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Subepidermal Schwann cell counts correlate with skin innervation – an exploratory study

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Abstract

Introduction/Aims: Schwann cell clusters have been described at the murine dermis-epidermis border. We quantified dermal Schwann cells in the skin of patients with small-fiber neuropathy (SFN) compared with healthy controls to correlate with the clinical phenotype.

Methods: Skin punch biopsies from the lower legs of 28 patients with SFN (11 men, 17 women; median age, 54 [range, 19-73] years) and 9 healthy controls (five men, four women, median age, 34 [range, 25-69] years) were immunoreacted for S100 calcium-binding protein B as a Schwann cell marker, protein-gene product 9.5 as a pan-neuronal marker, and CD207 as a Langerhans cell marker. Intraepidermal nerve fiber density (IENFD) and subepidermal Schwann cell counts were determined.

Results: Skin samples of patients with SFN showed lower IENFD ($P < .05$), fewer Schwann cells per millimeter ($P < .01$), and fewer Schwann cell clusters per millimeter ($P < .05$) than controls. When comparing SFN patients with reduced (n = 13; median age, 53 [range, 19-73] years) and normal distal ($n = 15$, median age, 54 [range, 43-68] years) IENFD, the number of solitary Schwann cells per millimeter ($p < .01$) and subepidermal nerve fibers associated with Schwann cell branches ($P < .05$) were lower in patients with reduced IENFD. All three parameters correlated positively with distal IENFD ($P < .05$ to $P < .01$), whereas no correlation was found between Schwann cell counts and clinical pain characteristics.

Discussion: Our data raise questions about the mechanisms underlying the interdependence of dermal Schwann cells and skin innervation in SFN. The temporal course and functional impact of Schwann cell presence and kinetics need further investigation.

KEYWORDS
innervation, intraepidermal nerve fiber density, neuropathic pain, Schwann cell, skin punch biopsy, small-fiber neuropathy

Abbreviations: BSA, bovine serum albumin; CD207, Langerin, marker of Langerhans cells; DAPI, 4′,6-diamidino-2-phenylindole; ErbB4, erb-B2 receptor tyrosine kinase 4; IENFD, intraepidermal nerve fiber density; PBS, phosphate-buffered saline; PGP, protein-gene product; ROI, region of interest; S100B, S100 calcium-binding protein B; SFN, small-fiber neuropathy.

1 | INTRODUCTION

Small-fiber neuropathies (SFNs) are a subgroup of painful sensory neuropathies with predominant impairment of the thinly myelinated A-delta and unmyelinated C nerve fibers.[1] The clinical phenotype of SFN is characterized by acral burning pain and par-/dysesthesias, whereas large nerve fiber functions remain preserved. The etiology of SFN is diverse: Although most cases remain idiopathic at first examination,[2,3] acquired and hereditary forms can be distinguished, with impairment of glucose metabolism being one of the most frequent reasons for acquired SFN.[4] Although a diagnostic "gold standard" is missing, a typical pain history with signs of small-fiber impairment on neurological examination and evidence of skin denervation may help to make the diagnosis.[3]

Although histologically classified as thinly myelinated and unmyelinated, A-delta and C nerve fibers are ensheathed by nonmyelinating Schwann cells, which do not generate a compact myelin, but form Remak bundles in the dermis.[5,6] When entering the epidermis, A-delta and C nerve fibers lose their Schwann cell ensheathment and are surrounded by keratinocytes.[7,8]

Reduced skin innervation is one morphological hallmark in subgroups of patients with SFN, but intraepidermal nerve fiber density (IENFD) may also be normal.[3,9,10] Although the exact mechanisms of small nerve fiber degeneration in SFN remain to be elucidated, the recent description of Schwann cells at the dermis-epidermis border of transgenic mice and in human skin has attracted attention.[11] In contrast to previous reports,[7,8] the authors proposed that these Schwann cells accompany and nurture subepidermal and epidermal nerve fibers.[11,12] Furthermore, the studies suggest that cutaneous Schwann cells may also be involved in the development of neuropathic pain and lead to the manifestation of SFN in transgenic animal models.[11,12] In this context, structural and functional disturbance of Schwann cells may cause progressive small-caliber nerve fiber degeneration and sensory neuropathy.[13,14] These findings indicate an interdependence between Schwann cells and nerve fibers in skin, as has been shown for the central and peripheral nervous systems.[15-17] Reduced IENFD is also found in hereditary neuropathies based on Schwann cell deficiency, such as Charcot-Marie-Tooth disease type 1.[18]

In this exploratory study, we aimed to determine whether Schwann cell counts differ between patients with SFN and healthy controls, and whether they correlate with the clinical pain phenotype.

2 | METHODS

2.1 | Subjects and baseline characterization

In this exploratory study, we included randomly selected patients with SFN, recruited as part of a large study[3] between 2015 and 2019. As described in previous work, patients were included if they reported symptoms indicative of SFN (eg, acral, localized and/or widespread pain and/or dys-/paresthesias, and autonomic dysfunction) and if there was no evidence of polyneuropathy or any other neurological disease during neurological examination and nerve conduction studies. We further enrolled healthy controls who reported no neurological diseases or neuropathic pain. Controls underwent neurological examinations and nerve conduction studies of the sural nerve that did not show evidence of polyneuropathy or other neurological disorders. The study was approved by the ethics committee of the University of Würzburg Medical Faculty (#135/15) and study subjects gave written informed consent for participation.

2.2 | Skin punch biopsy

2.2.1 | Skin innervation

Skin punch biopsies were taken from the right lateral lower leg and were processed to determine the IENFD, as described elsewhere.[19] In brief, 40-μm cryosections were immunoreacted with antibodies against the pan-neuronal marker protein-gene product (PGP) 9.5 (1:1000; Zytomed, Berlin, Germany). Cy3 was used as fluorescent secondary antibody (1:100; Dianova, Hamburg, Germany). IENFD was determined following standardized counting rules[20] by an investigator blinded to subject allocation. For IENFD quantification, a fluorescence microscope (Axiophot 2; Zeiss, Oberkochen, Germany) equipped with a 14.2 Color Mosaic camera (Diagnostic Instruments, Sterling Heights, Michigan) and SPOT software (Diagnostic Instruments) was used. To categorize IENFD as normal or reduced, we compared data with the normative values from our laboratory based on 180 healthy controls (124 women; median age, 50 [range, 20-84] years; and 56 men; median age, 53 [range, 22-76] years) in skin biopsies obtained from the lower leg (women: $n = 109$; men: $n = 46$).

2.2.2 | Assessment of Schwann cells

For the investigation of skin Schwann cells, we used 40-μm cryosections. Immunohistochemical triple-staining was performed by applying the following antibodies: anti–PGP 9.5 (1:200; Bio-Rad, Hercules, California) as a neuronal marker, anti–S100 calcium-binding protein B (S100B; 1:200; Abcam, Cambridge, UK) as a marker for Schwann cells, and anti-CD207 (1:500; Novus Biologicals, Centennial, Colorado) as a marker for Langerhans cells. After blocking (10% bovine serum albumin [BSA]/phosphate-buffered saline [PBS]), sections were first incubated with the primary antibodies in 1% BSA/PBS with 0.3% Triton overnight at room temperature and then with appropriate fluorescent secondary antibodies (Cy3-conjugated rabbit anti-mouse, 1:100, Jackson ImmunoResearch, Cambridge, UK; Alexa Fluor 647–conjugated donkey anti-rabbit, 1:200, Jackson ImmunoResearch; Alexa Fluor 488 donkey anti-rat, 1:100, Jackson ImmunoResearch) for 2 hours. After washing, sections were mounted with mounting medium (VectaShield; Vector Laboratories; Burlingame, California) with 4′,6-diamidino-2-phenylindole (DAPI). Three sections per subject were assessed under a fluorescence microscope (AxioImager M.2;

Zeiss, Oberkochen, Germany) with a mono-camera (Axiocam 506; Zeiss) at 400× total magnification for Schwann cells and 630× magnification for Schwann cell branches and detailed analyses. To analyze only Schwann cells and/or their branches within the epidermis or subepidermal plexus, the region of interest (ROI) was defined as an area of less than 40 μm beneath the dermis-epidermis border (Figure S1). Schwann cells were identified by S100B positivity with typical ellipsoid nuclei along the nerve fibers at the dermis-epidermis

TABLE 1 Main characteristics of the study cohort

	All SFN	SFN normal IENFD	SFN reduced IENFD	Healthy controls
Age (years)	58 (19-73)	53 (19-73)	54 (43-68)	34 (25-69)
Sex (M:F)	11:17	2:13	9:4	5:4
Distal IENFD (fibers/mm)	6 (1-13)	8 (6-13)	4 (1-5)	9 (6–18)
Current pain intensity (NRS)	4 (1-8)	4 (1-8)	5 (1-8)	NA
Duration of pain (years)	4 (0.3-24)	5 (0.3-16)	3.5 (1-24)	NA
Time since diagnosis (years)	1 (0.1-8)	0.6 (0.1-4)	1.5 (0.1-8)	NA
Pain distribution				NA
Acral	11 of 28	4 of 15	7 of 13	
Generalized	9 of 28	5 of 15	4 of 13	
Other (eg, proximal, back)	8 of 28	6 of 15	2 of 13	
Presence of par-/dysesthesias	3 of 28	1 of 15	2 of 13	NA
Etiology of SFN				NA
Idiopathic	19 of 28	12 of 15	7 of 13	
Impaired glucose tolerance	4 of 28	1 of 15	3 of 13	
Hypothyroidism	3 of 28	2 of 15	1 of 13	
Vitamin B_{12} deficiency	2 of 28	0 of 15	2 of 13	

Note: Data expressed as median (range).
Abbreviations: F, female; IENFD, intraepidermal nerve fiber density; M, male; NA, not applicable; NRS, numeric rating scale; SFN, small-fiber neuropathy.

TABLE 2 Synopsis of quantified parameters

	All SFN (I)	SFN normal IENFD (II)	SFN reduced IENFD (III)	Healthy controls (IV)	*P* value effect size			
					I vs IV	II vs III	II vs IV	III vs IV
(1) IENFD fibers/mm (Figure 2A,D)	5.7 [1.0-12.5], CI, 4.9-7.2	7.5 [5.7-12.5], CI, 6.9-9.3	3.9 [1.0-5.3], CI, 2.7-4.6	9.0 [4.5-18.5], CI, 6.4-12.3	<.05 −.964	<.001 −2.381	.379 −.398	<.001 −1.922
(2) Solitary SC/mm (Figure 2F,H)	3.6 [0.6-6.0], CI, 3.2-4.2	4.6 [2.2-6.0], CI, 3.9-5.0	2.9 [0.6-4.7], CI, 2.2-3.5	4.5 [3.1-5.7], CI, 4.0-5.2	.073 −.798	<.001 −1.534	.907 −.141	<.001 −1.835
(3) SC clusters/mm (Figure 2J,L)	1.1 [0-3.3], CI, 1.0-1.6	1.0 [0.4-3.3], CI, 0.9-1.8	1.2 [0-2.2], CI, 0.8-1.6	2.3 [0.5-4.1], CI, 1.3-3.2	<.05 −.948	.650 −.271	.084 −.840	<.05 −1.084
(4) SC/cluster (Figure 2J,L)	2.1 [0-3.0], CI, 1.8-2.3	2.1 [0.7-2.9], CI, 1.8-2.5	2.2 [0-3.0], CI, 1.5-2.5	2.6 [1.4-3.2], CI, 2.1-3.0	.053 −.759	.717 −.238	.108 −.709	.071 −.793
(5) Total number of SC/mm	6.9 [0.6-13.2], CI, 5.8-7.7	7.5 [4.9-13.2], CI, 6.4-8.9	6.5 [0.6-8.6], CI, 4.3-7.1	9.3 [5.8-16.4], CI, 7.6-13.7	<.01 −1.206	.052 −.870	.055 −.920	<.01 −1.544
(6) Dermal nerve fibers associated with SC branches/mm (Figure 2I-L)	6.4 [3.2-14.9], CI, 6.0-8.2	8.0 [3.7-14.9], CI, 6.61-10.0	5.7 [3.2-9.0], CI, 4.7-6.58	7.5 [5.1-13.8], CI, 6.1-9.9	.213 −.359	<.01 −1.103	.861 −.108	<.01 −1.144
(7) Dermal nerve fibers not associated with SC branches/mm (Figure 2A-D)	0.4 [0-1.3], CI, 0-0.2	0.1 [0-0.4], CI, 0-0.2	0 [0-1.3], CI, 0-0.4	0.1 [0-0.8], CI, 0-0.5	.144 −.423	.683 .308	.108 −.728	.324 −.224

Note: Data expressed as median [range].
Abbreviations: CI, confidence interval; IENFD, intraepidermal nerve fiber density; SC, Schwann cell; SFN, small-fiber neuropathy.

border. S100B-positive Langerhans cells were excluded by CD207 co-positivity. Melanocytes expressing S100B were excluded by their characteristic morphology and location at the basal layer. The following parameters were determined by the same investigator blinded to sample allocation (Figure S1):

1. Intraepidermal nerve fibers per millimeter.
2. Number of solitary Schwann cells per millimeter (ie, single Schwann cells not located in the vicinity of another Schwann cell).
3. Number of Schwann cell clusters per millimeter (ie, ≥2 Schwann cells that are adjacent to each other and get in contact by their somas and/or branches).
4. Number of Schwann cells per cluster.
5. Total number of Schwann cells per millimeter (ie, sum of parameters 2 and 4).
6. Number of dermal nerve fibers associated with Schwann cell branches per millimeter.
7. Number of dermal nerve fibers not associated with Schwann cell branches per millimeter.
8. Number of Schwann cell branches associated with epidermal nerve fibers (ie, ascending Schwann cell processes accompanying epidermal nerve fibers that cross the basement membrane).
9. Number of Schwann cell branches not associated with epidermal nerve fibers per millimeter (ie, ascending Schwann cell processes not accompanying nerve fibers).

2.2.3 | Microscopy

Photomicrographs were acquired using an Apotome.2 device for optical sectioning with the microscope just described at 400× and 630× total magnification. Exposure time was calculated automatically by Zen Blue software (all from Zeiss, Oberkochen, Germany) to ensure proper illumination. Due to an overall low signal intensity in the Alexa Fluor 647 channel (labeling S100B), the grid of the Apotome device was still visible after reconstruction. Photomicrographs were also acquired using a spinning disk microscope, consisting of an AxioImager.M2 (Zeiss), a Spot Xplorer charge-coupled device camera (SPOT Imaging), and an X-Light V1 spinning disk device (CrestOptics, Rome, Italy). The stack range was set to 35 µm, with a 1-µm step-size. Postprocessing was performed using ImageJ (National Institutes of Health, Bethesda, Maryland) with maximum-intensity *z* projections for the full stack, selected focal planes, or a single focal plane.

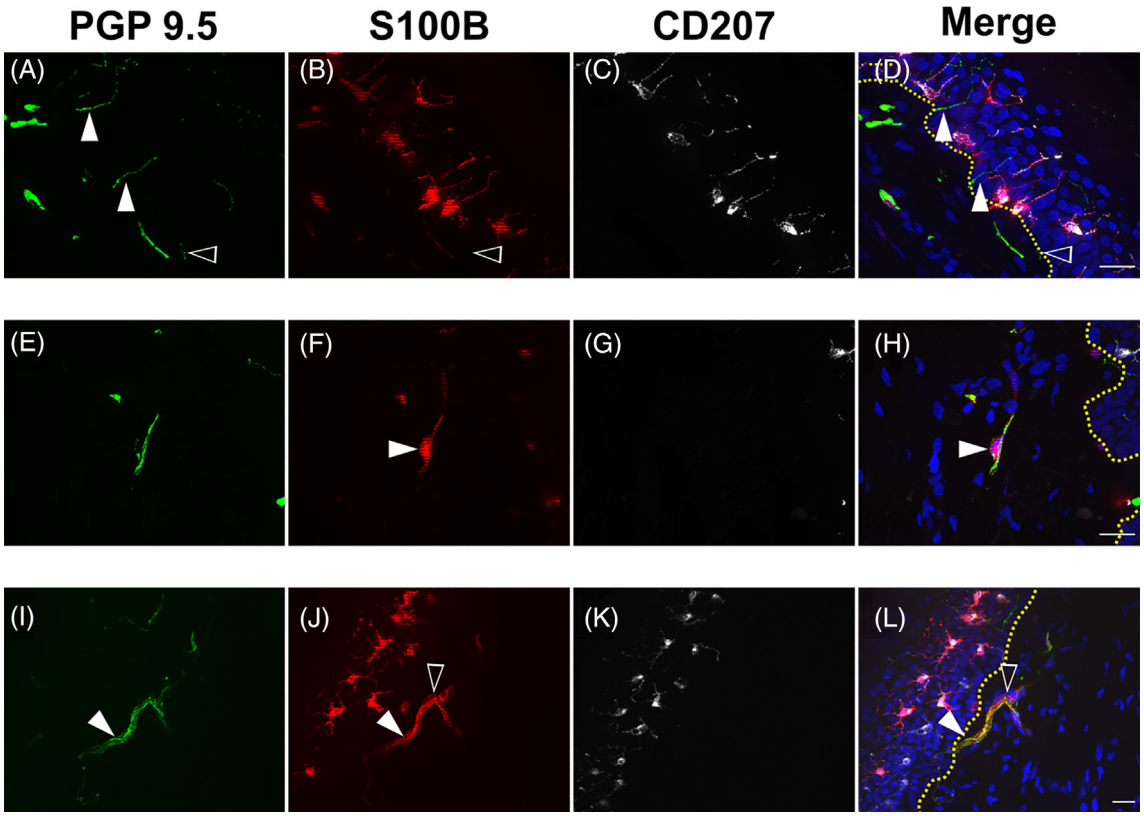

FIGURE 1 Histological parameters in human skin. A-D, Nerve fibers (filled arrowheads), crossing the basement membrane (yellow dotted line), are in some cases not associated with S100B (empty arrowheads). E-H, Solitary SCs (filled arrowheads) were identified by S100B signal in the dermis. I-L, SC branches, associated with dermal nerve fiber branches (filled arrowheads), can have their origin in SC clusters (empty arrowhead). Yellow dotted line marks the basement membrane. CD207, Langerin; PGP 9.5, protein-gene product 9.5; S100B, S100 calcium-binding protein B; SC, Schwann cell. Scale bars: 25 µm

2.3 | Statistical analysis

We used SPSS version 26 (IBM Corp, Armonk, New York). For non-normally distributed data, we applied the nonparametric Mann-Whitney *U* test and expressed data as median, range, and confidence interval. Eta-squared was calculated to determine effect size for all tests performed and then converted to Cohen's *d* using free online software from Psychometrica (https://www.psychometrica.de/effect_size.html). Data are presented as scatterplots with integrated boxplots, which were created with GraphPad Prism version 7 (GraphPad, Inc, La Jolla, California). Median values are marked with a horizontal line, and the 25th and 75th percentile are indicated by whiskers. Data were used as collected without transformation. Statistical significance was set at $P < .05$.

3 | RESULTS

3.1 | Characterization of the study cohort

Table 1 presents the main characteristics of the study cohort. We enrolled 28 patients with SFN. The cohort consisted of 11 men and 17 women with a median age of 54 (range, 19-73) years. We further enrolled nine healthy controls (five men, four women; median age, 34 [range, 25-69] years). In 19 patients, SFN etiology was unknown (categorized as idiopathic). Causes of SFN in the other nine patients are provided in Table 1. IENFD was reduced in 13 of 28 SFN patients (Table 1) when comparing fiber counts with our laboratory's normative values.

3.2 | Subepidermal Schwann cells are less frequent in SFN patients than in healthy controls

Table 2 provides a synopsis of the quantified parameters just detailed above and intergroup comparisons between patients with SFN and healthy controls. Figure 1 presents these parameters histologically. First, we compared the entire group of patients with SFN with healthy controls. IENFD was lower in patients with SFN than in controls. Patients with SFN had fewer Schwann cells per millimeter and fewer Schwann cell clusters per millimeter compared with healthy controls. We did not observe Schwann cell branches associated or not associated with epidermal nerve fibers in either study group. The fluorescent signal of Schwann cell branches vanished at

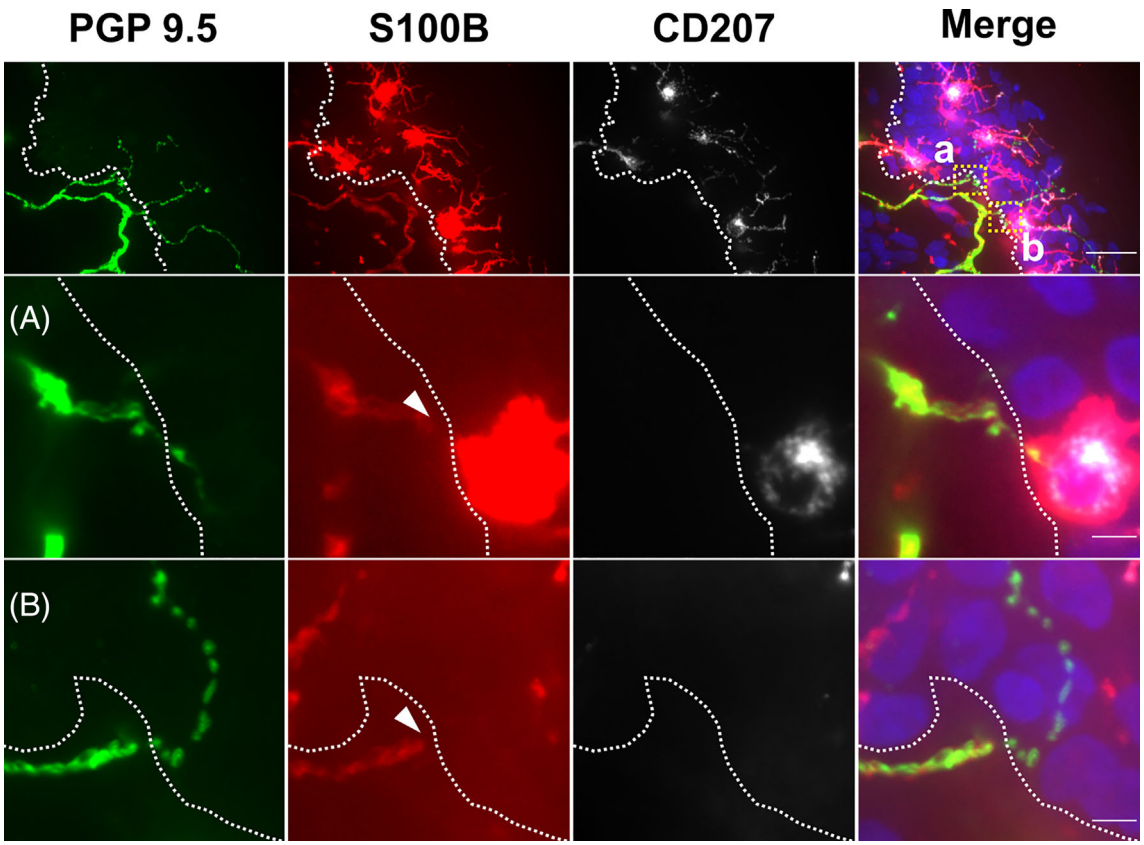

FIGURE 2 Comparison of z projections and single focal planes. A, Maximum intensity z projections for PGP 9.5, S100B, CD207, and the corresponding merge photomicrograph. a, Digitally magnified region of interest from a single plane of the stack from (A). The PGP 9.5 signal crosses the basement membrane, whereas the S100B signal is lost (filled arrowhead) near the dermis-epidermis border (yellow dotted line). In the epidermal part, a $CD207^{+}$ cell can be seen. Note that the course of the basement membrane differs greatly from (A). b, Digitally magnified region of interest from a maximum z projection spanning 5 μm in total. PGP 9.5 crosses the border, whereas the $S100B^{+}$ structure loses its immunoreactivity (filled arrowhead) close to the border (yellow dotted line). CD207, Langerin; PGP 9.5, protein-gene product 9.5; S100B, S100 calcium-binding protein B; SC, Schwann cell. Scale bars: 25 μm in (A) and 5 μm in (a) and (b)

the basement membrane upon further analysis, using spinning-disk confocal methodology (Figure 2)

3.3 | Number of dermal Schwann cells associated with IENFD

The assessment of IENFD revealed two patient subgroups: 15 patients had a normal distal IENFD (median 8 [range, 6-13] fibers/mm) and 13 patients had reduced distal IENFD (median, 4 [range, 1-5] fibers/mm; Figure 3A). Healthy controls had normal distal IENFD (median, 9 [range, 6-18] fibers/mm). We compared the parameters determined between these two patient groups and with healthy controls and found lower numbers of solitary Schwann cells per millimeter in SFN patients with reduced IENFD vs those with normal IENFD (Figure 3B). The total number of Schwann cells per millimeter was not different between SFN patients with reduced IENFD and SFN patients with normal IENFD (Figure 3C). The number of subepidermal nerve fibers associated with Schwann cell branches per millimeter was lower in skin samples of patients with SFN and reduced IENFD than in those with normal IENFD (Figure 3D). The other parameters assessed showed no difference between the two subgroups (Table 2).

3.4 | Schwann cell counts correlate with skin innervation

There were no relevant correlations between distal IENFD and Schwann cell parameters in any of the three study subgroups when assessed separately (data not shown). However, distal IENFD of the entire group of study subjects (n = 37) showed a strong positive correlation with the number of solitary Schwann cells per millimeter (Figure 4A), the total number of Schwann cells per millimeter (Figure 4B), and the number of subepidermal nerve fibers associated with Schwann cell branches (Figure 4C).

3.5 | Schwann cell counts are not associated with clinical pain characteristics

The Schwann cell parameters just described did not correlate with the intensity, distribution (acral, focal, generalized), or duration of pain. Further, par- and/or dysesthesias accompanying pain did not correlate with the Schwann cell parameters. We also did not find male-female differences among our patients for clinical phenotype, skin innervation, and the Schwann cell parameters assessed, which may be due to the overall small number of study participants.

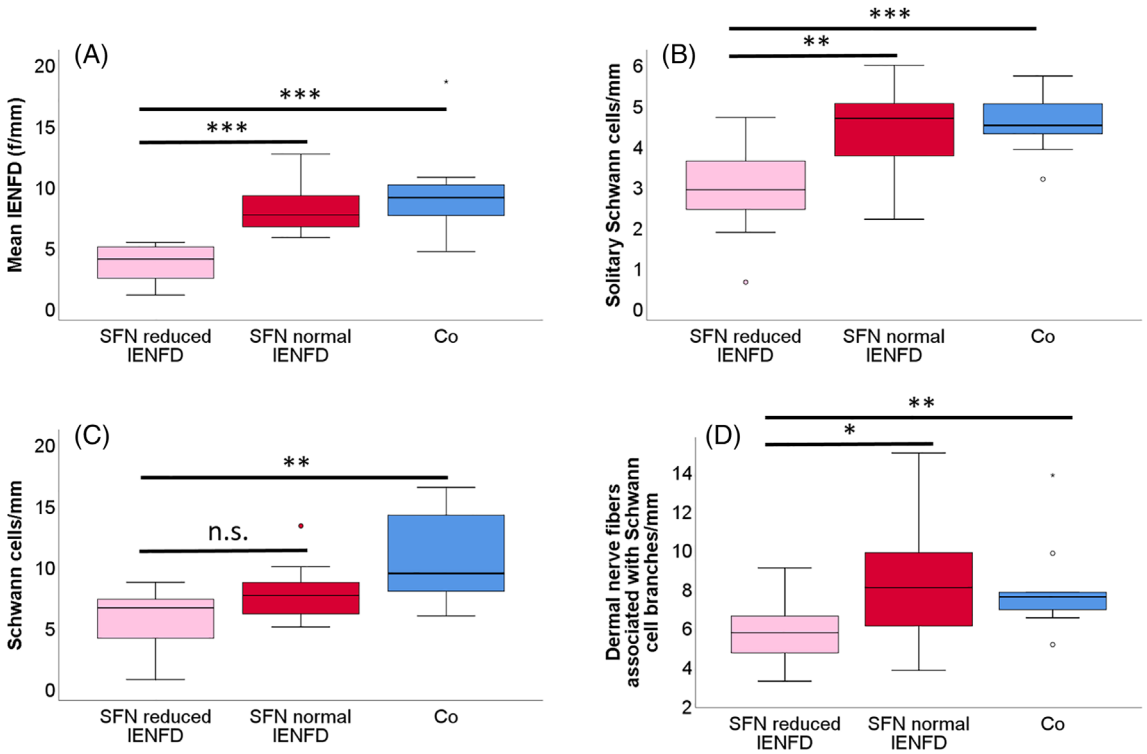

FIGURE 3 Innervation and SC quantification in skin of SFN patients with reduced IENFD compared with SFN patients with normal IENFD and Co. A, IENFD was lower in SFN patients with reduced IENFD compared with SFN patients with normal IENFD and Co. B, SFN patients with reduced IENFD had less solitary SCs per millimeter compared with SFN patients with normal IENFD and Co. C, Total number of SCs per millimeter was lower in SFN patients with reduced IENFD compared with Co, and the difference did not reach significance between the two patient groups with SFN. D, Dermal nerve fibers associated with SCs per millimeter per millimeter were fewer in SFN patients with reduced IENFD compared with SFN patients with normal IENFD and Co. Co, healthy controls; IENFD, intraepidermal nerve fiber density; n.s., not significant; SC, Schwann cell; SFN, small-fiber neuropathy. *$P < .05$, **$P < .01$, ***$P < .001$

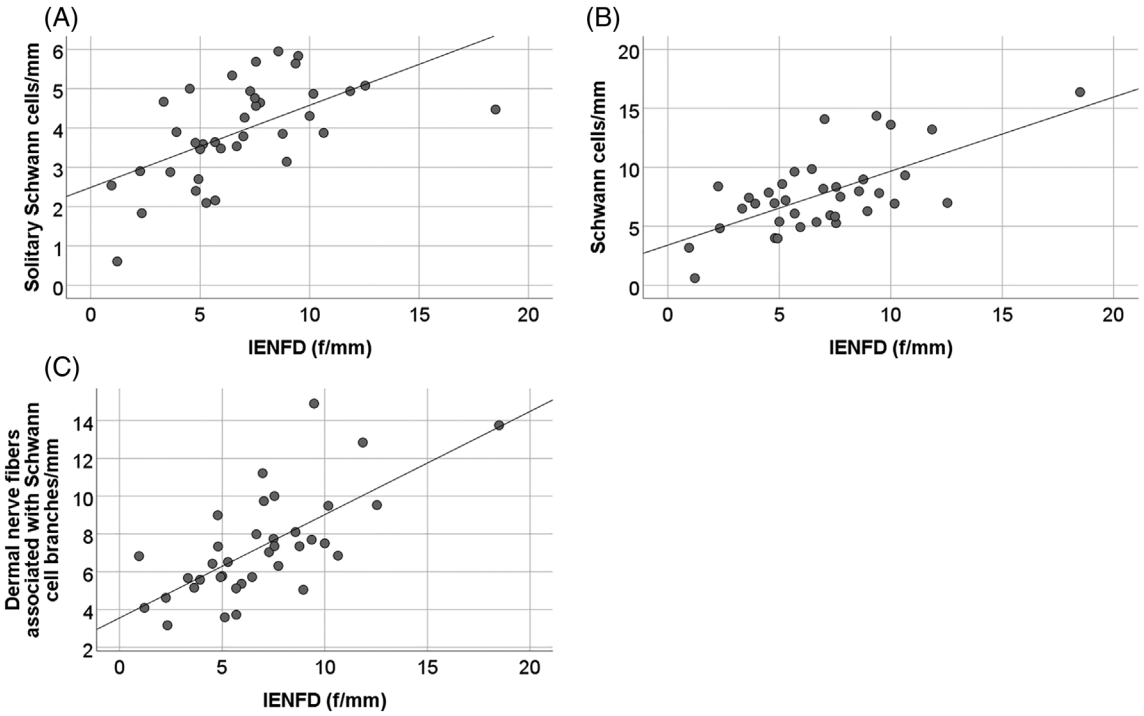

FIGURE 4 Correlation analysis with skin innervation and SC parameters. Linear regression analyses demonstrating positive correlation of IENFD with: A, solitary SCs/mm (correlation coefficient = 0.619; $P < .001$); B, total number of SCs/mm (correlation coefficient = 0.511; $P < .01$); and C, dermal nerve fibers associated with SCs/mm (correlation coefficient = 0.651; $P < .001$). IENFD, intraepidermal nerve fiber density; SC, Schwann cell

4 | DISCUSSION

We found lower numbers of Schwann cells mainly in the subgroup of patients with reduced IENFD compared with healthy controls. Although the quantity of Schwann cells correlated positively with skin innervation, no correlations were found with clinical parameters.

Previous studies indicated that Schwann cells are crucial for the survival[21] and regeneration of peripheral sensory neurons.[17] It has been assumed that dermal unmyelinated nociceptors are abutted by Schwann cells and lose their myelin sheath when entering the epidermis.[7,22] In recent studies, specialized nociceptive Schwann cells were characterized that form a meshlike network with nerve fibers at the dermis-epidermis border accompanying nerve fibers into the epidermis in the murine model[11] and also in human skin when assessed using electron microscopy.[12] Interestingly, selective depletion of intraepidermal nerve fibers resulted in a reduction of Schwann cell counts in murine skin, whereas ablation of Schwann cells led to nerve fiber retraction.[12] In line with these findings, we found lower numbers of Schwann cells in skin samples of SFN patients with reduced distal IENFD, but our study design did not allow conclusions about causality. Also, using fluorescent microscopy, we did not observe epidermal Schwann cell branches, but found them to vanish at the basement membrane.

Although the physiological function of human cutaneous Schwann cells is not yet clear, murine data suggest that these glial cells are highly mechanosensitive and transmit nociceptive information.[11,12] Previous studies also showed that genetic ablation of the transmembrane protein usherin, which is physiologically found in dermal Schwann cells, impairs mechanoperception and vibration sense in mice.[23]

When considering the entire study population, we found strong positive correlations between skin innervation and subepidermal Schwann cell counts. It is known that, similar to IENFD, the density of Schwann cells within the upper dermis varies in different regions of the human body.[7]

Loss of predominantly intraepidermal nerve fibers combined with the loss of Schwann cells suggests a Wallerian-like mechanism focused on small, unmyelinated nerve fibers. Wallerian degeneration is associated with axotomy, and disruption of axonal contact triggers the reprogramming of both myelinating and nonmyelinating (Remak) Schwann cells to adopt a repair Schwann cell phenotype. These repair Schwann cells are specialized to support regeneration, and injured neurons activate a gene program that facilitates axon growth.[24] The transcription factors c-Jun,[25] signal transducer and activator of transcription 3,[26] and lysine 27 onto histone H3 trimethylation[25,27] control this repair program. These proteins are upregulated quickly after injury and increase the expression of genes implicated in the regulation and trophic support of neurons.[28] These repair Schwann cells also recruit macrophages to degrade myelin debris by activating the innate immune response via cytokines.[29-31] Although the peripheral nervous system is unique by its regeneration capacity, inadequate recovery may result in peripheral neuropathy.

We cannot determine from our studies whether damage in SFN first occurs in the peripheral axons or their supportive Schwann cells. Schwann cells respond to loss of axonal interaction by cellular alterations that involve dedifferentiation.[32] Assessing the presence of repair Schwann cells by immunohistochemical staining of Schwann cell markers, together with c-Jun, may provide hints about whether Schwann cell degeneration may be the consequence of nerve fiber loss or a preceding factor.

Our findings suggest that a reduced number of Schwann cells is associated with epidermal denervation, but no further association was found. SFN patients with a normal IENFD were similar to our healthy controls regarding Schwann cell parameters, implying that Schwann cells residing at the dermis-epidermis junction may have only a minor direct impact on other SFN symptoms, if any at all.

Although the exact role of Schwann cells in neuropathic pain remains elusive, Schwann cells are increasingly recognized as potentially pro-nociceptive.[11,12,33] Various receptors and ion channels expressed by Schwann cells are reported to be involved in different pain conditions.[33] The functional disturbance of Schwann cells that express truncated erb-B2 receptor tyrosine kinase 4 (ErbB4) receptors causes progressive C-fiber degeneration and sensory neuropathy as well as marked heat and pain insensitivity in transgenic mice.[13] Loss of the low-density lipoprotein receptor–related protein, a potent regulator of Schwann cell migration and survival,[34,35] resulted in Schwann cell ensheathment deficits and slight hypomyelination, leading to mechanical allodynia in the absence of injury.[36] Deletion of *GRIN1* encoding the *N*-methyl-D-aspartate receptor GluN1 subunit in Schwann cells caused ultrastructural changes in Remak bundles and decreased IENFD in mice. These mice had increased mechanical and thermal sensitivity in the absence of nerve injury.[14] The loss of nociceptive Schwann cells can by itself lead to the manifestation of SFN and a sensitization to both mechanical and thermal stimuli in mice.[12] These findings indicate that Schwann cells are crucial for the survival of C fibers and that they may play a role in the development of neuropathic pain. Although we found an interdependence between the skin innervation and Schwann cell counts, there were no correlations between the quantity of Schwann cells and clinical characteristics in our study groups.

In our study, approximately half of the patient group had normal distal skin innervation. This is not surprising when compared with the literature[3]: many patients with SFN do not have reduced IENFD in skin punch biopsies and, conversely, healthy controls may have reduced skin innervation. This supports the conclusion that skin punch biopsy is not a gold standard to make the diagnosis of SFN-as we and others have shown before.[2,3,10] It is also noteworthy that patients may report characteristic SFN symptoms but present with unremarkable clinical and small-fiber tests.[37] In turn, SFN may be asymptomatically present, as reported in patients with diabetes mellitus.[38]

In collecting mere morphological data, the question remains as to which roles Schwann cells play in SFN patients with normal and reduced IENFD. One major question that needs to be answered is the time course of denervation; that is, whether the reduction of Schwann cells causes denervation, or denervation causes the reduction of Schwann cells. If the former is true, and Schwann cells are initially affected by the disease, it may be possible that a reduction of IENF is caused by a lack of glial-cell-line–derived neurotrophic factor, which is crucial for axonal maintenance[39] and is secreted by Schwann cells. In one report, denervation upon axotomy developed within 50 days in an epidermis-to-dermis direction. Also, p75 and S100b immunostaining for Schwann cells was more pronounced within the first month of axotomy than at later time-points when Schwann cells disappeared first from the superficial dermis and then from deeper parts.[8] For further elucidation of the functional role of Schwann cells in SFN, prospective follow-up studies are needed to investigate the mechanisms of Schwann cell reduction and skin denervation.

In this exploratory study, we could only investigate a small number of study subgroups using this demanding and time-consuming manual assessment of Schwann cells. The healthy control group was younger than the patient cohort, which may have impacted our results. Also, using wide-field microscopy techniques, it is challenging to determine the exact location of PGP 9.5^{+}/S100B^{+} in the vicinity of the border, making investigation of epidermal Schwann cell branches especially difficult.

Despite these limitations, our study offers a detailed analysis of subepidermal Schwann cells in SFN patients with either reduced or normal IENFD. Although the pathophysiological mechanisms remain unclear, our findings underscore that Schwann cells are altered in the skin of patients with SFN and demonstrate the interdependence of nerve fibers and Schwann cells in human skin. Further studies investigating the temporal course and functional impact of the Schwann cell decrease would contribute to a better understanding of SFN.

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CONFLICT OF INTEREST

None of the authors has any conflict of interest to disclose.

ETHICAL PUBLICATION STATEMENT

The authors report that they have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

DATA AVAILABILITY STATEMENT

Data available on request from the authors

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SUPPORTING INFORMATION

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