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Epidermal Neurite Density in Skin Biopsies From Patients With Juvenile Fibromyalgia

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ABSTRACT. ***Objective.*** Fibromyalgia (FM) is defined by idiopathic, chronic, widespread musculoskeletal pain. In adults with FM, a metaanalysis of lower-leg skin biopsy demonstrated 45% pooled prevalence of abnormally low epidermal neurite density (END). END < 5th centile of the normal distribution is the consensus diagnostic threshold for small-fiber neuropathy. However, the clinical significance of END findings in FM is unknown. Here, we examine the prevalence of small-fiber pathology in juvenile FM, which has not been studied previously.

Methods. We screened 21 patients aged 13–20 years with FM diagnosed by pediatric rheumatologists. Fifteen meeting the American College of Rheumatology criteria (modified for juvenile FM) underwent lower-leg measurements of END and completed validated questionnaires assessing pain, functional disability, and dysautonomia symptoms. The primary outcome was proportion of FM patients with END < 5th centile of age/sex/race-based laboratory norms. Cases were systematically matched by ethnicity, race, sex, and age to a group of previously biopsied healthy adolescents with selection blinded to biopsy results. All 23 controls matching demographic criteria were included.

Results. Among biopsied juvenile FM patients, 53% (8/15) had END < 5th centile vs 4% (1/23) of healthy controls ($P < 0.001$). Mean patient END was 273/mm^2 skin surface (95% CI 198–389) vs 413/mm^2 (95% CI 359–467, $P < 0.001$). As expected, patients with FM reported more functional disability, dysautonomia, and pain than healthy controls.

Conclusion. Abnormal END reduction is common in adolescents with FM, with similar prevalence in adults with FM. More studies are needed to fully characterize the significance of low END in FM and to elucidate the clinical implications of these findings.

Key Indexing Terms: adolescent, epidermis/innervation, fibromyalgia, nerve fibers/pathology

Juvenile fibromyalgia (JFM) is characterized by persistent, widespread musculoskeletal pain, fatigue, and other somatic symptoms[1]. It affects 2–6% of school-aged children, predominantly adolescent females[2]. Patients often report impaired physical, school, social, and emotional functioning[3]. The causes of fibromyalgia (FM) are unknown, and the relative contributions of central vs peripheral neurological abnormalities are debated. Brain imaging studies show altered regional blood flow and gray matter volume, changed activation and connectivity of pain processing regions[4], and altered levels of several neurotransmitters[5]. However, no primary brain pathology has been identified as causal, and similar abnormalities are seen in various acute and chronic pain conditions. These central findings may be nonspecific responses to changed peripheral input, pain, deconditioning, mood, or medications[6].

Small-fiber neuropathy (SFN) is a peripheral polyneuropathy that, like FM, causes chronic widespread pain, exertional intolerance, gastrointestinal symptoms, and chronic headaches[7,8].

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Common causes in mature adults include diabetes, chemotherapy, inflammation, and dysimmunity, with genetic variants and other toxins being rare causes[8]. SFN is characterized by electrical hyperactivity and distal degeneration of the peripheral neurons that mediate pain, heat and cold sensation, and internal autonomic functions. Electrophysiologists report excess, spontaneous, and prolonged firing of C and Aδ pain fibers in adult FM and SFN[9,10].

Measuring epidermal neurite density (END) within protein gene product 9.5 (PGP9.5)-immunolabeled lower-leg skin biopsies has become standard for confirming suspected cases of SFN[8]. A systematic review of early skin biopsy studies of adult FM generated 45% pooled prevalence of pathological biopsies (95% CI 32–59%)[11]. A subsequent study of 117 skin-biopsied adults with FM identified correlations between severity of multiple FM symptoms and extent of cutaneous denervation, and documented abnormal proximal as well as distal skin biopsies in some patients[10]. Nerve conduction studies have also identified correlations between low medial plantar and sural nerve amplitudes, and low END in adult FM[12]. Brain imaging studies show reduced functional connectivity between the anterior cingulate, amygdala, and precuneus in SFN, with severity paralleling skin denervation, supporting the hypothesis that SFN can produce postsynaptic brain effects similar to those reported in FM[13]. Given the increasing importance of skin biopsies in adult FM, we undertook this novel study to examine END in JFM.

MATERIALS AND METHODS

Patients with FM aged 13–20 years were recruited at the Rutgers-Robert Wood Johnson and Columbia University Medical Centers between December 2016 and November 2018. Inclusion for screening required clinical diagnosis by a pediatric rheumatologist. Patients with disorders associated with SFN were excluded. This study was approved by the institutional review boards of the participating centers (Rutgers IRB Pro20160000631, Columbia IRB AAAR3311). Participants over 18 years provided consent; those below 18 years provided assent plus parental permission.

Participants who did not meet the 2010 American College of Rheumatology (ACR) FM diagnostic criteria (modified for JFM) were excluded[1]. Included participants completed validated surveys of pain, functional disability, and dysautonomic symptoms. Self-reported pain during the prior week was assessed by a 0–10 numeric rating scale (0 = no pain; 10 = worst pain). We administered the Functional Disability Inventory (FDI), a 60-point self-report scale with higher scores indicating more disability[14]. Dysautonomia symptoms were assessed by Composite Autonomic Symptom Score 31 (COMPASS 31), with a 0–100 range encompassing orthostatic, vasomotor, secretomotor, gastrointestinal, bladder, and pupillomotor symptoms; higher scores indicate worse symptoms[15]. COMPASS 31 is validated in adolescent as well as adult SFN[16]. Symptom duration was calculated as months between symptom onset and skin biopsy.

Participants underwent 3-mm skin punch biopsy from the standard site above the lateral malleolus. Biopsies mailed in Zamboni's fixative to the Massachusetts General Hospital (MGH) clinically accredited neuropathology lab were processed, PGP9.5-immunolabeled, and analyzed according to clinical standards. MGH is one of very few diagnostic labs with skin biopsy data from healthy children and adolescents, providing pediatric normative values. All biopsies were blindly evaluated by the same morphometrist. MGH reports END as per mm^2 skin surface area to control for section thickness variability between laboratories; the correction factor is 20 for comparing to reports from laboratories that use 50 μm sections but report linear END.

We constructed the control group by screening the 65 previously biopsied healthy controls between 13–20 years of age. Thirteen were excluded for unobtainable ethnicity. The remaining 52 were evaluated for inclusion using a nonbiased algorithm that matched race, ethnicity, sex, and age of the JFM cohort. Matching was blind to all other results including skin biopsies. To match race and ethnicity, every eligible Black, Hispanic, and Asian control was included, then all other males were excluded to match the 95% female JFM group; this yielded 23 healthy controls. With MGH IRB approval (1999P009042/MGH), all 23 healthy controls were recontacted and invited to complete the study questionnaires.

The primary outcome was END < 5th centile of age/sex/race-based norms. Survey question outcomes were secondary. After verifying normality, categorical outcomes were summarized by proportions and continuous outcomes by means. Fisher exact test compared the prevalence of abnormal results of categorical variables, *t*-test compared means of continuous variables, and Wilcoxon rank-sum test compared non-normal variables.

RESULTS

Twenty-one adolescents with clinical JFM completed the surveys, and the 18 meeting ACR diagnostic criteria were offered skin biopsies. Fifteen underwent uncomplicated biopsies; 3 declined. The JFM and control samples were well matched by sex (7% vs 9% male, $P = 0.99$), age (17.2 vs 17.9 yrs, $P = 0.13$), ethnicity (Hispanic: 4 vs 3, $P = 0.4$), and race (Black: 2 vs 3 Black, $P = 0.99$; Asian: 1 vs 0). Ten of the 23 healthy controls completed surveys; 13 did not respond.

Fifty-three percent (8/15, 95% CI 26–79%) of JFM participants had END < 5th centile of the age/sex/race-based normal distribution vs 4% (1/23, 95% CI 0–22%) of healthy controls ($P < 0.01$; Figure 1). Mean END among participants with FM was 273/mm^2 skin surface (95% CI 198–389) vs 413/mm^2 (95% CI 359–467) for controls ($P < 0.001$). JFM participants had markedly higher median pain scores (7 vs 1), FDI (33 vs 1), and COMPASS 31 (49 vs 14) than healthy controls (Table 1), and no controls had scores consistent with FM. There were no evident differences between JFM participants with END above vs below the 5th centile with respect to median age (17 vs 17 yrs), symptom duration (26 vs 21 months), pain scores (7 vs 7), FDI (33 vs 33), or COMPASS 31 scores (43 vs 53).

DISCUSSION

To our knowledge, this case-control study provides the first prevalence data for small-fiber pathology in adolescents with FM, with the 53% prevalence of abnormally low END comparable to the 45% pooled prevalence in adult patients[11]. Juvenile and adult FM share many clinical characteristics, and most adolescents and young adults continue to experience symptoms into adulthood, suggesting a common spectrum of disease.

These objective neuropathologic findings may have potential for guiding treatment and measuring posttreatment axonal regeneration[8]. Some investigators hypothesize that undiagnosed SFN is a cause of FM symptoms[10], whereas others believe peripheral changes in FM to be epiphenomena of centralized chronic pain[17]. Many patients with SFN have presentations that do not resemble FM, including exclusively distal or autonomic symptoms, and small-fiber pathology can develop in

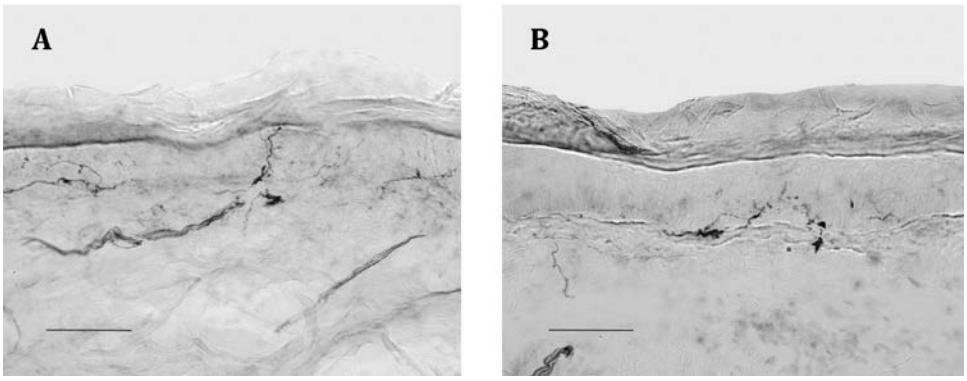

Figure 1. Representative skin biopsies from 10 cm above the lateral malleolus, bright-field, 40×. Scale bar = 100 μm. (A) Healthy control: Biopsy from 17.9-year-old White female with END of 500/mm² skin surface area (at the 64.3 percentile of the age, sex, and race-adjusted normal distribution). (B) Juvenile fibromyalgia patient: Biopsy from a 17.8-year-old White patient with END of 242/mm² skin surface area (at the 4.7 centile of predicted). END: epidermal neurite density.

Table 1. Characteristics of patients with JFM and healthy controls.

Characteristic	JFM Patients, n = 15	Healthy Controls, n = 23	*P*
Age, yrs, median (IQR)	17.2 (15.7–18.2)	17.9 (16.3–19.3)	0.13
Female sex, n (%)	14 (93)	21 (91)	0.99
Race/ethnicity, n (%)			
Hispanic	4 (26)	3 (13)	0.4
Black	2 (13)	3 (13)	0.99
Asian	1 (7)	0	
Skin biopsy data			
END < 5th centile, n (%)	8 (53)	1 (4)	< 0.001
END, neurite/mm², mean ± SD	273 ± 149	413 ± 132	< 0.001
Surveys, median (IQR)	n = 15	n = 10	
Pain score	7 (6–8)	1 (0–2)	< 0.001
FDI	33 (26–39)	1 (0–4)	< 0.001
COMPASS 31			
Total	49 (41–59)	14 (3–19)	< 0.001
Orthostatic intolerance	24 (22–32)	12 (0–19)	< 0.001
Vasomotor	0 (0–3)	0 (0–0)	0.08
Secretomotor	6 (4–10)	0 (0–0)	< 0.001
Gastrointestinal	12 (8–16)	1 (0–3)	0.001
Bladder	1 (0–2)	0 (0–0)	0.03
Pupilomotor	3 (2–3)	0 (0–0)	< 0.001

COMPASS 31: Composite Autonomic Symptom Score 31; END: epidermal neurite density; FDI: Functional Disability Inventory; JFM: juvenile fibromyalgia.

other neurodegenerative conditions, suggesting that decreased END in patients with FM could be an incidental, nonspecific finding[18].

A strength of our study is the strict inclusion criteria requiring clinical diagnosis of JFM plus best-available diagnostic criteria[1]. Our results corroborate previous reports of abnormal skin biopsies and autonomic function testing in youth with more loosely defined chronic widespread pain, in whom JFM diagnoses were not captured[19,20]. Given barriers to recruiting healthy children for research requiring invasive procedures, another strength of our study is the availability of comparator biopsies from demographically matched healthy controls. This approach contrasts with a recent publication that used a convenience control sample of younger children undergoing skin biopsies for other chronic neuromuscular diseases[20].

Our study's main limitation was a lack of neurologist evaluations to determine which JFM patients met criteria for SFN. Questionnaires and other metrics for diagnosing and tracking SFN should be validated in children, along with forthcoming research diagnostic criteria for adult SFN[7,16]. Also, our small sample may not have fully represented the JFM population, providing an inexact population prevalence. Controls were recruited at different times and from a different geographic region than JFM participants, though they were demographically matched. Another limitation is that the healthy controls' participation in surveys was limited, and their surveys were

completed at different times than skin biopsies. The survey results from controls, however, reflected values expected from healthy youth. Finally, the small sample size limited our ability to compare JFM patients with vs without abnormal END. Despite this, we still observed striking differences in END between cases and controls that were highly consistent with studies of adults with FM.

In summary, our study offers the first description of END measurements in JFM, to our knowledge. Our data demonstrate abnormally low END in half of JFM participants, in marked contrast to the expected 5% prevalence in healthy adolescents. These findings parallel those from adult FM patients. More research is needed to fully characterize the significance of END findings in FM and to elucidate the clinical implications of these findings.

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