

Multicentric dermatofibrosarcoma protuberans in patients with adenosine deaminase–deficient severe combined immune deficiency

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Background: Dermatofibrosarcoma protuberans (DFSP) is a rare malignant skin tumor associated with a characteristic chromosomal translocation (t(17;22)(q22;q13)) resulting in the *COL1A1*-platelet-derived growth factor β (*PDGFB*) fusion gene. This malignancy is rarely diagnosed in childhood.

Objective: We observed a high incidence of this tumor in children affected with adenosine deaminase–deficient severe combined immunodeficiency (ADA-SCID).

Methods: Twelve patients with ADA-SCID were evaluated with a complete dermatologic examination and skin biopsy when indicated. Conventional cytogenetic and molecular analyses (fluorescence *in situ* hybridization, RT-PCR, or both) were performed when possible.

Results: Eight patients were found to have DFSP. Six patients had multicentric involvement (4–15 lesions), primarily of the trunk and extremities. Most lesions presented as 2- to 15-mm, round atrophic plaques. Nodular lesions were present in 3 patients. In all cases CD34 expression was diffusely positive, and diagnosis was confirmed either by means of cytogenetic analysis, molecular testing, or both. The characteristic DFSP-associated translocation, t(17;22)(q22;q13), was identified in 6 patients; results of fluorescence *in situ* hybridization were positive for fusion of the *COL1A1* and *PDGFB* loci in 7 patients; and RT-PCR showed the *COL1A1*-*PDGFB* fusion transcript in 6 patients.

Conclusions: We describe a previously unrecognized association between ADA-SCID and DFSP with unique features, such as multicentricity and occurrence in early age. We hypothesize that the t(17;22)(q22;q13) translocation that results in dermal overexpression of *PDGFB* and favors the development of fibrotic tumors might arise because of the known DNA repair defect in patients with ADA-SCID. Although the natural course of DFSP in the setting of ADA-SCID is unknown, this observation should prompt regular screening for DFSP in patients with ADA-SCID. (J Allergy Clin Immunol 2011;■■■:■■■-■■■.)

Key words: Severe combined immunodeficiency, adenosine deaminase, dermatofibrosarcoma, adenosine, fibrosis

Adenosine deaminase–deficient severe combined immunodeficiency (ADA-SCID) is a rare genetic disorder with an estimated incidence between 1:200,000 and 1:1,000,000. Both humoral and cellular immunity are affected, which results in increased risk of recurrent and life-threatening infections. Unlike most other forms of severe combined immunodeficiency, adenosine deaminase (ADA) deficiency is a metabolic disorder with clinical complications beyond the immune system that include skeletal, endocrine, and central nervous system abnormalities.^{1,2} Several pathophysiologic mechanisms have been implicated in the disease, including DNA repair defects caused by cellular nucleotide pool imbalance and inhibition of DNA polymerase activity. In addition, deoxyadenosine, one of the substrates that accumulate in ADA-deficient cells, has been reported to result in chromosome breakage.^{1,2} Treatment options include enzyme replacement therapy with pegylated bovine adenosine deaminase (PEG-ADA), hematopoietic cell transplantation (HCT), and gene therapy.³

Dermatofibrosarcoma protuberans (DFSP) is an uncommon mesenchymal tumor of the skin and subcutaneous tissues with low-to-intermediate malignant potential and low risk for metastasis (<2% of cases).^{4,5} The incidence of DFSP is 4.2 per million, accounting for approximately 0.1% of all cancers.⁶ Because of its slow growing characteristics, the clinical diagnosis of DFSP can be delayed for 2 to 3 decades.^{7,8} The occurrence of DFSP is even more uncommon in the pediatric setting, with fewer than 200 cases reported.^{9–13} Although DFSP typically presents as a solitary subcutaneous nodule, other forms exist, including atrophic, anetoderma-like, and morpheaform variants. The characteristic

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Abbreviations used

ADA:	Adenosine deaminase
ADA-SCID:	Adenosine deaminase–deficient severe combined immune deficiency
DFSP:	Dermatofibrosarcoma protuberans
FISH:	Fluorescence <i>in situ</i> hybridization
GCF:	Giant cell fibroblastoma
HCT:	Hematopoietic cell transplantation
PDGFβ:	Platelet-derived growth factor β
PEG-ADA:	Pegylated bovine adenosine deaminase

histologic finding is a spindle cell tumor with a storiform pattern. These lesions are CD34⁺ and are negative for Factor XIIIa and S100. The giant cell fibroblastoma (GCF) variant, which is more commonly seen in children, demonstrates characteristic pseudovascular spaces and giant cell florets. DFSP grows in an infiltrative manner, requiring wide local excision or microscopic margin control to achieve tumor clearance. Depending on margin widths and surgical techniques, local recurrence has been reported in 0% to 50% of cases.^{8,14,15} In the spectrum of fibrous tumors of the skin, DFSP is considered more worrisome than benign fibrous histiocytoma and less aggressive than malignant fibrous histiocytoma. At the cytogenetic level, DFSP is characterized by abnormal rearrangement of chromosomes 17 and 22, often leading to ring chromosomes or unbalanced t(17;22)(q22;q13).^{16,17} This characteristic rearrangement is described in more than 95% of cases when tested by using karyotype, fluorescence *in situ* hybridization (FISH), or RT-PCR.¹⁸ The resulting chimeric *COL1A1*–platelet-derived growth factor β (*PDGFB*) gene produces a functional and constitutively active PDGFβ protein.

Here we present a cohort of 12 unselected cases of ADA-SCID, 8 of whom had atrophic, nodular, or both DFSP lesions. This remarkable increased risk for DFSP in patients with ADA-SCID has not previously been reported, to our knowledge, and suggests an important pathophysiologic link between these 2 rare diseases.

METHODS**Patients**

All human subject research procedures were approved by the Institutional Review Board of the National Human Genome Research Institute (clinical research protocols registered with ClinicalTrials.gov as NCT00018018 and NCT00006319).

Twelve patients with ADA-SCID were followed at our institution between 2006 and 2009. The diagnosis of DFSP in 2 consecutive subjects prompted us to routinely screen for this rare malignancy. At the time of screening, patients were between 2 and 27 years old. In each case ADA-SCID was confirmed by sequencing of the *ADA* gene. Patients' clinical characteristics are reported in Table I. All patients underwent comprehensive physical, immunologic, and dermatologic examinations. When medically indicated, punch skin biopsy, surgical excision, or both were performed.

Histologic and immunohistochemical evaluation

Formalin-fixed, paraffin-embedded biopsy specimens were sectioned and stained with hematoxylin and eosin. Immunohistochemical analysis was performed with antibodies against CD34, Factor XIII, and S100 protein by using standard procedures.

See additional information in the [Methods](#) section in this article's Online Repository for cytogenetics, FISH analyses, and RT-PCR.

RESULTS**Clinical findings**

On examination, 11 of 12 patients presented with 1 or more skin lesions that warranted biopsy. The majority of lesions were small, atrophic, red-brown plaques less than 1 cm in greatest dimension. Plaques were either raised, flat, or depressed. One lesion presented as a slowly healing ulceration in the context of diaper dermatitis. One other lesion was detected as a violaceous, painless, subcutaneous mass (Fig 1). Only a minority of lesions had a nodular appearance. In one case a nodule developed within a previously identified plaque. In another case the lesion was nodular at diagnosis. Lesions were distributed on the trunk and extremities and ranged in number from 1 to 15 per patient. No facial or scalp lesions were observed.

Typically, patients, their parents, or both were unable to define the time of onset of the lesions that, in some cases, were reported as present from birth. In patients in whom serial examinations were performed, growth of some lesions and appearance of new lesions within 1 to 2 years of the initial examination could be documented. In 1 patient (ADA14) a nodular lesion was only ascertained after computed tomographic radiologic staging.

At diagnosis, patients were typically lymphopenic (range, 184–478 lymphocytes/μL) but showed conserved lymphocyte proliferation responses to PHA at levels varying between 91% and 581% of the lower limit of normal, with the exception of patient ADA7, who showed PHA responses at only 14% of the lower limit of normal.

Histology and immunohistochemistry

Eight of 11 patients in whom a biopsy of at least 1 lesion was performed showed a uniformly CD34⁺ spindle cell proliferation consistent with a diagnosis of DFSP. As reported in Table I and Fig 2, various histologic features were appreciated. Lesions from 2 patients (ADA12 and ADA16) showed classic findings of giant cell fibroblastoma with evidence of floret cells, multinucleated giant cells, loose myxoid stroma, and pseudovascular spaces (Fig 2, C and F). The classic DFSP storiform pattern was described only in 1 patient (ADA1; Fig 2, B and E). All other CD34⁺ lesions showed a nonspecific pattern of spindle cell proliferation. Cellularity ranged from paucicellular to densely cellular, and mitotic activity was low (<2 per high-powered field). In all cases tumor borders were indistinct, often infiltrating into the subcutaneous tissue. In several lesions this infiltration was best appreciated by using immunohistochemistry evaluation for CD34 (Fig 1, G). All CD34⁺ lesions were negative for Factor XIII and S100. DFSP was ruled out from biopsy material in 3 patients (Table I).

Cytogenetic and molecular findings

Eight lesions from 6 patients with a histologic diagnosis consistent with DFSP yielded sufficient fresh tissue for conventional cytogenetics. An abnormal karyotype revealing the characteristic t(17;22)(q22;q13) translocation was evident in all but 1 lesion from 1 patient (ADA4; Table I and Fig 3, A). However, another lesion from the same patient showed the characteristic translocation t(17;22)(q22;q13). No ring chromosomes were identified. Biopsy specimens from 2 patients (ADA6 and ADA9) with inconclusive histologic findings showed normal karyotype.

Nineteen lesions from the 8 patients with histologic findings consistent with DFSP were studied by using FISH. In 14 lesions from 7 patients, fusion of the *COL1A1* and *PDGFB* loci was identified (Table I). FISH analysis of 4 lesions yielded equivocal results or failed because of insufficient cellularity of the samples, exhaustion of the lesional tissue, or possibly prolonged formalin exposure. In 1 patient (ADA7) no additional samples were available to study. In the other 3 cases (patients ADA12, ADA14, and ADA16) additional biopsy specimens were found to be positive for the *COL1A1-PDGFB* fusion signal. In patient ADA4, 2 CD34⁺ spindle cell lesions were studied by using FISH, only one of which was found to harbor fusion of the *COL1A1* and *PDGFB* loci. As expected, FISH results were negative for a *COL1A1-PDGFB* fusion in the 2 lesions studied from patients ADA6 and ADA9, in whom histologic findings did not support the diagnosis of DFSP (Table I).

Twenty lesions from the 8 patients with histologic diagnosis consistent with DFSP were studied by using RT-PCR for the presence of *COL1A1-PDGFB* fusion transcripts. Nine lesions from 6 patients returned positive results, including 1 lesion that had resulted in equivocal FISH findings (patient ADA7). On the contrary, RT-PCR returned negative results in 1 lesion from patient ADA5, who was given a diagnosis of being positive for the presence of fusion of the *COL1A1* and *PDGFB* loci by means of FISH. Noninformative RT-PCR results were obtained from 10 lesions of 5 patients. Of the 10 lesions, 4 had been found to harbor a *COL1A1-PDGFB* fusion by using FISH, and 1 was found to be negative by using FISH (ADA4, Table I). Discordant FISH and RT-PCR findings can be explained by differences in sampling, sensitivity, or both between the 2 technical approaches or possibly unusual molecular variants not detectable with the primer set or sets used.

In summary, 26 skin biopsy specimens were performed in 11 patients with ADA-SCID. A total of 22 lesions from 8 patients showed CD34⁺ spindle cell proliferation consistent with DFSP. The diagnosis of DFSP was unequivocally established in all 8 patients by means of cytogenetic testing, molecular testing, or both. Eight lesions were positive for the presence of the *COL1A1-PDGFB* fusion by using both FISH and RT-PCR, 6 were determined to be positive by using FISH and not informative or negative by using RT-PCR, and 1 was determined to be positive by using RT-PCR but equivocal by using FISH. Importantly, in those cases in which multiple lesions from a single patient were studied, different breakpoints were identified by means of sequence analysis, thus indicating independently originating events (data not shown).

DISCUSSION

We report here the association of 2 rare conditions: ADA-SCID and DFSP. Of 12 patients studied, 8 had DFSP, and in 7 patients the tumor had multicentric presentation.

The occurrence of DFSP in patients with ADA-SCID is remarkable in several ways. At diagnosis, our cohort of affected patients had an average age of 8.9 years (range, 2-22 years), which points to ADA-SCID as a condition that predisposes to childhood presentation of DFSP, a rare occurrence in itself. More remarkable still is the finding of multiple DFSP lesions in 7 (87.5%) of 8 patients. In addition, most DFSP lesions presented in the preprotuberant morpheaform plaque stage, which might be overlooked by parents and clinicians alike. Finally, the histology

of lesions differed from that of classic DFSP by the absence of the classic storiform histologic pattern. Only in one of the 8 patients in whom DFSP was diagnosed did the lesion present as a protuberant nodule and show the typical microscopic storiform features (Figs 1, C, and 2, B and E).

Two case reports have described single DFSP and GCF lesions in isolated cases of ADA-SCID.^{19,20} A third case has been reported in abstract form.²¹ However, our observations are unprecedented because they establish a marked increase in the incidence of such tumors in patients with ADA-SCID. The previous lack of reporting of this finding might reflect underdiagnosis because of the benign appearance of the plaques, the very slow growth of the tumors, and the limited life expectancy of children with ADA-SCID. In contrast to the previous reported cases, our series also demonstrates a remarkable phenomenon of multiple DFSP tumors (up to 15) in patients with ADA-SCID. The finding of different *COL1A1-PDGFB* junction breakpoints in different tumors argues that these lesions are multiple primary malignant events and not cutaneous metastases. No correlations were noted between locations of the breakpoints and the clinical/pathologic features of the tumors.

The natural history of DFSP tumors in the setting of ADA-SCID is unknown. Most of the lesions found in our patients were atrophic plaques rather than nodular tumors at the time of diagnosis and lacked the classic storiform histology. One potential explanation is that lesions were detected earlier than typical DFSP because of the heightened scrutiny of our patient population.

Our findings are relevant to the clinical management of ADA-SCID and should prompt physicians to have a high clinical suspicion for DFSP. Importantly, small DFSP lesions were difficult to distinguish from small scars and areas of benign macular pigmentation. Furthermore, the atypical histology of DFSP in the setting of ADA-SCID might lead to histologic misdiagnosis in the absence of clinical correlation. Our observations that not all DFSP lesions were positive by using conventional cytogenetics, FISH, and RT-PCR suggest that a combination of immunohistochemical, cytogenetic, and/or molecular studies should be considered to rule out DFSP in any spindle cell lesion identified in patients with ADA-SCID.

The high frequency of a rare tumor in patients with a separate rare disease argues for the existence of a common pathophysiologic mechanism. Given the underlying immunodeficient state of patients with ADA-SCID, one possible hypothesis would be an infectious cause of DFSP tumors. Although we cannot formally rule out this possibility, results of preliminary investigations of *in situ* hybridization using degenerate primers targeting herpes virus sequences were negative. Similarly, electron microscopic studies of biopsy samples did not detect viral particles (data not shown). In addition, we have not observed any correlation between the immunologic and metabolic status of our patients at the time of screening or the type of therapeutic intervention used to treat their underlying ADA deficiency and the occurrence of DFSP. However, it is worth noting that because it was not possible to clearly define the time of occurrence of the tumors in our patients, the immunologic and metabolic details of the subjects at the time of tumor appearance remain unknown. Of note, occurrence and relapse of DFSP have been reported after successful HCT and immunologic correction with PEG-ADA,¹⁹⁻²¹ which allows us to postulate that DFSP can develop in ADA-deficient patients despite successful therapeutic intervention.

TABLE I. Patients' characteristics and skin findings

Patient identifier	Age at screening/diagnosis of DFSP; sex	Prior therapies	Treatment at screening or diagnosis of skin lesions	Skin lesions (no.)	Size range (mm)	Lesion(s) biopsied
ADA1	22 y; F	PEG-ADA Genetically corrected autologous lymphocytes	PEG-ADA	Slightly raised right lower leg plaque (1)	11	1
ADA3	12 y; M	T cell-depleted haploidentical BMT	PEG-ADA	Atrophic plaques (4)	4-11	3
ADA4	9 y; M	T cell-depleted haploidentical BMT	PEG-ADA	Atrophic plaques (5)	3-10	2
ADA5	7 y; M	PEG-ADA	PEG-ADA	Atrophic plaques (2)	5-7	1
ADA7	5 y; M	T cell-depleted haploidentical BMT	PEG-ADA	Atrophic plaques (12)	3-16	1
ADA12	12 y; F	Genetically corrected autologous BMT	PEG-ADA	Left groin subcutaneous lump/nodule (1) Atrophic plaques (7)	NA 0.5-20	1 2
ADA14	2 y; F	PEG-ADA	Genetically corrected autologous BMT	Atrophic plaques (14)	2-14	6
ADA16	2 y; M	Genetically corrected autologous BMT	Genetically corrected autologous BMT	Left groin lump (1) Atrophic plaques (5)	10 3-11	1 3
ADA6	7 y; M	PEG-ADA T cell-depleted haploidentical BMT	PEG-ADA	Atrophic plaques (2)	5-6	1
ADA9	4 y; M	PEG-ADA	PEG-ADA	Slightly raised plaque	8	1
ADA10	3 y; M	PEG-ADA	Genetically corrected autologous BMT	Papules (2)	3	2
ADA13	28 y; F	PEG-ADA Genetically corrected autologous lymphocytes Genetically corrected autologous BMT	PEG-ADA	None	NA	NA

BM, Bone marrow; BMT, bone marrow transplantation; NA, not applicable.

TABLE I. (Continued)

Location, size	Histologic findings	Karyotype	FISH	RT-PCR	Final diagnosis
Right lower leg, slightly raised plaque, 10 × 11 mm	CD34 ⁺ spindle cell lesion	Not done	<i>COL1A1-PGFB</i> fusion signal	<i>COL1A1-PDGFB</i> fusion transcript	DFSP
Right lower leg, depressed plaque, 11 × 6 mm	CD34 ⁺ spindle cell lesion	Not done	Not done	Not informative	
Left upper abdomen, depressed plaque, 6 × 4 mm	CD34 ⁺ spindle cell lesion	t(17;22)(q11;q13)	<i>COL1A1-PGFB</i> fusion signal	Not informative	DFSP
Right gluteal, 5 × 3 mm	CD34 ⁺ spindle cell lesion	Not done	<i>COL1A1-PGFB</i> fusion signal	Not informative	DFSP
Right anterior thigh, depressed plaque, 8 × 4 mm	CD34 ⁺ spindle cell lesion	Normal	Negative	Not informative	
Left axillary depressed plaque, 7 × 5 mm	CD34 ⁺ spindle cell lesion	t(17;22)(q11;q13)	<i>COL1A1-PGFB</i> fusion signal	<i>COL1A1-PDGFB</i> fusion transcript	DFSP
3 depressed plaques, 3-10 mm	Clinically suspicious for DFSP. No biopsy	NA	NA	NA	
Right inner arm plaque, 5 × 5 mm	CD34 ⁺ spindle cell lesion	t(17;22)(q11;q13)	<i>COL1A1-PGFB</i> fusion signal	Negative	DFSP
Right lower abdomen, slightly depressed plaque, 7 × 4 mm	Clinically suspicious for DFSP; no biopsy	NA	NA	NA	
Right lateral abdomen, depressed plaque, 13 × 10 mm	CD34 ⁺ spindle cell lesion	Not done	Equivocal	<i>COL1A1-PDGFB</i> fusion transcript	DFSP
11 depressed plaques from 3-16 mm	Clinically suspicious for DFSP. No biopsy	NA	NA	NA	
Left inguinal subcutaneous fibrotic lump, size not available	GCF	Not done	Not informative	Not informative	GCF
Right lateral chest wall, depressed plaque, 10 × 12 mm	CD34 ⁺ spindle cell lesion	Not done	Not done	Not done	
Right anterior shoulder, 0.6 × 0.5 mm	CD34 ⁺ spindle cell lesion	t(17;22)(q11;q13)	<i>COL1A1-PGFB</i> fusion signal	<i>COL1A1-PDGFB</i> fusion transcript	DFSP
5 flat to depressed plaques, 3-20 mm	Clinically suspicious for DFSP; no biopsy	NA	NA	NA	DFSP
Right chest wall nodule within a depressed plaque, 5 × 5 mm	CD34 ⁺ spindle cell lesion	t(17;22)(q11;q13)	<i>COL1A1-PGFB</i> fusion signal	Not informative	DFSP
Medial right buttock, flat to slightly raised plaque, 13 × 8 mm	CD34 ⁺ spindle cell lesion	t(17;22)(q11;q13)	<i>COL1A1-PGFB</i> fusion signal	<i>COL1A1-PDGFB</i> fusion transcript	DFSP
Right upper abdomen, depressed plaque, 10 × 5 mm	CD34 ⁺ spindle cell lesion	Not done	<i>COL1A1-PGFB</i> fusion signal	Not informative	DFSP
Right lower abdomen, depressed, 8 × 3 mm	CD34 ⁺ spindle cell lesion	Not done	Equivocal	Not informative	
Left abdomen (flank), depressed plaque, 8 × 3 mm	CD34 ⁺ spindle cell lesion	Not done	<i>COL1A1-PGFB</i> fusion signal	<i>COL1A1-PDGFB</i> fusion transcript	DFSP
Left calf, depressed plaque, 10 × 10 mm	CD34 ⁺ spindle cell lesion	Not done	<i>COL1A1-PGFB</i> fusion signal	<i>COL1A1-PDGFB</i> fusion transcript	DFSP
Left waistband, violaceous soft flat plaque, site of BM, 14 × 5 mm	Clinically suspicious for DFSP; no biopsy	NA	NA	NA	
8 depressed plaques from 2-8 mm	Clinically suspicious for DFSP; no biopsy	NA	NA	NA	
Left thigh, subcutaneous lump, 10 × 10 mm	GCF	Not done	<i>COL1A1-PGFB</i> fusion signal	<i>COL1A1-PDGFB</i> fusion transcript	GCF
Right inner arm, depressed plaque, 5 × 3 mm	CD34 ⁺ spindle cell lesion	t(17;22)(q11;q13)	<i>COL1A1-PDGFB</i> fusion signal	Not informative	DFSP
Right abdominal wall, depressed plaque, 5 × 4 mm	CD34 ⁺ spindle cell lesion	Not done	Not informative	Not informative	
Left supraclavicular depressed plaque, 9 × 3 mm	CD34 ⁺ spindle cell lesion	Not done	<i>COL1A1-PDGFB</i> fusion signal	<i>COL1A1-PDGFB</i> fusion transcript	DFSP
2 depressed plaques, right lateral upper thigh, 6 × 4 mm and midsuprapubic, 11 × 5 mm	Clinically suspicious for DFSP; no biopsy done	NA	NA	NA	
Right buttock	Dermal spindle cells, mildly CD34 ⁺	Normal	Negative	Not informative	Paucicellular spindle cell lesion
Right thigh	Dermal fibrosis	Normal	Negative	Not done	Scar
2 papules, right shin, left hand	Large aggregates of mast cells	Not done	Not done	Not done	Mastocytoma
					NA



FIG 1. Clinical presentation of DFSP in patients with ADA-SCID. **A**, Flat plaque (ADA12). **B**, Depressed plaque (ADA7). **C**, Slightly raised plaque (ADA1; see also Fig 2, *B*, *E*, and *H*). **D**, Subcutaneous nodule (ADA16; see also Fig 2, *C*, *F*, and *I*).

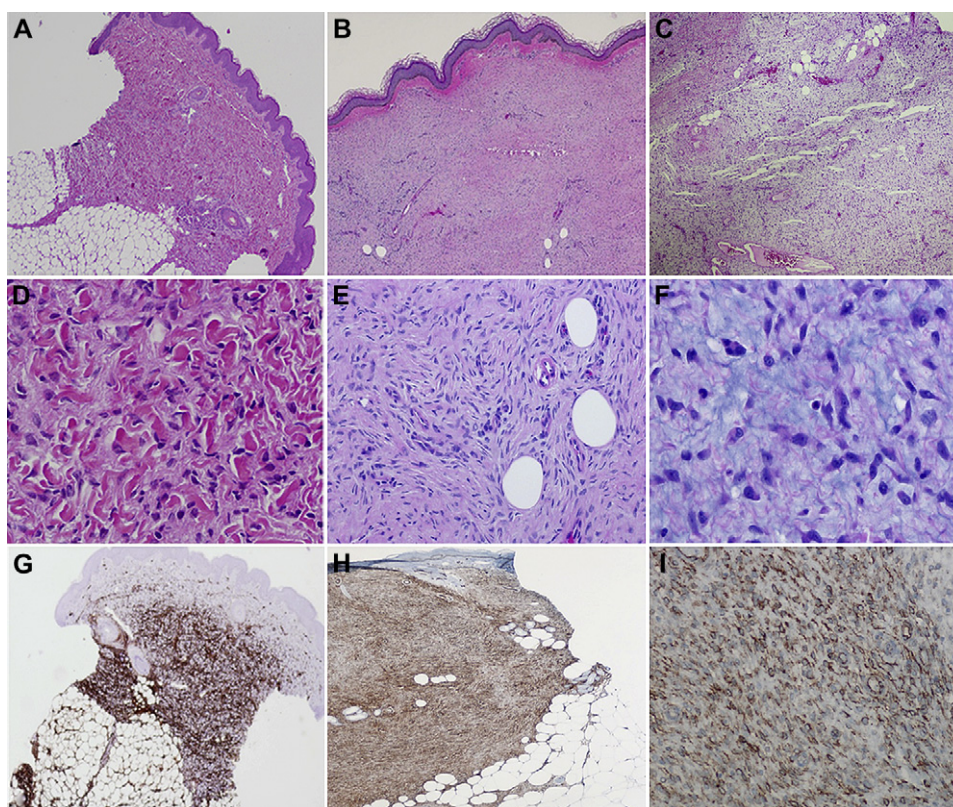


FIG 2. Histologic features of DFSP in patients with ADA-SCID. **A** and **D**, Low cellularity from a depressed plaque (ADA14). **B** and **E**, Storiform pattern from a raised plaque (ADA1). **C** and **F**, Vascular spindle cell lesion in a myxoid background with floret cells characteristic of GCF (ADA16). **G-I**, CD34 stain decorating spindle cells in all 3 lesions.

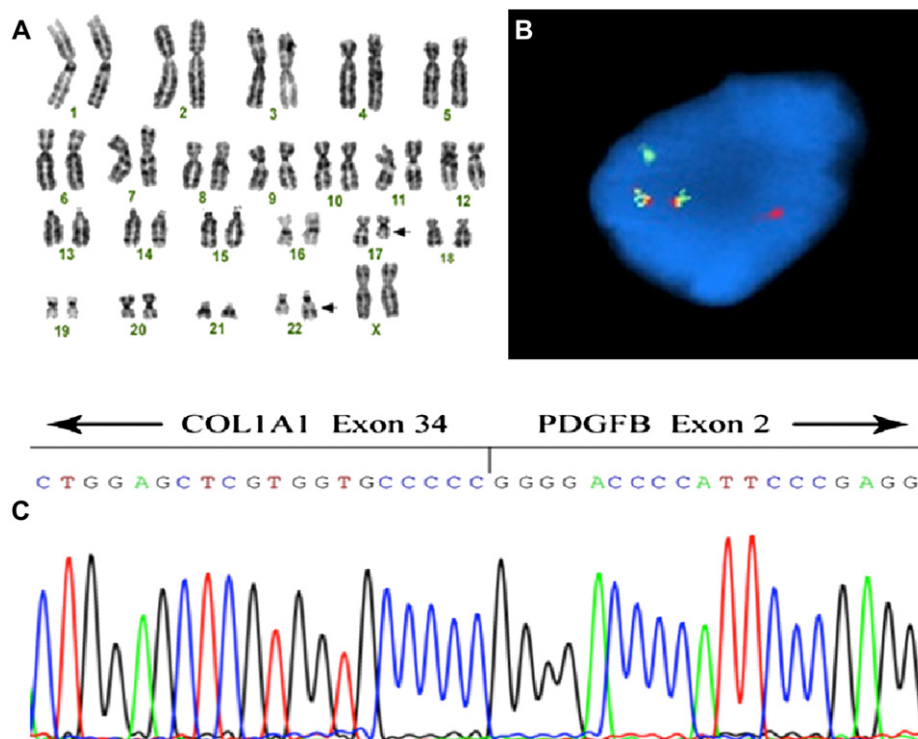


FIG 3. Molecular characteristics of DFSP in patients with ADA-SCID. **A**, Karyotyping from a lesion (ADA14) showing t(17;22)(q22;q13). **B**, FISH of a lesion (ADA16) revealing 2 fused red/green signals consistent with the presence of the *COL1A1*-*PDGFB* fusion. **C**, Sequence of the RT-PCR product from a lesion (ADA7) showing the junction of the *COL1A1* and *PDGFB* transcripts.

An alternative explanation is that the presence of DFSP in our patients is related to the metabolic consequences of ADA deficiency. ADA deficiency causes increased concentrations of adenosine and deoxyadenosine in the plasma of affected patients.^{1,2} Detailed information regarding adenosine/deoxyadenosine levels in tissues of patients with ADA-SCID is not available; however, studies in ADA-deficient mice have shown increased adenosine and deoxyadenosine levels in multiple organs, including the thymus, spleen, liver kidney, lung, and skin.^{22,23} In these models increased tissue adenosine levels have been associated with pulmonary and dermal fibrosis.^{22,24,25} Interestingly, a number of profibrotic mediators, including TGF- β 1, IL-16, IL-13, connective tissue growth factor, and PDGF-AA, are significantly increased in skin homogenates from ADA-deficient mice.²² Although there are no reports of a general increase in dermal fibrosis in patients with ADA deficiency, it can be postulated that patients with ADA-SCID might be exposed to the fibrogenic effects of adenosine in the skin, as well as in other organs. Therapeutic interventions that replace ADA activity, such as HCT, enzyme replacement therapy, and gene therapy, are expected to decrease the concentrations of ADA substrates. In fact, administration of PEG-ADA has been shown to prevent and reverse lung fibrosis in mice.²⁵ Data on adenosine tissue levels in human subjects treated with PEG-ADA are not available. However, plasma adenosine levels have been reported to remain high after HCT,^{1,26} which could lead to the presence of a profibrotic milieu in patients with ADA-SCID both before and after the implementation of measures aimed at the correction of their immune function.

It is also important to note that deoxyadenosine is known to cause single-strand DNA breaks and that deoxyadenosine

triphosphate accumulation can interfere with DNA repair mechanisms.²⁷ Therefore it is possible that the increased propensity for DNA strand breaks in ADA deficiency coupled with a fibrogenic dermal environment creates unique conditions amenable to the development of fibrous tumors, such as DFSP.

In conclusion, we show that DFSP is commonly found in patients with ADA-SCID. The association of the 2 disorders and the frequent multicentricity of DFSP are previously unreported observations and have important implications for further studies of the pathogenesis and pathophysiology of both diseases.

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Clinical implications: Patients affected by ADA deficiency should be screened for the presence and followed for the possible occurrence of DFSP.

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METHODS

Cytogenetic and FISH analyses

Conventional cytogenetic analysis was performed on representative fresh tissue according to standard procedures of chromosomal preparations. For each case, 15 to 25 Giemsa-trypsin-Giemsa-banded metaphase cells were analyzed. FISH analysis was performed either on cytologic touch preparations or on formalin-fixed, paraffin-embedded tissue sections by using a homebrew dual-color (a spectrum orange-labeled *COL1A1* probe and a spectrum green-labeled *PDGFB*), dual-fusion probe set that spans the *COL1A1* and *PDGFB* loci and has been validated for use in the clinical laboratory of the University of Nebraska Medical Center.^{E1} Hybridization signals were assessed in 100 to 200 interphase nuclei; strong well-delineated signals were independently scored by 2 subjects. An interphase cell specimen was interpreted as abnormal if juxtaposed *COL1A1-PDGFB* probe set signals were detected in greater than 10% of cells (>2 SDs greater than the average false-positive rate). Images were acquired with the CytoVision Image Analysis System (Applied Imaging, Santa Clara, Calif).

RNA extraction and RT-PCR

RNA extraction from either two 20- μ m paraffin-embedded representative tumor tissue sections or from 3 to 6 unstained formalin-fixed, paraffin-

embedded tissue sections was carried out with the Optimum FFPE RNA Isolation Kit (Ambion Diagnostics, Austin, Tex), according to the manufacturer's instructions. RT-PCR to detect *COL1A1-PDGFB* fusion transcripts was performed by using sets of specific *COL1A1* forward primers and a single *PDGFB* reverse primer, as previously described.^{E2} PCR products were sequenced with the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit and the ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, Calif). Sequence and breakpoint analyses were performed by using BLAST sequence similarity searches.

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