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RESEARCH ARTICLE

Identification of small fiber neuropathy in neuronal intranuclear inclusion disease: A clinicopathological study

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Abstract

INTRODUCTION: Neuronal intranuclear inclusion disease (NIID) manifests as dementia combined with other neurological symptoms. However, small fiber neuropathy (SFN) and pathology remain unknown in NIID.

METHODS: A total of 294 subjects, including patients with NIID, Parkinson's disease, Alzheimer's disease, diabetic peripheral neuropathy, and healthy controls (HCs), were included. Clinical scales, sensory and autonomic function testing, and skin biopsy were performed.

RESULTS: NIID patients had more severe sensory and autonomic dysfunction than other groups. Substantial reductions in intraepidermal, sweat gland, and pilomotor nerve fiber densities were observed in NIID patients, with a non–length dependent pattern. Detailed analysis revealed marked reductions in noradrenergic, cholinergic, peptidergic, and regenerative nerve fibers. Small fiber densities showed high diagnostic accuracy in distinguishing NIID from HCs and other diseases.

DISCUSSION: This study is the first to reveal wide and severe loss of small fibers in NIID, suggesting the involvement of SFN in the pathogenesis of NIID.

KEYWORDS
autonomic dysfunction, dementia, intraepidermal nerve fiber density, neuronal intranuclear inclusion disease, skin biopsy, small fiber neuropathy

Minglei Liu, Ruoyu Liu, and Yanpeng Yuan have contributed equally to this work.

Highlights

- Our study is the first to identify wide and severe non–length dependent small fiber neuropathy in neuronal intranuclear inclusion disease (NIID) patients.
- Approximately 50% of NIID patients exhibited pure small fiber neuropathy without large fiber or mixed neuropathy.
- NIID patients showed a significant reduction in noradrenergic, cholinergic, peptidergic, and regenerative fiber innervation.
- Small fiber densities, especially intraepidermal nerve fiber density, demonstrated high diagnostic accuracy in distinguishing NIID patients from healthy controls and other disease groups.
- Findings suggest that small fiber neuropathy may play a role in the pathogenesis of NIID.

1 | BACKGROUND

Neuronal intranuclear inclusion disease (NIID) is a progressive neurodegenerative disorder that is more prevalent in Asia than in other regions, such as China and Japan.[1–4] NIID is characterized by the presence of eosinophilic intranuclear inclusions in neurons and other cell types across the central and peripheral nervous systems, as well as in various visceral organs and the skin.[5,6] The clinical presentation of NIID is remarkably heterogeneous, encompassing a spectrum of symptoms like dementia, peripheral neuropathy, movement disorders, episodic symptoms, and autonomic dysfunction.[5] Among all the clinical symptoms, dementia is considered the most common phenotype in adult-onset NIID.[5,7] The determination of NIID is traditionally dependent on brain autopsy, and the identification of intranuclear inclusions in skin cells facilitates the *ante mortem* diagnosis of NIID.[6,8]

Notably, peripheral neuropathy, including marked weakness and numbness in the distal limbs, as well as subclinical neuropathy detectable by electrophysiological tests, is common in patients with NIID.[9–11] While significant damage to large fibers has been well documented, the changes in small fibers in NIID remain largely unknown. Clinically, patients with NIID report symptoms indicative of small fiber involvement, such as numbness, sweating disorders, and constipation, suggesting potential small fiber neuropathy (SFN) in patients with NIID. Therefore, the involvement of small fiber pathology warrants investigation.

To address this, we conducted a clinicopathological study to investigate whether pathological changes in skin small fibers exist in patients with NIID and to assess their correlations with clinical phenotypes. To better characterize the small fiber alterations in the skin of patients with NIID, we included neurological diseases with known small fiber involvement, such as Parkinson's disease (PD) and diabetic peripheral neuropathy (DPN), as well as conditions without small fiber involvement, such as Alzheimer's disease (AD), and healthy controls (HCs).

2 | METHODS

2.1 | Study participants

This clinicopathological study consisted of five different groups: (1) patients with NIID, (2) patients with PD, (3) patients with AD, (4) patients with DPN, and (5) HCs. All participants were consecutively recruited from the Department of Neurology at the First Affiliated Hospital of Zhengzhou University from June 2019 to December 2023. The diagnosis of NIID was confirmed through both pathological and genetic evidence, including the presence of ubiquitin-positive intranuclear inclusions in the skin and GGC repeat expansion in the *NOTCH2NLC* gene ($n = 56$).[12] Patients were diagnosed with clinically confirmed PD according to the 2015 Movement Disorder Society Clinical Diagnostic Criteria for PD ($n = 85$).[13] Patients were diagnosed with AD based on cerebrospinal fluid/positron emission tomography biomarker profiles fulfilling the US National Institute on Aging and Alzheimer's Association research framework criteria ($n = 68$).[14] Patients were diagnosed with DPN according to the consensus definition of DPN proposed by the Toronto Diabetic Neuropathy Expert Group ($n = 45$).[15] Moreover, age- and sex-matched HCs, who were examined by two neurologists and found to have no neurological symptoms, were included in this study ($n = 40$).

All patients were screened to identify and exclude participants with other possible causes of SFN, such as abnormal thyroid function; vitamin B12 and folate deficiencies; hepatic or renal failure; and human immunodeficiency virus or connective tissue disorders.[16] Additionally, patients with NIID, PD, and AD were confirmed to have no coexisting diabetes mellitus.

Given the heterogeneity in the clinical manifestations of NIID, we conducted a subgroup analysis by categorizing patients into four subgroups based on their predominant symptoms: muscle weakness-dominant, dementia-dominant, movement disorder-dominant, and paroxysmal symptom-dominant types.[7,17]

The study was approved by the ethics committee of the First Affiliated Hospital of Zhengzhou University, China (Approval No. 2019-KY-294), and all the subjects signed an informed consent form in accordance with the Declaration of Helsinki.

2.2 | Assessments of small fiber symptoms

Sensory and autonomic symptoms were assessed using two well-established self-assessments: the Small-Fiber Neuropathy Symptoms Inventory Questionnaire (SFN-SIQ) and the Composite Autonomic Symptom Score-31 items (COMPASS-31), which target symptoms associated with small fiber involvement. The SFN-SIQ is a multi-item composite measure designed to assess 13 symptoms associated with SFN.[18] These symptoms include changes such as sweating, diarrhea, constipation, micturition problems (incontinence and/or retention), dry eyes, dry mouth, dizziness standing, palpitations, hot flashes, sensitive skin, burning feet, sheet intolerance, and restless legs. Each item is scored on a scale of 0 to 3, where 0 indicates "never," 1 indicates "sometimes," 2 indicates "often," and 3 indicates "always." The total score ranges up to 39, with higher scores indicating more severe small fiber symptoms. The COMPASS-31 is a concise quantitative measure of autonomic symptoms that is widely used in both clinical research and practice to assess the severity of autonomic dysfunction.[19] This tool evaluates subjective symptoms across six domains: orthostatic intolerance, vasomotor, secretomotor, gastrointestinal, bladder, and pupillomotor functions. It consists of a total of 31 questions, with each domain weighted differently to reflect its impact. The final score ranges from 0 to 100, with higher scores indicating more severe autonomic symptoms.

RESEARCH IN CONTEXT

1. **Systematic review**: The authors conducted a comprehensive review of existing literature on neuronal intranuclear inclusion disease (NIID) and small fiber neuropathy (SFN), using traditional sources such as PubMed, as well as recent meeting abstracts and presentations. Despite reports of small fiber symptoms, such as abnormal sweating and constipation, in patients with NIID, these aspects have not been thoroughly investigated, and the pathological changes associated with small fibers remain largely unknown.
2. **Interpretation**: This study provides the first evidence of extensive, severe, non-length dependent SFN across all NIID patient subgroups, with ≈ 50% showing exclusive small fiber involvement. Our findings highlight that intraepidermal nerve fiber density offers high diagnostic accuracy for differentiating NIID from other conditions, supporting the role of SFN in NIID pathogenesis.
3. **Future directions**: Future research should aim at further elucidating the mechanisms underlying SFN in NIID. Potential areas of investigation include: (a) the exploration of mechanisms contributing to small fiber degeneration in NIID, (b) the development of targeted therapeutic strategies to address small fiber symptoms, (c) the longitudinal study of small fiber density changes in response to treatment interventions, and (d) the comparison of small fiber pathology in NIID with other neurodegenerative disorders to better understand its unique clinical features.

2.3 | Nerve conduction study

A nerve conduction study (NCS) was conducted on the lower extremities to measure large fiber function, with a focus on the common peroneal and sural nerves. Abnormal sural nerve function was indicated by a sensory nerve action potential (SNAP) < 5 μV and a sensory conduction velocity (SCV) < 40 m/s. For the common peroneal nerve, abnormalities were defined by a compound muscle action potential (CMAP) in the extensor digitorum longus of < 3 mV and a muscle conduction velocity (MCV) < 40 m/s. Among them, the sural SNAP amplitude was considered the NCS main outcome variable. Skin sympathetic responses (SSRs) were elicited for autonomic nervous system assessment. All tests were performed using a Nicolet Viking electromyography system under controlled temperature conditions.

2.4 | Quantitative sensory testing and autonomic function testing

Quantitative sensory testing was performed on the subjects' feet to evaluate small fiber function. Quantitative sensory testing included thermal and current perception testing. Thermal testing was conducted using a Modular Sensory Analyzer thermal stimulator (Somedic MSA NG, Somedic SenseLab) equipped with fluid-cooled Peltier elements with an active surface area of 25 mm × 50 mm. The cold detection threshold (CDT) and warm detection threshold (WDT) were determined using the "method of limits" through sensory threshold measurements. The cold pain threshold (CPT) and heat pain threshold (HPT) were subsequently measured. By pressing the stop button connected to the computer unit, the thresholds could be determined based on the temperature of the thermal contact surface (with a temperature change rate of 1°C/second) starting from a baseline temperature of 32°C. To ensure compliance with appropriate safety guidelines, the unit automatically stopped measurement when the temperature reached 0°C or 50°C and returned to the starting temperature of 32°C to avoid skin irritation. The mean calculated from three consecutive individual measurements was used as the actual pain threshold.[20,21] The current perception threshold was measured with a neurometer (Neurotron Inc.) equipped with two gold-plated electrodes with a diameter of 1 cm. One electrode was placed on the lateral aspect of the toe,

whereas the other was placed on the medial aspect. The current perception threshold was measured at three predetermined frequencies: 5, 250, and 2000 Hz, with a range of 0.01 to 9.99 mA. By measuring the current perception thresholds at 2000, 250, and 5 Hz, we detected and quantified the functional status of three distinct types of nerve fibers: large myelinated (Aβ) fibers, medium-sized myelinated (Aδ) fibers, and unmyelinated (C) fibers. The intensity of the electrical stimulus was gradually increased until the patient reported a specific sensation of tingling or pricking. Subsequently, lower amplitude short stimuli were applied until a minimal but consistent threshold was detected.[22]

Autonomic function testing included tilt table testing and sudomotor function testing. Tilt table testing was also operationalized through the assessment of neurogenic orthostatic hypotension, which was conducted either by a 3 minute head-up tilt test at 65° or by standing. Orthostatic hypotension was identified when there was a decrease in blood pressure of at least 20/10 mmHg (systolic/diastolic) without a significant change in heart rate.[23] Sudomotor function assessment was conducted using the Sudoscan device (Impeto Medical), which evaluates sweat gland function by measuring sweat chloride concentrations through reverse iontophoresis and chronoamperometry. The device generated a current proportional to the chloride concentration, which was measured as the electrochemical skin conductance (ESC) in microSiemens (μS). During the test, patients placed their feet on the electrodes and remained still for 2 to 3 minutes. The device provided average ESC values for the feet.[24]

2.5 | Skin biopsy and histology

Skin biopsies (3 mm in diameter) were performed at distinct anatomical sites: the distal leg, proximal thigh, and cervical area.[12] Continuously frozen sections were cut with a freezing microtome (Leica CM1950) at a thickness of 50 μm.[25] The remaining tissues were embedded in paraffin, and continuous sections were cut with a Leica clinical microtome (HistoCore BIOCUT) at a thickness of 4 μm. In accordance with previously published procedures, four serial 50 μm sections were immunostained with the pan-neuronal marker protein-encoding gene product 9.5 (Mouse PGP 9.5, 1:1000, Cat# MCA4750, Bio-Rad or Rabbit PGP 9.5, 1:1000, Cat# ab108986, Abcam) for skin innervation. Four additional 50 μm sections were double-immunostained to detect different types of small nerve fibers using a large panel of antibodies, including primary antibodies against vasoactive intestinal peptide (Rabbit VIP, 1:200, Cat# 20077, ImmunoStar) as a marker of cholinergic fibers, tyrosine hydroxylase (Rabbit TH, 1:1000, Cat# NB300-109, Novus Biologicals) as a marker of noradrenergic fibers, substance P (Rabbit SP, 1:1,000, Cat# 20064, ImmunoStar), and calcitonin gene–related peptide (Rabbit CGRP, 1:500, Cat# 24112, ImmunoStar) as markers of peptidergic nerve fibers, growth-associated protein 43 (Rabbit GAP-43, 1:1000, Cat# NB300-143, Novus Biologicals) as a marker of regenerative fibers, and collagen type IV (Mouse ColIV, 1:800, Cat# MAB1910, Sigma-Aldrich).[26–28] The secondary antibodies used were anti-mouse Alexa Fluor 594 (1:600, Cat# ZF-0513, ZSGB-BIO) and anti-rabbit Alexa Fluor 488 (1:800, Cat# ZF-0511, ZSGB-BIO). The resulting 4 μm sections were stained with an anti-ubiquitin antibody (Rabbit ubiquitin, 1:400, Cat# ab7780, Abcam) to detect intranuclear inclusions.[8] Photomicrographs were captured with a confocal laser scanning microscope (Zeiss LSM 980 with Airyscan 2) or an automatic digital slide scanner (3D-HISTECH Pannoramic MIDI). All immunostaining, measurement, and statistical analysis of skin biopsy sections were performed by two independent neuropathologists who were completely blinded to the clinical diagnoses of the participants.

2.6 | Quantification of cutaneous small fiber innervation

2.6.1 | Intraepidermal nerve fiber density

According to the guidelines of the European Federation of Neurological Societies, PGP 9.5–positive nerve fibers at the epidermis–dermis junction were counted manually, and the length of the epidermal basement membrane was measured to calculate the Intraepidermal nerve fiber density (IENFD; fibers/mm).[29]

2.6.2 | Sweat gland nerve fiber density

PGP 9.5–positive nerve fibers around sweat glands were photographed. The nerve fibers intersecting a series of standardized circles around each sweat gland were counted to calculate the sweat gland nerve fiber density (SGNFD) as a percentage of intersected circles.[30]

2.6.3 | Pilomotor nerve fiber density

Slides were scanned in 30 μm Z-stack mode, PGP 9.5–positive nerve fibers around the arrector pili muscles were counted, and the pilomotor nerve fiber density (PNFD) was calculated as the number of fibers per mm along a line perpendicular to the muscle's long axis.[31]

2.6.4 | Quantification of noradrenergic, cholinergic, peptidergic, and regenerative nerve fiber innervation

Noradrenergic and cholinergic fibers in the arrector pili muscles and sweat glands were assessed using TH and VIP staining. The density of TH-positive nerves was represented as PNFD, whereas the density of VIP-positive nerves was represented as SGNFD. Peptidergic innervation, indicated by SP and CGRP, was quantified by counting fibers intersecting lines on a grid, with results expressed in intercepts/mm^2.[27] Additionally, GAP-43 staining was used to measure the density of regenerative nerve fibers in both the somatosensory and the autonomic domains, expressed as IENFD and SGNFD.

2.7 | Quantification of cutaneous intranuclear inclusion deposition

According to previous studies, intranuclear inclusions in patients with NIID were found primarily in fibroblasts, sweat gland cells, and adipocytes within the skin tissue.[8] Therefore, we quantified the frequency of intranuclear inclusions in these three cell types. The frequency of intranuclear inclusions was calculated as the number of intranuclear inclusion-positive cells divided by the total number of cells.[12]

2.8 | Statistical analysis

For descriptive statistics on the sample, quantitative data that conformed to a normal distribution are expressed as the mean ± standard deviation (SD), whereas data that did not conform to a normal distribution are presented as the median (interquartile range [IQR]). The Shapiro–Wilk test was used for testing normality. For inferential statistics, the two independent-sample *t* tests were applied for data that followed a normal distribution. For data that were not normally distributed, the Mann–Whitney *U* test was utilized. Multiple logistic regression analysis was conducted to adjust for the effects of age and sex on small fiber density. For comparisons of multiple samples, one-way analysis of variance was used. Count data are presented as percentages (%) and were compared using the χ^2 test or Fisher exact test. The diagnostic accuracy of indicators was evaluated using the receiver operating characteristic (ROC) curve and the area under the curve (AUC). The correlation of continuous variables was analyzed using Spearman correlation analysis. A p value of < 0.05 was considered statistically significant. Statistical analyses were conducted using SPSS Statistics 21.0 software (IBM Corporation).

3 | RESULTS

3.1 | Demographics and clinical features

A total of 294 subjects, including patients with NIID ($n = 56$), PD ($n = 85$), AD ($n = 68$), DPN ($n = 45$), and HCs ($n = 40$), were included in this clinicopathological study. The study design is shown in Figure 1. The patient demographics and auxiliary examination results are shown in Table 1. There were no significant differences in age or sex among patients with NIID, PD, DPN, or AD or the HCs.

Among patients with NIID, the median age was 63.0 years (IQR 56.0–67.5), and the median disease duration was 2.0 years (1.0–5.0). Twelve patients had a family history of NIID (12/56, 21.4%), and 44 were sporadic cases (44/56, 78.6%). Patients with NIID were divided into four subgroups according to their dominant clinical symptoms: 27 with the paroxysmal symptom-dominant type (27/56, 48.2%), 15 with the dementia-dominant type (15/56, 26.8%), 9 with the movement disorder–dominant type (9/56, 16.1%), and 5 with the muscle weakness–dominant type (5/56, 8.9%). All patients with NIID presented with ubiquitin-positive intranuclear inclusions in their skin cells. Additionally, all patients with NIID carried abnormal *NOTCH2NLC* GGC repeat expansions (repeats > 60 repeats), with a median GGC repeat size of 112.0 (101.0–124.0).

3.2 | Small fiber symptoms in patients with NIID

Further evaluation of small fiber symptoms was conducted (Table 1). The median SFN-SIQ score in the NIID group was 6.0 (3.5–9.0), which was not different from those of the PD (6.0 vs. 5.0, $p = 0.061$) and DPN (6.0 vs. 7.0, $p = 0.340$) groups but was higher than those of the AD (6.0 vs. 2.0, $p < 0.001$) and HCs (6.0 vs. 0.0, $p < 0.001$) groups. The most common small fiber symptom was changed sweating (56.9%), followed by symptoms such as dry mouth (54.9%), micturition problems (51.0%), constipation (43.1%), dizziness standing (35.3%), and dry eyes (35.3%). Sensory symptoms such as sensitive skin and sheet intolerance were reported in < 5% of patients with NIID. The median COMPASS-31 score in the NIID group was 17.1 (8.1–27.0), which was similar to that in the PD group (17.1 vs. 14.1, $p = 0.169$) but greater than those in the AD (17.10 vs. 3.6, $p < 0.001$), DPN (17.1 vs. 10.0, $p = 0.005$), and HC (17.1 vs. 0.0, $p < 0.001$) groups. The commonly affected domains were gastrointestinal (76.0%), secretomotor (74.0%), bladder (66.0%), pupillomotor functions (58.0%), and orthostatic intolerance (30.0%), with vasomotor disturbances (6.0%) being less reported. In general, the NIID group presented more severe bladder and pupillomotor symptoms than the other groups did (all $p < 0.05$).

3.3 | Assessment of nerve fiber function in NIID

Large fiber function was assessed by NCS in 30 patients with NIID. Among these patients, the median CMAP and MCV of the common peroneal nerve were 4.0 (2.3–5.6) mV and 38.0 (34.0–40.0) m/s, respectively. The median SNAP and SCV of the sural nerve were 5.0 (3.0–7.0) and 37.0 (34.0–39.0) m/s, respectively. Notably, 10 patients (33.3%) had CMAP values below the normal range, 21 patients (70.0%) had reduced MCV, 16 patients (53.3%) had decreased SNAP, and 24 patients (80.0%) presented lower-than-normal SCVs. An abnormal SSR was found in 36.8% of patients with NIID.

Small fiber function was assessed by quantitative sensory testing in all participants (Table 2). Both the WDT and HPT were greater and the CDT and CPT were lower in patients with NIID than in the other groups (all $p < 0.001$), indicating that patients with NIID had impaired thermal sensation function. Additionally, at frequencies of 2000, 250, and 5 Hz, the current perception thresholds in patients with NIID were greater than those in the other groups (all $p < 0.05$), indicating that patients with NIID had impaired pain sensation function.

The autonomic nerve function tests included the tilt table test to assess vasomotor function and the Sudoscan device to evaluate sudomotor function (Table 2). The results of the tilting table test revealed that the decreases in systolic blood pressure and diastolic blood pressure in patients with NIID were greater than those in the other groups

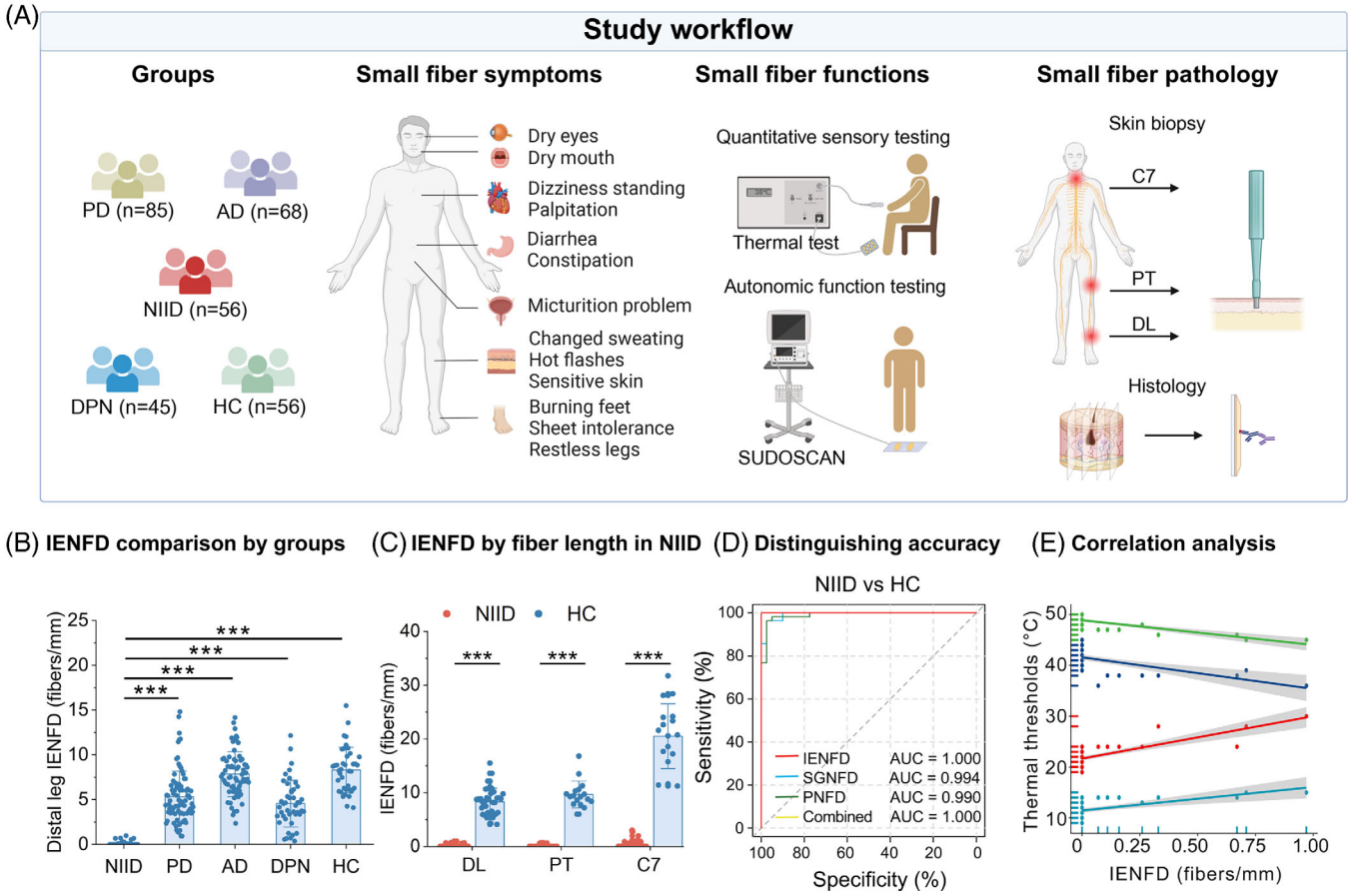

FIGURE 1 Study design. A, This study included a total of 294 participants, including patients with NIID ($n = 56$), PD ($n = 85$), AD ($n = 68$), and DPN ($n = 45$) and HCs ($n = 40$). All participants completed detailed small fiber symptom self-report questionnaires, assessments of quantitative sensory and autonomic function testing, and evaluations of small fiber pathology. Further analysis included IENFD comparison by group (B), IENFD by fiber length (C), diagnostic accuracy (D), and correlation analysis (E). AD, Alzheimer's disease; AUC, area under the curve; C7, seventh cervical paravertebral area; DL, distal leg; DPN, diabetic peripheral neuropathy; HC, healthy control; IENFD, intraepidermal nerve fiber density; NIID, neuronal intranuclear inclusion disease; PD, Parkinson's disease; PNFD, pilomotor nerve fiber density; PT, proximal thigh; SGNFD, sweat gland nerve fiber density.

(all $p < 0.05$), and the heart rate was lower in patients with NIID than in the other groups (all $p < 0.05$). Sudomotor function assessment revealed that the NIID group had worse ESCs than the other groups did (all $p < 0.01$). These results indicate that patients with NIID have more severe autonomic dysfunction than other patients do.

3.4 | Assessments of small fiber innervation in NIID

Morphological analysis of skin samples revealed that HCs and patients with AD had abundant intraepidermal, sweat gland, pilomotor, and vasomotor nerve fibers and dermal nerve bundles, whereas these nerves were less dense in patients with PD and DPN. In patients with NIID, a substantial reduction in nerve fiber density was observed, with intraepidermal nerve fibers completely absent in most cases, indicating severe damage to somatosensory nerve integrity (Figure 2).

Quantitative analysis of nerve fiber density revealed that the IENFD was reduced in patients with NIID, regardless of the distal leg, proximal thigh, or cervical area (all $p < 0.001$, Table 3), suggesting that small fiber damage is not length dependent. Notably, all patients with NIID had distal leg IENFDs below the age-normalized reference value and were diagnosable with SFN. Autonomic nerve fiber densities, including SGNFD and PNFD, were lower in the NIID group than in the other groups (all $p < 0.05$, Table 3). Additionally, there were no significant differences in IENFD, SGNFD, or PNFD among the different subgroups of patients with NIID (Figure S1 in supporting information).

Further analysis of axonopathy patterns combined with NCS revealed that 53.3% (16/30) of these patients exhibited mixed neuropathy patterns involving both large and small nerve fibers. In contrast, 46.7% (14/30) of the patients had pure small fiber involvement. The damage of small fibers was more extensive than that of large fibers in NIID (100 .0% vs. 53.3%, $p < 0.001$). These findings highlight the complexity and diversity of neuropathic injury patterns in NIID and underscore the significant involvement of the SFN in this condition.

TABLE 1 Demographic and clinical data in study participants.

	NIID	PD	AD	DPN	HC	p^a	p^b	p^c	p^d
Age (years)	63.0 (56.0–67.5)	64.0 (55.0–69.0)	65.0 (57.0–71.0)	62.0 (55.0–66.0)	61.0 (52.5–65.0)	0.859	0.217	0.433	0.086
Sex ratio (male/ female)	23/33	36/49	26/42	21/24	17/23	0.880	0.748	0.573	0.889
Age at onset (years)	58.5 (49.5–54.0)	57.0 (52.0–65.0)	60.0 (53.0–68.5)	58.0 (51.0–63.0)	/	0.619	0.100	0.771	/
Disease duration (years)	2.0 (1.0–5.0)	2.0 (1.0–4.0)	3.0 (1.0–4.0)	2.0 (1.0–4.0)	/	0.736	0.895	0.570	/
Ubiquitin–positive intranuclear inclusions, %	56/56 (100%)	0/85 (0%)	0/68 (0%)	0/45 (0%)	0/40 (0%)	<0.001	<0.001	<0.001	<0.001
***NOTCH2NLC* GGC repeats**	111.0 (96.0–123.0)	19.0 (14.0–24.0)	18.5 (14.0–20.0)	19.0 (18.0–21.0)	18.0 (14.0–21.0)	<0.001	<0.001	<0.001	<0.001
SFN-SIQ total	6.0 (3.5–9.0)	5.0 (3.0–7.0)	2.0 (0.0–6.0)	7.0 (4.0–10.0)	0.0 (0.0–1.0)	0.061	<0.001	0.340	<0.001
Changed sweating	1.0 (0.0–2.0)	0.0 (0.0–2.0)	0.0 (0.0–1.5)	0.0 (0.0–2.0)	0.0 (0.0–0.0)	0.166	0.007	0.733	<0.001
Diarrhea	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.004	0.034	0.337	0.261
Constipation	0.0 (0.0–1.0)	1.0 (0.0–2.0)	0.0 (0.0–0.5)	1.0 (0.0–2.0)	0.0 (0.0–0.0)	0.156	0.083	0.013	<0.001
Micturition problem	1.0 (0.0–3.0)	0.0 (0.0–1.0)	0.0 (0.0–0.0)	0.0 (0.0–1.0)	0.0 (0.0–0.0)	0.000	<0.001	0.005	<0.001
Dry eyes	0.0 (0.0–1.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–1.0)	0.0 (0.0–0.0)	0.139	0.002	0.486	0.002
Dry mouth	1.0 (0.0–2.0)	0.0 (0.0–1.0)	0.0 (0.0–1.0)	1.0 (0.0–2.0)	0.0 (0.0–0.0)	0.136	0.013	0.981	<0.001
Dizziness standing	0.0 (0.0–1.0)	0.0 (0.0–1.0)	0.0 (0.0–0.0)	1.0 (0.0–1.0)	0.0 (0.0–0.0)	0.708	0.002	0.039	0.004
Palpitation	0.0 (0.0–1.0)	0.0 (0.0–1.0)	0.0 (0.0–0.0)	0.0 (0.0–1.0)	0.0 (0.0–0.0)	0.989	0.180	0.083	0.001
Hot flashes	0.0 (0.0–1.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–1.0)	0.0 (0.0–0.0)	0.066	0.031	0.775	0.001
Sensitive skin	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.291	0.248	0.261	0.376
Burning feet	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.070	0.404	0.549	0.041
Sheet intolerance	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.012	0.620	0.089	0.208
Restless legs	0.0 (0.0–0.0)	0.0 (0.0–1.0)	0.0 (0.0–1.0)	1.0 (0.0–1.0)	0.0 (0.0–0.0)	0.333	0.374	0.002	0.004
COMPASS-31 total	17.1 (8.1–27.0)	14.1 (7.3–22.4)	3.6 (0.0–11.7)	10.0 (0.0–16.2)	0.0 (0.0–2.7)	0.169	<0.001	0.001	<0.001
Orthostatic intolerance	0.0 (0.0–8.0)	0.0 (0.0–12.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.735	0.001	0.086	0.002
Vasomotor	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	>0.999	0.989	0.751	0.117
Secretomotor	6.4 (0.0–8.6)	6.4 (0.0–8.6)	0.0 (0.0–2.1)	2.1 (0.0–6.4)	0.0 (0.0–0.0)	0.355	<0.001	0.108	<0.001
Gastrointestinal	4.5 (0.9–6.3)	3.6 (0.0–6.2)	0.0 (0.0–3.6)	0.9 (0.0–6.2)	0.0 (0.0–0.0)	0.252	<0.001	0.069	<0.001
Bladder	2.2 (0.0–6.7)	0.0 (0.0–2.2)	0.0 (0.0–0.0)	0.0 (0.0–1.1)	0.0 (0.0–0.0)	<0.001	<0.001	<0.001	<0.001
Pupillomotor	1.3 (0.0–2.3)	0.0 (0.0–1.7)	0.0 (0.0–0.2)	0.0 (0.0–1.7)	0.0 (0.0–0.0)	0.020	<0.001	0.018	<0.001

Note: All the quantitative variables are expressed as median (IQR). Proportions are expressed as percentages.
Abbreviations: AD, Alzheimer's disease; COMPASS-31, Composite Autonomic Symptom Score 31 items; DPN, diabetic peripheral neuropathy; HC, healthy control; IQR, interquartile range; NIID, neuronal intranuclear inclusion disease; PD, Parkinson's disease; SFN-SIQ, Small Fiber Neuropathy Symptoms Inventory Questionnaire.
[a]NIID versus PD.
[b]NIID versus AD.
[c]NIID versus DPN.
[d]NIID versus HC.

3.5 | Assessment of damage to different nerve fiber types

To determine the extent of nerve fiber involvement in patients with NIID, we meticulously examined the innervation of various nerve fiber types, including noradrenergic, cholinergic, peptidergic, and regenerative fibers. Compared to those in HCs, quantitative analysis of TH-positive autonomic fibers in the arrector pili muscles revealed a marked decrease in noradrenergic nerves in patients with NIID ($p < 0.001$, Figure 3A–C). Similarly, a reduction in cholinergic nerves was observed in sweat glands, as indicated by decreased VIP-positive autonomic nerves ($p < 0.001$, Figure 3D–F). The distributions of peptidergic nerve fibers, reflected by CGRP and SP in the dermis, were also notably reduced in patients with NIID (all $p < 0.001$, Figure 3G–L). Moreover, the application of GAP-43 staining underscored a decrease in nerve fiber regeneration in patients with NIID, with sensory nerve regeneration being almost undetectable ($p < 0.001$, Figure 3M–O), whereas autonomic nerve regeneration remained present but was reduced in patients with NIID compared to HCs ($p < 0.001$, Figure 3P–R).

3.6 | Nerve fiber density as a diagnostic index in NIID

We evaluated the diagnostic utility of distal leg IENFD, SGNFD, and PNFD in distinguishing patients with NIID from patients with PD, AD, and DPN and the HCs (Figure 4A–C). ROC curve analysis demonstrated that all three metrics (IENFD, SGNFD, and PNFD) exhibited high diagnostic accuracy in differentiating patients with NIID from the other groups (all AUCs > 0.800, Figure 4D–G). Among them, IENFD consistently showed the highest diagnostic value across different groups (NIID vs. PD: AUC = 1.000, 95% confidence interval [CI]:

TABLE 2 Assessments of small fiber nerve functions in study participants.

	NIID	PD	AD	DPN	HC	p^a	p^b	p^c	p^d
Quantitative sensory testing									
Thermal testing									
WDT (°C)	41.0 (40.0–43.0)	36.0 (35.0–41.0)	36.0 (35.0–37.0)	37.0 (36.0–38.0)	35.0 (33.5–36.0)	<0.001	<0.001	<0.001	<0.001
CDT (°C)	22.0 (21.0–23.0)	27.0 (25.0–28.0)	28.0 (25.0–30.0)	25.0 (23.0–27.0)	27.0 (26.0–29.0)	<0.001	<0.001	<0.001	<0.001
HPT (°C)	49.0 (48.0–50.0)	42.0 (41.0–45.0)	43.0 (41.0–44.0)	45.0 (42.0–46.0)	43.0 (40.0–45.0)	<0.001	<0.001	<0.001	<0.001
CPT (°C)	12.0 (10.0–13.0)	16.0 (10.0–20.0)	16.0 (10.5–21.0)	15.0 (10.0–19.0)	16.0 (12.0–20.5)	0.002	<0.001	0.007	<0.001
Current perception threshold									
2000 Hz (mA)	373.5 (341.0–445.0)	305.0 (259.0–366.0)	305.5 (251.0–369.5)	324.0 (292.0–420.0)	305.0 (260.0–353.5)	<0.001	<0.001	0.022	<0.001
250 Hz (mA)	799.0 (799.0–799.0)	155.0 (115.0–226.0)	154.5 (121.0–199.5)	180.0 (140.0–799.0)	163.0 (135.5–202.5)	<0.001	<0.001	<0.001	<0.001
5 Hz (mA)	799.0 (799.0–799.0)	139.0 (93.0–185.0)	113.0 (83.5–158.5)	122.0 (99.0–164.0)	130.5 (98.0–192.0)	<0.001	<0.001	<0.001	<0.001
Autonomic function testing									
Tilt table testing									
SBP decrease (mmHg)	13.0 (6.0–18.5)	3.5 (0.0–17.5)	0.5 (0.0–5.0)	7.0 (0.0–11.0)	5.0 (3.0–7.0)	0.006	<0.001	0.013	<0.001
DBP decrease (mmHg)	6.5 (4.0–9.0)	2.0 (0.0–7.5)	2.5 (0.0–4.0)	0.0 (0.0–4.0)	3.0 (2.0–5.0)	0.002	<0.001	<0.001	<0.001
HR increase (bpm)	5.5 (2.0–7.0)	8.5 (5.0–15.5)	8.5 (3.0–16.0)	9.0 (7.0–12.0)	16.0 (13.0–21.0)	<0.001	0.012	0.002	<0.001
Sudomotor function assessment									
ESC (μS)	40.0 (22.0–51.0)	50.0 (40.0–58.0)	81.0 (76.0–86.0)	48.0 (33.0–54.0)	82.5 (75.0–88.0)	<0.001	<0.001	0.037	<0.001

Note: All the quantitative variables are expressed as median (IQR).

Abbreviations: AD, Alzheimer's disease; CDT, cold detection threshold; CPT, cold pain threshold; DBP, diastolic blood pressure; DPN, diabetic peripheral neuropathy; ESC, electrochemical skin conductance; HC, healthy control; HPT, heat pain threshold; HR, heart rate; IQR, interquartile range; NIID, neuronal intranuclear inclusion disease; PD, Parkinson's disease; SBP, systolic blood pressure; WDT, warm detection threshold.

[a]NIID versus PD.

[b]NIID versus AD.

[c]NIID versus DPN.

[d]NIID versus HC.

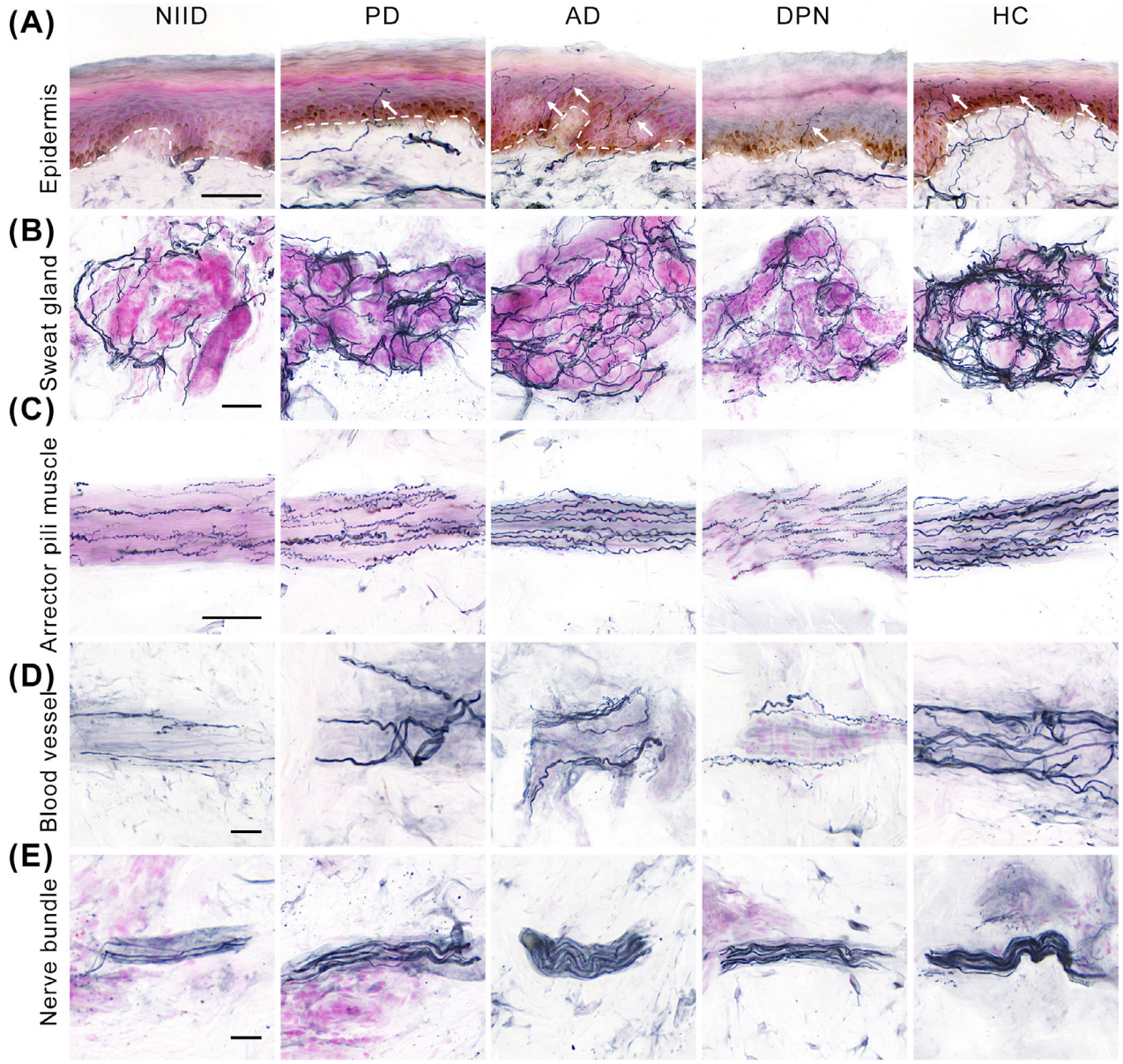

FIGURE 2 Cutaneous innervation across different groups. Representative images taken with an optical microscope showing PGP9.5-labeled nerve fibers, including those passing through the basement membrane to form intraepidermal nerve fibers (A), those surrounding the sweat gland to form sweat gland nerve fibers (B), those surrounding the arrector pili muscle to form pilomotor nerve fibers (C), those surrounding the blood vessel to form vasomotor nerve fibers (D), and those surrounding the dermal nerve bundle (E). These images sequentially depict conditions, including NIID, PD, AD, DPN, and HC. Scale bars = 50 μm in A–C and 20 μm in D and E. AD, Alzheimer's disease; DPN, diabetic peripheral neuropathy; HC, healthy control; NIID, neuronal intranuclear inclusion disease; PD, Parkinson's disease.

0.998–1.000, $p < 0.001$; NIID vs. AD: AUC = 1.000, 95% CI: 1.000–1.000, $p < 0.001$; NIID vs. DPN: AUC = 0.994, 95% CI: 0.986–1.000, $p < 0.001$; NIID vs. HCs: AUC = 1.000, 95% CI: 1.000–1.000, $p < 0.001$).

3.7 | Correlations of nerve fiber density with clinical and functional indicators

Correlation analysis of skin nerve fiber density in patients with NIID revealed a positive correlation between SGNFD and PNFD ($\rho = 0.734$, $p < 0.001$). However, no significant correlation was detected between IENFD and either SGNFD ($\rho = -0.239$, $p = 0.075$) or PNFD ($\rho = -0.031$, $p = 0.823$). Further analysis linking nerve fiber densities with clinical scales revealed no correlations between IENFD and SFN-SIQ scores ($\rho = 0.169$, $p = 0.241$) or COMPASS-31 scores ($\rho = 0.128$, $p = 0.377$; Figure 5A). In contrast, SGNFD was negatively correlated with SFN-SIQ ($\rho = -0.483$, $p < 0.001$) and COMPASS-31 scores ($\rho = -0.677$, $p < 0.001$; Figure 5B), as was PNFD (SFN-SIQ: $\rho = -0.458$, $p < 0.001$; COMPASS-31: $\rho = -0.657$, $p < 0.001$; Figure 5C), when these clinical scales were used.

TABLE 3 Qualifications of small fiber nerve densities in study participants.

	NIID	PD	AD	DPN	HC	p^a	p^b	p^c	p^d
Distal leg									
IENFD (fibers/mm)	0.0 (0.0–0.0)	4.7 (3.5–6.5)	7.6 (6.2–9.4)	4.4 (3.2–6.0)	8.3 (6.1–9.8)	<0.001	<0.001	<0.001	<0.001
SGNFD (%)	19.8 (16.5–24.0)	30.0 (24.4–35.0)	42.6 (32.6–50.3)	32.6 (27.4–36.0)	42.1 (38.9–50.6)	<0.001	<0.001	<0.001	<0.001
PNFD (fibers/mm)	32.4 (22.6–36.9)	50.6 (37.3–57.3)	66.1 (51.6–80.1)	46.3 (40.7–55.1)	69.9 (58.0–79.2)	<0.001	<0.001	<0.001	<0.001
Proximal thigh									
IENFD (fibers/mm)	0.0 (0.0–0.0)	9.8 (6.2–10.5)	9.6 (6.8–11.9)	6.5 (5.0–9.1)	9.3 (8.4–10.3)	<0.001	<0.001	<0.001	<0.001
SGNFD (%)	23.4 (20.3–27.0)	31.3 (29.2–33.0)	42.9 (42.1–44.5)	27.9 (25.1–35.2)	45.6 (39.7–49.2)	0.009	0.002	0.032	<0.001
PNFD (fibers/mm)	40.0 (24.9–40.3)	56.4 (49.4–68.3)	56.3 (49.4–60.6)	52.0 (41.3–59.2)	72.3 (58.6–77.8)	0.001	0.014	0.015	<0.001
Cervical area									
IENFD (fibers/mm)	0.0 (0.0–0.5)	19.1 (15.3–20.6)	17.2 (11.2–19.5)	17.9 (13.3–18.6)	20.7 (17.2–24.0)	<0.001	<0.001	<0.001	<0.001
SGNFD (%)	31.8 (17.4–34.9)	44.6 (42.6–45.4)	51.9 (42.6–62.0)	42.7 (36.5–48.0)	67.4 (56.9–76.1)	<0.001	<0.001	0.034	<0.001
PNFD (fibers/mm)	52.1 (39.4–64.3)	71.1 (63.6–77.9)	79.1 (59.5–94.2)	82.0 (65.0–86.9)	90.4 (79.9–99.1)	0.003	0.042	0.002	<0.001

Note: All the quantitative variables are expressed as median (IQR).
Abbreviations: AD, Alzheimer's disease; DPN, diabetic peripheral neuropathy; HC, healthy control; IENFD, intraepidermal nerve fiber density; IQR, interquartile range; NIID, neuronal intranuclear inclusion disease; PD, Parkinson's disease; PNFD, pilomotor nerve fiber density; SGNFD, sweat gland nerve fiber density.
[a]NIID versus PD.
[b]NIID versus AD.
[c]NIID versus DPN.
[d]NIID versus HC.

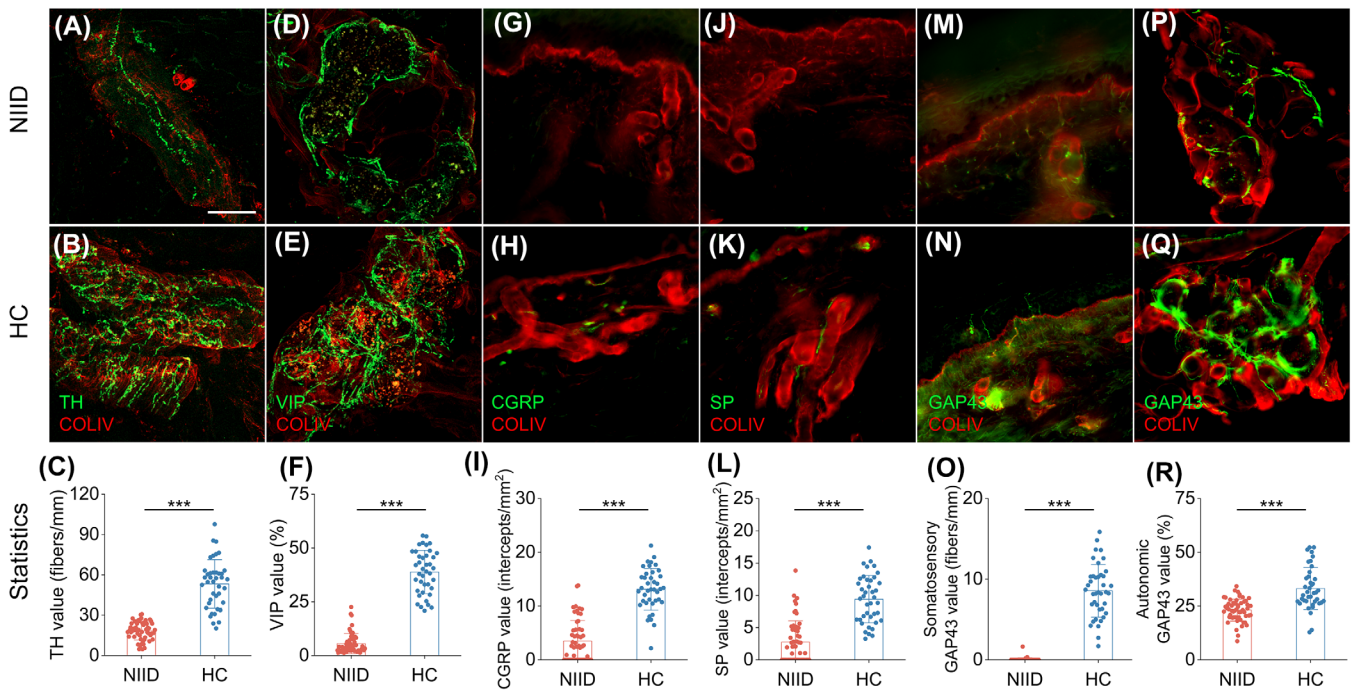

FIGURE 3 Noradrenergic, cholinergic, peptidergic, and regenerative nerve fibers in patients with NIID and HCs. Confocal microscopy images of skin sections paired with corresponding quantitative analyses revealing a comparative investigation of different nerve fibers in patients with NIID and HCs, including TH-labeled noradrenergic nerve fibers (A–C), VIP-labeled cholinergic nerve fibers (D–F), CGRP (G–I)- and SP (J–L)-labeled peptidergic nerve fibers, and GAP-43-labeled somatosensory (M–O) and autonomic regenerative nerve fibers (P–R). Scale bars = 50 μm. ***, $p < 0.001$. CGRP, calcitonin gene–related peptide; GAP-43, growth-associated protein 43; HC, healthy control; NIID, neuronal intranuclear inclusion disease; SP, substance P; TH, tyrosine hydroxylase; VIP, vasoactive intestinal peptide.

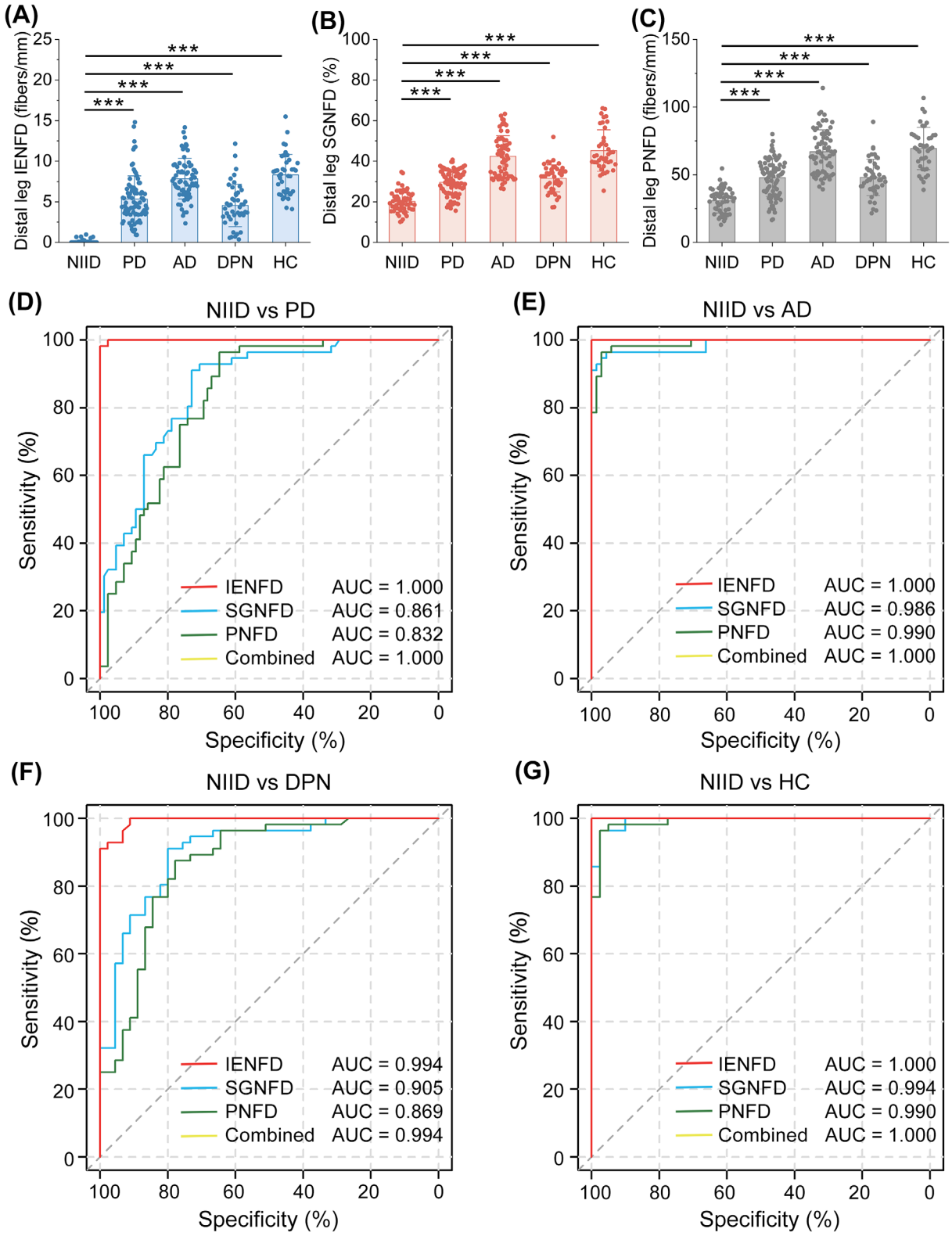

FIGURE 4 Diagnostic value of small fiber density. The bar plots quantitatively compare the IENFD (A), SGNFD (B), and PNFD (C) at the distal leg across the study groups. ***, $p < 0.001$. ROC curve analysis of IENFD, SGNFD, PNFD, and their combination in distinguishing NIID from PD (D), AD (E), DPN (F), and HC (G). AD, Alzheimer's disease; AUC, area under the curve; DPN, diabetic peripheral neuropathy; HC, healthy control; IENFD, intraepidermal nerve fiber density; NIID, neuronal intranuclear inclusion disease; PD, Parkinson's disease; PNFD, pilomotor nerve fiber density; ROC, receiver operating characteristic; SGNFD, sweat gland nerve fiber density.

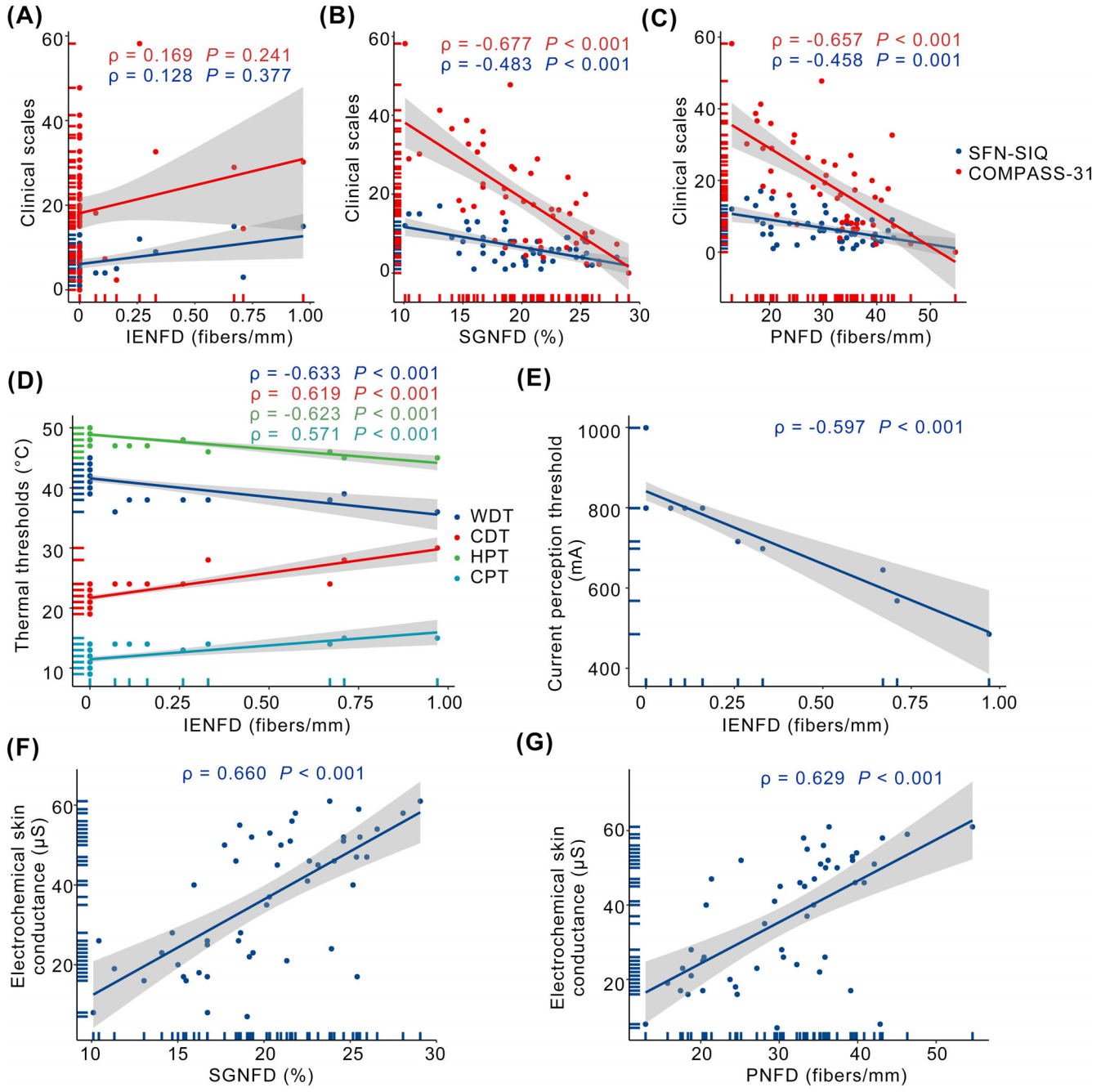

FIGURE 5 Correlations of nerve fiber density with clinical and functional indicators. Correlation analysis of IENFD (A), SGNFD (B), and PNFD (C) with SFN-SIQ and COMPASS-31 scores. Correlation analysis of IENFD with the results of quantitative sensory testing, including thermal thresholds (D) and current perception thresholds at a frequency of 5 Hz (E). Correlation analysis of SGNFD (F) and PNFD (G) with the results of the sudomotor function assessment. COMPASS-31, Composite Autonomic Symptom Score-31; IENFD, intraepidermal nerve fiber density; PNFD, pilomotor nerve fiber density; SFN-SIQ, Small-Fiber Neuropathy Symptoms Inventory Questionnaire; SGNFD, sweat gland nerve fiber density; ρ, Spearman correlation coefficient.

There was also an association between nerve fiber density and function in patients with NIID. IENFD was negatively correlated with WDT ($\rho = -0.633$, $p < 0.001$) and HPT ($\rho = -0.623$, $p < 0.001$) and positively correlated with CDT ($\rho = 0.619$, $p < 0.001$) and CPT ($\rho = 0.571$, $p < 0.001$; Figure 5D) in the thermal test. Additionally, IENFD was negatively correlated with the current perception thresholds at a frequency of 5 Hz ($\rho = -0.597$, $p < 0.001$; Figure 5E). SGNFD ($\rho = 0.660$, $p < 0.001$; Figure 5F) and PNFD ($\rho = 0.629$, $p < 0.001$; Figure 5G) were positively correlated with ESCs in the sudomotor function test.

3.8 | Relationship between small fiber density and intranuclear inclusion deposition

We further investigated the relationship between small fiber involvement and intranuclear inclusion formation in the skin (Figure S2 in supporting information). Correlation analysis revealed no significant associations between the frequency of intranuclear inclusions and IENFD ($\rho = 0.248$, $p = 0.187$), SGNFD ($\rho = -0.179$, $p = 0.345$), or PNFD ($\rho = -0.133$, $p = 0.482$). Additionally, the quantification of inclusions within fibroblasts and sweat gland cells revealed no significant correlation with IENFD ($\rho = 0.197$, $p = 0.298$) or SGNFD ($\rho = -0.253$, $p = 0.213$). These findings suggest that small fiber reduction in NIID may not be directly linked to the formation of intranuclear inclusions in the skin.

4 | DISCUSSION

This study systematically investigated SFN in patients with NIID, including assessments of small fiber symptoms, electrophysiology, and pathology. The main findings indicate that patients with NIID present with a range of small fiber symptoms and wide and severe small nerve fiber damage. Furthermore, small fiber densities have been shown to accurately distinguish patients with NIID from those with other diseases and HCs. The reduction in small fiber density was correlated with clinical symptoms and electrophysiological indicators of SFN.

Peripheral neuropathy is a common symptom in patients with NIID.[9–11] In a recent study, although central nervous system symptoms were predominant, subclinical peripheral neuropathy was also common in patients with NIID even when clinical symptoms were not initially observable.[9] Another study in Japan, which analyzed *NOTCH2NLC* repeat expansion in many patients clinically diagnosed with inherited peripheral neuropathy/Charcot–Marie–Tooth (CMT) disease, identified *NOTCH2NLC* repeat expansions in several cases. The findings included a mean median motor nerve conduction velocity of 41 m/s and a classification of 69% of cases as intermediate CMT. These findings suggest that the differences in the dominant phenotype of non–length-dependent motion and prominent autonomic nervous system involvement are attributed to the clinical heterogeneity of *NOTCH2NLC*-associated diseases.[32] Sural nerve biopsy in patients with NIID revealed a mild to moderate loss of myelinating fibers, with some thinly myelinating fibers, whereas a few cases presented severe losses. Electron microscopy corroborated these findings, revealing a decreased density of unmyelinated fibers.[9] These findings indicate that primary demyelinating impairment with subtle axonal degeneration is the main pathogenic mechanism of peripheral neuropathy in patients with NIID. Our research thereby expands the understanding of axonopathy patterns present in NIID. All patients with NIID presented IENFD in the distal legs below age-standardized reference values, confirming the presence of SFN. Furthermore, combined analysis with nerve conduction studies revealed a mixed neuropathy pattern involving both large and small nerve fibers in more than half of the patients with NIID, although some exhibited pure SFN. It is important to further investigate whether peripheral neuropathy in patients with NIID follows a progression from small to large fiber involvement, akin to patterns observed in diabetes and other neurodegenerative diseases.[33,34]

In this study, we assessed skin nerve fiber morphology in patients with NIID, demonstrating for the first time a significant reduction in both somatosensory and autonomic nerve fiber density in patients with NIID compared to those with other neurological disorders. SFN characteristically affects small-diameter, thinly myelinated Aδ fibers and unmyelinated C fibers.[35] Previous studies have documented a reduction in small fiber density in neurodegenerative diseases with inclusion bodies, such as PD and multiple system atrophy, suggesting that small fiber damage might be an intrinsic pathological feature of these diseases.[26,36] Intriguingly, our findings indicate a reduction in skin nerve fiber density in both the distal and proximal areas in patients with NIID, suggesting a non–length-dependent pattern of small fiber nerve damage. This pattern contrasts sharply with the length-dependent pattern observed in traditional SFNs, such as diabetic neuropathy and PD-related neuropathy, possibly reflecting a unique pathologic mechanism in NIID.[16,36–38] For biopsy site selection, the distal leg and proximal thigh can reflect length-dependent patterns of small fiber nerve damage, while the neck is commonly chosen for neurodegenerative diseases like PD, representing the most proximal skin. The lumbar skin can also be selected as an option.

In patients with NIID, significant decreases in SGNFD and PNFD were also observed, indicating extensive autonomic nerve damage during the disease course. However, no correlation was found between SGNFD and IENFD, suggesting that sensory and autonomic nerve damage in patients with NIID may not occur in parallel. This finding is consistent with previous reports on SFN.[39] Quantitative assessments of somatic and autonomic symptoms in patients with NIID revealed fewer sensory symptoms typical of SFN, such as sheet intolerance, sensitive skin, burning feet, and restless legs. In contrast, autonomic symptoms were common in patients with NIID in our study, which aligns with the findings of previous studies on the clinical features of NIID.[17,40] Despite the significant decline in IENFD, the clinical sensory symptoms were mild, suggesting potential subclinical stages or the absence of subjective symptoms due to excessive nerve damage. However, functional and pathological damage, which are objective indices reflecting damage to sensory nerves, are parallel. Conversely, reductions in autonomic nerve densities, such as SGNFD and PNFD, correlated with clinical and functional indicators, demonstrating a clinicopathological correlation that explains the broad manifestation of autonomic symptoms.

Our study also focused on diverse types of small fiber nerve damage in NIID, including significant reductions in cholinergic and noradrenergic nerve fibers, which typically reflect postganglionic sudomotor function.[26] Additionally, the numbers of pain-associated fibers (SP and CGRP) were markedly reduced or absent, which were correlated with minimal pain symptoms in patients with NIID. These findings suggest disease-specific declines in these fibers, akin to that in the somatosensory nerves. Furthermore, GAP-43, which is highly expressed in neuronal growth cones and is involved in axon growth and regeneration,

particularly during developmental stages and after nerve damage, was upregulated.[41,42] However, in our study, we did not observe regeneration of somatosensory nerve axons in patients with NIID, suggesting that the loss of somatosensory nerves could be partially attributed to insufficient regeneration. Nevertheless, some regeneration of autonomic nerve structures suggests a retained regenerative capacity in these areas, possibly explaining the partial preservation of autonomic nerves in patients with NIID.

Our study highlights the diagnostic value of measuring small fiber densities in NIID, demonstrating their high diagnostic potential in differentiating NIID from other conditions. Consequently, we propose that severe SFN, identified in routine skin biopsies for the diagnosis of neurological disorders, should prompt further inclusion staining to avoid misdiagnosis. IENFD proved to be a useful marker for SFN presence in patients with NIID, as it is significantly lower in patients with NIID than in those with other conditions.

The mechanisms underlying the involvement of small fibers in NIID remain unclear. Phenotypic grouping of NIID patients did not reveal differences in small fiber density, suggesting that SFN maybe a common pathologic change in NIID disease and, like inclusion deposition, is not affected by the clinical subtype of the disease. Additionally, attempts to link skin small fiber nerve damage with inclusion were unsuccessful, indicating that peripheral inclusion toxicity might not be the cause of nerve fiber damage. Recent studies have shown that GGC expansion in the *NOTCH2NLC* gene can translate into abnormal polyG proteins, whose aggregation and deposition are the primary mechanisms behind intranuclear inclusion formation in patients with NIID.[43,44] Additionally, polyG may interfere with the nuclear–cytoplasmic transport process, potentially causing neuronal cell damage and neurodegeneration.[44] However, further investigations are needed to determine whether neuronal polyG directly contributes to peripheral neurodegeneration. Additionally, because the SFN in NIID patients is more extensive than large fiber injury, the cause of small fiber damage may also stem from axonal degeneration itself, rather than being secondary to proximal neuronal degeneration.

Our study has several limitations. First, the inclusion of other neurological disorders as controls might introduce variability in outcomes. The specificity of nerve fiber density still requires further confirmation. A recent study revealed that instances of almost zero IENFD and reduced autonomic innervation also occur in patients with cerebellar ataxia with neuropathy and vestibular areflexia syndrome.[45] This similarity suggests that including a broader range of rare diseases could enhance our understanding of the diagnostic value of small fiber nerve density. Additionally, for a comprehensive assessment of nerve damage, it is necessary to perform sural nerve biopsies, which can elucidate the extent of large nerve fiber involvement.

In conclusion, our study advances the understanding of NIID by illustrating the non–length-dependent involvement of small nerve fibers and demonstrating the diagnostic potential of small fiber density measurements, providing new insights from skin biopsy. Further studies are needed to investigate the mechanisms underlying damage to the structure and function of small nerve fibers in patients with NIID, which could offer further insights into disease pathogenesis and therapeutic targets.

AUTHOR CONTRIBUTIONS

Jing Yang and Yuming Xu contributed to the conception and design of the study; Minglei Liu, Ruoyu Liu, Yanpeng Yuan, Xiaojing Liu, Lanjun Li, Yangyang Wang, Jing Yuan, Ke Zhang, Shuo Li, Ting Yang, Yanlin Wang, Yuan Gao, Han Liu, Yinge Xue, Chen Liu, Tianyuan Yang, Ying Kong, and Lin Cheng contributed to the acquisition and analysis of data; Minglei Liu, Ruoyu Liu, and Yanjiang Wang contributed to drafting the text or preparing the figures.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest. Author disclosures are available in the supporting information.

DATA AVAILABILITY STATEMENT

The data are available upon reasonable request after ethics clearance and approval from the corresponding authors.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the ethics committee of the First Affiliated Hospital of Zhengzhou University, China (Approval No. 2019-KY-294), and all the subjects signed an informed consent form in accordance with the Declaration of Helsinki.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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