Autoantibodies to Dense Fine Speckles in Pediatric Diseases and Controls

Heinrike Schmeling, Michael Mahler, Deborah M. Levy, Katharine Moore, Anne M. Stevens, James Wick, Jacob D. McMillan, Gerd Horneff, Shervin Assassi, Julio Charles, Gloria Salazar, Maureen D. Mayes, Earl D. Silverman, Marissa Klien-Gitelman, Tzelan Lee, Hermine I. Brunner, Ann M. Reed, and Marvin J. Fritzler

ABSTRACT. ***Objective.*** Autoantibodies to the dense fine speckled 70 kDa antigen (DFS70) are reported to be more common in individuals who do not have an antinuclear antibody (ANA)-associated rheumatic disease (AARD) than in patients with AARD. The frequency of anti-DFS70 antibodies has been thoroughly studied in adult but not in pediatric populations. The primary objective of this observational study was to determine the frequency of anti-DFS70 in pediatric AARD and reference cohorts.

Methods. Sera from 743 children with AARD and related conditions, and 345 samples from reference cohorts (healthy children and those being investigated for AARD) were studied for anti-DFS70 autoantibodies as measured by a chemiluminescence immunoassay. A de-identified administrative database was used to retrieve demographic, serologic, and clinical data.

Results. Anti-DFS70 antibodies were seen in 2.1% of healthy children and in 4.5% of sera from pediatric individuals referred for ANA testing. The frequency of anti-DFS70 was highest in juvenile localized scleroderma (LS; 4/29, 13.8%), juvenile dermatomyositis (JDM; 2/11, 18.2%), childhood systemic lupus erythematosus (cSLE; 19/331, 5.7%), diffuse cutaneous systemic sclerosis (1/22, 4.5%), celiac disease (2/49, 4.1%), and juvenile idiopathic arthritis (JIA; 5/202, 2.5%). Of note, anti-DFS70 antibodies were observed in 3/26 children (11.5%) with uveitis and JIA-associated uveitis.

Conclusion. The frequency of anti-DFS70 autoantibodies in healthy pediatric subjects is within the lower range of that reported in adults. Anti-DFS70 antibodies can be found in childhood SSc and cSLE, but has a remarkably high frequency in children with LS, JDM, and uveitis. (J Rheumatol First Release October 15 2015; doi:10.3899/jrheum.150567)

Key Indexing Terms:
AUTOANTIBODIES ANTINUCLEAR ANTIBODIES DENSE FINE SPECKLES
AUTOIMMUNE DISEASES PEDIATRICS

From the Department of Paediatrics, Alberta Children's Hospital, and McCaig Institute for Bone and Joint Health, and Faculty of Medicine, University of Calgary; Alberta Children's Hospital Research Institute, Calgary, Alberta; Hospital for Sick Children; University of Toronto, Toronto, Ontario, Canada; Inova Diagnostics Inc., San Diego, California; Stanford University/Lucile Packard Children's Hospital, Stanford, California; Seattle Children's Research Institute, Department of Pediatrics, University of Washington, Seattle, Washington; Division of Rheumatology, University of Texas Houston Medical School, Houston, Texas; Northwestern University/Lurie Children's Hospital, Chicago, Illinois; University of Cincinnati; Cincinnati Children's Hospital and Medical Center, Cincinnati, Ohio; Department of Pediatrics, Duke University, Durham, North Carolina, USA; Department of General Paediatrics, Centre of Paediatrics and Neonatology, Asklepios Clinics, Sankt Augustin, Germany.

Funded and supported by resources from Mitogen Advanced Diagnostics Laboratory, the Dawson Jarrock Research Award (Calgary, Alberta, Canada) administered by the Alberta Children's Foundation/Alberta Children's Hospital Research Institute, and funds allocated to the Arthritis Society Research Chair by the University of Calgary, Division of Rheumatology, University of Calgary. M. Fritzler is a paid consultant, has received honoraria or gifts in kind from ImmunoConcepts Inc. and Inova Diagnostics Inc. M. Mahler is an employee of Inova Diagnostics Inc., a manufacturer of autoantibody diagnostic kits.

H. Schmeling, MD, Department of Paediatrics, Alberta Children's Hospital, University of Calgary, and Alberta Children's Hospital Research Institute, and McCaig Institute for Bone and Joint Health; M. Mahler, PhD, Inova Diagnostics Inc.; D.M. Levy, PhD, Hospital for Sick Children, and University of Toronto; K. Moore, MD, Seattle Children's Research Institute, Department of Pediatrics, University of Washington; A.M. Stevens, MD, PhD, Seattle Children's Research Institute, Department of Pediatrics, University of Washington; J. Wick, BSc, Faculty of Medicine, University of Calgary; J.D. McMillan, Faculty of Medicine, University of Calgary; G. Horneff, MD, Department of General Paediatrics, Centre of Paediatrics and Neonatology, Asklepios Clinics; S. Assassi, MD, Division of Rheumatology, University of Texas Houston Medical School; J. Charles, BSc, MSc, Division of Rheumatology, University of Texas Houston Medical School; G. Salazar, MD, Division of Rheumatology, University of Texas Houston Medical School; M.D. Mayes, MD, MPH, Division of Rheumatology, University of Texas Houston Medical School; E.D. Silverman, MD, Hospital for Sick Children, and University of Toronto; M. Klien-Gitelman, MD, MPH, Northwestern University/Lurie Children's Hospital; T. Lee, MD, Stanford University/Lucile Packard Children's Hospital; H.I. Brunner, MD, University of Cincinnati, and Cincinnati Children's Hospital and Medical Center; A.M. Reed, MD, Department of Pediatrics, Duke University; M.J. Fritzler, MD, PhD, Faculty of Medicine, University of Calgary.

Address correspondence to Dr. M.J. Fritzler, Cumming School of Medicine, University of Calgary, 3330 Hospital Dr. NW, Calgary, Alberta T2N 4N1, Canada. E-mail: fritzler@ucalgary.ca

Accepted for publication July 30, 2015.

In 1994, Ochs, *et al* first reported autoantibodies that had an antinuclear antibody (ANA) indirect immunofluorescence (IIF) pattern described as dense fine speckles (DFS) in patients with interstitial cystitis[1]. Initially, the target antigen was identified and named "lens epithelium-derived growth factor" (LEDGF)[2]. Because sera with the DFS ANA IIF pattern were shown to bind a 70 kDa protein in immunoblots, the target autoantigen was named DFS70[3,4]. Today, both names are used interchangeably with the main difference being that in the context of autoimmunity, DFS70 is the most commonly used name, whereas in the context of human immunodeficiency virus, cancer, and other diseases, the preferred name is LEDGF[2]. Within a few years, anti-DFS70 antibodies were reported in a variety of other conditions[3,4,5], including atopic dermatitis[5], prostate cancer[6], and even in healthy individuals[4,7]. In a study of 25,516 consecutive sera referred for an ANA test to an Italian laboratory, 0.8% (n = 172) were positive for anti-DFS by IIF, and in a cohort of 334 patients with neoplastic diseases, the prevalence was 1.8%[8]. A study of 597 sera by Watanabe, *et al*[9] found a frequency of 10.7% in healthy Japanese subjects compared with a frequency of only 1.5% in 200 patients with ANA-associated rheumatic disease [AARD; childhood-onset systemic lupus erythematosus (cSLE), childhood-onset systemic sclerosis (cSSc), Sjögren syndrome (SS), idiopathic inflammatory myopathies (IIM), and mixed connective tissue disease]. In another landmark study, Dellavance, *et al* used IIF and immunoblotting techniques to show that 34% of 13,641 ANA-positive sera had anti-DFS70 antibodies, but the anti-DFS70–positive individuals had no evidence of an AARD, although autoimmune thyroiditis was particularly common[10]. Moreover, a subsequent report noted that none of the anti-DFS70–positive healthy individuals developed AARD over an average of 4 years of clinical followup[11]. Although the spectrum of clinical associations of anti-DFS70 antibodies continues to widen, there is emerging consensus that in adults, anti-DFS70 antibodies are more prevalent in apparently healthy individuals than in patients with AARD[3,9,12]. Based on these observations and a report of anti-DFS70 antibodies in an 8-year-old female with acute poststreptococcal glomerulonephritis[13], it has been suggested that the presence of isolated anti-DFS70 antibodies could be used as a biomarker to help exclude the diagnosis of AARD, such as SLE[2,7,9,11,12,14].

To date, studies on anti-DFS70 antibodies have almost exclusively been in adults. A report by Kuwabara, *et al* described an autoantibody, initially referred to as anti-Sa, that recognized DFS70 in 40% of ANA-positive children with chronic fatigue syndrome (CFS) and other complaints[15]. A subsequent study of 21 Japanese children that fulfilled CFS criteria demonstrated a homogeneous and speckled ANA IIF pattern of staining consistent with anti-DFS70 reactivity[16]. Immunoblotting confirmed that 85.7% of those samples and only 6% of ANA-positive sera from children with fibromyalgia reacted with a 70 kDa protein presumed to be DFS70. While these findings are remarkable, they have not been confirmed in other studies, and the frequency of anti-DFS70 antibodies is not reported in healthy children or children with other conditions, particularly AARD[17].

To our knowledge, our report is the first observational study of children with the primary goal of determining the prevalence of anti-DFS70 antibodies in a spectrum of childhood AARD and related diseases. We also examined sera from children referred to a diagnostic laboratory for autoantibody testing [i.e., ANA and extractable nuclear antigens (ENA)]. We used a highly specific chemiluminescence immunoassay (CIA) to detect anti-DFS70 antibodies and specific diagnostic autoantibody assays useful in clinical investigations of the various diseases.

MATERIALS AND METHODS

Ethics. Our study was approved by the University of Calgary Conjoint Health Research Ethics Board (Ethics ID#: E22534), the Hospital for Sick Children (Toronto, Ontario, Canada) Research Ethics Board (Approval #1000037383), the Seattle Children's Hospital internal review board (#11671), and the Division of Rheumatology, University of Texas at Houston. Under the terms of this approval, all patient information was de-identified prior to analysis, precluding the requirement of written informed consent. All clinical investigation was conducted according to the Declaration of Helsinki.

Patient samples and study population. In accord with previous publications[18], the age range for children in our study was set at 18 years and younger. Plasma or serum from the children were collected at 9 pediatric medical centers and analyzed for anti-DFS70 and AARD-related autoantibodies. Children from Germany came from a geographical area of minimal immigration (all children were German) whereas the Calgary, Seattle, Houston, and Toronto cohorts represented a mixed ethnicity group. Sera from children who had 4 or more American College of Rheumatology or Systemic Lupus International Collaborating Clinics criteria for SLE[19,20] were classified as cSLE. Sera from children with SSc were diagnosed according to international criteria for that disease[21]. All children with juvenile idiopathic arthritis (JIA) met the International League Against Rheumatism classification for JIA[22]. Patients classified as localized scleroderma (LS) had subtypes of morphea or linear scleroderma. The diagnosis associated with sera derived from other conditions and reference cohorts are identified in Table 1. Sera were also obtained at the time of routine blood workup from children with confirmed autoimmune diseases who were followed at the Pediatric Rheumatology Clinic at the Alberta Children's Hospital (Calgary, Alberta, Canada) or were referred for autoantibody testing (i.e., ANA and ENA) to Mitogen Advanced Diagnostics Laboratory (Calgary, Alberta, Canada; www.mitogen.ca). The samples referred for autoantibody testing were called "query connective tissue disease" (qCTD). Reference cohort sera were from healthy children who were recruited by word of mouth and determined not to have autoimmune diseases as self-reported on questionnaires, which included yes/no questions regarding thyroid disease and celiac disease. Children were grouped into pre- (< 8 yrs), transition (8–13 yrs), and postpubertal (> 13 yrs). All sera in our study were de-identified, aliquoted, and stored at –80°C or –20°C until analysis.

Our study population of 743 (Table 1) included 200 sera (18.3%) referred for ANA testing (qCTD) to the diagnostic laboratory or were healthy children (n = 145, 13.3%). There were 383 children (51.5%) who had an AARD: cSLE (n = 331, 51.3%), juvenile dermatomyositis (JDM; n = 11, 1.5%), and SSc [n = 41, 6.4%; 19 limited cutaneous SSc (lcSSc) and 22 diffuse cutaneous SSc]. The others had JIA and JIA/uveitis (n = 202, 31.3%), celiac disease (n = 49, 7.6%), or other inflammatory diseases (n = 65, 6.0%).

Table 1. Frequency of anti-DFS70 antibodies in pediatric cohorts.

Cohort	n	DFS70-positive, n	DFS70-positive, %	p†
Reference cohorts, n = 345				
ANA referrals, qCTD*	200	9	4.5	NS
Healthy	145	3	2.1	NA
AARD, n = 383				
cSLE	331	19	5.7	NS
JDM	11	2	18.2	NS
Diffuse cSSc	22	1	4.5	NS
Limited cSSc	19	0	0.0	NS
Other inflammatory conditions, n = 360				
JIA without uveitis	183	3	1.6	NS
JIA with uveitis	19	2	10.5	NS
Arthralgia	32	0	0.0	NS
Celiac disease	49	2	4.1	NS
Localized scleroderma	29	4	13.8	0.0310
Idiopathic uveitis	7	1	14.3	NS
Reactive arthritis	21	0	0.0	NS
Other**	20	0	0.0	NS

* Sera referred for an ANA/ENA test as part of the investigations for qCTD. Sera were from children ≤ 18 years of age. ** Cases of Crohn disease, Reiter syndrome, panniculitis, sarcoidosis, primary antiphospholipid syndrome, viral myopathy, eosinophilic fasciitis. † P value is calculated against healthy individuals by Student t test. DFS70: dense fine speckled 70 kDa antigen; ANA: antinuclear antibody; AARD: ANA-associated rheumatic disease; qCTD: query connective tissue disease (children being investigated for an AARD); cSLE: childhood systemic lupus erythematosus; cSSc: childhood systemic sclerosis; JIA: juvenile idiopathic arthritis; NA: not applicable; NS: not statistically significant; JDM: juvenile dermatomyositis.

ANA and ENA testing. ANA, anti-DFS70, AARD-related autoantibodies, and anticyclic citrullinated protein antibodies (ACPA) testing were performed by Mitogen Advanced Diagnostics Laboratory as previously described[12,23,24]. ANA testing included pattern and titer assessment by IIF on HEp-2 cell substrates (HEp-2000, ImmunoConcepts Inc.) at initial screening serum dilutions of 1/40 and 1/160[25]. Samples were tested for other autoantibodies primarily based on their diagnostic categories: cSLE samples were tested for ENA [chromatin, ribosomal P, Sm, U1RNP, SS-A/Ro60, Ro52/TRIM21, SS-B/La, Scl-70 (topoisomerase I), Jo1 (histidyl tRNA synthetase)] by addressable laser bead immunoassay (FIDIS, TheraDiag) and dsDNA by the *Crithidia luciliae* IIF test (ImmunoConcepts)[26]. Sera from patients with cSSc were tested by an SSc line immunoassay (LIA; Euroline Scleroderma Assay, Euroimmun GmbH), which includes testing for centromere protein (CENP)-A, -B, PM/Scl-75, -100, RP-11, -155, topoisomerase I/Scl-70, antinucleolar organizer NOR90, Th/To, Ku, and platelet-derived growth factor receptor. Anti-SP100 antibodies were detected by LIA (Euroimmun). ACPA in JIA sera was tested by ELISA (QUANTA Lite, CCP3, Inova Diagnostics Inc.) with protocols and cutoff values (absorbance units; AU) as recommended by the manufacturer.

Antibodies to DFS70 were detected by CIA (QUANTA Flash DFS70, Inova Diagnostics Inc.)[12,27]. The CIA uses recombinant DFS70/LEDGF coated onto paramagnetic beads designed for the BIO-FLASH instrument, a previously described diagnostic assay platform[12,28]. The assay values, expressed as chemiluminescence units (CU), are proportional to the amount of isoluminol conjugate that is bound to the human immunoglobulin G, which is proportional to the number of anti-DFS70 antibodies bound to the antigen on the beads. The cutoff for a positive test, established at 20 CU, was based on the comparison to a DFS70 ELISA yielding the highest percentage agreement between the assays.

Data analysis. The data were evaluated using the Analyse-it software (Version 1.62; Analyse-it Software Ltd.). Positive and negative likelihood ratios were calculated for the individual autoantibodies. For statistical analysis, patients were grouped into AARD and non-AARD. Fisher's exact test was used to analyze differences between groups, and p values < 0.05 were considered significant.

RESULTS

Frequency of anti-DFS70 antibodies in different pediatric cohorts. Anti-DFS70 antibodies as detected by CIA were seen in 2.1% of the 145 healthy children and in 4.5% of the 200 sera from the qCTD cohort (Table 1). In sera that had ANA testing done, there was a high agreement (91.0%) of anti-DFS70 antibodies as detected by CIA and the DFS IIF ANA pattern on HEp-2 cells (Figure 1). In the cohorts with an established diagnosis, the highest frequency of anti-DFS70 antibodies was observed in juvenile LS (4/29, 13.8%), cSLE (19/331, 5.7%), JIA with uveitis (2/19, 10.5%), and JDM (2/11, 18.2%; Table 1). The difference in prevalence was significant for LS versus healthy children (Student t test, p = 0.0310), but not for the other cohorts (potentially attributable to the small cohort sizes). Anti-DFS70 antibodies were also seen in sera of celiac disease (2/49, 4.1%), JIA without uveitis (3/183, 1.6%), and idiopathic uveitis (1/7, 14.3%). When a separate analysis of sera from children with uveitis (idiopathic or JIA/uveitis) was done, a high frequency of anti-DFS70 antibodies was seen (3/26, 11.5%), but this was not statistically different from that of the healthy children. When analyzing the anti-DFS70 antibody results in the context of ANA and the DFS pattern, 55/200 (27.5%) of qCTD sera had a positive ANA and 9 (4.5%) of these had anti-DFS70 antibodies. Further,

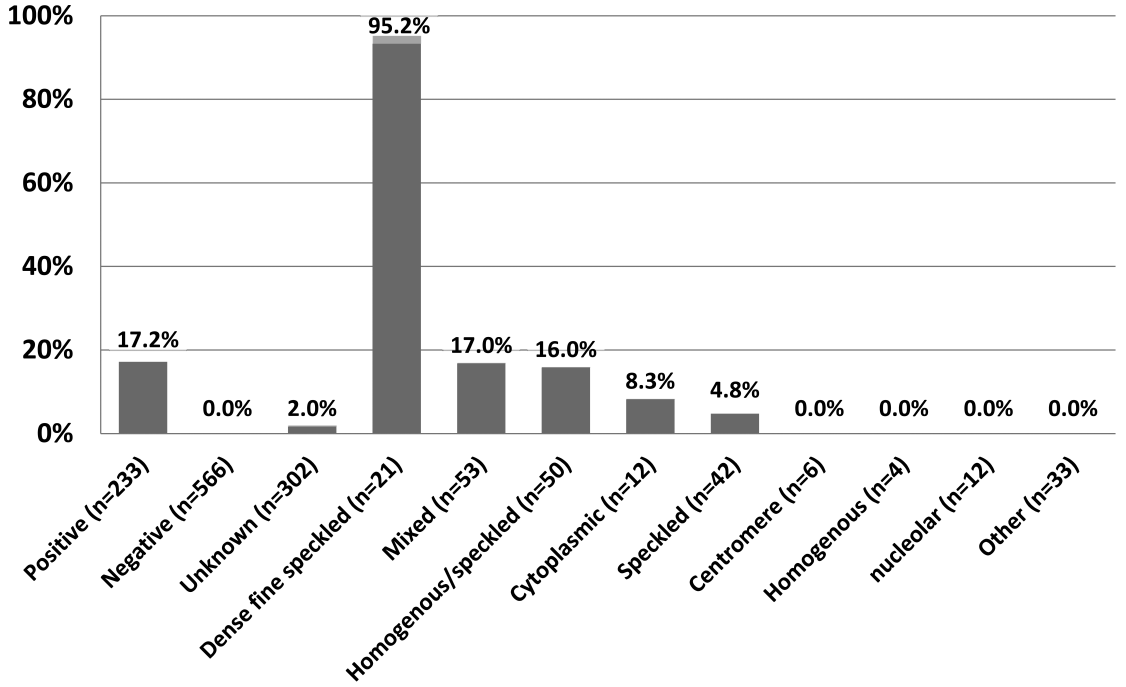

Figure 1. Prevalence of anti-DFS70 autoantibodies as detected by CIA in samples grouped according to the ANA IIF patterns on HEp-2 cell substrates. DFS70: dense fine speckled 70 kDa antigen; CIA: chemiluminescence immunoassay; ANA: antinuclear antibody; IIF: indirect immunofluorescence.

9/55 (16.4%) of those that were ANA-positive had anti-DFS70.

Demographic associations (Table 2). The majority (64.8%) of children in the entire cohort were white, 18.9% were Asian, and the remainder were from a variety of other racial extractions. The ethnicity of the anti-DFS70 antibody-positive group was not significantly different from the negative group (data not shown). Sex information was available for 1007 individuals, of whom 43 (4.3%) had anti-DFS70 antibodies. The prevalence of anti-DFS70 antibody positivity among females (31/699, 4.4%) and males (12/308, 3.9%) was similar (p = not significant). Among the anti-DFS70 antibody-positive children with a recorded age, the age range was 4–18 years and the median age was 13.2 years, which was not statistically different from the anti-DFS70 antibody-negative group (14.1 yrs, p = 0.1893). However, the prevalence of anti-DFS70 antibodies was higher in prepubertal as compared with postpubertal children (10.0% vs 4.2%, p = 0.0415; Supplementary Figure 1 is available from the authors on request).

Serologic associations (Table 2). In the cSLE group, 6/19 (31.6%) of anti-DFS70 antibody-positive samples had no other autoantibodies detected in the routine serological tests (i.e., ENA profile for cSLE, SSc LIA for cSSc). Among the patients with cSLE with additional autoantibodies, anti-U1RNP antibodies were the most commonly observed specificity (11/13, 84.6%), whereas anti-dsDNA antibodies were only seen in 3/19 (15.8%). In the single anti-DFS70 antibody-positive sample from a patient with diffuse cSSc, antibodies to CENP-B were also detected. By comparison, 2/4 (50%) of the samples from localized patients with SSc had ANA and 1/4 (25%) had a multiple nuclear dots pattern, but no detectable antibodies to SP100. Of interest, of the 9 anti-DFS70 antibody-positive sera referred because of qCTD, 2 had another autoantibody detected: 1 anti-dsDNA and 1 antinucleolar organizer (NOR90, human upstream binding factor) antibodies.

DISCUSSION

To our knowledge, our study is the first published study of anti-DFS70 antibodies in pediatric AARD, a spectrum of pediatric diseases, pediatric individuals with qCTD referred for ANA/ENA testing, and healthy children. A rapidly growing body of literature in adults indicates that anti-DFS70 antibodies are frequently observed in ANA-positive individuals who have no evidence of an AARD, including apparently healthy people[10,11,12,27,29]. Explanations for the decreased prevalence of anti-DFS70 autoantibodies in adult AARD are unclear, but may relate to sociodemographic, genetic, therapeutic[30], and/or technical variables[4]. It should be noted that the presence of these autoantibodies does not necessarily imply the absence of disease because they have also been reported in a variety of atopic and inflammatory conditions and in children with chronic fatigue syn-

Table 2. Overview of patients with anti-DFS70 antibodies.

Patient No.	Diagnosis	Age, yrs	Sex	Racial Group	DFS70 Reactivity by CIA, CU	ANA Pattern/ titer by IIF	ENA/LIA/other Autoantibodies*
1	JIA	10	M	White	36.3	NT	Negative
2	JIA	13	M	White	49.9	NT	Negative
3	JIA	13	F	White	27.9	DFS 1/320	Negative
4	JIA uveitis	6	F	White	131.5	DFS 1/5120	Negative
5	JIA uveitis	9	M	Asian	38.2	DFS 1/1280	Negative
6	Uveitis	16	M	White	78.2	DFS 1/2560	Negative
7	cSLE	15	F	White	212.0	DFS 1/1280	Sm, U1RNP weak positive
8	cSLE	16	F	White	138.4	DFS 1/1280	dsDNA, U1RNP
9	cSLE	18	F	Asian	88.6	DFS, MND	dsDNA, U1RNP
10	cSLE	10	F	Mixed	140.1	DFS 1/2560	Sm, U1RNP, Ro52
11	cSLE*	15	F	White	59.1	DFS 1/2560	U1RNP, weak dsDNA
12	cSLE	14	F	Black	45.6	DFS, NLR, cytoplasmic 1/1280	U1RNP, Ro60, weak Rib P, Ro52
13	cSLE	13	F	Asian	39.1	DFS 1/320	Negative
14	cSLE*	15	F	White	81.5	DFS, NLR 1/1280	U1RNP
15	cSLE	13	F	Asian	30.7	DFS 1/2560	Sm, U1RNP, Ro60
16	cSLE	7	F	Hispanic	71.1	DFS 1/2560	Sm, U1RNP, Ro60, Ro52, weak SS-B
17	cSLE	12	F	White	194.0	DFS 1/2560	Negative
18	cSLE	14	M	White	312.3	DFS 1/2560	Negative
19	cSLE	5	F	Asian	140.7	DFS 1/1280	Negative
20	cSLE	18	F	Asian	232.1	DFS 1/320	U1RNP
21	cSLE	17	F	Unknown	> 450.8	DFS 1/5120	Low dsDNA
22	cSLE	16	M	Unknown	209.6	DFS 1/1280	Negative
23	cSLE	16	F	White	22.1	Cytoplasmic 1/160	Negative
24	cSLE	16	F	Asian	26.7	Homo, NLR 1/1280	Low U1RNP
25	cSLE	15	F	Asian	35.6	DFS 1/640	Low U1RNP
26	LS	8	M	White	120.5	DFS 1/640	Negative
27	LS	14	F	White	60.1	MND, NLR, CS 1/1280	Negative
28	LS	15	M	Mixed	35.9	DFS 1/320	Negative
29	LS	12	F	Mixed	57.2	DFS, NLR 1/1280	Negative
30	cdSSc	18	F	White	67.8	DFS, CENP 1/1280	CENP-B
31	JDM	6	F	Asian	408.3	Negative	Negative
32	CD	12	F	White	51.4	DFS 1/1280	Anti-tTG–positive
33	CD	14	M	White	67.1	DFS 1/1280	Anti-tTG–negative
34	qCTD	6	M	White	115.8	DFS 1/2560	Negative
35	qCTD	7	F	White	72.8	DFS 1/5120	Negative
36	qCTD	13	F	White	286.3	DFS 1/5120	Anti-dsDNA
37	qCTD	7	F	White	79.4	DFS 1/5120	Negative
38	qCTD	14	F	White	326.9	DFS 1/5120	Negative
39	qCTD	5	M	White	87.6	DFS 1/640	Negative
40	qCTD	10	M	White	23.5	DFS 1/160	Negative
41	qCTD	7	F	White	80.3	DFS 1/640	Negative
42	qCTD	9	F	White	32.3	DFS 1/1280	NOR90
43	Healthy	9	M	White	21.7	DFS	NT
44	Healthy	7	F	Asian	59.1	MND	NT
45	Healthy	14	F	White	26.1	DFS	Negative

* Patients 11 and 14 are identical twins. DFS70: dense fine speckled 70 kDa antigen; CIA: chemiluminescence immunoassay; CU: chemiluminescence units; ANA: antinuclear antibody; IIF: indirect immunofluorescence; ENA: extractable nuclear antigens; LIA: line immunoassay; JIA: juvenile idiopathic arthritis; cSLE: childhood systemic lupus erythematosus; LS: localized scleroderma (morphea, linear scleroderma); cdSSc: childhood diffuse cutaneous systemic sclerosis; JDM: juvenile dermatomyositis; CD: celiac disease; qCTD: query connective tissue disease, children being investigated for an AARD; AARD: ANA-associated rheumatic disease; NT: not tested; MND: multiple nuclear dots; NLR: nucleolar; CS: centrosome; CENP: centromere protein; tTG: tissue transglutaminase.

drome[2,4,29]. In our study, the frequency of anti-DFS70 autoantibodies in pediatric healthy individuals was low (2.1%). This is consistent with the low end of the range reported in adults, where the frequency of anti-DFS70 antibodies has reportedly ranged from 2–10%[10,12,27,29]. In 2 published studies, up to 15% of positive ANA test results were attributed to anti-DFS70 antibodies[12,29]. In our study, 55/200 (27.5%) of qCTD sera had a positive ANA and 9/200 (4.5%) had anti-DFS70 antibodies. Further, 9/55 (16.4%) of those who were ANA-positive had anti-DFS70. By comparison, in an Italian study, anti-DFS70 autoantibodies were detected in only 0.8% of sera referred for ANA testing[8],

which is in contrast to a study of Brazilian adult sera in which it was reported that 37% (5081/13,641) of ANA-positive samples had anti-DFS70 antibodies[10]. A recent study reported that 4% of adult sera in a Canadian ANA-positive cohort referred for evaluation by a rheumatologist had anti-DFS70 antibodies[31].

Of interest, Sperotto, *et al* found the prevalence and persistence of ANA (titer ≥ 1:80) among 261 prepubertal Italian school children to be 12.3%, and 44.8% among postpubertal children[32]. In the Sperotto, *et al* study, the dramatic increase in postpubertal ANA prevalence and titers, especially in females, had no correlation with chronic non-inflammatory musculoskeletal pain. Unfortunately, the investigators did not test for anti-DFS70 antibodies, leaving open the question whether the increase in ANA positivity can be attributed to hormonal factors in postpubertal children[17]. Our study suggests that anti-DFS70 antibodies are not a common confounding ANA test result in pediatric samples referred for ANA and/or ENA testing (qCTD cohort) and that their presence is not associated with the age or the sex (females 4.4%, males 3.9) of the children. However, in contrast to the Sperotto, *et al* study, in which the prevalence of ANA significantly increased with age from pre- to postpuberty[32], the frequency of anti-DFS70 antibodies in our cohort was lower in post- compared with prepubertal sera.

Based on studies in adults, it has been suggested that the presence of anti-DFS70 antibodies is a useful biomarker that helps to rule out the diagnosis of AARD such as SLE, SSc, mixed connective tissue disease, SS, and IIM[2,29]. In a study of 200 ANA-positive samples, 100 with DFS ANA IIF pattern and 100 with other ANA IIF patterns occurring in a hospital setting, only 13.4% with a DFS70 IIF pattern and anti-DFS70 antibodies as detected by CIA had an AARD[27]. Further, only 5.5% of these patients had an AARD when their sera were negative by an ANA screening ELISA. These observations indicated that although isolated (monospecific) anti-DFS70 antibodies cannot completely rule out the presence of an AARD, the likelihood is significantly lower than in patients with other autoantibodies and IIF patterns. Since not all samples in our study were tested for a wide spectrum of autoantibodies, we were not able to meaningfully determine whether monospecificity of anti-DFS70 antibodies differentiated between AARD and non-AARD in children as it does in adults. Thus, it appears that the interpretation of an anti-DFS70 result that excludes the diagnosis of an AARD in a pediatric setting needs to be taken with some caution until future studies have thoroughly and systematically analyzed the sera for a broad spectrum of autoantibodies in all AARD sera.

A recent case report[13] of a child with poststreptococcal disease is consistent with the prevailing paradigm that the presence of anti-DFS70–isolated autoantibodies can be used to rule out the diagnosis of systemic autoimmune rheumatic disease; it also points to other considerations of environmental factors that may be at play in the generation of anti-DFS70 B cell responses. In our study, the frequency of anti-DFS70 antibodies in related pediatric conditions was wide, ranging from 0% in lcSSc to a maximum of 18.2% in JDM. By comparison, 31.6% of the cSLE sera with anti-DFS70 reactivity had anti-DFS70 as the only detectable autoantibody (i.e., anti-dsDNA and ENA profile were negative). It should be noted that all anti-DFS70–positive cSLE sera were from a single clinical center, which suggests unanticipated differences in the demographic profiles, disease activity, or concomitant therapies. Because of the different sizes of the patient cohorts and the difference in age in our study, it remains unclear whether the different prevalence of anti-DFS70 antibodies in pre- versus postpubertal status is related to factors accompanying pubertal status or vice versa.

Although we segregated localized, diffuse cutaneous SSc, and lcSSc into separate clinical subsets, we did not segregate SLE or any of the other pathological conditions based on disease severity, clinical status, or concomitant therapies. The available evidence in adult studies to date is that the presence and titers of anti-DFS70 are relatively stable over time and do not fluctuate with immunosuppression or disease activity[10,29]. However, studies of inception cohorts of pediatric AARD and other conditions followed by longitudinal and clinical outcome studies are needed to determine whether this paradigm is consistent.

As noted earlier, some studies have suggested that anti-DFS70 antibodies may be associated with autoimmune thyroiditis[10], atopy[5], and chronic fatigue syndrome[15]. We did not systematically evaluate all children in our study for these conditions, although in the healthy children there was no self-reported evidence for these conditions. The association of anti-DFS70 with these conditions has not been apparent in numerous studies to date. In a study of 3263 sequential serum samples from patients with various diseases and healthy individuals[12], the prevalence of anti-DFS70 antibodies as measured by CIA was 8.9% in healthy individuals, 2.8% in SLE, 2.6% in rheumatoid arthritis, 4.0% in asthma, 5.0% in interstitial cystitis, 1.7% in Graves disease, and 6.0% in Hashimoto thyroiditis. Hence, the frequency of anti-DFS70 antibodies in autoimmune thyroid disease and atopic conditions do not appear to be higher than in apparently healthy individuals.

The higher frequency of anti-DFS70 antibodies in children with idiopathic uveitis and JIA with uveitis in our study is of particular interest because children with JIA and a positive ANA are known to be at risk to develop severe uveitis, which can lead to blindness if unchecked[33,34,35]. Antigens detected with commonly used immunoassays do not include the DFS70 antigen[36] and thus cannot be used to screen patients with JIA at potential risk for uveitis. Because the target autoantigen of anti-DFS70 antibodies was initially called LEDGF[3,7,29], the finding of a possible link between idiopathic uveitis and anti-DFS70 antibodies

is of particular interest. Accordingly, previous reports indicated that sera from Vogt–Koyanagi–Harada disease, an inflammatory condition associated with acute panuveitis, as well as other ocular pathologies and a cutaneous disorder, had the highest prevalence of anti-DFS70 antibodies (ranging from 67–100%)[37,38], suggesting that a strong association exists between anti-DFS70 antibodies and eye-related pathologies. Further, some studies[8] showed that a certain subset of anti-DFS70 patients had antibodies that reacted with ocular tissue. Speculation that this might be related to an alternatively spliced isoform of DFS70[2,3,5] has yet to be conclusively verified[8]. Because not all children with uveitis in our study had antibodies to DFS70, it seems unlikely that DFS70 can serve as a sensitive or specific biomarker for that condition. Nevertheless, given that the uveitis cohort is small, a frequency of ~10% (3/27) for anti-DFS70 antibodies in children with uveitis is of value because it is higher than that found in comparator pediatric cohorts.

Finally, it is important to comment on the technical aspects of anti-DFS70 antibody testing. It is noted that patients 5 and 15 had low CU values measured by CIA, but very high DFS IIF titers (Table 2). By comparison, patient 31 had a high CU value (> 400 CU) but a negative ANA IIF. It should be understood that there is no *a priori* reason to expect 100% agreement between 2 different immunoassays, namely CIA and IIF ANA. IIF ANA may represent the summation of reactivity to more than 1 autoantigen or epitope, such as an isoform of DFS70 or another component of the DFS macromolecular complex[2]. CIA, which used a purified recombinant DFS70 covalently bound to paramagnetic beads, detects autoantibodies to that specific protein, but not to any interacting partners of DFS70. Examples of such disparate findings between IIF patterns and other immunoassays are very well known. As only 1 example, < 60% of sera with high titer antiribosomal P antibodies as detected by single analytes immunoassays have the corresponding "classical" cytoplasmic pattern of staining[39]. That being said, the agreement between IIF and CIA for anti-DFS70 antibodies was > 90% in our study.

The frequency of anti-DFS70 antibodies in pediatric sera from apparently healthy children is within the lower range of that reported in adults. In childhood AARD and other conditions, the frequency of anti-DFS70 has a broad range, but in this clinical setting it was accompanied by another autoantibody in the majority of sera. The high frequency of anti-DFS70 antibodies in other conditions such as JDM, JIA with uveitis, and LS requires further multicenter studies with attention to demographic, racial, and environmental factors that may account for these findings. Longitudinal studies of anti-DFS70 antibodies are required to determine the relationship between the levels of these autoantibodies and disease activity or treatment compared with other autoantibodies typically found in AARD.

ACKNOWLEDGMENT

The authors acknowledge the technical support of Haiyan Hou, Jane Yang, Meifeng Zhang, and Danielle Schmidt. The HEp-2 slides were a gift from ImmunoConcepts Inc. (Sacramento, California, USA) and the antidense fine speckled 70 kDa antigen chemiluminescence immunoassay kits were a gift of Inova Diagnostics Inc. (San Diego, California, USA). Pediatric samples from Seattle were made possible through the generous efforts of the Childhood Arthritis and Rheumatology Research Alliance group.

REFERENCES

1. Ochs RL, Stein TW Jr, Peebles CL, Gittes RF, Tan EM. Autoantibodies in interstitial cystitis. J Urol 1994;151:587-92.
2. Ochs RL, Mahler M, Basu A, Rios-Colon L, Sanchez TW, Andrade LE, et al. The significance of autoantibodies to DFS70/LEDGFp75 in health and disease: integrating basic science with clinical understanding. Clin Exp Med 2015 Jun 19 (E-pub ahead of print).
3. Ganapathy V, Casiano CA. Autoimmunity to the nuclear autoantigen DFS70 (LEDGF): What exactly are the autoantibodies trying to tell us? Arthritis Rheum 2004;50:684-8.
4. Mahler M, Fritzler MJ. The clinical significance of the dense fine speckled immunofluorescence pattern on HEp-2 cells for the diagnosis of systemic autoimmune diseases. Clin Dev Immunol 2012;2012:494356.
5. Ochs RL, Muro Y, Si Y, Ge H, Chan EK, Tan EM. Autoantibodies to DFS 70 kd/transcription coactivator p75 in atopic dermatitis and other conditions. J Allergy Clin Immunol 2000;105:1211-20.
6. Daniels T, Zhang J, Gutierrez I, Elliot ML, Yamada B, Heeb MJ, et al. Antinuclear autoantibodies in prostate cancer: immunity to LEDGF/p75, a survival protein highly expressed in prostate tumors and cleaved during apoptosis. Prostate 2005;62:14-26.
7. Basu A, Sanchez TW, Casiano CA. DFS70/LEDGFp75: An enigmatic autoantigen at the interface between autoimmunity, AIDS, and cancer. Front Immunol 2015;6:116.
8. Bizzaro N, Tonutti E, Visentini D, Alessio MG, Platzgummer S, Morozzi G, et al. Antibodies to the lens and cornea in anti-DFS70-positive subjects. Ann N Y Acad Sci 2007;1107:174-83.
9. Watanabe A, Kodera M, Sugiura K, Usuda T, Tan EM, Takasaki Y, et al. Anti-DFS70 antibodies in 597 healthy hospital workers. Arthritis Rheum 2004;50:892-900.
10. Dellavance A, Viana VS, Leon EP, Bonfa ES, Andrade LE, Leser PG. The clinical spectrum of antinuclear antibodies associated with the nuclear dense fine speckled immunofluorescence pattern. J Rheumatol 2005;32:2144-9.
11. Mariz HA, Sato EI, Barbosa SH, Rodrigues SH, Dellavance A, Andrade LE. Pattern on the antinuclear antibody-HEp-2 test is a critical parameter for discriminating antinuclear antibody-positive healthy individuals and patients with autoimmune rheumatic diseases. Arthritis Rheum 2011;63:191-200.
12. Mahler M, Parker T, Peebles CL, Andrade LE, Swart A, Carbone Y, et al. Anti-DFS70/LEDGF antibodies are more prevalent in healthy individuals compared to patients with systemic autoimmune rheumatic diseases. J Rheumatol 2012;39:2104-10.
13. Fabris M, Zago S, Tosolini R, Melli P, Bizzaro N, Tonutti E. Anti-DFS70 antibodies: a useful biomarker in a pediatric case with suspected autoimmune disease. Pediatrics 2014;134:e1706-8.
14. Muro Y, Sugiura K, Morita Y, Tomita Y. High concomitance of disease marker autoantibodies in anti-DFS70/LEDGF autoantibody-positive patients with autoimmune rheumatic disease. Lupus 2008;17:171-6.
15. Kuwabara N, Itoh Y, Igarshi T, Fukunaga Y. Autoantibodies to lens epithelium-derived growth factor/transcription co-activator P75 (LEDGF/P75) in children with chronic nonspecific complaints and with positive antinuclear antibodies. Autoimmunity 2009;42:492-6.
16. Itoh Y, Shigemori T, Igarashi T, Fukunaga Y. Fibromyalgia and

chronic fatigue syndrome in children. Pediatr Int 2012;54:266-71.
17. Mahler M, Fritzler MJ. Antinuclear antibodies in children. J Rheumatol 2014;41:1260-2.
18. Mina R, Brunner HI. Update on differences between childhood-onset and adult-onset systemic lupus erythematosus. Arthritis Res Ther 2013;15:218.
19. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.
20. Petri M, Orbai AM, Alarcón GS, Gordon C, Merrill JT, Fortin PR, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum 2012;64:2677-86.
21. Zulian F, Woo P, Athreya BH, Laxer RM, Medsger TA Jr, Lehman TJ, et al. The Pediatric Rheumatology European Society/American College of Rheumatology/European League against Rheumatism provisional classification criteria for juvenile systemic sclerosis. Arthritis Rheum 2007;57:203-12.
22. Petty RE, Southwood TR, Manners P, Baum J, Glass DN, Goldenberg J, et al; International League of Associations for Rheumatology. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol 2004;31:390-2.
23. Hanly JG, Su L, Farewell V, Fritzler MJ. Comparison between multiplex assays for autoantibody detection in systemic lupus erythematosus. J Immunol Methods 2010;358:75-80.
24. Hudson M, Fritzler MJ, Baron M; Canadian Scleroderma Research Group (CSRG). Systemic sclerosis: establishing diagnostic criteria. Medicine 2010;89:159-65.
25. Tan EM, Feltkamp TE, Smolen JS, Butcher B, Dawkins R, Fritzler MJ, et al. Range of antinuclear antibodies in "healthy" individuals. Arthritis Rheum 1997;40:1601-11.
26. Stinton LM, Barr SG, Tibbles LA, Yilmaz S, Sar A, Benedikttson H, et al. Autoantibodies in lupus nephritis patients requiring renal transplantation. Lupus 2007;16:394-400.
27. Miyara M, Albesa R, Charuel JL, El Amri M, Fritzler MJ, Ghillani-Dalbin P, et al. Clinical phenotypes of patients with anti-DFS70/LEDGF antibodies in a routine ANA referral cohort. Clin Dev Immunol 2013;2013:703759.
28. Mahler M, Radice A, Yang W, Bentow C, Seaman A, Bianchi L, et al. Development and performance evaluation of novel chemiluminescence assays for detection of anti-PR3 and anti-MPO antibodies. Clin Chim Acta 2012;413:719-26.
29. Mahler M, Hanly JG, Fritzler MJ. Importance of the dense fine speckled pattern on HEp-2 cells and anti-DFS70 antibodies for the diagnosis of systemic autoimmune diseases. Autoimmun Rev 2012;11:642-5.
30. Muro Y, Ogawa Y, Sugiura K, Tomita Y. HLA-associated production of anti-DFS70/LEDGF autoantibodies and systemic autoimmune disease. J Autoimmun 2006;26:252-7.
31. Fitch-Rogalsky C, Steber W, Mahler M, Lupton T, Martin L, Barr SG, et al. Clinical and serological features of patients referred through a rheumatology triage system because of positive antinuclear antibodies. PLoS ONE 2014;9:e93812.
32. Sperotto F, Cuffaro G, Brachi S, Seguso M, Zulian F. Prevalence of antinuclear antibodies in schoolchildren during puberty and possible relationship with musculoskeletal pain: a longitudinal study. J Rheumatol 2014;41:1405-8.
33. Hu-Torres S, Foster CS. Disease of the year: juvenile idiopathic arthritis-differential diagnosis. Ocul Immunol Inflamm 2014; 22:42-55.
34. Heiligenhaus A, Heinz C, Edelsten C, Kotaniemi K, Minden K. Review for disease of the year: epidemiology of juvenile idiopathic arthritis and its associated uveitis: the probable risk factors. Ocul Immunol Inflamm 2013;21:180-91.
35. Zierhut M, Heiligenhaus A, deBoer J, Cunningham ET, Tugal-Tutkun I. Controversies in juvenile idiopathic arthritis-associated uveitis. Ocul Immunol Inflamm 2013;21:167-79.
36. Xu M, Roberts BB, Busby BA, Jack RM, Finn LS, Emery HM, et al. Evaluation of multiplex antinuclear antibody assay in pediatric patients. Lab Med 2007;38:671-5.
37. Yamada K, Senju S, Shinohara T, Nakatsura T, Murata Y, Ishihara M, et al. Humoral immune response directed against LEDGF in patients with VKH. Immunol Lett 2001;78:161-8.
38. Ayaki M, Ohoguro N, Azuma N, Majima Y, Yata K, Ibaraki N, et al. Detection of cytotoxic anti-LEDGF autoantibodies in atopic dermatitis. Autoimmun 2002;35:319-27.
39. Mahler M, Ngo JT, Schulte-Pelkum J, Luettich T, Fritzler MJ. Limited reliability of the indirect immunofluorescence technique for the detection of anti-Rib-P antibodies. Arthritis Res Ther 2008;10:R131.