Original Article

Skeletal phenotype in patients with Shwachman–Diamond syndrome and mutations in *SBDS*

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Pancreatic exocrine and bone marrow dysfunctions are considered to be universal features of Shwachman–Diamond syndrome (SDS) whereas the associated skeletal dysplasia is variable and not consistently observed. The genetic defect in SDS has recently been identified; causative mutations have been shown in the *SBDS* gene. The aims of this study were to characterize the nature, frequency, and age-related changes of radiographic skeletal abnormalities in patients with *SBDS* mutations and to assess genotype–phenotype correlation. Fifteen patients (mean age 9.7 years) with a clinical diagnosis of SDS and documented *SBDS* gene mutations were included. Review of their skeletal radiographs showed abnormalities in all patients. The skeletal changes were variable, even in patients with identical genotypes. The typical features were (1) delayed appearance of secondary ossification centers, (2) variable widening and irregularity of the metaphyses in early childhood, followed by progressive thickening and irregularity of the growth plates, and (3) generalized osteopenia. There was a tendency towards normalization of the epiphyseal maturation defect and progression of the metaphyseal changes with age. The results suggest that the characteristic skeletal changes are present in all patients with SDS and *SBDS* mutations, but their severity and localization varies with age. No phenotype–genotype correlation was observed.

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Shwachman–Diamond syndrome (SDS, MIM 260400) is an autosomal recessive disorder characterized by exocrine pancreatic dysfunction, bone marrow failure, skeletal abnormalities, short stature, and a variety of other less common features (1–4). The pancreatic dysfunction presents as impaired enzyme output and low serum amylase and trypsinogen levels; imaging studies show a small, fatty pancreas (5, 6). Bone marrow dysfunction is characterized in the peripheral blood count by persistent or intermittent neutropenia, anemia, or thrombocytopenia, and in bone marrow studies by hypocellular marrow, aplastic anemia, myelodysplasia, and occasionally leukemia (7, 8).

Linkage and haplotype analyses of families with SDS identified a single-gene locus at chromosome 7q11 (9). Mutations were identified in an uncharacterized gene, *SBDS,* which has a 1.6 kb transcript and encodes a predicted protein of 250 amino acids (10). While the function of the gene remains unknown, it was proposed that SDS results from deficient RNA-processing in a pathway which is essential for development of the exocrine pancreas as well as for hematopoiesis and skeletal development (10).

The skeletal features are reported to be variable and may not be present in all patients with SDS (3–5). Abnormal development of growth plates and metaphyses, delayed bone age as well as progressive deformities and pathological fractures have been reported (3–5). However, previous studies have been based solely on cross-sectional data and incomplete skeletal assessments; the

possible age-related changes in the skeletal manifestations have not been evaluated. To further clarify the skeletal phenotypes, we analyzed the radiographs of 15 patients with SDS and identified *SBDS* mutations for the nature, frequency, and evolution with age of the skeletal abnormalities. The results showed that skeletal changes are present in all patients, but their severity and localization vary with age. No phenotype–genotype correlation was observed, and the severity of the skeletal findings varied even in patients with identical *SBDS* mutations.

Patients and Methods

Patients

Causative mutations in *SBDS* and skeletal radiographs were available in 15 of a cohort of 30 patients with a clinical diagnosis of SDS who had been seen at the Division of Gastroenterology and Nutrition, The Hospital for Sick Children, Toronto, Canada (5, 10). The clinical diagnosis of SDS was based on the identification of pancreatic and hematologic dysfunction, as described previously (4, 5), and exclusion of other pancreatic or hematologic conditions with a phenotypic resemblance to SDS. Metaphyseal changes in skeletal radiographs were used only as a positive criterion for diagnosis. Fifteen of the 30 patients were excluded from the study because their skeletal radiographs were unavailable for review (12 patients), because no DNA was obtained for mutational analysis (two patients), or no mutations were identified in *SBDS* (one patient). The study was approved by the Research Ethics Board of the Hospital for Sick Children, Toronto, Canada.

Clinical data

Clinical data were collected from the hospital records and correlated with the skeletal findings. Pancreatic function was classified on the basis of serum trypsinogen concentrations (4). Patients with values $<6\,\mu g/l$ were classified as having pancreatic insufficiency (PI). Patients with intermediate $(6–16.7\,\mu g/l)$ or normal $(>16.7\,\mu g/l)$ values were classified as having pancreatic sufficiency (PS). Heights and weights at the time of the last radiographic review were compared with age- and sex-specific norms and expressed as z scores (11). Hematological dysfunction was assessed using peripheral blood counts and bone marrow findings. Anemia was defined as a hemoglobin $<10\,g/dl$, neutropenia as a white cell count $<1500 \times 10^9$ cells/l, and thrombocytopenia as a

platelet count of $<150 \times 10^9$ cells/l. Abnormal bone marrow findings were classified as bone marrow hypoplasia or hyperplasia, bone marrow dysplasia, myelodysplastic syndrome (MDS) $\pm$ clonal cytogenetics, and leukemia (8).

Mutational analysis

SBDS coding regions were screened for mutations by restriction enzyme digestion of amplified exon 2 or by direct sequencing of polymerase chain reaction (PCR)-amplified products of genomic DNA, as previously described (10).

Radiographic analyses

A total of 247 skeletal radiographs (median 11 per patient) were available for review. Longitudinal data, with two or more skeletal examinations over a minimum of 5 years, were available for 10 patients; in five of these patients longitudinal radiologic studies extended to skeletal maturity. Cross-sectional data was available for the remaining five patients. All radiographs were first evaluated cross-sectionally to characterize typical features in different age groups. Longitudinal radiographs were then used to see whether similar sequence of changes with age occurred in individual patients. The radiographic findings were correlated with the other phenotypic features of SDS and with the patterns of *SBDS* mutations.

Results

Clinical phenotypes

The 15 patients (8 male) included in the study were diagnosed with SDS at a median age of 1.2 years (range 0.25–3.8 years). Twelve patients were of European ancestry and two originated from the Indian subcontinent. The remaining patient was a native Canadian. Their median age at the last radiographic examination was 9.3 years (0.9–19.9 years). The clinical findings at the time of the last radiographic examination are summarized in Table 1. The cohort included one pair of sibs (Patients 15 and 16); three other patients had an affected sib who had been excluded from the study because of unavailability of radiographs. All patients were PI at the time of diagnosis and received pancreatic enzyme supplements. Subsequently, four patients became pancreatic sufficient on the basis of serum trypsinogen levels and discontinued enzyme replacement therapy at the age of 2 years (Patient 5), 4 years (Patient 9), 5 years (Patient 12), and 9 years (Patient 1). All patients had hematological abnormalities both

Table 1. Clinical and genetic characteristics of the 15 patients with Shwachman–Diamond syndrome

Patient					Hematology		
Number	Sex	Age	Height (SDS)	Pancreatic function	PB	BM	*SBDS* mutations
2	Female	16.2	−1.5	PI	N	Hypocellular	D/C
4	Female	12.7	−1.4	PI	N, T	MDS	D/C
9	Male	19.9	−4.8	PS	N, T	Hypocellular	D/C
11	Male	2.3	−2.1	PI	N	Hypocellular	D/C
12	Female	17.2	−2.6	PS	N	Hypocellular	D/C
13	Male	16.3	−1.0	PI	N	Hypocellular	D/C
14	Female	1.3	−4.5	PI	N	Hypercellular	D/C
1	Male	14.7	−1.0	PS	N	Hypocellular	DC/C
10	Female	1.3	−2.1	PI	N	Blasts	DC/C
5	Male	4.9	−0.0	PS	N	Hypocellular	C/119delG
6	Male	9.3	−3.2	PI	N	Clonal abnormality	C/119delG
15	Female	5.5	−3.2	PI	N	Hypocellular	C/131A>G
16	Female	0.9	−2.9	PI	N	Hypocellular	C/131A>G
3	Male	16.8	−1.5	PI	N	Hypocellular	C/260T>C
18	Male	6.9	−1.0	PI	N	Clonal abnormality	C/X

Age, age at the last radiographic assessment; PB, peripheral blood; BM, bone marrow; PI, pancreatic insufficiency; PS, pancreatic sufficiency (based on serum trypsinogen level); N, neutropenia; T, thrombocytopenia; MDS, myelodysplastic syndrome. *SBDS* mutations: D, 183–184TA>CT; C, 258+2T>C; DC, 183–184TA>CT+258+2T>C; X, unknown.

in the peripheral blood and in the bone marrow. In three patients, bone marrow abnormalities progressed to MDS or clonal changes; one patient had blasts (Table 1). None of the patients required specific treatment for these changes. The median z score for height was −2.1 SD (−4.79–0.0 SD); eight patients (53%) had height z score greater than two SD below normal mean. Five patients had clinically narrow rib cage; one of them (Patient 9) had severe thoracic dystrophy. This patient also had bilateral genu varum and leg length discrepancy which were surgically corrected at the age of 16 years; no other orthopedic surgeries had been performed.

SBDS mutations

The observed DNA sequence changes in the *SBDS* gene are shown in Table 1. All patients had one copy of the major mutation 258+2T>C and seven patients of the other common mutation 183–184TA>CT. Seven patients were heterozygous for both common mutations. None of the patients were homozygous for either of these changes. Other uncommon mutations included 119delG (two patients), 131A>G (two patients), 183–184TA>CT+258+2T>C (two patients) and 260T>C (one patient) (Table 1). One patient who was heterozygous for 258+2T>C carries a yet unidentified sequence change in the other allele.

Radiographic findings

Skeletal changes were variable but present in all patients. The typical features were (1) delayed appearance followed by normal development of secondary ossification centers, (2) variable widening and irregularity of the metaphyses which were commonly observed in early childhood in the ribs as well as proximal and distal femora, (3) progressive thickening and irregularity of the growth plates associated frequently with asymmetrical growth, and (4) thin cortices with generalized osteopenia of the long bones (Table 2). Longitudinal studies showed an age-related tendency towards normalization of epiphyseal maturation whereas the abnormalities in the metaphyses and growth plates worsened with age (Table 2). The cross-sectional and longitudinal findings showed that the right and left limbs were affected similarly. The results also showed that the legs were usually more severely affected than the arms, and that in any given patient, the hips and knees were affected to a similar extent.

Chest. Chest radiographs were available for 13 of the 15 patients. Marked expansion of the anterior metaphyses of the ribs at the costochondral junctions were observed in all 10 patients who had chest radiographs performed before 2 years of age (Fig. 1). Similar expansion was observed, but was less obvious, in most of the older patients. The expanded rib metaphyses were observed as early as 4 days of age in one patient. In six patients, the ribs appeared abnormally short (Fig. 1), consistent with the clinical observation of narrowing of the thorax in five of them.

Wrist and hand. Wrist and hand radiographs were available for 12 of the 15 patients; longitudinal data were available for four patients. The secondary ossification centers were shaped normally but were delayed in their ages of

Table 2. The characteristic skeletal findings in relation to age in Shwachman–Diamond syndrome

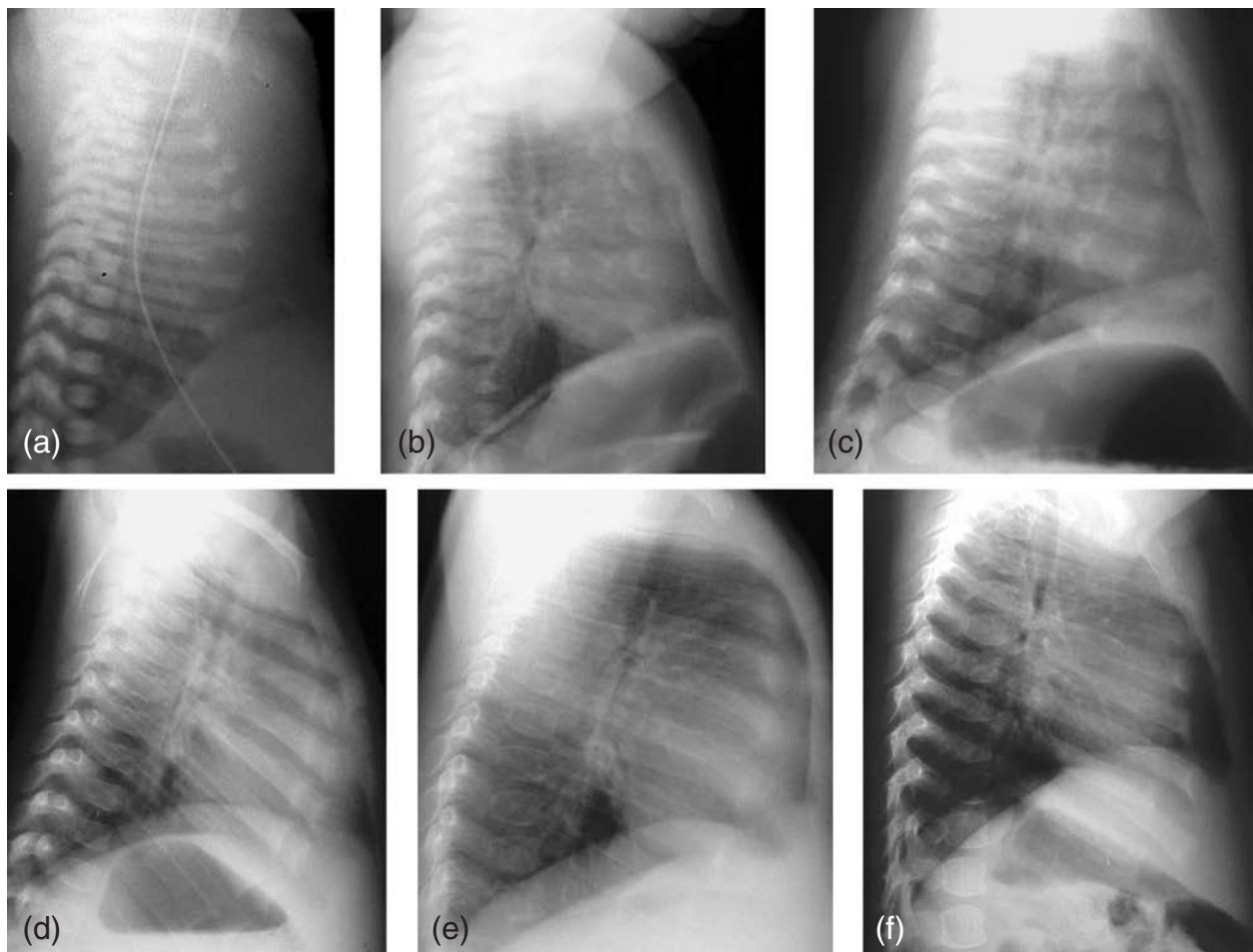| Age	Localization	Features
Infancy	Secondary ossification centers	Delayed appearance (wrist, proximal femur)
	Metaphyses	Widening, irregularity (costochondral junctions, distal radius and ulna)
	Other	Osteopenia (tubular bones, skull)
		Wormian bones (skull)
		Oval and globular vertebral bodies (spine)
Early childhood	Secondary ossification centers	Delayed appearance (wrist)
		Slow development (wrist, phalanges, proximal and distal femur)
	Metaphyses	Widening, irregularity (proximal and distal femur)
	Other	Osteopenia (tubular bones, skull)
		Coxa valga (femoral neck)
		Oval vertebral bodies (spine)
Mid-childhood	Secondary ossification centers	Slow development (carpal bones)
	Metaphyses	Irregularity, sclerosis (proximal and distal femur)
		Thickening of growth plate (proximal and distal femur)
	Other	Osteopenia (tubular bones, spine)
		Coxa valga (femoral neck)
Late childhood	Secondary ossification centers	Normal
	Metaphyses	Irregularity, sclerosis (proximal and distal femur)
		Thickening of growth plate (proximal and distal femur)
		Asymmetrical growth (proximal and distal femur)
		Stress fractures (proximal femur)
	Other	Osteopenia and end plate deformity (spine)

Fig. 1. Metaphyseal changes in the costochondral junctions in lateral chest radiographs in six infants with Shwachman–Diamond syndrome (SDS). (a) Patient 9 (age 4 days), (b) Patient 15 (age 2 months), (c) Patient 2 (age 3 months), (d) Patient 13 (age 4 months), (e) Patient 18 (age 9 months), and (f) Patient 16 (age 11 months). The ribs are short and the metaphyseal ends are flared and irregular. Note also the oval shape and globular appearance of vertebral bodies.

appearance (Fig. 2). Skeletal maturation of the carpal bones and metacarpals was delayed in all patients especially in early childhood, but tended to normalize by mid-childhood (Fig. 2). Determination of bone ages on the basis of maturation of the phalangeal ossification centers showed that retardation of bone age in relation to chronological age was not significant after the first year of life. However, the dissimilar degree of maturation defect of carpal bones, metacarpals, and interphalangeal epiphyses impaired the assessment of bone ages by the standard methods. The growth plates of the phalanges, metacarpals, and the distal radius and ulna were of normal thickness except in 3 cases. In the latter cases, the growth plate of the distal ulna was thickened and irregular.

Expansion, cupping, and irregularity of the metaphyses of the metacarpals as well as of the distal ulna and radius were observed in all seven patients in whom radiologic images were available before 2 years of age (Fig. 2). The metaphyses tended to normalize in appearance as the secondary ossification centers developed. One patient had post-axial polydactyly of the thumb (reported in [12]) but no other malformations were observed.

Pelvis and hips. Radiographs of the pelvis and hips were available in 12 of the 15 patients. Two or more serial radiographs were available in seven of the 12 children. The pelvis of each child was normal in shape at all ages although the cortices were thin. During the first 2 years of life, the proximal femur showed abnormal features in all 10 patients who had hip radiographs available before 2 years of age. The femoral necks were more vertical than normal producing coxa valga deformities. The junction of the proximal metaphysis with the cartilaginous head of the femur was horizontal and often irregular (Fig. 3). The age of appearance of the proximal femoral ossification center was delayed but the femoral head developed normally from a single ossification center. In mid-childhood, radiographs showed that the secondary ossification centers of the femoral heads were smaller than normal as well as slightly abnormal in shape.

When the secondary ossification centers of the femoral heads had reached the width of the metaphyses of the femoral heads three patterns of development of the proximal femur were observed. In the first pattern (one patient), the development of the proximal femur appeared to be normal. In the second pattern (two patients), there was evidence of asymmetrical growth of the proximal femoral growth plate so that the superior margin of the femoral neck was shorter than the inferior margin. The asymmetrical growth resulted in progressive valgus tilting of the proximal femoral growth plate and femoral head on the femoral neck. At skeletal maturity, the valgus femoral heads were poorly covered by the acetabula. In the third pattern, which was most commonly observed (five patients), the femoral necks were short and the metaphyses contained lucent areas that were continuous with the growth plate (Fig. 4). There was also asymmetrical growth of the proximal femoral growth plate resulting in a valgus deformity of the femoral head on the femoral neck. Patients with extensive areas of lucency in the metaphysis also showed stress fractures resulting in varus deformities of the femoral neck (Figs 3 and 4).
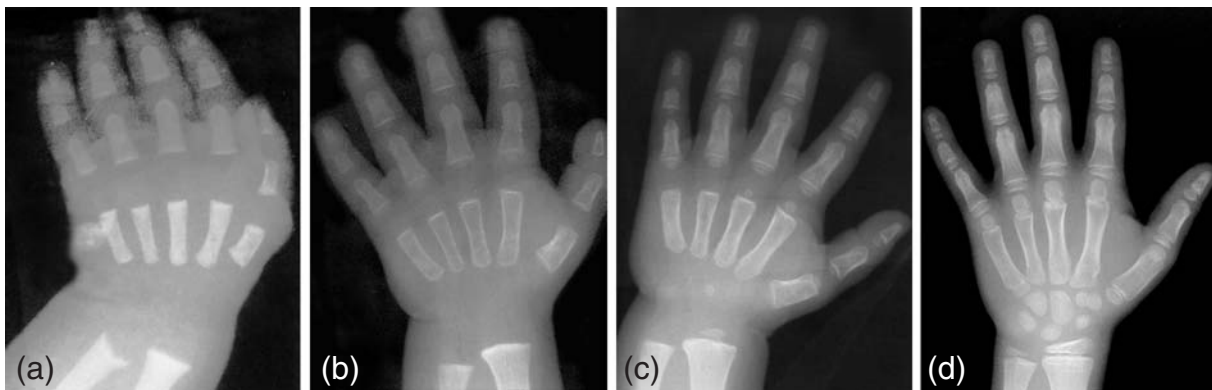
Fig. 2. Longitudinal changes in skeletal abnormalities in the wrist and hand in Patient 9. (a) At the age of 3 days metaphyseal cupping and irregularity is seen in the distal ulna and radius as well as in the metacarpals. (b) The appearance of all secondary ossification centers is delayed and the tubular bones are osteopenic. The metaphyseal changes are less remarkable (age 1 year 4 months). (c) The appearance and development of carpal bones and metacarpal epiphyses are delayed whereas the interphalangeal epiphyses are normal (age 2 years 7 months). (d) Normal secondary ossification centers and metaphyses (age 9 years 6 months).
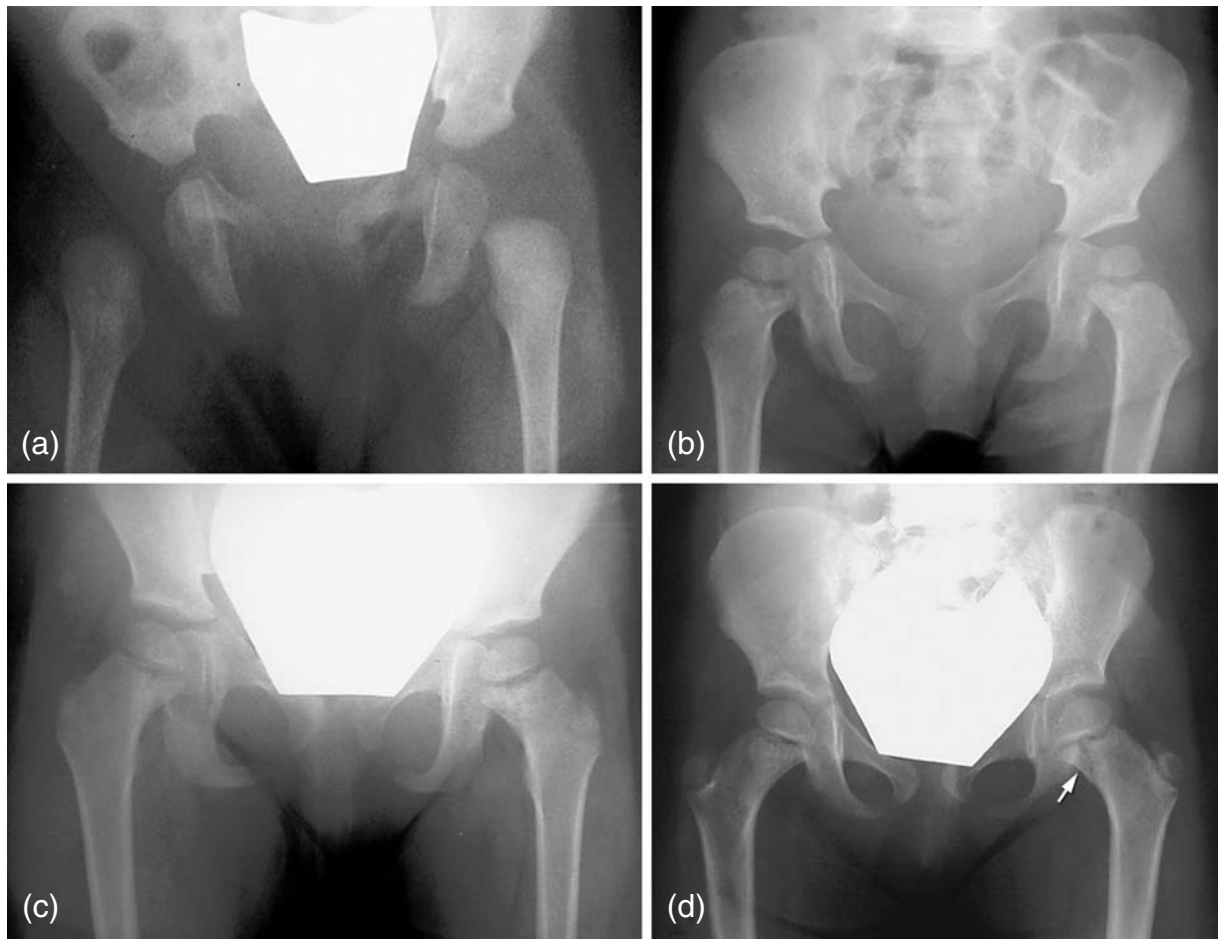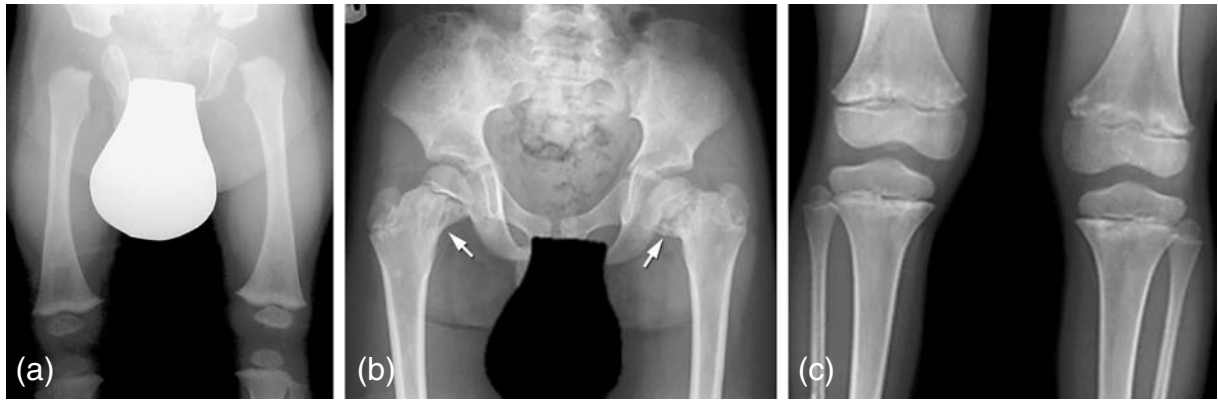
Fig. 3. Skeletal changes in the hip in Patient 15 (anterior–posterior view of pelvis). (a) The metaphyseal ends of proximal femurs are slightly widened, and the femoral necks are vertical (age 4 months). (b) The proximal femoral epiphyses are small. The metaphyseal irregularity is more pronounced. The femoral necks are widened and in vertical position, and the growth plate is horizontal (age 2 years 3 months). (c) Wide proximal femoral metaphyses with medial beaking (age 4 years 3 months). (d) The proximal femoral epiphyses are normal, and the femoral necks in normal position. The femoral metaphyses are wide, irregular and thickened. Left femoral metaphysis shows a lucent area (arrow), probably representing a stress fracture (age 5 years 6 months).

Knees. Knee radiographs were available for 12 of the 15 patients, six of whom had two or more radiographs for longitudinal evaluation. During the first 2 years of life, the secondary ossification centers of the distal femur and proximal tibia were small and developed slowly (Figs 4 and 5). The metaphyses of the distal femur and proximal tibia were often expanded with beaking of the medial and lateral corners. During late-childhood and adolescence, the secondary centers of ossification became larger and more normal in shape. At this age, thickening of the growth plates was often associated with lucent areas and irregular areas of metaphyseal ossification (Figs 4 and 5). At skeletal maturity, the metaphyses contained coarse trabecular bone with vertical striations. Some of the latter cases had asymmetrical growth producing varus deformities of the knee

and premature closure of the growth plate and a short leg (Fig. 6). Similar changes were seen in the proximal tibia as well, but the abnormalities were less pronounced.

Spine. Radiographs of the thoracic spine were analyzed from chest radiographs of 13 patients. In addition, six patients had thoracolumbar spinal radiographs available for analysis. The vertebral bodies were oval and globular in early childhood (Fig. 1), but their shape normalized in mid-childhood. Diffuse osteopenia was present in 11 patients in early childhood, and early signs of osteoporosis with end plate deformity and mild decrease in vertebral height were observed in two adolescent patients.

Skull. Skull radiographs were available for five patients. All showed normal shape of the skull but reduced thickness of the cortical bone in the

Fig. 4. Skeletal changes in the hips and knees in Patient 5 (anterior–posterior views of pelvis and knees). (a) The appearance of proximal femoral epiphyses is delayed, the secondary ossification centers at the knees are normal in size and shape. The proximal femoral metaphyses are wide and the growth plate horizontal. The distal femoral and the proximal tibial metaphyses are flared with medial beaking (age 8 months). (b) The proximal femoral epiphyses are smaller than normal, and the metaphyses show significant irregularity with lucent areas. The femoral necks are wide and short; asymmetrical growth has resulted in valgus deformity. Stress fractures are seen on both sides (arrows) (age 7 years 5 months). (c) Normal size, shape, and contour of the secondary ossification centers. The distal femoral and proximal tibial metaphyses are irregular with patchy sclerosis and thickened growth plate (age 7 years 5 months).

cranium. Wormian bones were seen in three patients (Fig. 7).

Other features. Unlike the growth plates, the tubular bones were normal in shape. However, osteopenia with reduced cortical thickness was consistently observed especially during the first 5 years of life.

Phenotype–Genotype correlation

The skeletal phenotype varied considerably not only with age but also from individual to individual. Variability in severity was observed even among patients with identical mutations and within family (Fig. 6). The seven patients heterozygous for both 258+2T>C and 183–184TA>CT
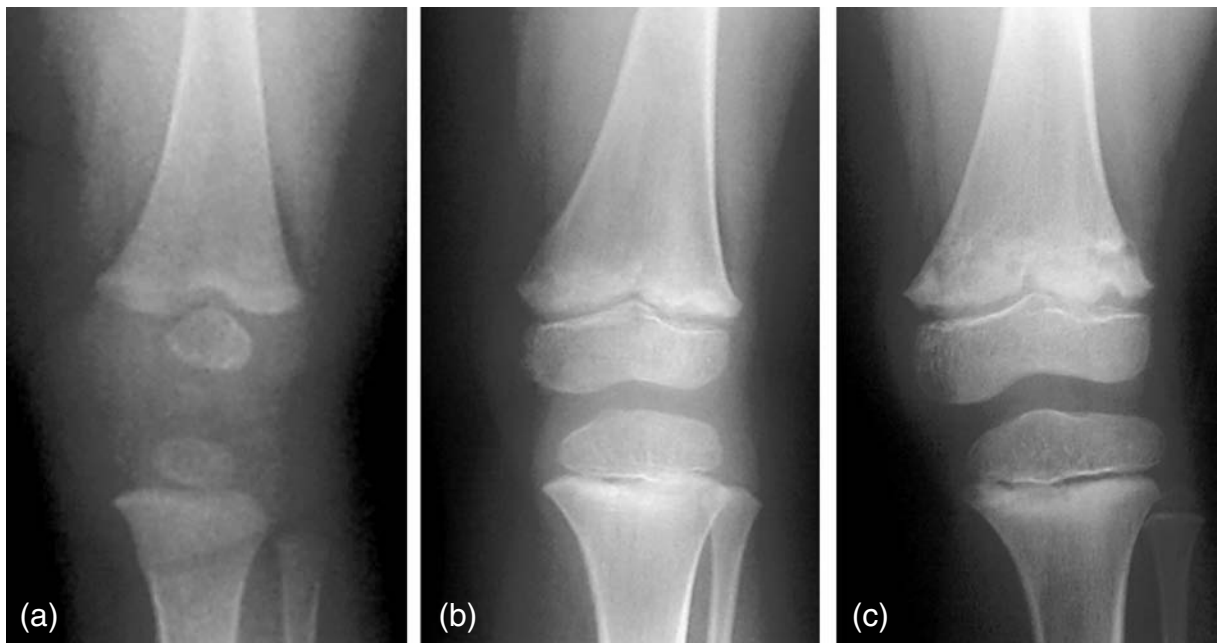
Fig. 5. Longitudinal changes in the knee in Patient 15 (anterior–posterior views). (a) Small secondary ossification centers and mild metaphyseal widening with medial beaking (age 5 months). (b) Normal secondary ossification centers. The distal femoral and proximal tibial metaphyses show mild metaphyseal irregularity and thickening (age 4 years 2 months). (c) The metaphyseal changes have progressed to significant irregularity with sclerosis, more marked in the femoral metaphysis (age 5 years 6 months).
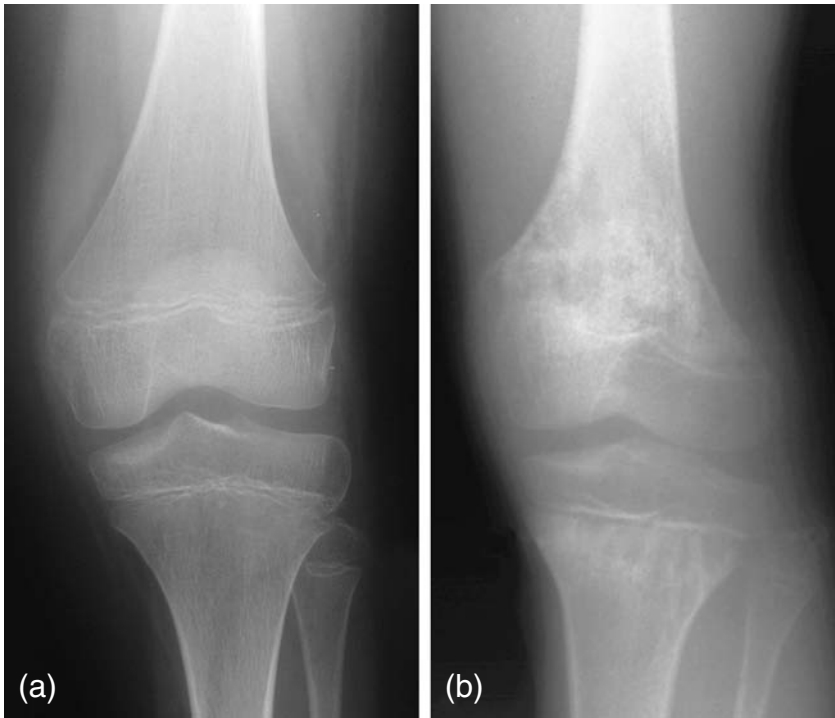
Fig. 6. Knee radiographs (anterior–posterior view) of Patients 4 and 9 at age 12 years 4 months. Patients have identical *SBDS* mutations (compound heterozygotes for the two major mutations). (a) Patient 4 has normal epiphyses and very mild metaphyseal irregularity and no sclerosis. There is mild osteopenia. (b) Patient 9 has significant metaphyseal sclerosis and irregularity in the distal femur and proximal tibia. Asymmetrical growth arrest in the distal femoral growth plate has resulted in varus deformity.

ranged in height from −1.0 SDS to −4.8 SDS and the severity of their skeletal changes varied significantly. Patients carrying a 'rare' mutation were within the same spectrum of variability. No gender differences in the phenotypic presentation were observed. Similarly, there was a lack of

Fig. 7. Skull radiograph (lateral view) of Patient 14 shows osteopenia and Wormian bones (arrow) (age 1 year 2 months).

correlation between the skeletal abnormalities and other clinical features, including pancreatic function, hematological manifestations, and growth. For example, Patients 5 and 6 with identical mutations were discordant for all these clinical features (Table 1). Therefore, no obvious genotype–phenotype correlation was observed in this cohort.

Discussion

Shwachman–Diamond Syndrome (SDS, MIM 260400) is a pleiotropic disorder in which the symptoms arising from PI and bone marrow dysfunction dominate. The recent discovery of the SDS gene, *SBDS*, is a significant advance towards understanding the causative pathogenetic mechanisms in SDS (10). *SBDS* is widely expressed not only in pancreas, bone marrow, and leukocytes but also in many other tissues (10); this and *SBDS*'s speculated function in RNA metabolism are in accordance with the wide spectrum of clinical manifestations seen in patients with SDS. The association of metaphyseal chondrodysplasia with SDS was first reported by Burke et al. (13) and confirmed in subsequent reports (3, 5, 14–17). These studies, which have been limited to cross-sectional data only, have reported metaphyseal dysostosis in 40–80% of the patients and rib and/or thoracic cage abnormalities in 30–50% of the patients (3–5); delayed skeletal maturation was observed in all patients studied by Aggett et al. (3).

The present study is the first systematic evaluation of age-related skeletal changes in a longitudinal analysis. Furthermore, only patients with identified *SBDS* mutations were included. The results suggest, in contrast to the previous observations, that the skeletal changes are in fact present in all patients examined. However, the presence or absence of abnormalities in a specific region of the skeleton appears to be dependent on the age of the patient. During the first 2 years of life, chest radiographs were universally abnormal with evidence of flaring and irregularity of the anterior ribs. Also wrist radiographs consistently showed metaphyseal irregularity and/or cupping of the distal radius and ulna as well as of the metacarpals. In older patients, these findings were more variable and less consistently present. In contrast, hip radiographs taken after age 2 years always demonstrated metaphyseal changes at the proximal femur. These observations strongly suggest that both the metaphyseal changes in the ribs and other tubular bones are invariably present, but vary in terms of location and severity with age.

The appearance and development of secondary ossification centers was delayed in all patients. However, when the ossification centers appeared they were single (as opposed to fragmented or multiple as seen in epiphyseal dysplasias and some other forms of skeletal dysplasia) and with advancing age their growth and development tended to normalize more rapidly in the hand than in the hip and knees. This variable degree of severity in upper and lower limbs and the slow normalization of the maturation defect with age suggest that it is due to the underlying cellular pathology and not secondary to nutritional problems. The distal radius and ulna as well as the metacarpals showed mild metaphyseal flaring and irregularity in early childhood, but these abnormalities also tended to normalize with age. In contrast, the metaphyseal changes in the proximal and distal femur became more prominent and severe after age 3–5 years. The metaphyseal findings were characterized by progressive thickening of the growth plate with mild flaring, variable irregularity, and patchy sclerosis of the metaphysis. Evidence of persistent asymmetrical growth was observed in some older patients; this resulted in valgus alignment of the femoral necks and valgus tilting of the femoral heads on the femoral necks, and in the distal femur, in varus deformities of the knees. Some patients also had evidence of structural failure of the metaphyseal bone of the femoral necks giving rise to stress fractures and varus deformities.

The skeletal abnormalities and age-related alterations were very similar in all patients and showed no obvious correlation with the underlying *SBDS* mutations. Furthermore, there was considerable variability in the location and severity of the skeletal abnormalities even among patients with identical mutations. Thus, the range and variability of skeletal abnormalities are very similar to the considerable phenotypic variability that has previously been reported between affected sibs in other organs affected by SDS especially in regards to the hematological abnormalities and growth failure. This contrasts with a high degree of familial concordance observed for the presence of PI or PS (4). Although only one pair of siblings was included in this cohort, the results suggest that in addition to the *SBDS* genotype other modifying genes and/or environmental factors determine the phenotypic presentation of SDS in an individual patient.

Osteopenia was observed in almost all the patients and spinal radiographs of some patients showed early signs of osteoporotic vertebral deformities. Osteoporotic spinal changes and

compression fractures have previously been reported in occasional patients with SDS (3, 4, 18). It is possible that these changes were secondary to compromised nutrition and vitamin D deficiency. Unfortunately, no data on biochemical parameters of calcium homeostasis, bone turnover markers, or bone mineral densities were available for the present patient cohort. However, Aggett et al. (3) found no abnormalities in serum calcium, phosphate, or alkaline phosphate levels and McLennan et al. (16) observed no improvement in the bony abnormalities with intensive vitamin D therapy. The exocrine PI is most significant between birth and 2 years of age and shows improvement with increasing age and may reverse in up to 45% of patients to PS later in childhood (3, 5). In the present cohort, four patients became pancreatic sufficient after diagnosis. Because osteopenia persisted in treated patients and in patients who became PS, osteopenia probably reflects a primary defect in bone metabolism. Bone mineral density measurements and careful monitoring of vitamin D levels and radiographic spinal changes are recommended to prevent osteoporotic fractures.

The metaphyseal changes differed from those seen in rickets, Jansen type of metaphyseal chondrodysplasia (MIM 156400), or Schmid type of metaphyseal chondrodysplasia (MIM 156500). Unlike nutritional rickets in infancy, the degree of metaphyseal flaring and irregularity in SDS is more prominent in the costochondral junctions than in the metaphyses of long bones. At a later age, the metaphyseal changes, with characteristic thickening and irregular calcification of the growth plate, is easily differentiated from those typical for rickets (19). Jansen type of metaphyseal chondrodysplasia is characterized by extreme disorganization of the metaphyses and by severe short stature (20). While rickets and Jansen type of metaphyseal chondrodysplasia are associated with biochemical abnormalities, serum calcium, phosphorus, and alkaline phosphatase levels are usually normal in SDS (3). Schmid type of metaphyseal chondrodysplasia is an autosomal dominant chondrodysplasia resulting from mutations in *COL10A1*, the gene encoding for the α1(X) chains of type X collagen molecules (21–24). Typical features include disproportionate short stature, bowed legs, coxa vara, and metaphyseal flaring and irregularity. The radiographic changes are most pronounced at the hip with enlarged capital femoral epiphysis, short femoral neck with significant metaphyseal irregularity, and severe coxa vara deformity with vertically oriented growth plates (22). These changes are different from those seen in SDS, and more

importantly, there are no extra-skeletal manifestations in the other disorders.

The ability to distinguish between cartilage-hair hypoplasia (metaphyseal chondrodysplasia, McKusick type, MIM 250250), another metaphyseal chondrodysplasia with autosomal recessive inheritance, and SDS may be more challenging. Hematological abnormalities, defective immunity, short stature, and metaphyseal skeletal changes are present in both disorders (25–28). Malabsorption has been observed in some patients with cartilage-hair hypoplasia (29), but this complication is due to gastrointestinal infections instead of pancreatic failure. In addition, majority of cartilage-hair hypoplasia patients can be distinguished from SDS because they have severe short-limbed short stature and hair hypoplasia which is not observed in SDS (28, 29). The metaphyseal changes are most prominent at knees with significant flaring and irregularity of the metaphyses. Unlike the observations in SDS, however, there is no thickening or sclerosis of the growth plate. Also the proximal femurs and other long bones are affected but to lesser extent (30). Interestingly, cartilage-hair hypoplasia is caused by mutations in *RMRP*, the gene coding for the untranslated RNA component of the RNAse MRP complex (mitochondrial RNA-processing endoribonuclease), which is involved in multiple cellular and mitochondrial functions (31). It remains possible that basic molecular abnormalities of *RMRP* and *SBDS* both involve related pathogenetic pathways, which might explain the concordance of some of the clinical manifestations.

The expression and role of *SBDS* in the bone and in the various cell types essential for normal bone formation and resorption remain unknown. Histologically, SDS growth plate shows markedly abnormal ossification with very poor vascularization of epiphyseal cartilage, irregular columniation of chondroblasts, and fibroblastic proliferation across the edge of the epiphyseal plate (15, 32). The electron microscopic appearance of the chondrocytes shows markedly dilated rough endoplasmic reticulum and Golgi apparatus (32). This may be due to defective processing of proteins leading to intracellular protein accumulation and premature cell death. It is interesting that whereas pancreatic and hepatic dysfunction in SDS tend to normalize with age, the skeletal and hematological changes are progressive. *SBDS* is likely to have different role in these organ systems, and also the compensatory mechanisms may vary. Further studies on *SBDS*'s role in intracellular protein processing will shed light on the pathogenetic pathways in SDS.

In conclusion, this cross-sectional and longitudinal radiographic evaluation of skeletal phenotypes in 15 patients with SDS suggests that abnormal endochondral and intramembranous ossification of the skeleton is a universal feature of SDS. The characteristic skeletal changes, delayed appearance but normal shape of epiphyses, progressive thickening of metaphyses, and osteopenia, are present in all patients with SDS and *SBDS* mutations, but their severity and localization vary with age. These skeletal manifestations, in addition to the pancreatic and bone marrow insufficiency, should be considered as an integral diagnostic feature of the syndrome and carefully assessed with complete skeletal survey at presentation. Due to the progression of the skeletal abnormalities with advancing age, regular radiographic reassessment is recommended in all patients even if the early skeletal changes are subtle. In order to gain a more complete understanding of the occurrence, extent and natural history of the skeletal phenotype in childhood and beyond skeletal maturity, a wider range of patients with SDS need to be studied in a prospective and preferably multicenter setting. In addition, more detailed evaluations of skeletal development, bone mineral density, fracture healing, parameters of calcium homeostasis and bone turnover, and expression of *SBDS* are required.

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