decreased percentage of circulating natural killer cells has also been observed (Dror et al. 2001). B cell defects comprise low IgG or IgG subclasses, low percentage of circulating B lymphocytes, decreased in vitro B lymphocyte proliferation, and an inability to produce specific antibodies against polysaccharide antigens. This permanent inability to T cell-independent B cell responses, also called specific antibody deficiency, is seen in all clinically important antibody deficiencies (Bonilla et al. 2005). In its most severe forms, specific antibody deficiency closely resembles the clinical phenotype of the entity called common variable immunodeficiency. Recently, a 59-year-old male, treated with intravenous immunoglobulin for common variable immunodeficiency over 12 years was recognized to carry a heterozygous SBDS mutation (Khan et al. 2008).

Skeletal manifestations
Compromised longitudinal growth leading to short stature is considered an integral feature of SDS. Patients may present with proportionate mild to moderate growth retardation which may be clinically evident even at birth (Shwachman et al. 1964, Bodian, Sheldon & Lightwood 1964, Aggett et al. 1980, Mack et al. 1996, Ginzberg et al. 1999). The height deviation becomes more evident during early childhood and, despite adequate pancreatic enzyme supplementation, growth remains impaired and results usually in subnormal adult height (Ginzberg et al. 1999). However, short stature cannot be used to reliably establish a diagnosis because patients with normal height and weight have been described and half of the patients are within 2 standard deviations (SD) of the normal mean for height (Mack et al. 1996, Ginzberg et al. 1999).

SDS-associated metaphyseal chondrodysplasia affecting the ribs and the knees was the first recognized skeletal feature (Burke et al. 1967). Other bone abnormalities were later described including delayed skeletal matu-
ration and narrow rib cage which occasionally leads to thoracic dystrophy (Fellman, Kozlowski & Senger 1972, McLennan, Steinbach 1974, Danks et al. 1976, Aggett et al. 1980, Mack et al. 1996, Ginzberg et al. 1999). Electron microscopic examination of growth plate chondrocytes in SDS showed marked dilatation of rough endoplasmic reticulum and Golgi apparatus (Spycher et al. 1974). Systematic analysis of longitudinal radiographic data on 15 genetically confirmed patients revealed that skeletal abnormalities are present in all patients with SDS but the severity and the localization of bone changes vary with age and even with identical genotype (Mäkitie et al. 2004). SDS-associated skeletal dysplasia is characterized by delayed appearance but subsequent normal development of secondary ossification centers, and by variable metaphyseal widening and irregularity most often seen in the ribs and in the proximal and distal femora at early age. Although metaphyseal changes may become undetectable over time, they may also progress and result in knee and hip deformities (Mäkitie et al. 2004). A recent case report described two children with SBDS mutations who presented with respiratory failure and severe skeletal abnormalities. In these severely affected children, the skeletal phenotype resembled spondyloepimetaphyseal dysplasia Sedaghatian type with markedly short stature, platyspondyly, megaepiphyses of the long bones, and severe metaphyseal changes (Nishimura et al. 2007).

In addition to skeletal dysplasia, generalized osteopenia and osteoporosis have occasionally been observed in children and adults with SDS (Nezelof & LeSec 1979, Aggett et al. 1980, Mäkitie et al. 2004, Rosendahl et al. 2006).

Liver involvement
Hepatic abnormalities have been described in about 50-75% of the patients (Brueton et al. 1977, Mäki et al. 1978, Liebman et al. 1979, Aggett et al. 1980, Mack et al. 1996, Ginzberg et al. 1999). Liver involvement in SDS manifests primarily in infancy with mild to moderate derangement in biochemical parameters of liver function, with or without hepatomegaly. However, even severe manifestations with massive hepatosplenomegaly and markedly elevated liver enzyme levels have occasionally been described (Havlíkova, Vychytil & Jelinek 1967, Wilschanski et al. 1994). Percutaneous liver biopsies and autopsy findings have shown micro- and macrovesicular hepatosteatosis, non-specific portal inflammation and variable periportal fibrosis in SDS (Bodian, Sheldon & Lightwood 1964, Aggett et al. 1980, Mack et al. 1996, Kuijpers et al. 2005). Like the symptoms of exocrine pancreatic insufficiency, hepatic involvement tends to regress with age. In one patient, necropsy findings six years after percutaneous liver biopsy suggested decrease of the fatty infiltration in the liver parenchyma over time (Aggett et al. 1980). Natural course of liver involvement in SDS is unknown, but in the majority of cases liver disease at early age is believed to be rather inconsequential.
clinically. Patients with SDS, however, do appear particularly susceptible to organ toxicity, and liver failure in conjunction with induction for bone marrow transplantation has been described (Ritchie et al. 2002, Dror 2005).

Central nervous system involvement
Although cognitive function in SDS was initially reported normal (Shwachman et al. 1964), other early studies suggested variable cognitive impairment in some patients with SDS (Bodian, Sheldon & Lightwood 1964, Aggett et al. 1980). Mild to moderate developmental retardation or reduced IQ, or both, were noted in 85% of the patients studied by Aggett et al. (1980). Another study showed that patients with SDS had significantly lower IQ compared to their unaffected siblings and to the patients with CF (Kent, Murphy & Milla 1990). Subsequent studies have reported a wide variability in IQ (Mack et al. 1996) as well as developmental delay, attention deficit disorders, and learning difficulties in some patients (Cipolli et al. 1999, Ginzberg et al. 1999). Preliminary findings in children with SDS and verified SBDS mutations suggest that affected children perform lower than their unaffected siblings and healthy controls in most cognitive domains (Kerr et al. 2005).

Little is known about imaging features or histopathology of the brain in SDS. Hitherto, brain malformations in autopsy have not been described in SDS. Early reports describe pontine myelinolysis and focal pontine leukencephalopathy in few patients with SDS who had succumbed due to sepsis (Steinsapir & Vinters 1985, Mah, Nelson & Vinters 1987, Anders et al. 1993). Recently, delayed myelination on MRI in a neonate (Kamoda et al. 2005), and agenesis of the corpus callosum on a CT scan of another infant (Todorovic-Guid et al. 2006) have been reported.

Cardiac manifestations
Fatal neonatal cardiac manifestations have been associated with SDS and with pancreatic lipomatosis in several early reports (Sacrez et al. 1969, Nivelon et al. 1978, Guerrero et al. 1979, Nezelof & LeSec 1979, Graham et al. 1980, Savilahti & Rapola 1984, Rybojad et al. 1996). In most of these cases histopathology has shown myocardial necrosis or fibrosis (Nezelof, Bouvier & Dijoud 2002). In a series of 16 Finnish patients with SDS, eight had deceased with cardiomyopathy in early infancy. Autopsies showed, in addition to pancreatic lipomatosis, myocardial necrosis and/or left ventricular and interventricular septum fibrosis but no signs of myocardial inflammation or coronary artery changes (Savilahti & Rapola 1984).

More recently, patients with SDS have appeared susceptible to excessive cardiac toxicity with stem cell transplantation (Tsai et al. 1990, Fleitz et al. 2002, Donadieu et al. 2005, Dror 2005). Hitherto, the only observation of cardiomyopathy in SBDS-mutation verified patient is that of an adolescent
diagnosed with progressive dilated cardiomyopathy who was considered a candidate for heart transplantation by the age of 19 (Danesino et al. 2005).

**Oral disease**
Patients with SDS have high prevalence oral diseases. Extensive caries of both primary and permanent teeth and delayed dental maturation are common; up to 72% of the patients have tooth enamel defects ranging from hypomaturation to hypoplasia (Aggett et al. 1980, Ho et al. 2007). Periodontal disorders, such as recurrent oral ulcerations and gingival bleeding are commonly seen in patients with SDS (Ho et al. 2007).

**Kidney involvement**
Shwachman et al. reported unimpaired renal function but slight galactosuria in SDS (Shwachman et al. 1964), and variable glycosuria was noted in 50% of the patients in the study by Aggett et al. (1980). Subsequent studies, however, have not demonstrated glycosuria (Cipolli et al. 1999, Ginzberg et al. 1999). Tubular acidosis, calciuria, nephrocalcinosis, urolithiasis and renal malformations such as a duplex ureter have occasionally been observed (Aggett et al. 1980, Mack et al. 1996, Cipolli et al. 1999, Ginzberg et al. 1999).

**Skin manifestations**
Skin involvement ranging from slight scaling of the skin to severe ichthyosis has been observed in about 50% of the patients (Aggett et al. 1980, Savilahti & Rapola 1984). Skin lesions present at early age, sometimes even at birth, but they tend to improve and resolve after the first year of life (Savilahti & Rapola 1984). Skin biopsy has shown non-specific dermatitis (Liebman et al. 1979), and histological and ultrastructural analysis of the skin in patients suffering from severe ichthyosis demonstrated marked diffuse hyperkeratosis with variable parakeratosis, thickening of the granular layer, slight perivascular lymphocyte infiltration in the papillary dermis, and the presence of solitary or multiple droplets of varying size in the cytoplasm of the keratinocytes (Goeteyn et al. 1991, Rybojad et al. 1996).

**Other organ involvement**
Single cases of growth hormone deficiency (Kornfeld et al. 1995), hypogonadotropic hypogonadism (Raj et al. 2003), and hypothyroidism (Shimamura 2006) have been observed in SDS. An autopsy report described testicular fibrosis and cardiomegaly in a child with SDS (Graham et al. 1980).

Hirschsprung disease (Burke et al. 1967), ulcerative colitis (Ginzberg et al. 1999) and mild intestinal villous atrophy (Mack et al. 1996) have been described only in a few cases. Occasional observations of eye involvement such as strabismus, retinitis pigmentosa, coloboma and punctate keratitis have been reported (Aggett et al. 1980, Ginzberg et al. 1999).
3.1.5. Differential diagnosis

The diagnosis of SDS is based on clinical diagnostic criteria of exocrine pancreatic and bone marrow dysfunction. Other diseases with similar clinical presentation should be excluded at the time of diagnosis. Celiac disease may sometimes mimic the clinical symptoms of SDS such as failure to thrive, diarrhea, short stature, and anemia. Sometimes patients with cow's milk protein intolerance with erythematous skin rash and gastrointestinal symptoms may have similar early symptoms. Those patients, however, do not have exocrine pancreatic insufficiency. Cystic fibrosis (MIM # 602421), the most common cause for exocrine pancreatic insufficiency in the developed countries, can be excluded with repeated normal sweat chloride test results. Pearson syndrome (MIM # 557000) with exocrine pancreatic dysfunction, sideroblastic anemia and vacuolization of erythroid and bone marrow precursor cells is confirmed by the demonstration of mitochondrial DNA deletions. Johanson-Blizzard syndrome (MIM # 243800), also causing pancreatic insufficiency, can be excluded by typical phenotypic features that include congenital deafness, hypoplasia of alae nasi and scalp defects, and by genetic confirmation. Various forms of severe congenital neutropenias maybe also be diagnostic alternatives. Other bone marrow failure syndromes such as Fanconi anemia (MIM # 227650) and Diamond-Blackfan anemia (MIM # 105650), may have to be considered; Fanconi anemia can be diagnosed with chromosomal breakage test. Cartilage-hair hypoplasia (MIM # 250250), another disease with underlying ribosomal dysfunction, presents with short stature and metaphyseal dysplasia, variable cytopenia and immunological deficiency; genetic defect lies in the RMRP gene at chromosome 9p21.

3.1.6. Treatment and follow-up

Pancreatic insufficiency is treated with pancreatic enzyme substitution with meals and snacks and with supplements for fat-soluble vitamins A, D, and E. With clinically improved pancreatic function over time, some of the patients may no longer require pancreatic enzyme substitution (Mack et al. 1996).

Neutropenic patients with SDS and fever should be evaluated without delay and treated promptly with antibiotics bearing in mind the risks of serious, potentially life-threatening infections. Impaired neutrophil chemotaxis adds to the risk of infection beyond the low neutrophil count. Therefore, a patient with a severe chemotactic defect, even with a normal neutrophil count, may be at significant risk of infection (Dror
et al. 2001, Shimamura 2006). This should be taken into account even during minor dental or surgical procedures. Severe neutropenia in SDS has been successfully treated with low doses of granulocyte colony stimulating factor, G-CSF (Donadieu et al. 2005). Leukofiltered red blood cell and thrombocyte transfusions have been used in cases of severe anemia or thrombocytopenia; leukofiltration reduces the risks of developing graft rejection after possible bone marrow transplant.

Unlike pancreatic function, bone marrow involvement does not improve with age, and stem cell transplantation is the only curative treatment for bone marrow dysfunction in SDS (Dror 2005). However, due to transplantation-related organ toxicity and increased risk of refractory disease, the outcome of chemotherapy for SDS-related hematological malignancies is poor (Tsai et al. 1990, Fleitz et al. 2002, Donadieu et al. 2005, Dror 2005). Preparative regimens that include cyclophosphamide and total body irradiation are associated with an increased risk of organ-toxicity. Recently, reduced-intensity condition protocol has been shown to substantially improve transplantation outcome (Bhatla et al. 2008).

Detection of asymptomatic myelodysplastic syndrome with excess blasts and acute myeloid leukemia with low blast count may prompt early treatment and improve prognosis. Therefore, in a stable patient with mild to moderate cytopenia, complete blood count and peripheral blood smear three to four times a year as well as annual bone marrow testing to look for occult transformation have been suggested. In cases of severe bone marrow aplasia and myelodysplastic syndrome, stem cell transplantation should be considered. (Shimamura 2006).

Bone deformities due to metaphyseal chondrodysplasia, usually located in the hips or the knees, may require surgical interventions. A single case report of bisphosphonate (alendronate) treatment for SDS-related osteoporosis describes an increase in bone mineral density (BMD) in an adult patient with several mutations in SBDS, severe growth failure, hypophosphatemia and osteoporosis (Rosendahl et al. 2006).

Because multiple organ systems are affected in SDS, multidisciplinary approach to clinical care is expected to improve the treatment and quality of life of these patients.
3.2. Imaging studies in the assessment of various body systems

3.2.1. Basic principles of imaging modalities

X-ray is a form of electromagnetic ionizing radiation which can be used in medical imaging for plain radiographs but also in other imaging modalities, such as dual-energy X-ray absorptiometry (DXA) and computed tomography (CT). DXA uses low doses of X-rays to assess BMD; the radiation dose corresponds to approximately 10% of the dose of a standard chest X-ray. Two X-ray beams with different energy levels are aimed at the assessed area, and tissue absorption of each beam is recorded. CT is based on X-ray beams that pass through the patient from various directions: X-ray beam that has the shape of a thin fan rotates around the patient and detectors measure the intensity of the attenuated radiation as it emerges from the body. The local attenuation is calculated at each point within the CT section with a mathematical image reconstruction, and these data are converted into a CT image. Multidetector-row CT (multislice CT) technique enables fast acquisition of image data in a short time and allows for three-dimensional imaging and multiplanar reformats. The radiation from a CT examination is considerably high; for example, radiation dose from an abdominal CT corresponds to that of 400 standard chest X-rays. (Silverman & Kuhn 1993).

Sonography i.e. ultrasound is based on mechanical vibration that creates high frequency sound waves (usually 1-30 MHz). Brief bursts of ultrasound waves are transmitted into the body where they travel through tissues. Different tissues have different acoustic properties i.e. acoustic impedances. Whenever the sound pulse encounters an interface between tissues of different acoustic impedance, reflection or scattering of the sound occurs. As these echoes of the transmitted sound waves return to the receiver, an ultrasound image is generated. One of the important features of sonography is the lack of ionizing radiation. (Silverman & Kuhn 1993).

Magnetic resonance imaging (MRI) is based on signals obtained from the protons in the tissue hydrogen atoms within an external magnetic field. The protons possess an intrinsic spin and a positive electrical charge, and thus generate a small magnetic field. As the patient is placed into
a strong external field in the MRI scanner, all protons align with this external magnetic field; this is called longitudinal magnetization. After the application of an excitation radiofrequency energy pulse, a subset of protons that have the same frequency absorb energy and tilt to a higher energy level in a phenomenon called resonance and start to spin synchronously; this is called transverse magnetization. When the radiofrequency energy pulse is turned off, the protons return to equilibrium and release their absorbed energy producing an MRI signal. Two magnetization relaxation processes can be used to characterize substances. First, the longitudinal magnetization will get back to its original position; the time needed for the process is called longitudinal relaxation (T1) time. Secondly, as the protons move, they experience varying magnetic fields due to the surrounding environment. This causes a spread of resonant frequencies and a dephasing of magnetization in the transverse plane. The time needed for this process is called transverse relaxation (T2) time. Both T1 and T2 values are tissue-specific and thus enable effective tissue characterization. MRI provides a versatile, non-invasive method that causes no radiation exposure and enables effective evaluation of soft tissue organs and their disorders. Dynamic contrast-agent enhanced imaging allows for tissue characterization and assessment on vascular supply in different phases (e.g. arterial and venous phases). Heavily T2-weighted sequences produce high signal of stagnant fluid (e.g. fluid in the biliary system and pancreatic duct). Ultrafast sequences may be used for data collection during a single cardiac cycle. Advances in MRI technology have enabled the development of novel sequences and techniques, including three-dimensional volumetric imaging methods. (Bushberg et al. 2002).

Imaging in children presents special challenges in view of their greater sensitivity to radiation and, in younger age groups, of their lack of cooperation. One important goal in pediatric imaging is to obtain diagnostic information with the least amount of radiation exposure. Therefore, in pediatric imaging, ultrasound and MRI without radiation exposure are preferable modalities rather than CT with a high dose of ionizing radiation. Disadvantages of MRI include the need for anesthesia in young children, its limited availability, and the financial cost.
3.2.2. Pancreatic imaging

Sonography is considered the primary imaging modality of the pancreas in pediatric patients (Nijs, Callahan & Taylor 2005). Several reports describe hyperechogenic pancreas in patients with SDS (Robberecht et al. 1985, Schneider, Harms & Fendel 1987, Berrocal et al. 1995, Adachi et al. 2005). In young patients, however, sonography is insensitive to detect lipomatosis, and even entirely fatty pancreas can be overlooked (Adachi et al. 2005). CT can more reliably demonstrate lack of normal pancreatic tissue and its replacement by fat in SDS (Bom et al. 1993, Robberecht et al. 1985) but its major disadvantage is high dose of ionizing radiation.

MRI is an ideal technique for pancreatic imaging in both children and adults (Pamuklar & Semelka 2005). The use of MRI in detection of pancreatic involvement in SDS has been described in only a few reports (Bom et al. 1993, Lacaille et al. 1996, Ruggiero et al. 2008). On MRI, pancreatic parenchyma and fat can be detected with high specificity and sensitivity. Advanced techniques such as chemical shift imaging (in-phase, out-of-phase imaging) even enable visualization of intracellular tissue fat accumulation of microscopic scale. Magnetic resonance cholangiopancreatography (MRCP) allows for precise non-invasive visualization of the biliary system and pancreatic duct morphology. Secretin-stimulated MRCP enables dynamic functional assessment of the pancreatic duct and improves visualization of the entire duct including its side branches as secretin increases exocrine production of fluid and bicarbonate in the pancreas (Brice 1999, Lee et al. 2006).

3.2.3. Bone assessment

Conventional radiographs are the gold standard in the assessment of skeletal dysplasia and vertebral morphology (Genant et al. 1993, Mäkitie et al. 2005).

DXA is the most widely used non-invasive method to measure BMD. It allows for the quantification of bone mass and BMD and can also be used to measure total body composition and fat content. In DXA, bone mineral content (BMC), measured as the attenuation of the X-ray by the scanned bones, is divided by the area of the site being scanned to obtain areal BMD (g/cm²). Thus it does not measure true BMD, which is bone mass divided by bone volume (g/cm³). Despite problems with bone volume estimation, DXA is still a fairly accurate measure of BMC. T-score, the number of SD that an individual BMD measurement differs from the mean when a historical, sex-matched normal young adult population is used as the reference, has
proved useful in defining osteoporosis in adults (Genant et al. 1999). With low radiation dose and short imaging time, DXA can be used to assess bone mass also in young children (Njeh et al. 1997). Special considerations must be taken into account when interpreting DXA measurements in pediatric population (Bishop et al. 2008). First, the comparison of BMD of a child to the adult reference data (T-scores) would lead to an overestimation of bone mineral deficits, since children have less bone mass than adults. Therefore, children’s BMD scores are commonly compared with reference data for the same gender and age (Z-score). In addition to age, other variables such as bone size and bone maturation may confound the interpretation of DXA-derived BMD. Therefore, correction with bone age and lean tissue mass (LTM) have been proposed to obtain more accurate and comparable BMD results in children (Högler et al. 2003).

3.2.4. Brain imaging

MRI is the modality of choice for the evaluation of myelination, neurodegenerative disorders, and developmental malformations of the brain. However, individuals with intellectual disabilities of unexplained etiology often have, in the visual assessment of MRI data, either unremarkable or mild, non-specific findings (Decobert et al. 2005). More accurate computer-assisted measurements of different brain structures can reveal changes that are not detectable in the visual analysis (Ashburner et al. 2003).

Voxel-based morphometry is a fully automated computed method that allows for the segmentation of the imaging data voxel by voxel into different tissue types. With the voxel segmentation process, the volumes of gray and white matter as well as total brain volume and cerebrospinal fluid volume can be accurately determined, and regional and global brain matter volume changes can be assessed (Ashburner & Friston 2000).

3.2.5. Cardiac imaging methods

Conventional echocardiography is an expedient non-invasive method to acquire baseline information on intracardiac anatomy, myocardial contractility, and diastolic function. However, basic functional measurements may be insensitive to detect subtle changes in the systolic and diastolic properties of the heart. Tissue Doppler velocity imaging has recently been applied to measure the systolic ejection wave (S-wave) and isovolumic acceleration of the ventricular myocardium at the level of the mitral valve annulus. Isovolumic acceleration is sensitive to changes

Compared to echocardiography, MRI can more precisely assess the right side of the heart, myocardium, and the pericardium (White & Patel 2007). 3-D computer-based model as a quantitative segmentation tool in MRI image analysis yields accurate quantitative information on volumetric changes within different phases of the cardiac cycle (Lötiönen et al. 2004).
4. AIMS OF THE STUDY

The aims of the study were to assess

1. the clinical and genetic features
2. the pancreatic phenotype and imaging findings
3. the prevalence and the nature of osteoporosis
4. the MRI characteristics of the brain, and
5. the cardiologic characteristics

of the Finnish patients with SDS.
5. METHODS

5.1. Study subjects

5.1.1. Patients with Shwachman-Diamond syndrome

The pediatric and gastroenterological units of all university hospitals in Finland were contacted to obtain information on patients diagnosed with SDS or with features suggestive of SDS. Fourteen patients with a previous diagnosis of SDS were identified in Finland; the diagnosis was based on verified exocrine pancreatic insufficiency and hematological dysfunction (permanent or intermittent neutropenia with or without other hematological abnormalities) and on the exclusion of other pancreatic and hematological conditions with a phenotypic resemblance to SDS. One patient, a 50-year-old male, was unwilling to participate in the study. Thirteen patients consented and were subsequently invited to the out-patient clinic of the Hospital for Children and Adolescents, Helsinki University Hospital. Five adult patients in the present study, aged 24-37 years, had been previously assessed as infants (Savilahti & Rapola 1984). In addition to the previously diagnosed patients, four children investigated in our institution during the study in 2004-2007, who fulfilled the clinical criteria of SDS, were included.

The Research Ethics Board, Helsinki University Hospital, approved the study protocol and written informed consent was obtained from each patient and/or guardian.

Study I: Pancreatic phenotype and abdominal MRI in Shwachman-Diamond syndrome

All patients with a clinical diagnosis of SDS who did not require anesthesia for abdominal MRI were included in prospective abdominal MRI examination. In addition, clinically indicated abdominal MRI data which had been obtained under anesthesia in young patients prior to or at the time of the study were included.

Study II: Evaluation of bone health in Shwachman-Diamond syndrome

All patients with a clinical diagnosis of SDS and verified mutations in the SBDS gene, and over 4 years of age, in order to have the required co-operation for the DXA examination, were included in the study on bone health.
**Study III: Neuroradiological assessment of Shwachman-Diamond syndrome**

Patients with a clinical diagnosis of SDS and verified mutations in the SBDS gene, without history of brain damage or serious head trauma with loss of consciousness, and with age over 5 years in order to avoid anesthesia during the brain MRI, were included in the brain MRI study.

**Study IV: Myocardial function in Shwachman-Diamond syndrome**

Patients with SBDS mutation-verified SDS and over 6 years of age, in order to enable pedal ergometry during tissue Doppler examination, were included in the study on cardiac features. Cardiac MRI was performed only on those patients who did not require anesthesia.

### 5.1.2. Control subjects

**Study III**

For each patient with SDS, two healthy right-handed age- and gender-matched hospital workers or their family members were ascertained for the control group of the brain MRI study. History of neuropsychological symptoms, systemic illnesses, antenatal or perinatal insults, or serious head trauma with loss of consciousness were excluded by patient/guardian interview. Altogether 18 age- and gender-matched control subjects for nine patients with SDS underwent study protocol brain MRI in 2005-2006.

**Study IV**

The control group for tissue Doppler echocardiography consisted of 26 healthy volunteers studied at the Hospital for Sick Children, Toronto, Canada in 2003-2004 using similar equipment and study protocol. All had normal cardiac structure and systolic function in baseline echocardiography. A family history of sudden death or prolongation of the corrected QT interval was excluded by a patient/parent interview.

Control imaging data for the cardiac MRI were obtained from the previously reported examinations performed in 2002 on 12 healthy Finnish subjects without a history of diabetes, coronary artery disease, valvular, or hypertensive disease (Kivistö et al. 2004, Kivistö et al. 2006). The imaging protocol was similar to that used for the patients except for late enhancement sequence which was only obtained for the patients with SDS. The number and the age range of the patients and the controls are summarized in Table 4.
### TABLE 4. Numbers of patients with Shwachman-Diamond syndrome and controls that participated in the studies.

<table>
<thead>
<tr>
<th>N:O OF PATIENTS/ CONTROLS</th>
<th>AGE RANGE (YEARS)</th>
<th>INCLUSION CRITERIA</th>
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<tr>
<td>Study cohort</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 patients</td>
<td>2.1-37</td>
<td>Clinical diagnosis of SDS, exclusion of cystic fibrosis by sweat tests</td>
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<td>Healthy age- and gender-matched subjects</td>
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### 5.2. Assessment methods

#### 5.2.1. Clinical, biochemical, and histomorphometric assessment

*Clinical and auxological assessment*

A detailed family history and history for growth and health, exercising habits, and medications including the use of pancreatic enzyme and vitamin substitutions were obtained by patient/parent interview and from hospital records. Patients were clinically assessed for growth, puberty, as well as gastrointestinal and skeletal symptoms by a pediatrician. In addition, a pediatric cardiologist performed clinical cardiac assessment on all the patients who were included in Study IV. Height, weight and occipitofrontal head circumference (OFC) were measured; values were compared with normative data (Sorva et al. 1990). Height and OFC SD scores (Z-scores) were defined as deviation, in SD units, from the mean value for age and sex (Sorva et al. 1990, Pere 2000). Weight was expressed as body mass index, and the body surface area was calculated as previously described (Mosteller 1987).
Biochemical assessment

Peripheral blood counts were obtained to assess hematological dysfunction. Anemia was defined as a hemoglobin concentration below the normal age- and sex-specific reference range (Dallman & Siimes 1979), neutropenia as a neutrophil count <1500 x 10⁶ cells/L in repeated measurements, and thrombocytopenia as a platelet count of <150 x 10⁹ cells/L.

Serum concentrations of cationic trypsinogen (reference range 16.6 - 45.6 µg/L) and pancreatic isoamylase activity (reference range 13 - 53U/L) were determined. The presence or absence of pancreatic insufficiency was classified on the basis of serum trypsinogen concentration (Moore et al. 1986, Ginzberg et al. 1999); patients with values <6 µg/L were classified as having pancreatic insufficiency and patients with intermediate (6 - 16.6 µg/L) or normal (>16.6 µg/L) values were classified as having pancreatic sufficiency. Fecal pancreatic elastase 1 (reference range >200 µg/L) was quantified by ELISA (Pancreatic Elastase ELISA, Bioserv Analytics and Medical Devices Ltd. Rostock, Germany). Serum concentrations of 25-OH-vitamin D (S-25-OHD), vitamin A using retinol (Sigma R-7632, UK) as standard and vitamin E using alfa-tocopherol (Sigma T-3634, UK) as standard were determined by HPLC followed by UV detection (HP 1100 Liquid Chromatograph for 25-OHD and HP1090 Liquid Chromatograph for vitamins A and E). S-25-OHD values lower than 38 nmol/L were regarded consistent with hypovitaminosis D. The reference range for vitamin A was 1-3 µmol/L and for vitamin E 12-40 µmol/L.

Plasma concentrations of alkaline phosphatase (reference range age-dependent), aspartate aminotransferase (reference range <50 U/L), alanine aminotransaminase (reference range <40 U/L), gamma glutamyltransferase (reference range sex- and age-dependent), total bilirubin (reference range 5 - 25 µg/L), conjugated bilirubin (reference range <2 µg/L), creatinine (reference range 50 - 90 µg/L), phosphate (reference range age-dependent), prothrombin time (normal >70%), and serum concentrations of fasting bile acid (reference range <6µg/L), and base excess (reference range −2.5 - +2.5) were determined by standard assays.

Mutation analyses of the SBDS gene

A blood sample for DNA extraction was obtained from the patient and the parents for mutational analysis of the SBDS gene. Mutational analyses of the DNA samples for 16 patients were performed in Dr. J. Rommens’ laboratory at the Hospital for Sick Children, Toronto, Canada. In one patient, mutational analysis was performed in Dr Shiro Ikegawa’s laboratory in Tokyo, Japan. SBDS coding regions were screened for mutations by restriction enzyme digestion of amplified exon 2 or by direct sequencing of PCR amplified products of genomic DNA, as previously
described (Boocock et al. 2003). DNA samples of patients without two identified mutations in exon 2 underwent direct sequencing of all coding regions of the SBDS gene.

**Bone histomorphometry**

A transiliac bone biopsy for histological and histomorphometric studies was obtained from selected consenting patients with a bone biopsy needle with an inner diameter of 7.5 mm (Rochester Bone Biopsy, Medical Innovations Incorporation Inc, USA) following a double-labeling course with oral tetracycline, with a 10-day interval period. The biopsy was performed four days after the end of tetracycline administration. Analyses for bone histomorphometry were conducted at the Bone and Cartilage Research Unit, University of Kuopio, Kuopio, Finland. All parameters were analyzed using a semiautomatic image analyzer (Bioquant Osteo, Bioquant Image Analysis Corporation, Nashville, TN, USA) and the results were compared with normative data (Glorieux et al. 2000, Recker et al. 1988, Rehman et al. 1994). All nomenclature, abbreviations, and standard formulas follow the recommendations of the American Society for Bone and Mineral Research.

5.2.2. Radiographic, bone densitometric, and echocardiographic assessment

**Radiographs**

Radiographic assessment included plain radiographs of the chest (posterior-anterior and lateral views), thoracic and lumbar spine (anterior-posterior and lateral views), pelvis (anterior-posterior view), knees (anterior-posterior view), and the hands (anterior-posterior view). All radiographs were reviewed by two radiologists, and skeletal abnormalities were recorded. Thoracic and lumbar radiographs were assessed and changes in vertebral morphology were graded by inspection of digitized images (AGFA ImPacs System). The grading methods of Genant (Genant et al. 1993) for adults and of Mäkitie (Mäkitie et al. 2005) for children were used to identify vertebral morphological changes suggestive of osteoporosis. Compression of more than 20% in the anterior, middle, or posterior vertebral height was considered significant.

**Bone Densitometry**

Areal BMD for the lumbar spine (L1-L4), proximal femur, and the whole body were measured with DXA (Hologic Discovery A, Bedford, USA). The areal BMDs were transformed into Z-scores by using age- and sex-specific reference data for the equipment. In addition, T-scores were calculated for adult patients by comparing the areal BMD results with sex-specific reference data for the equipment. Body composition, includ-
ing BMC, fat mass and LTM, was obtained with the same DXA scanner. Height-adjusted Z-scores for BMC/LTM were calculated using previously published reference data for children and young adults (Högler et al. 2003).

**Echocardiography with tissue Doppler assessment**

ECG was recorded at rest and baseline echocardiography (Acuson Sequoia 512 Siemens, Mountain View, CA) was performed. The study subject was then exercised for the tissue Doppler examination on the cycle ergometer by increasing resistance of the pedals by 15-25 W at intervals of 1-2 minutes in order to achieve, during each interval, an increase in heart rate of 20 beats/min from the previous level; the target heart rate was 160 beats/min. The heart was imaged through an apical four-chamber view with the most optimal angle to the left ventricle free wall; myocardial velocities of the left ventricular basal free wall were measured as previously described with simultaneous ECG recording (Vogel et al. 2003). The left ventricle Tei-index that describes combined systolic and diastolic function was derived from the Doppler-measurements (Tei et al. 1995). The left ventricular rate-corrected velocity of circumferential fiber shortening was calculated as previously described (De Wolf et al. 1998). The acquired tissue velocity data were analysed using Echopac software (GE Medical Systems, GE Vingmed Ultrasound AS, Horten, Norway). Isovolumic acceleration and the systolic wall movement velocity were measured during tissue Doppler echocardiography from a minimum of three cardiac cycles and averaged (Vogel et al. 2003).

5.2.3. MRI assessments

**Abdominal MRI**

The prospective MRI studies were performed with three 1.5 T scanners (Philips Intera Achieva for the children, and Siemens Sonata and Vision for the adults). Imaging protocol included axial images of the upper abdomen with gradient echo sequence (FFE/TrueFISP)-, fat-saturated T1 (T1 fs)- and fat-saturated T2 (T2 fs)-weighted images, gadolinium-enhanced (intravenous Dotarem 279.3 mg/mL, 0.2 mL/kg) fat-saturated T1-weighted (THRIVE/VIBE) images at arterial, venous and late venous phases, chemical shift (in-phase/opposed-phase) imaging, MRCP sequence, and, in four adult patients, secretin-stimulated MRCP images after intravenous administration of secretin (Secretlux, Goldham Bioglan, Zusmarshausen, Germany, 1 IU/ kg body weight).

All prospective and previously performed MRI studies were reviewed independently by two radiologists who were blinded to the genetic findings. The final conclusions were reached by consensus. The size of the
pancreas was graded as small, normal or enlarged visually and using previously reported measurements for age (Heuck et al. 1987). Signal intensity, parenchymal structure, ductal anatomy, and contrast enhancement of the pancreas were assessed. The presence or the absence of identifiable pancreatic tissue was recorded.

**Brain MRI**
The patients and their matched controls underwent brain MRI with the same 1.5T imager (Philips Intera Achieva). T2-weighted axial and coronal slices with a turbo-spin-echo sequence (TR 5000 ms, TE 100 ms, slice thickness 3 mm), an axial fluid-attenuated inversion recovery (FLAIR) sequence (TR 11000 ms, TE 140 ms, IR 2800 ms, slice thickness 4 mm), and a 3-dimensional magnetization-prepared rapid acquisition gradient echo (3D MPRAGE) sequence (TR 25 ms, TE 4.6 ms, flip angle 30º, slice thickness 1 mm) were obtained.

An experienced neuroradiologist who was blinded to the subjects’ genotype assessed all T2-weighted and FLAIR images for visually detectable brain changes. The brain signal intensities, gray-white matter differentiations, the sizes of the ventricles and cortical cerebrospinal fluid spaces were recorded. 3D MPRAGE image data were segmented into white matter, gray matter, cerebrospinal fluid, and non-brain tissues, using automated segmentation method (SPM2 software package, available at http://www.fil.ion.ucl.ac.uk/spm/). After segmentation, the volumes of global brain matter, white matter, gray matter, and cerebrospinal fluid were automatically calculated for each studied subject.

Midsagittal MRI slice was defined by the visualization of cerebral aqueduct. Midsagittal areas of internal skull surface, cerebral surface, posterior fossa surface, vermis, mesencephalon, pons, medulla, the length and the surface area of corpus callosum, and the midsagittal diameters of genu, body and splenium of corpus callosum, and of mesencephalon, pons, and medulla were measured by a radiologist from 3D MPRAGE sagittal images (Figure 1 A) with a digital radiological work station (Agfa Impax) measurement tools (Hashimoto et al. 1993, Laissy et al. 1993, Raininko et al. 1994). The following ratios were calculated from the midsagittal area measurements: posterior fossa/vermis, cerebrum/vermis, cerebrum/corpus callosum. The bifrontal ratio, obtained from the ratio of the longest distances of the anterior horns of lateral ventricles at the level of the third ventricle and the brain width at the same level (Aylward et al. 1991), was calculated from T2-weighted axial images (Figure 1 B).
FIGURE 1. Brain MRI measurements.
A. Midsagittal areal measurements from a 3D MPRAGE (TR 25 ms, TE 4.6 ms, flip angle 30°, 1 mm slice) sagittal image. CC, corpus callosum; ME, mesencephalon; M, medulla; P, pons; V, vermis. b, callosal body; g, callosal genu; s, callosal splenium, t, cerebellar tonsil.
B. The measurement of bifrontal ratio (a-b distance/c-d distance) from an axial TSE T2 (TR 5000 ms, TE 100 ms image, 3 mm slice) image.

Cardiac MRI
Cardiac MRI was performed with a 1.5T MRI imager (Philips Intera Achieva) using a cardiac coil. To assess cardiac anatomy and function, cine images were obtained with an ECG-gated breath-hold balanced TFE sequence (TR 3.27 ms, TE 1.63 ms, flip angle 60°) which produces a series of images within the cardiac cycle every 40 ms. All cine sections were acquired at the same end-expiration phase to minimize the effects of respiratory variation. The heart was covered from ventricular base to apex with two- and four-chamber views, and short-axis sections of 8 mm thickness 8 mm apart. Late enhancement images at the same sections were obtained to assess myocardial fibrosis 15 minutes after intravenous Gadolinium-DTPA-BMA (Dotarem 0.15 mmol/kg) with IR balanced FFE pulse sequence (TR 3.53 ms, TE 1.72 ms, section thickness 6 mm). Inversion time was individually set to null signal from normal myocardium (250-300 ms).

The long- and short-axis cine images were used to obtain accurate description of cardiac anatomy and function. Atrial and ventricular volumes and the left ventricular mass were semi-automatically segmented from each frame of the cine MRI data (Figure 2) using a three-dimensional segmentation method developed for this purpose (Lötjönen et al. 2004).
The largest and the smallest volumes of the atria and cyclic atrial volume changes were determined (Järvinen et al. 1994). End diastolic and end systolic volumes, stroke volume and ejection fraction, the maximal slope during diastole (=peak filling rate) and the volume change from early diastole to the peak filling rate (=early diastolic filling) of both ventricles were determined. The left ventricle mass and the ventricular volumes were adjusted for body surface area. The myocardial wall enhancement was visually analyzed by two radiologists.

**FIGURE 2. Volumetric assessment of cardiac MRI data.**
A-B. Slices of the two-chamber and the four-chamber view of the heart obtained with cardiac MRI cine images (Balanced TFE sequence, TR 3.27 ms, TE 1.63 ms, flip angle 60°, 8 mm slice). Each compartment is outlined for volumetric assessment of the atria and the ventricles

### 5.3. Statistical analyses

*Studies I and II:* Simple regression analysis, Student’s paired t-test, and Chi square test were used, as appropriate, for statistical analysis (Statview 5.0.1 for Macintosh, 1992-1998 SAS Institute Inc., USA). A *P*-value of less than 0.05 was considered statistically significant.

*Study III:* Paired samples t-test, Wilcoxon signed rank test, Spearman’s correlation test, Fisher’s test, and regression analysis were used, as appropriate, for statistical analysis (SPSS for Windows, version 13.0, SPSS, Chicago, USA). The mean measurement values of the two matched controls for each patient were used in paired analyses; the results were considered significant when paired samples t-test and Wilcoxon signed rank test both agreed.

*Study IV:* Independent samples t test, Mann-Whitney-U, Fischer’s exact test, Tukey post hoc test, and one way analysis of variance (ANOVA) tests were used, as appropriate, for statistical analysis (SPSS for Windows, version 13.0, SPSS, Chicago, USA).
6. RESULTS

6.1. Clinical and genetic findings

Seventeen Finnish patients who fulfilled the clinical diagnostic criteria for SDS participated in the study. There were two sets of siblings. The age range of the patients at the study entry was 2.1-37.0 years (mean age 17.4 years). Eleven out of the 17 patients (65 %) were males. The age of diagnosis of SDS ranged from 4 months to 14.5 years. All except the four patients diagnosed after 5 years of age had started pancreatic enzyme substitution at the time of the diagnosis, and all patients had suffered from recurrent infections in infancy. Eleven patients had neutropenia (neutrophil count <1500 x10^6/L), and three of them had severe neutropenia (neutrophil count <500 x10^6/L). Three had subnormal hemoglobin levels (age-dependent), and three had thrombocytopenia (thrombocyte count <150 x10^6/L). In one patient all three cell lines were affected. None of the patients had evidence of MDS or malignant bone marrow transformation at the time of the study.

The radiographic assessment showed several bone abnormalities such as expansion of costochondral junctions and rib cage narrowing (Figure 3 A-C), metaphyseal changes in the wrists, hips, and the knees (Figure 3 D-H), and delayed bone age (Figure 3 H-K). Two patients had undergone surgery due to limb deformities caused by metaphyseal dysplasia; one of them had had hemiepiphysodesis of the knee because of genu valgum (Figure 3 G), the other bilateral femoral osteotomy because of Legg-Calvé-Perthes disease of the hips.

Genetic analysis revealed mutations in SBDS in twelve patients. Nine patients were compound heterozygotes for the two common SBDS mutations 258+2T>C and 183TA>CT. Three additional patients were heterozygous for 258+2T>C; a second mutation was identified on the other allele in two of these patients. In the third patient, no mutations could be identified by complete gene sequencing of the second allele. In five patients, complete gene sequencing failed to identify any SBDS mutations. Clinical and genetic findings are summarized in Table 5.
FIGURE 3. Skeletal radiographic features of Shwachman-Diamond syndrome.

A-B. Short ribs with marked cupping and widening of the anterior ends in chest X-ray at 11 months.
C. CT slice shows narrow and deformed rib case, as well as cupping and irregular widening of the costochondral junctions.
D. Broad pelvis, short iliac notches, horizontal acetabuli, and wide proximal metaphyses of the femora are noted in pelvic X-ray at 11 months.
E. X-ray of the lower limbs at 6 months shows mild bowing of the femoral shafts, metaphyseal widening in the proximal and the distal femora and tibiae, and cupping in the distal metaphyses of the tibiae and the fibulae. Femoral necks are short and in valgus position.
F-G. Marked metaphyseal changes with striated abnormal bony structure in the hips and the knees at 14 years. Medial hemiepiphyseodesis was performed on the right distal femur because of genu valgum.
H-I. Delayed bone age at 11 months with missing ossification of the carpal (H) and tarsal (I) bones. The middle phalanges in the 5th fingers (MP V) are short and the wrist metaphyses are irregular.
J-K. The hand radiograph at 4.5 years (J) shows markedly delayed bone maturation particularly in the carpal bones, and short MP V. At 11 years (K), the bone age of the same patient is slightly delayed, and MP V has grown in length.
L. The bone age in the hand radiograph at 14.5 years is within normal limits. MP V with cone-epiphysis is short.
TABLE 5. Clinical, biochemical, and SBDS mutation data on 17 patients with a clinical diagnosis of Shwachman-Diamond syndrome.

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S-Tryp, serum cationic trypsinogen; S-Isoamyl, serum pancreatic isoamylase activity; Height Z-score, deviation of height, in standard deviation units, from mean height for age and sex; BMI, body mass index; na, not assessed; PI, pancreatic insufficient (S-Tryp < 6 µg/L); C, 258+2T>C; D, 183TA>C/T; X, IVS2-124G>A. Values marked with * are below the reference range.
6.2. Study I: Pancreatic biochemistry and imaging findings in patients with clinical diagnosis of Shwachman-Diamond syndrome

Altogether fourteen patients (10 males, 2.1 – 37.0 years) were included: eleven had prospective abdominal MRI and three children had undergone MRI for clinical indications. Two children were too young to undergo MRI without anesthesia, and parental consent was lacking for one child: these three patients were thus excluded from the study cohort. At the time of the study, three patients, all with different mutations in SBDS, were pancreatic insufficient based upon S-trypsinogen levels. Four patients, three with SBDS mutations and one without any mutations, had intermediate values. In the remaining six patients, values were within normal range (not assessed in one).

At the study assessment, all SBDS mutation-positive patients except one had neutropenia, and all had low serum isoamylase levels. On MRI, all patients with SBDS mutations had small to normal size fat-replaced pancreas with occasional scattered enhancing foci of pancreatic parenchyma, and subtle enhancement along the pancreatic duct (Figure 4 A-F). With the exception of the patient previously diagnosed with type 1 diabetes mellitus, the endocrine function of the pancreas, based upon HbA1c measurements, was normal in mutation-positive patients.

All five SBDS mutation-negative patients had normal neutrophil counts at the time of the study assessment. MRI demonstrated normal pancreatic anatomy and parenchyma without fatty infiltration (Figure 4 G) in four patients, and a rare pancreatic anomaly (agenesis of dorsal pancreas with associated polysplenia) in an adolescent who was investigated due to a clinical suspicion of SDS (Figure 4 H). Serum pancreatic isoenzyme levels were normal in the mutation-negative patients, except for the patient with a rare developmental anomaly of the pancreas. Endocrine pancreatic function was normal in all mutation-negative patients.
RESULTS
FIGURE 4. Pancreatic MRI findings in patients with a clinical diagnosis of Shwachman-Diamond syndrome.

A-B. In a 2-year-old SBDS-positive patient, T1 FFE (A; TR 3.35 ms, TE 1.67 ms, 5 mm slice) and T2 fs (B; TR 1200 ms, TE 70 ms, 5 mm slice) images at the level of the pancreatic body and tail show fatty replacement of the pancreas.

C-E. In a 33-year-old SBDS mutation-positive patient, in-phase (C; TR 100 ms, TE 5.04 ms, flip angle 70°, 5 mm slice) and out-of-phase (D; TR 100 ms, TE 2.38 ms, flip angle 70°, 5 mm slice) images demonstrate small, lipomatous pancreas. Gadolinium-enhanced (Gd) T1 fs (E; TR 3.35 ms, TE 1.67 ms, 5 mm slice) image shows scattered parenchymal enhancement particularly in the tail of the pancreas.

F. Enhancing duct (arrows) within a small fat-replaced pancreas in a 31-year-old patient with SBDS mutations (Gd T1 fs, TR 3.35 ms, TE 1.67 ms, 5 mm slice).

G. Normal pancreatic anatomy and structure in a 24-year-old mutation-negative patient (Gd T1 fs, TR 3.35 ms, TE 1.67 ms, 5 mm slice).

H. Pancreatic head (long arrow) but missing body and tail and one of the multiple spleens (arrowhead) are demonstrated in the abdominal T2 fs (TR 1200 ms, TE 70 ms, 5 mm slice) image in a 13-year-old patient without mutations in SBDS. The imaging findings are consistent with polysplenia-dorsal pancreas agenesis anomaly.

6.3. Study II: Radiographic, bone mineral density, and bone histomorphometry findings in SBDS mutation-positive patients with Shwachman-Diamond syndrome

All eleven SBDS mutation-positive patients (8 males, 5.2 – 37.0 years) that fulfilled the inclusion criteria participated. The median height Z-score at the time of the study was -2.3, ranging from -3.1 to -0.7. None of the patients had motor disabilities and their physical activity was considered normal. Nine patients had received pancreatic enzyme supplements, and eight had been given supplementation for vitamin D, six for vitamins E and A, and two for calcium. One patient had sustained a vertebral fracture after a low-energy fall, and three patients had sustained peripheral fractures; the fracture sites being the wrist, the hip and the femoral shaft.

Blood biochemistry suggested mild deficiencies of vitamin D (median 37 nmol/L, range 17 - 76 nmol/L) and vitamin K (median 73%, range 58 - 109%). Serum concentrations of vitamins E and A and plasma levels of Ca, Pi and alkaline phosphatase were normal in all patients. Three patients had hyperparathyroidism, interpreted to be secondary to vitamin D deficiency in two patients. In the third patient, the cause of hyperparathyroidism remained unknown.

In spinal radiographs three patients had altogether eight compressed vertebrae, all in the thoracic spine. Three patients had scoliosis and one had kyphosis. BMD Z-scores (Figure 5) were significantly reduced at the
lumbar spine (median -2.1, ranging from -4.4 to -0.8), the proximal femur (median -1.3, ranging from -2.2 to -0.7), and at the whole body (median -1.0, ranging from -2.8 to 0.6). Lumbar BMD Z-score was below -2.0 SD in 63% of the patients. The median height-adjusted BMC/LTM Z-score was -0.9, ranging from -3.6 to 1.1. Four out of five adults had BMD T-scores suggestive of osteopenia (T-score < -1.0) or of osteoporosis (T-score < -2.5). No correlation was observed between neutrophil levels and any of the bone density values.

FIGURE 5. Bone densitometry in Shwachman-Diamond syndrome.
Low bone density in the lumbar spine and the femoral neck
A-B. in a 5-year-old patient with pancreatic enzyme supplementation from age 18 months.
C-D. in a 14.5-year-old patient without any pancreatic enzyme supplementation.
E-F. in a 26-year-old patient with pancreatic enzyme substitution from age 4 months.

Bone biopsy was performed on four patients (age range 14.6 – 31.1 years). Three samples were suitable for histomorphometric analysis. Bone trabecular structure was broken in the biopsy material of the fourth patient who had the most severe osteoporosis based on BMD values (Figure 5 E-F) making it impossible to perform complete histomorphometric analysis. Both osteoblasts and osteoclasts were reduced in number in all bone biopsy samples, but mineralization of osteoid was deemed normal. Three technically optimal samples all showed reduced amounts of osteoid and low bone turnover. Histomorphometric findings typical of osteomalacia were not observed in any of the bone samples.
6.4. Study III: Brain magnetic resonance imaging findings in patients with Shwachman-Diamond syndrome and SBDS gene mutations

Nine consenting SBDS mutation-verified patients (7 males, 7 - 37 years) that fulfilled the inclusion criteria were included in the study. The auxological data on height and OFC at birth, at the age of 1 year, and at study assessment are presented in Table 6. Patients with SDS had smaller head circumferences than the controls (mean OFC Z-score -1.3 vs. +0.3). In a subset of patients, head circumferences had already been small at birth (mean OFC Z-score -0.9, range -2.5 - 0.3) and in early infancy (mean OFC Z-score at the age of 1 year -1.0, range -2.0 - 0.0), but no consistent pattern of OFC growth during development was noted.

TABLE 6. Height and OFC Z-scores at birth, at age 1 year, and at study assessment.

<table>
<thead>
<tr>
<th>GENDER, AGE AT DG/AT STUDY (years)</th>
<th>AT BIRTH</th>
<th>AT 1 YEAR</th>
<th>AT STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Height Z-score</td>
<td>OFC Z-score</td>
<td>Height Z-score</td>
</tr>
<tr>
<td>M 6/7</td>
<td>-0.9</td>
<td>+0.3</td>
<td>-2.5</td>
</tr>
<tr>
<td>F 5/13</td>
<td>-2.3</td>
<td>-2.5</td>
<td>-3.2</td>
</tr>
<tr>
<td>M 15/15</td>
<td>-0.6</td>
<td>-1.0</td>
<td>-2.3</td>
</tr>
<tr>
<td>M 1.1/17</td>
<td>-0.3</td>
<td>-0.5</td>
<td>-2.5</td>
</tr>
<tr>
<td>M 1.5/25</td>
<td>-1.5</td>
<td>-0.5</td>
<td>-3.5</td>
</tr>
<tr>
<td>M 0.3/27</td>
<td>-2.0</td>
<td>-2.5</td>
<td>-3.0</td>
</tr>
<tr>
<td>F 0.5/32</td>
<td>-1.8</td>
<td>na</td>
<td>-3.5</td>
</tr>
<tr>
<td>M 1.2/23</td>
<td>+1.2</td>
<td>+0.3</td>
<td>-1.3</td>
</tr>
<tr>
<td>M 1.1/37</td>
<td>-2.0</td>
<td>na</td>
<td>-2.5</td>
</tr>
</tbody>
</table>

OFC, occipitofrontal circumference; na, not available.

According to parent reports, five patients had had delayed speech or motor development, or of both. Compared to their healthy siblings, the majority had lower academic achievement. Eight out of nine patients had learning difficulties, mathematics and foreign languages were the most challenging subjects at school. Two patients had undergone neuropsychological evaluation due to significant learning difficulties. Four patients had received special education services.

Visual assessment of the brain MRI was considered normal in all patients with SDS and the controls. Global brain volume was smaller in patients with SDS than in controls (1.74 L vs. 1.94 L, P = 0.019), both gray matter (0.72 L vs. 0.79 L, P = 0.042) and white matter (0.40 L vs. 0.46 L, P = 0.007).
volumes were reduced. In voxel-based analysis, regional brain matter volume changes failed to reach significance. Midsagittal measurements revealed that posterior fossa (35.6 cm² vs. 43.5 cm², P <0.0001) and cerebellar vermis (10.1 cm² vs. 12.5 cm², P = 0.002), corpus callosum (5.5 cm² vs. 6.7 cm², P = 0.010), and brain stem (4.1 cm² vs. 5.13 cm², P <0.0001) were structurally smaller in patients with SDS than in the healthy controls. The ratios of cerebrum/vermis (15.1 vs. 9.99, P <0.0001) and cerebrum/corpus callosum (21.1 vs. 18.7, OFC-adjusted P <0.029) were increased.

6.5. Study IV: Myocardial function and imaging findings in patients with SBDS mutation-verified Shwachman-Diamond syndrome

Eight consenting patients that fulfilled the inclusion criteria were included in the study. The mean age at the time of the study was 24.1 years (range 7-37 years). Clinical cardiac and echocardiographic assessment was performed on all the patients, and exercise tissue Doppler examination was performed on all except the youngest patient who was too small to reach the ergometer pedals. Six adult patients (5 males, mean age 28 years, range 17-37 years) underwent the cardiac MRI protocol.

None of the patients had cardiac symptoms prior to or at the time of the study. The patient with type 1 diabetes mellitus had hypertension; he was treated with enalaprin and insulin. Other patients were normotensive and had not received cardiac medication. The 37-year-old male had two siblings who had succumbed to SDS and cardiac failure as neonates; in one of them, autopsy had shown myocardial necrosis (Savilahti & Rapola, 1984).

No abnormalities in cardiac anatomy or function were observed at baseline clinical assessment. All patients had sinus rhythm and normal parameters on ECG. Conventional echocardiographic measurements were within normal range in all of them. In tissue Doppler examination during exercise, maximum isovolumic acceleration value was significantly lower in patients with SDS (3.6 cm/sec² vs. 8.1 cm/sec², P < 0.001) than in controls; no differences were noted in S-wave velocities between these groups.

Myocardial structure assessed with MRI was normal in patients with SDS, and left ventricular mass did not differ from that of the controls. No evidence of myocardial fibrosis was noted in late-enhancement contrast-enhanced MRI images. Atrial functions and left ventricle systolic and diastolic parameters were normal. On MRI, the mean right ventricle
ejection fraction (66% vs. 58%, \( P = 0.02 \)) and the mean right ventricle peak filling rate (520 ml/s vs. 320 ml/s, \( P = 0.008 \)) as well as the mean left ventricle peak filling rate (520 ml/s vs. 410 ml/s, n.s) at rest were higher in patients with SDS than in controls.
7. DISCUSSION

The recent discovery of disease-causing mutations in the \textit{SBDS} gene has opened new insight into the pathogenetic and molecular mechanisms behind SDS. The clinical presentation of this pleiotropic disorder has appeared more diverse and complex than previously believed. Careful evaluation of a genetically verified group of patients may reveal previously unrecognized features and expand our understanding of the full phenotypic spectrum of SDS. Accordingly, this study was able to characterize novel phenotypic features of SDS such as low-turn-over osteoporosis and structural brain alterations, both of which are likely to have clinical implications for patients with this disease.

7.1. Study I: Pancreatic phenotype in Shwachman-Diamond syndrome

In the present study, abdominal MRI proved to be an accurate method in differentiating patients with true SDS and \textit{SBDS} mutations from those with an SDS-like phenotype but no identifiable mutations in the \textit{SBDS} gene. At the time of the diagnosis, all five of our mutation-negative patients had a typical presentation of SDS with exocrine pancreatic insufficiency and neutropenia, yet no mutations in the \textit{SBDS} gene were detected at the time of this study. Clinically, the normalization of pancreatic function over time was similar in patients with and in those without mutations in \textit{SBDS} but the MRI findings differed significantly between these two groups. In line with previous observations on SDS patients without genotypic verification (Ip et al. 2002), the pancreatic phenotype in patients with \textit{SBDS} mutations was characterized biochemically by constantly low serum pancreatic isoamylase levels. MRI invariably showed a fat-replaced pancreatic gland with occasional scattered foci of parenchyma and enhancement of the pancreatic duct, regardless of variable clinical exocrine pancreatic sufficiency state.

Patients with SDS phenotype but with no mutations in the \textit{SBDS} gene had normal pancreatic structure and parenchyma without any fat accumulation in the gland. In one \textit{SBDS} mutation-negative patient, MRI was able to reveal a rare developmental pancreatic anomaly of heterotaxy syndrome group. All other \textit{SBDS}-negative patients had normal levels of serum pancreatic isoamylase.

Sonography, considered the primary imaging modality for children, can provide evidence of lipomatosis but its visibility may be compromised, especially in infants (Nijs, Callahan & Taylor 2005). Furthermore, due to
operator-dependency, hyperechogenic pancreas may be overlooked, especially if the sonographer is unaware of the clinical suspicion of SDS (Adachi et al. 2005). Accordingly, in the present study, a retrospective image analysis of sonographic examinations performed prior to or at the time of diagnosis showed in a large proportion of patients hyperechogenic pancreas that had primarily been reported normal. CT can reliably demonstrate pancreatic lipomatosis (Bom et al. 1993, Robberecht et al. 1985) but the associated heavy radiation load makes it less advisable in children - even more so in this specific group of patients known to have high propensity for malignant bone marrow transformation. MRI provides a useful tool for pancreatic imaging (Pamuklar & Semelka 2005). With standard abdominal MRI sequences, it was possible to reliably differentiate the SDS patients with SBDS mutations from the mutation-negative patients. Furthermore, MRI may reveal differential diagnostic alternatives, as was demonstrated by the detection of a rare pancreatic anomaly in one patient. Therefore, it is advisable to include abdominal MRI in the clinical diagnostic work-up of children with unexplained exocrine pancreatic dysfunction.

Endocrine pancreatic function was affected in only one patient; a male with a combination of one common mutation and one complex mutation in SBDS was diagnosed with type 1 diabetes at age 15. SDS has occasionally been associated with type I and type II diabetes, even neonatal-onset diabetes has been reported (Shmerling et al. 1969, Aggett et al. 1980, Mack et al. 1996, Ginzberg et al. 1999, Filippi et al. 2002, Mäkitie et al. 2004, Kawakami et al. 2005, Kamoda et al. 2005, Kawashima et al. 2006, Rosendahl et al. 2006). The association of SDS and diabetes is presently obscure, but it is possible that in the future deeper understanding of the SBDS protein function in pancreatic cells may shed light on new potential pathogenetic mechanisms in diabetes.

The mechanisms and the time of acinar cell damage – or lack of cell development – in SDS that results in pancreatic lipomatosis as well as the apparent improvement of exocrine pancreatic function later in life remain unclear. Imaging and histopathology have shown fatty pancreas even in infancy. Hitherto, there is no evidence of in utero presentation of pancreatic lipomatosis in SDS. On the other hand, evidence of normal pancreatic tissue conversion into fat is currently also lacking. The pancreas is composed of endocrine and exocrine (ductal and acinar) compartments, but during embryonic development all pancreatic cells differentiate from ductal epithelium. Adult ductal epithelium retains its ability to give rise to all cell types of the pancreas, and it has been suggested that the main progenitor source for pancreatic growth and regeneration are the mature duct cells that have regressed or dedifferentiated (Bonner-
Weir et al. 2004). The exocrine pancreas has a large functional capacity; only 2-10% of residual function suffices to render patients clinically pancreatic sufficient (DiMagno, Go & Summerskill 1973, Gaskin et al. 1984). Whether the small foci of enhancing parenchyma in SDS patients represent preserved acini or foci of regeneration remains to be addressed in future studies. The observed duct enhancement in some of our patients demonstrates the presence of duct cells. Preservation of the duct and islet cells, and the absence of irreversible pancreatic fibrous degeneration may explain the recovery of the exocrine pancreatic function over time.

7.2. Study II: Expansion of the skeletal phenotype in Shwachman-Diamond syndrome

The findings of the present study expand the spectrum of SDS-related bone disease, in addition to earlier recognized metaphyseal chondrodysplasia, to include early-onset low-turnover osteoporosis. We were able to show that the skeletal phenotype of SDS is more complex than previously believed and that not only chondrocytes in growth plates but also osteoblasts and osteoclasts are involved.

The present study showed low bone density in all patients with SBDS mutation-verified SDS. The associated history of low-impact fractures with vertebral compression deformities in some patients suggests true impairment of bone quality. Bone biopsy findings with reduced trabecular bone volume, low numbers of osteoclasts and osteoblasts, and reduced amount of osteoid further indicate low turn-over osteoporosis. Bone biopsy results differ from bone histomorphometry findings in cystic fibrosis, which have shown decreased osteoblastic but increased osteoclastic activity (Haworth et al. 2000). All SBDS mutation-positive patients had consistently decreased bone density, indicating that osteopenia/osteoporosis is a universal feature of this disorder. According to bone biopsy findings, osteoporosis results from a primary defect in bone metabolism and not from any nutritional deficiencies. However, suboptimal levels of vitamin D and K, noted in a large proportion of patients in the present study, may further impair bone quality in these patients.

Low-turnover osteoporosis with reduced numbers of both osteoclasts and osteoblasts may be directly related to the SBDS defect in stem cell proliferation or differentiation. Macrophages, osteoclasts, and neutrophils have a common precursor stem cell, the granulocyte/macrophage colony-forming cell. Thus, the reduced number of osteoclasts and neutropenia in SDS may be due to marrow hypofunction at the stem cell level. The level of expression and the role of SBDS in bone and in various
cell types required for normal bone formation and turnover are currently unknown. The role of the SBDS protein in the function of chondrocytes, osteoblasts, osteoclasts and their precursors thus needs to be elucidated in further studies. While no therapeutic means to target the primary defect are presently available, efforts should be made to optimize general preventive measures such as nutrition and intake of fat-soluble vitamins, as well as to promote weight-bearing exercise. Although vitamin D deficiency is not the underlying cause for impaired bone health in SDS, low vitamin D values in a significant number of patients indicate that vitamin substitution was not optimal; higher doses of substitution should be used in these patients in order to prevent further impairment of bone quality.

7.3. Study III: Structural brain alterations in Shwachman-Diamond syndrome

Despite the evidence for mild neuropsychological dysfunction associated with SDS, previous systematic neuroimaging studies have not been performed. This study was able to demonstrate that patients with mutation-verified SDS had formerly unrecognized structural brain changes in the cerebellum and in the corpus callosum.

The majority of the patients in the present study had a small head circumference, although only two were microcephalic. Microcephaly was less frequent in the present study than in the only previous systematic study on SDS with OFC-assessment where half of the patients had head circumference below third percentile (Aggett et al. 1980). The head size was independent of SBDS genotype and ranged from a severely small head (-4.3 SD) to normal OFC (+1.3 SD). In line with an overall small head circumference, patients had smaller global brain volume: both white and gray matters were equally decreased. At birth, human brain size is one fourth of its adult volume and reaches three fourths of its final volume by the end of the first year. Small head size has been associated with several syndromes presenting with psychomotor retardation. Moreover, among children evaluated for developmental disabilities, many with normal or borderline IQ, microcephaly is a common finding (Watemberg et al. 2002). In the present study, no consistent pattern of head growth reduction was observed: some of the patients had small OFC already at birth, while in some patients with normal OFC at birth small head size was, however, evident at a later age.
In the present study, all except one patient reported learning difficulties and they had distinctively lower academic achievement than their siblings. Only two, however, had undergone formal neuropsychological evaluation. It appears that cognitive impairment is present in the majority of the patients but it is usually mild and often unrecognized. Therefore, only a few patients are referred to neuropsychological evaluation. It is possible that the two patients who had undergone neuropsychological testing represented the more severe end in the neuropsychological phenotype of this variable disorder. Possibly, in order to recognize mild cognitive disabilities, all children with SDS would benefit from formal neuropsychological evaluation. Subsequently, some of them may require special education services at school.

In voxel-based morphometry analysis, the method that has uncovered subtle changes in various brain regions in different patient groups (Ashburner et al. 2003), no significant focal alterations in gray or white matter were noted in patients with SDS. Failure to identify regional abnormalities may be due to the small cohort size. Midsagittal areal measurements, however, revealed significantly smaller callosal and posterior fossa structures in SDS patients, even when adjusted for head size. Several studies have demonstrated the role of the cerebellum in higher cognitive functions, including visuospatial task performance, verb generation, and verbal working memory (Paradiso et al. 1997, Schmahmann & Sherman 1998, Cabeza & Nyberg 2000). Posterior fossa malformations and volumetric or structural abnormalities of the cerebellum or vermis have been associated with developmental and cognitive problems in several syndromes (Tavano et al. 2007). Small corpus callosum has been described in children with learning impairment after perinatal adverse events (Njioiktijen, de Sonneville & Vaal 1994), and in fetal alcohol syndrome (Autti-Rämö et al. 2002). The vermis develops after fusion of cerebellar hemispheres by the end of the 15th gestational week; rapid cerebellar growth occurs at late gestation (Limperopoulos et al. 2005). The development of corpus callosum occurs between the 3rd and 5th months of pregnancy, but the thickening of corpus callosum is completed only after birth. It is unclear whether the observed alterations in brain structures in SDS result from changes in prenatal development or only after birth. Therefore, fat malabsorption and serious infections at an early age may also play a role in brain growth and cognitive function in SDS.
7.4. Study IV: Myocardial function in Shwachman-Diamond syndrome

The previous fatal myocardial complications in Finnish infants with SDS (Savilahti & Rapola 1984) and excessive stem cell transplantation-related cardiac toxicity, more recently reported, prompted us to study whether patients with SDS show signs of myocardial damage or other cardiac manifestations. All assessed patients with mutation-verified SDS had normal myocardial structure and left ventricle mass. None of them had evidence of myocardial fibrosis, when assessed by a sensitive method of late-enhancement cardiac MRI (Hunold et al. 2005). This finding, together with the lack of reports on myocardial necrosis in SDS during the past two decades, suggests that the necrotic and fibrous cardiac lesions are not common manifestations in SDS. It is possible that the combination of inadequate nutrition and serious infections may have contributed to the fatal neonatal outcome with myocardial necrosis and fibrosis in the early reports.

However, we observed some abnormalities in the dynamic properties of myocardial function in patients with SDS and mutations in SBDS. Tissue Doppler echocardiography, which measures the left ventricle systolic wall movement velocity during ejection phase and myocardial acceleration during isovolumic contraction was used to assess the reserve contractile capacity of the myocardium. Isovolumic acceleration has been shown to reflect contractility (Vogel et al. 2003, Cheung et al. 2005). Unlike conventional indices of systolic function, tissue Doppler may offer data that are more sensitive to early alterations in left ventricle function. In the present study, a significantly reduced maximum isovolumic acceleration value, but not systolic wall movement, was noted in the patients. On the other hand, three-dimensional analysis of the MRI data showed that both left and right ventricle peak filling rates were increased in patients with SDS, although a significant change was noted only in the right ventricle peak filling rate. The aforementioned observations on isovolumic acceleration suggest that the left ventricular contractile reserve may be depressed, whereas increased peak filling rate as a sign of diastolic dysfunction is commonly observed in mildly restrictive cardiomyopathy. In cardiomyopathies in general, diastolic dysfunction usually precedes systolic disturbances, and right ventricle dysfunction becomes evident prior to left ventricular changes (White & Patel 2007). Similarly, diastolic alterations in the right ventricle were noted in the present study. Considering all these data, young SDS patients without clinical cardiac manifestations have subtle changes in myocardial properties. These findings that possibly represent a hitherto occult cardiac involvement may deserve careful attention in future clinical studies.
Observed alterations, in combination with the earlier reports of myocardial involvement and the reported cardiac toxicity during stem cell transplantation, raise the question whether patients with SDS have myocardial properties that predispose them to cardiac complications in extreme conditions. The use of cardiotoxic conditioning regimens such as cyclophosphamide and total body irradiation may further increase the risk of cardiac disturbances in patients with SDS.

7.5. General discussion

Although SDS classically presents during the first years of life with steatorrhea, neutropenia, and skeletal changes, it is important to emphasize that patients with SDS may be recognized well beyond childhood with symptoms less characteristic than in those diagnosed at an early age. As the typical gastroenterological symptoms may be mild and even disappear after infancy in a significant number of patients, the diagnostic work-up becomes more challenging. Due to the intermittent nature of neutropenia, hematological presentation may be clinically confusing. Phenotypic variability even within a family may further avert the clinician from the correct diagnosis.

During the present study, three new patients were diagnosed with SDS. A child presented at the age of 2 with classical symptoms of pancreatic insufficiency, neutropenia, recurrent infections and skeletal dysplasia. Another patient, a clinically asymptomatic 6-year-old boy, was recognized to have SDS when his 17-year-old brother who was diagnosed with SDS in infancy participated in the present study; both brothers had the same genotype. The third patient, a 14-year-old boy, was referred to further investigations by an orthopedic surgeon because of knee deformities and metaphyseal changes. In addition to these three patients, another patient, a 13-year-old girl who was referred to investigations because of neutropenia and low fecal elastase values had an extremely rare pancreatic developmental anomaly that clinically mimicked SDS. Since two of the patients diagnosed during the study presented without characteristic symptoms of SDS there is a strong possibility that there are additional unrecognized SDS patients in Finland. Moreover, the patients illustrate the large phenotypic and genotypic spectrum of SDS. The identification of these patients enables correct support and treatment for the variable symptoms and organ involvement in SDS. Furthermore, a correct diagnosis can effectively alleviate parental anxiety in families with a chronically ill child with variable symptoms but without accurate diagnosis.
No genotype-phenotype correlations have been observed in skeletal dysplasia (Mäkitie et al. 2004) or in hematological features (Kawakami et al. 2005, Kuijpers et al. 2005) in SDS. The finding of two patients with mutations in \textit{SBDS} gene and with severe bone disease expands the skeletal phenotypic spectrum from usually mild to moderate bone changes to its severest end with phenotypic resemblance of neonatal spondylometaphyseal dysplasia (Nishimura et al. 2007). Our results were similar: no genotype-phenotype correlation was noted regarding osteoporosis and neuroradiological aspects. Phenotypic features have been shown to change over time. Despite the invariable imaging finding of lipomatosis, pancreatic dysfunction becomes clinically insignificant in a large proportion of patients. However, osteoporosis is likely to result in significant clinical sequelae over time. Since long-term follow-up on cardiac features is lacking, it is unclear whether patients with SDS develop clinically relevant cardiac manifestations at a later age.

As some of the clinical features of SDS, such as hematological dysfunction, require regular follow-up, accurate recognition and correct diagnosis of this disorder is of utmost importance. \textit{SBDS} mutation-negative SDS appears to have better prognosis at least regarding hematological and pancreatic presentations. Future studies may be able to recognize new genetic defects behind the described SDS phenotype without mutations in \textit{SBDS}.

The versatile exploitation of imaging modalities, and the use of advanced specialized imaging methods for different organ systems in genetically defined patient groups provide useful tools for the characterization of novel phenotypic features. This approach could thus potentially increase the ethiopathogenetic understanding in various disease entities.

\textit{Limitations of the study}

The major limitations of our study are the small number of the patients and their wide age range. Due to the limited number of patients and of controls, the possibility of statistical errors cannot be excluded. It is possible that, due to the variable ages of the subjects and the small size of our cohort, some brain and myocardial alterations failed to reach significance. The present study protocol did not include neuropsychological testing because the main focus of the study was on the imaging findings. In retrospect, neuropsychological data would have been of interest and of importance. The cardiac imaging studies of the patients and of the controls were performed at different times and cardiac MRI with varying equipment, nor were we able to match the groups for age, gender, or height.
7.6. Prospects of future research

Our observations of primary osteoporosis and morphological brain changes in SDS should be re-examined in larger patient cohorts in order to validate the consistency of the findings in the general SDS population and to better enable the assessment of potential phenotype-genotype correlations. Characterization of the neuropsychological phenotype in SDS is of utmost importance for the patients in order to provide them with appropriate support and therapy for cognitive challenges. Mild functional myocardial alterations warrant assessment in larger patient groups with longitudinal follow-up in order to elucidate whether these alterations evolve over time and whether a distinct cardiac SDS phenotype exists.

At the moment, in about 5-15% of subjects with clinical findings of SDS, mutations cannot be found, even after extensive laboratory testing. It is possible that further studies may reveal additional genes that result in clinical SDS. Genetic testing, presently available, will allow for characterization of the full clinical spectrum of SDS. Future studies may shed light on the role of defective SBDS protein and whether heterozygous mutations in SBDS are involved in the pathogenesis of multigenic disorders in the general population.

Animal models of SDS can be used to investigate processes in the affected organs such as the pancreas, the liver, bone marrow and the skeleton. Furthermore, the understanding of the essential role of SBDS in ribosomal biogenesis may provide information on the pathophysiology of pancreatic and skeletal diseases, hematopoietic dysfunction and malignant bone marrow transformation. Finally, the elucidation of the underlying impairment in biological pathways could allow for the development of molecular therapeutic interventions in the future.
8. CONCLUSIONS

Wide phenotypic variation, unrelated to genotype, was observed in the study subjects. Clinical presentation varied with age. Since the presenting symptoms are often atypical, a significant number of patients with SDS may escape correct diagnosis.

The study demonstrated that abdominal MRI can reliably differentiate between SBDS mutation-positive and mutation-negative patients among the Finnish patients with a clinical diagnosis of SDS. In addition, MRI may provide valuable information on differential diagnostic alternatives. Therefore, abdominal MRI should be included in the evaluation of children with exocrine pancreatic insufficiency.

The present study was able to expand the SDS phenotype to include low-turnover osteoporosis and structural brain alterations.

The study showed that SDS in patients without cardiac symptoms is not associated with major cardiac involvement or myocardial fibrosis but mild alterations in the myocardial function of unknown clinical importance were detected.
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Sanna Toiviainen-Salo
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