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Antibody deficiency, growth retardation, spondyloepiphyseal dysplasia and retinal dystrophy: a novel syndrome

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The clinical and laboratory combination of recurrent infections due to antibody deficiency, spondyloepiphyseal dysplasia, growth retardation and retinal dystrophy is novel. Four patients with strikingly similar phenotypes from three different families of diverse genetic backgrounds are described, suggesting a similar underlying genotype. Increased awareness of this syndrome will hopefully lead to the description of a larger number of affected individuals, which ultimately might be critical for its genetic characterization.

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Key words: antibody deficiency – B-cell development – retinal dystrophy – signal transduction – spondyloepiphyseal dysplasia

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Humoral immunodeficiency consists of a heterogeneous group of disorders characterized by reduced serum levels of one or more immunoglobulin isotypes, and/or an inability to produce specific antibodies (1). The most significant clinical entities include (Brutons') agammaglobulinemia, common variable immunodeficiency (CVID) and dysgammaglobulinemia. Typically, patients with agammaglobulinemia present early in infancy with recurrent microbial infections and a complete lack of lymphoid tissue, reduced to absent circulating B cells and only traces of serum immunoglobulins. Recently, the gene responsible for this X-linked disorder has been identified as protein tyrosine kinase (PTK), which was named Brutons' tyrosine kinase (BTK) (2). Unlike agammaglobulemia, CVID patients present with a variety of symptoms, most commonly during adulthood. Lymphadenopathy, recurrent bacterial infections, neutropenia, hemolytic anemia and thrombocytopenia are often presenting features of CVID. Immunoglobulin levels are variable yet specific antibody formation is always faulty. The mode of inheritance is believed to be autosomal recessive in most patients and the genotype remains obscure (3). Finally, dysgammaglobulemia is a term used to describe a lack of specific antibody production in patients that have normal to elevated levels of serum immunoglobulins (3). This condition is rare and its inheritance and genotype are unknown. In 1997, the first case of dysgammaglobulemia associated with bone dysplasia, retinal dystrophy and developmental delay was reported (4). Such a combination of humoral immunodeficiency and bone dysplasia is extremely rare and has only been reported in a few cases with short-limb dwarfism (5, 6). However, bone dysplasias are more commonly associated with T-cell deficiencies or severe combined immunodeficiencies such as adenosine deaminase (ADA) deficiency, cartilage hair hypoplasia, short-limb dwarfism and Schimke immuno-osseus dysplasia (7).

The novel association of dysgammaglobulinemia, spondyloepiphyseal dysplasia, retinal dystrophy, pre- and postnatal growth delay, limited mental ability and various dysmorphic features has now been identified in multiple patients of different ethnic backgrounds suggesting a distinct syndrome not previously described.

Materials and methods

Lymphocyte markers

The surface phenotypes of blood mononuclear cells obtained by Ficoll–Hypaque density gradient centrifugation were determined by direct immunofluorescence with an FITC-conjugated goad anti-human immunoglobulin antibody (Tago, Burlingame, CA) or FITC-conjugated MoAbs B1 and anti-CD3, CD4, CD8, and CD2 (Coulter Instruments, Mississauga, ON, Canada). Analysis was performed on a Coulter EPICS V flow cytometer (Coulter).

Serum concentration of immunoglobulin and specific antibodies

Serum concentrations of immunoglobulins were measured by nephelometry. Serum IgE concentration was measured by radioimmunoassay with the IgE PRIST kit (Pharmacia Diagnostics, Dorval, Quebec, Canada). Levels of serum antibodies to tetanus were measured by enzyme-linked immunosorbent assay (ELISA) and polio antibody titers were determined by complement fixation.

T- and B-cell proliferative response

Lymphocyte proliferative responses to mitogens including phytohemagglutinin (PHA) (in the presence and absence of IL-2), concanavalin A (Con A), pokeweed, and *Staphylococcus aureus* and to a panel of recall antigens (including Candida, purified protein derivative, herpes simplex, and cytomegalovirus) were determined by tritiated thymidine incorporation with the microtiter plate technique. All assays were performed in triplicate and were compared with those simultaneously performed on normal controls.

Western blots

Peripheral blood lymphocytes (2×10^6) were lysed in SDS-sample buffer, electrophoresed on SDS/8% polyacrylamide gels, and transferred to nitrocellulose membranes (Hybond-C, Amersham). After nonspecific blocking with 5% mild solids in PBS, immunoblots with antiphosphotyrosine, anti-Btk, anti-Lyn or anti-Syk antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) were performed in PBS containing 0.05% Tween-20 and 1% mild solids. Bound antibody was detected with the secondary reagent hoseradish peroxidase conjugated with donkey antibody to rabbit immunoglobulin (Amersham) and developed by enhanced chemiluminescence (ECL, Amersham).

Results

Growth and development

Four male patients aged 12, 19, 4 and 7 years presented with an almost identical multi-system disorder. All patients were born prematurely between 35 and 37 weeks gestation. Further, marked intrauterine growth retardation (IUGR) was documented in all 4 patients (Table 1). All patients had delayed milestones in development, sitting at 8–12 months and walking at 3.5–4 years of age. Linear growth was compromised with height below the third percentile. Head circumference was two standard deviations below the mean in 2 patients and on the third percentile in the remaining 2 patients.

Assessment of intelligence performed in 3 of 4 patients showed borderline to mild retardation. Common features included delayed and slow development of speech, articulation of fine motor co-ordination and math computation.

Table 1. Clinical and laboratory manifestations

Manifestations	Patients
Growth deficiency	
Prenatal (IUGR)	4/4
Postnatal	4/4
Performance	
Low intelligence	4/4
Hypotonia	4/4
Facial and extremities	
Long philtrum	4/4
Narrow upturned nose	3/4
Thin upper lip	4/4
Palpebral slant	4/4
Clinodactyly 5th finger	4/4
Simian crease	4/4
Dermatologic	
Eczema	4/4
Skeletal	
Epiphyseal dysplasis of hips	4/4
Epiphyseal dysplasis of other long bones	3/4
Changes in vertebral plates	4/4
Opthalmologic	
Retinal dystrophy	2/4
Immunologic	
Elevated eosinophil count	4/4
Normal IgG, IgA, IgM, IgE	3/4
Elevated IgA	1/4
Low antibody titers	4/4
Low number of sIg+B cells	1/4
Absent nitogenic response to SAC	4/4
Normal T-cell numbers and function	4/4
Metabolic/Genetic	
Normal karyotype	4/4
Normal metabolic screen	3/3

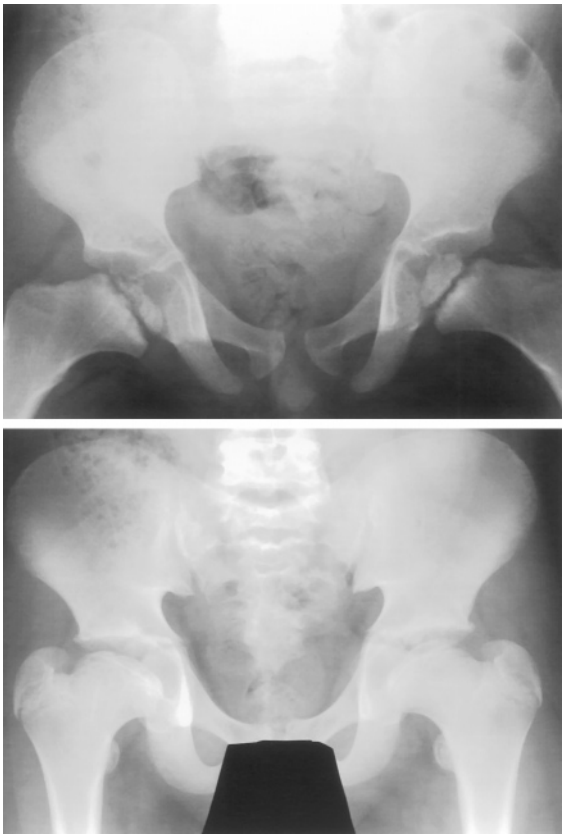

Fig. 1. Views of the pelvis demonstrate asymmetric ossification of the proximal femoral epiphyses in patient 1, with the right epiphysis demonstrating flattening and fragmentation. The changes are more symmetric in patient 2 (bottom).

Musculoskeletal features

All patients had hypotonia that was most pronounced during the first 3–4 years of life (Table 1). However, electromyography (EMG) and muscle biopsy analysis performed in 2 of 4 patients were normal. Joints showed a mild to moderate limitation of hip flexion in 3 of 4 patients. All patients were short, mainly because of a short trunk. Arm span, however, was normal. Hands and feet were slightly short and all patients had clinodactyly of the 5th digit.

Skeletal survey revealed epiphyseal changes in multiple joints in all patients. Three of 4 patients had markedly irregular and wavy vertebral endplates. The remaining patient had definite but more moderate changes. Other common features to all patients included various degrees of changes in the femoral heads, femoral condyles and tibiae. Femoral heads were broadened with overgrowth of the greater trochanters bilaterally and broadening of femoral necks (Fig. 1). Third and 4th metacarpals bilaterally were small and over-tubulous. Erosions of the medial femoral condyles and tibial plateaus were also noted.

Retinal abnormalities

Retinal changes were identified in 2 of 4 patients that were unrelated (Table 1). Fundoscopic analysis appeared consistent in these patients. One patient had bilateral speckled pigmentary changes present in the posterior pole with small retinal pigment epithelial atrophic spots in the mid-periphery. A fundoscopic inspection of the other patient showed similar pigmentary mottling bilaterally with an attenuated arteriolar tree. Angiography revealed punctuate-type transmission hyperfluorescence in the regions of pigmentary changes. No abnormal leakage was present and no nerve head straining was present.

Electroretinograms performed on both patients were completely extinguished, consistent with widespread degeneration of the rod and cone systems of the retinae.

Dysmorphic features, genetic and metabolic data

Facial features common to all patients include long and prominent eyelashes, down-slanting palpebral fissures, a long philtrum and a thin upper lip (Table 1). Three patients had a narrow and upturned nose; 2 patients had hypoplastic shallow mid-faces and epicanthic folds. Other signs in all 4 patients were clinodactyly of the 5th finger, slightly short tapered fingers and toes, hyperconvex nails and a simian crease.

The patients described include 2 siblings (currently 19 and 12 years) born to parents of Irish descent. The third patient's (4 years) parents are of Dutch and Maltese descent and the fourth patient's (7 years) parents are of Spanish and Italian descent. None of the parents were consanguineous.

Karyotype analysis and TORCH titer performed on all patients was normal up to $+550$ bands of resolution and Fragile-X analyses have been reported normal. Plasma amino acids, urine nitroprusside and dinitrophenylhydrazine were normal. Urine amino acids and organic acid were transiently elevated in 1 patient. Medium chain acyl CoA dehydrogenase gene was therefore analyzed and found normal. Urine oligosaccharide, mucopolysaccharides and very long chain fatty acids were normal in all patients.

Immunologic profile

All 4 patients had eczema with variable severity and 2 patients had asthma (Table 1). Accordingly, blood eosinophils were increased in 3 of 4 patients but surprisingly IgE levels were normal in all 4 patients. All patients had a history of multiple ear

infections, 2 had repeated episodes of pneumonia, 1 had septicemia and recurrent herpes reactivation.

All patients had lymphadenopathy and hepatosplenomegaly that varied from mild enlargement in 2 patients to more marked enlargement in the remaining 2 patients. Yet, enlargement of lymphoid tissue tends to exacerbate during infections, reminiscent of humoral immunodeficiency conditions. However, when serum immunoglobulin levels were measured (Table 2), IgG levels were within normal limits. IgA levels were normal in 3 patients and elevated in 1 patient. IgM levels were below normal in 2 patients and normal in the remaining 2 patients. Next, the ability of patients to form specific antibodies was measured. Isohemagglutinin levels (IgM-specific antibodies) were low to absent in all patients. Similarly, patients failed to mount an antibody response to polyvalent pneumococcal vaccine. Specific antibody titers to protein antigens such as tetanus, polio virus, mumps and rubella were all abnormally decreased or below detection. Re-immunization with tetanus and polio failed to produce sustained normal titers indicating a primary inability to produce specific antibodies.

Flow cytometry analysis of peripheral blood lymphocytes revealed normal numbers of T-cell receptor bearing cells and normal ratios of CD4 + and CD8 + T lymphocytes (Table 3). Proliferative responses to PHA and Con A and anti-CD3 antibodies were all within normal limits in all patients. B-cell numbers were determined by CD19 and CD20 antigens that appeared borderline low (range 2–7%). However, the proportion of antigen-receptor bearing B cells (sIg + B cells) was lower in all patients, yet 3 of 4 patients' sIg + B cells were still within normal limits. These results indicate that 20–50% of B lineage cells are immature, suggesting a partial block of B-cell maturation in these patients.

Mitogenic responses to *Staphylococcus aureus* Cowan A (SAC), a B-cell mitogen, were markedly diminished in all patients. This failure to proliferate cannot only be explained by the number of patient's B cells, because control peripheral blood leukocytes (PBL) containing 2% (N2-20) sIg + cells still recorded increased thymidine incorporation after SAC stimulation.

Aberrant antigen-receptor mediated tyrosine phosphorylation

The selective inability to respond to SAC by the patient's B cells is very uncommon in human immunodeficiency. Only in cases with X-linked agammaglobulinemia (XLA), which have no detectable B cells, such finding occurs. We have, therefore, studied the ability of patient's B cells to respond to ligation of the antigen receptor by anti-IgM. Normally, stimulation of the receptor induces a rapid increase in tyrosine phosphorylation on multiple substrates (5). The most strongly phosphorylated band is a 70–72-kDA protein. The identity of this protein remains unknown. Pre-clearing of lysates with anti-Syk antibody, which removes one of the antigen-receptor associated tyrosine kinases, Syk (70 kDA), does not significantly change phosphorylation of the 72-kDA protein, indicating that p72 was not a Syk kinase (not shown).

Immunoblotting with anti-phosphotyrosin antibody of lysates obtained from unstimulated and anti-IgM stimulated cells clearly shows that patients' B cells increased tyrosine phosphorylation on most substrates except for the 70–72-kDA protein (Fig. 2). The results suggest that the defect in these patients may be a signaling molecule asso-

Table 2. Studies of humoral and cellular immunity

	Patient 1	Patient 2	Patient 3	Patient 4	Control or normal range
Serum immunoglobulins					
IgG (g/l)	17.90	10.00	9.50	7.20	6.70–17.30
IgM (g/l)	0.20	0.83	1.10	0.75	0.50–3.10
IgA (g/l)	6.77	1.93	2.50	3.90	0.40–3.70
Specific antibodies					
Isohemagglutinins	1/1	1/8	1/1	1/1	>1/16
Tetanus	<0.01	<0.01	<0/01	<0.01	IU/ml, normal >0.04
Polio	<1/8	<1/8	<1/8	<1/8	>1/16
Pneumococcus	NR	NR	NR	NR	
Mitogenic responses (counts $\times 10^{-3}$)					
Phytohemagglutinin (PHA)	123.40	119.50	105.00	112.00	90–130
Anti-CD3	47.90	53.00	41.50	57.70	35–55
Staphylococcus aureus Cowan A (SAC)	3.30	2.60	4.10	1.60	14–21

NR, no response.

Table 3. Lymphocyte phenotyping

Markers (%)	Patient 1	Patient 2	Patient 3	Patient 4	Normal range
CD2	95.7	88.8	90.3	92.0	75–96
CD3	79.80	69.3	81.2	74.5	60–85
αβTcr	78.0	70.0	80.0	72.0	60–85
γδTcr	1.0	2.0	0.8	1.0	1–5
CD4	48.7	48.2	65.4	49.1	30–60
CD8	32.4	20.8	15.5	24.0	15–35
CD20	7.0	4.0	7.0	5.0	2–20
CD19	5.0	4.0	5.0	3.0	2–20
CD20/slg	2.8	2.6	2.0	0.8	2–20

ciated with the antigen receptor. The inability of patient's cells to increase phosphorylation suggests that one of the PTKs associated with the B-cell antigen receptor might be deficient. Lyn kinase of the src family and Syk were both shown to assist the antigen receptor in transmitting growth-related signals (6), while Btk, the protein tyrosine kinase associated with development of B-cell lineage (mutated in XLA), is also linked to the antigen-receptor signal transduction.

In order to determine whether one of the PTKs associated with the antigen receptor is abnormal, we have immunoblotted patient lysates with anti-Syk, anti-Btk and anti-Lyn antibodies. Fig. 3 shows that all three proteins are normally expressed in patient's cells, as compared with control samples. A mutation in any of these three enzymes is unlikely because null mutations of these genes in rodents or humans do not demonstrate the variety of multi-systems involvement observed in this syndrome.

Discussion

The association of humoral immunodeficiency with skeletal dysplasia, retinal dystrophy and developmental delay is described here in 4 patients, who are the products of three different sets of parents of different ethnic backgrounds. The striking similarity of clinical manifestations among this group of patients strongly suggests a common genotype.

The association of skeletal disorders with immunodeficiency has been observed in a few more defined diseases or syndromes (Table 4). Patients with ADA deficiency, a form of severe combined immunodeficiency, frequently have rib anomalies, flat iliae and changes of the scapular tips (8). Short-limb dwarfism that could be associated with predominantly cellular deficiency presents typically with metaphyseal widening of long bones, short pelvic bones and short ribs (9). A sometime overlapping syndrome is cartilage hair hypoplasia that is characterized by cystic changes at the metaphyseal plates (9). Other syndromes such as Schimke immuno-osseous dysplasia typically involve cellular immunity, nephrotic syndrome and hypoplastic pelvis (7). None of these disorders could be confused with the syndrome described here, as most features including the humoral deficiency, spondyloepiphyseal dysplasia and retinal dystrophy are distinct.

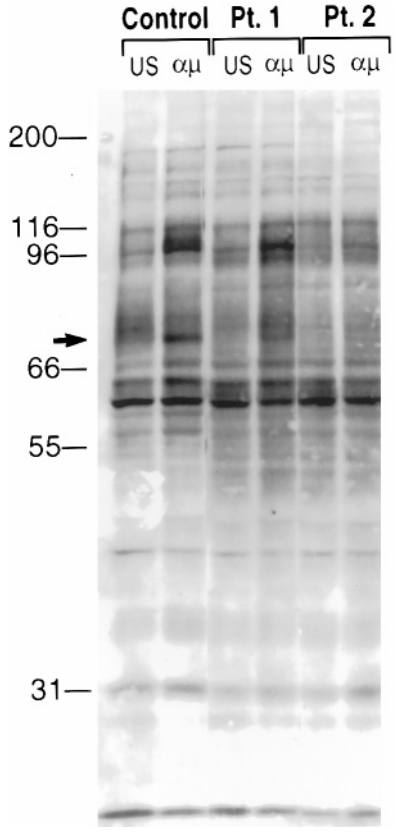

Fig. 2. Immunoblot of control and patient lysates with anti-phosphotyrosine antibody showing increased phosphorylation on multiple proteins after stimulation with anti-IgM (α,μ) antibody, as compared with unstimulated samples (US). Patient 1 (Pt1) showed reduced phosphorylation of p70–72, while patient 2 (Pt2) shows lack of increased phosphorylation of p70–72 and p96.

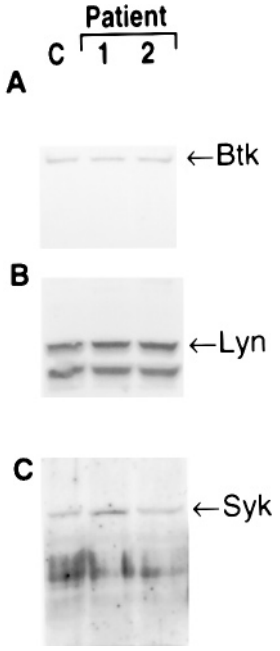

Fig. 3. Immunoblot with anti-Btk, anti-Lyn or anti-Syk antibodies of control and samples from both patients showing similar amounts of protein in control and patients samples for all three protein tyrosine kinases.

Several early reports described the association of antibody deficiency with skeletal abnormalities, which included short-limb dwarfism (9) or short-limb dwarfism with ectodermal dysplasia (6). Again, these cases are quite distinct from the cases reported here. Further, a recent review of a large member of multi-organ syndromes that are associated with immunodeficiency did not reveal a similar clinical and laboratory combination (Dr R Stein, personal communication).

The only measurable abnormalities observed *in vitro* are the borderline low B-cell numbers and their inability to respond to a mitogenic stimulus. SAC is the most potent, T-cell independent B-cell mitogen. Normal B cells, as low as 2–5% of total mononuclear cells, are sufficient to elicit a marked increase in thymidine incorporation. In contrast, patients' cells were unable to respond to this mitogenic stimulus suggesting that the antigen receptor on B cells fails to transmit normal signals. Interestingly, not all patients' peripheral blood derived B cells expressed the B-cell antigen receptor (BCR). In fact, only about 20–50% of patients' CD20 + B cells also expressed BCR (sIg), suggesting that the rest of these circulating B cells are immature. Yet, only 1 patient had sIg + cells below the normal range (2–20%) suggesting that the low SAC response may not be solely explained by the low number of mature BCR expressing B cells. Indeed, normal control peripheral blood mononuclear cells with comparable sIg + cells responded adequately to SAC stimulation.

One of the reasons for a failure to respond to mitogens could be attributed to a putative defect in transmitting intracellular signals from BCR to the nucleus. One of the early events following ligation of the BCR is a rapid increase in PTK activity by a group of enzymes including Syk, Lyn and Btk (10, 11). Immunoblot analysis of these PTKs in patient samples showed normal levels as compared with the control. Yet anti-phosphotyrosine immunoblots of whole lysates showed a selective lack of phosphorylation on a 72-kDA protein that is clearly identified in normal controls.

Together these results indicate that the underlying defect in this syndrome affects BCR-mediated signal transduction and possibly B-cell development. However, in the context of the other multi-system manifestations in this syndrome, it is more likely that a gene involved in ontogenesis might be mutated. Possible candidates include the Pax gene family. Pax genes are a family of developmental control genes that encode nuclear transcription factors. Evidence for this crucial role in morphogenesis, organogenesis and cell differentiation has been provided by rodent mutants and human diseases. Brain, eye, skeleton and the immune system are some of the organs dependent on Pax family genes for normal development. Pax2 deficiency provokes hypertrophia of the optic stalk and reduction of pigment epithelium in the retina (10, 11). Pax5 could have been a particularly attractive candidate as it was identified as a B-cell specific transcription factor that regulates the expression of the CD19-gene encoding in early B-cell surface antigens (12). In addition to involvement of the eye and brain, Pax5 deficiency arrests B-cell differentiation. However, sequence analysis of Pax5 gene in these patients was completely normal (Dadi and Roifman, unpublished). The recent explosive developments in understanding the mechanisms that govern ontogenesis improve the probability of identifying the genetic aberration causing this syndrome.

Further information on more patients should also assist in the search for the genotype of this syndrome. So far only male patients with the syndrome have been identified suggesting X-linked inheritance. However, other modes of inheritance cannot be excluded at this time.

Table 4. Clinical presentation, laboratory and radiologic changes in various syndromes that combine immunodeficiency and skeletal aberrations

	Antibody deficiency, *SDS retinopathy syndrome	Cartilage hair hypoplasia	Short-limb dwarfism	ADA deficiency	Schimke immuno-osseous dysplasia
Immunodeficiency	Antibody deficiency with normal immunoglobulins	Predominantly cellular	Cellular/normal or combined	Severe combined	Lymphopenia, low T cell, mitogenic responses, neutropenia
Skeletal abnormalities	Irregular vertebral endplates, broadening of femoral heads and femoral necks, small 3rd, 4th metacarpals	Cystic changes at metaphysealplates	Short stubby pelvic bones, flat acetabulae, metaphyseal widening and short ribs with splayed ends	Rib anomaly, flat ilia, 'squaring off' of scapular tips	Dorsal flattening of vertebrae, hypoplastic pelvis, small capital femoral epiphysis
Skin and hair manifestations	Eczema	Skin loose, hair sparse, lacks central pigmented core	Skin loose, occasionally total alopecia	Normal	Hyperpigmented spots, fine and thin hair
Joints	Flexion contraction	Hyperlaxity	Hyperlaxity	Normal	Normal
Eyes	Retinal dystrophy	Normal	Normal	Normal	Normal
Performance	Mental retardation	Normal	Normal	Normal	Normal
Others	Clinodactyly of the 5th digit, low-set ears, single palmar crease				Severe, nephrosis, mucopolysaccharidurie

SDS, spondyloepiphyseal dysplasia syndrome.

In conclusion, we have described a novel syndrome involving multiple organs and humoral immunity. The putative genetic defect is likely affecting critical signals of organ development as well as signal transduction in B cells.

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