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ORIGINAL ARTICLE

Skin Biopsy Findings in Patients With CMT1A: Baseline Data From the CLN-PXT3003-01 Study Provide New Insights Into the Pathophysiology of the Disorder

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Abstract

Charcot-Marie-Tooth disease type 1A (CMT1A), the most common form of Charcot-Marie-Tooth diseases, is a demyelinating neuropathy caused by a deletion encompassing the gene coding for PMP22, a myelin protein of the peripheral nervous system. Although myelinated fibers are mostly involved in CMT1A, some patients experience neuropathic pain. We thus investigated whether unmyelinated fibers are lost in CMT1A. Skin biopsies were taken from the distal portion of the leg of 80 patients with CMT1A as part of the PXT30003-01 study and processed for quantification of intraepidermal nerve fiber density (IENFD). Mean IENFD was significantly lower in CMT1A patients than in healthy controls. Although the data were highly dispersed, IENFD tended to decrease with age and was higher overall in female patients and controls than male patients and controls. This study shows that small nerve fibers are affected in CMT1A and that this correlates with pin sensitivity. The density of epidermal Langerhans cells (LCs) was also significantly reduced in CMT1A patients, suggesting the involvement of LCs in neuropathic pain processes. These findings raise several questions concerning the interactions of Schwann cells and LCs with unmyelinated fibers in CMT1A. Moreover, they suggest that factors other than PMP22 gene dosage are involved in small fiber pathology in CMT1A.

Key Words: Charcot-Marie-Tooth 1A, Langerhans cell, Neuropathic pain, Skin biopsy, Small nerve fiber.

INTRODUCTION

Charcot-Marie-Tooth (CMT) disease is the most frequent inherited neuropathy with a prevalence of ∼1 per 2500 births. Seventy-five percent of patients with CMT have CMT1A, which is transmitted in an autosomal dominant manner. CMT1A is caused by a 1.5-Mb duplication in 17p11.2, encompassing the gene coding for peripheral myelin protein 22 (PMP22). It causes demyelinating sensory and motor neuropathy with variable degrees of axon loss. CMT1A is considered to involve mainly myelinated fibers. Thus, few studies have focused on the impact of its mutation on small sensory C and Aδ fibers. However, 56%–96% of patients report positive sensory symptoms, including neuropathic pain (1). Two studies using electrophysiological techniques and questionnaires on pain evaluation have reported that pain in patients with CMT1A may be due to small fiber neuropathy (SFN) linked to the involvement of Aδ fibers (2, 3). Moreover, 1 study showed SFN in patients with CMT1A, using in vivo corneal confocal microscopy. In this study, loss of corneal C fibers was correlated with the severity of painful symptoms (4). The involvement of small fibers in CMT1A merits investigation because it may have a large impact on quality of life in these patients.

Several experimental studies have also suggested involvement of skin Langerhans cells (LCs) in neuropathic pain. For example, LCs become intensely immunoreactive to the protein PGP9.5 after sectioning the sciatic nerve (5) or in a chronic constriction model (6). Moreover, an increase in PGP9.5-immunoreactive LCs has been observed in an animal model of chemotherapy-induced neuropathy (7). In humans,

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Casanova-Molla et al reported an increase in the number of LCs in skin biopsies from patients with diabetes and pain due to SFN (8). Indeed, LCs may play a role during the process of axonal degeneration in SFN because of their pro-inflammatory properties and tight interactions with intraepidermal nerve fibers (IENFs) (9).

The main objectives of the present study were to investigate the potential involvement of unmyelinated fibers in CMT1A and assess whether there are alterations in the number and/or morphology of LCs, and finally, to link these results to clinical findings. The study is a phase 2 trial investigating the safety and efficacy of a new drug in CMT1A patients (10). As part of this study, a skin biopsy was taken from the distal region of the leg (10 cm above the lateral malleolus) of all enrolled patients. Two specimens were taken, one for biomarker analysis and the other for investigating IENF density (IENFD) and the evaluation of LCs.

MATERIALS AND METHODS

The entire protocol was approved by our local ethics committee and the Agence Nationale de la Sécurité du Médicament et des Produits de Santé (ANSM). The study was conducted in accordance with the declaration of Helsinki and according to Good Clinical Practice. Informed consent was obtained from all participating individuals.

Patients

Patients with CMT1A were recruited for the PXT3003-01 study (10). The diagnosis had to be genetically confirmed and patients had to have at least some weakness in foot dorsiflexion and a CMT neuropathy score (CMTNS) ≤ 20. Patients were excluded if they had any other neurological conditions or a concomitant systemic disease that could interfere with the neurological evaluation. Additionally, patients with abnormal liver or kidney function were also excluded. Age, sex, body mass index (BMI), and duration of illness were recorded for each patient.

Controls

Controls included in the study were patients that were initially referred for muscle biopsy or skin biopsy to the "Reference Center of Rare Peripheral Neuropathies" Limoges University Hospital and who were found to have either a normal muscle biopsy or a diagnosis of nonneuropathic pain. The exclusion criteria were all potential causes of SFN (e.g. metabolic syndrome, diabetes, neurotoxic treatment, human immunodeficiency virus, chronic alcoholism), as well as neuropathic pain of unknown origin.

Clinical Scales

The following scales were assessed at the screening phase for all patients: Charcot Marie Tooth neuropathy score (CMTNS); Overall Neuropathy Limitation Scale (ONLS); Visual Analogic Scale (VAS) for pain, fatigue, and global impression of health; Nine-Hole Peg Test (9HPT) for assessing manual dexterity; Quantitative Motor Testing (QMT) for tibialis anterior bilaterally, and bilateral Grip Test (Vigorimeter) for hand prehension. Superficial sensitivity was assessed at the legs by using a piece of cotton wool and vibration sense was assessed using a graduated 128 Hz tuning fork.

Nerve Conduction Studies

Nerve conduction parameters were assessed using standard techniques at a skin temperature of 32°C, as previously described (10). They included motor and sensory responses of median and ulnar nerves of the nondominant upper-limb.

Skin Biopsies

Skin specimens were obtained through a 3-mm punch skin biopsy 10 cm above the lateral malleolus in the region of the sural nerve. Samples were processed using established methodology (11) and 3 nonconsecutive vertical sections of 50 µm (for IENFD quantification) and 10 µm (for LCs analysis) thickness were prepared for each biopsy. Optical immunofluorescence was performed using the cytoplasmic axonal PGP 9.5 staining method (Protein Gene Product 9.5), with a polyclonal rabbit antihuman antibody (1/600, UltraClone, Isle of Wight, UK) for IENF and the monoclonal rat antihuman antibody antiLangerine/CD207 (1/200, Dendritics, Lyon, France).

IENFD was manually quantified (blindly, by 2 operators, at high magnification) with a NIKON DxM1200 light microscope. The density was calculated in at least 3 nonconsecutive sections as the number of IENF per length of section (fibers/mm) measured using NIS elements BR2.30 software (Nikon) at $\times 400$ magnification. Single fibers crossing the dermal-epidermal junction were counted according to established guidelines (11). Similarly, the number of positive LCs, for which the cell body was clearly visible, were counted at $\times 400$ magnification. The number of LCs was determined with respect to the length of the epidermis and their density expressed as the number of LCs/mm.

Statistics

Descriptive Analysis

Quantitative variables are reported for each population (controls and CMT1A) using the median, minimum, and maximum values. Correlations between clinical data and cutaneous biopsy results were determined using the Pearson correlation test.

Comparison Between Groups

We used normative values from our control population because of variation of the selection criteria between studies and heterogeneity of the methodology used for IENF staining. Thus, an IENFD below the threshold determined by the 5th percentile from the control population was considered to be abnormal. Data of CMT1A patients were compared with those of control subjects using Student parametric *t*-test if the values followed a normal distribution and a nonparametric Mann-Whitney test if they did not. All analyses were performed using Prism software version 6.04 (GraphPad Software, Inc.,

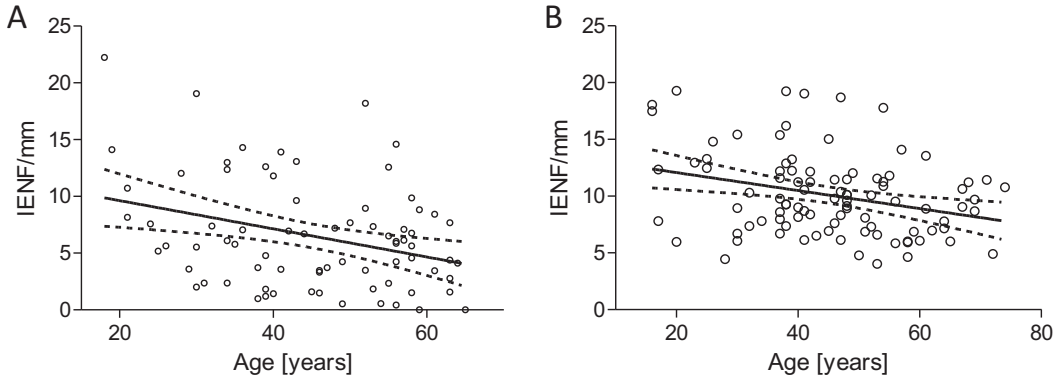

FIGURE 1. Correlations of IENFD with age in CMT1A **(A)** and control **(B)** populations. There was a significant inverse correlation between IENFD and age in CMT1A (n = 75; R = -0.33, p = 0.0041) and control (n = 94; R = -0.301, p = 0.0032) patients.

LaJolla, CA). The differences were considered to be statistically significant for $p < 0.05$.

RESULTS

Patients

Demographic Data

The CMT1A population was composed of 75 patients (31 men and 48 women). The mean age of the men was 45.78 ± 12.06 years (mean ± standard deviation) and that of the women 44.56 ± 13.29 years. The overall mean age was 45.05 ± 12.75 years. The distribution frequency of the IENFD was 5.8 [0–22.24] IENF/mm (median [minimum–maximum]). The IENFD was significantly higher in women than men (6.73 [0.54–22.24] vs 3.72 [0–12.62] IENF/mm, p = 0.0055). The IENFD significantly inversely correlated with age (Fig. 1A) and BMI (Fig. 2).

We assessed correlations between IENFD and age by gender. IENFD significantly inversely correlated with age in men and women (Fig. 3A, B).

IENFD and Clinical Parameters

There was no significant correlation between IENFD and the CMTNS, ONLS, NHPT, or VAS (Table 1). The CMTNS is a composite score of several items, including clinical and electrophysiological data on motor and sensory nerves. In order to determine whether there was a correlation between DFNIE and the various CMTNS clinical components, correlations were made with each item. There was no correlation with motor symptoms nor arm strength or leg strength. There was a slight correlation with sensitivity to vibrations and a weak, but significant, correlation with sensitivity to pinpricks (Table 2). There was a significant decrease of IENFD in CMT1A patients suffering from superficial sensitivity disorders (Fig. 4).

IENFD and Nerve Conduction Studies

We compared electrophysiological data, including motor and sensory nerve conduction parameters of the median and ulnar nerves on the nondominant side, with IENFD of CMT1A patients. There was no correlation with the motor (Table 3) or sensory parameters (Fig. 5).

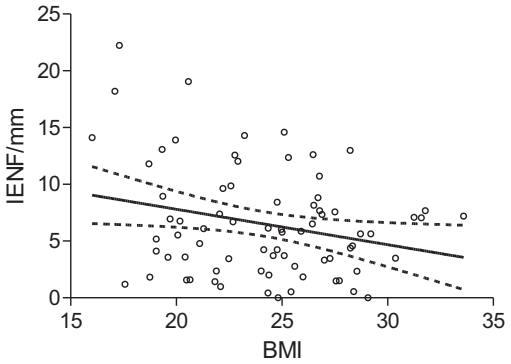

FIGURE 2. Correlation between IENFD and body mass index (BMI) in CMT1A population. There was a significant inverse correlation between IENFD and BMI in CMT1A patients (n = 75; R = -0.25, p = 0.0283).

Controls

Ninety-four control patients were included in the study (44 men and 50 women). The mean age of the men was 39.70 ± 10.62 years (mean ± standard deviation) and that of the women 50.06 ± 14.55 years. The overall mean age was 45.21 ± 13.8 years. The distribution frequency of the IENFD was 9.57 [4.04–19.29] IENF/mm (median [minimum–maximum]). There was no significant difference between men (8.82 [4.04–19.29] IENF/mm) and women (10.25 [4.64–19.04] IENF/mm). The IENFD significantly inversely correlated with age for women but not men (Fig. 3C, D).

CMT1A Versus Controls

IENFD Analysis

The normative values of the distal IENFD obtained from our control population allowed us to define a lower limit of the norm. We used the 5th percentile as the lower limit,

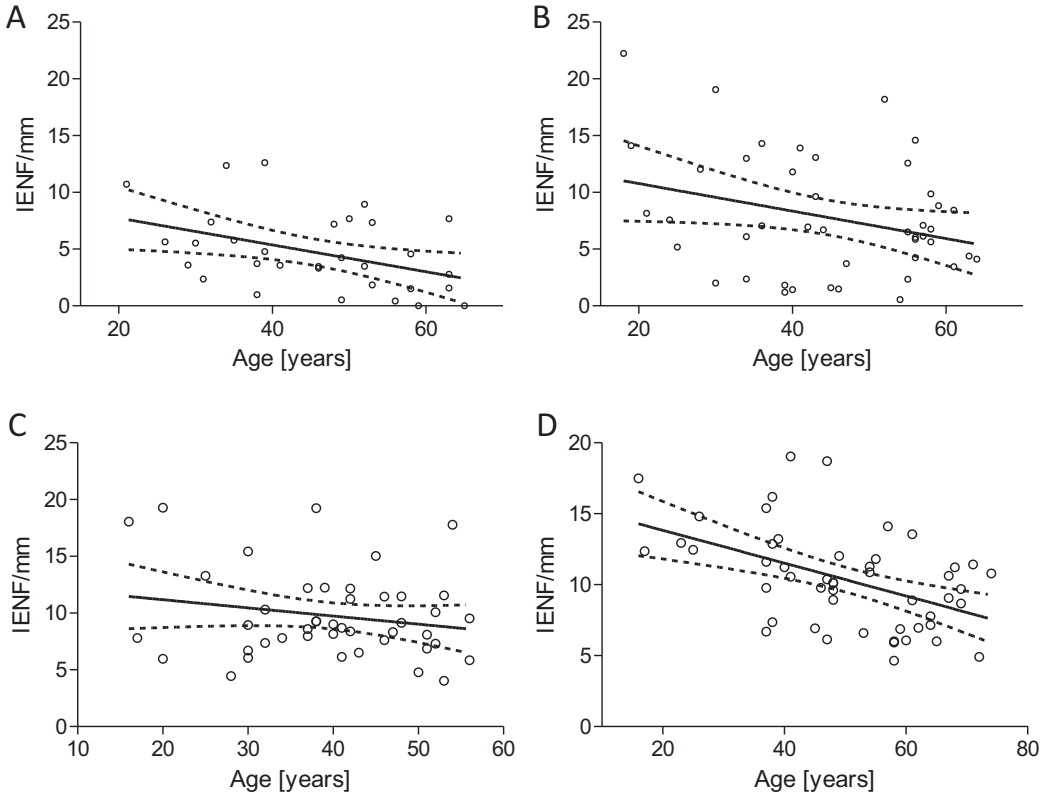

FIGURE 3. Correlation between IENFD and age by gender in CMT1A **(A, B)** and control **(C, D)** populations. In the CMT1A population, there was a significant inverse correlation between age and IENFD for men **(A)** (n = 31; R = -0.41, p = 0.0187) and women **(B)** (n = 44; R = -0.303, p = 0.046). In the control population, there was a significant and inverse correlation between IENFD and age for women **(C)** (n = 50; R = -0.484, p = 0.0004) but not men **(D)** (n = 44; R = -0.199, p = 0.1942).

TABLE 1. Correlation between IENFD and Clinical Parameters in the CMT1A Population

Clinical Parameters Versus IENFD		Pearson's Chi-Squared Test
CMTNS		n = 75; R = −0.002, p = 0.988
CMTES		n = 75; R = −0.059, p = 0.612
ONLS		n = 75; R = 0.167, p = 0.076
VAS	Pain	n = 75; R = −0.007, p = 0.476
	Tiredness	n = 75; R = 0.09, p = 0.073
	Overall	n = 75; R = 0.009, p = 0.468
NHPT	Dominant hand	n = 75; R = −0.09, p = 0.449
	Non-dominant hand	n = 75; R = 0.002, p = 0.987

TABLE 2. Correlations Between IENFD and Clinical Items of CMTNS in the CMT1A Population

Clinical Parameters of CMTNS Versus IENFD	Pearson's Chi-Squared Test
Motor symptoms	n = 75; R = 0.1746, p = 0.134
Arm strength	n = 75; R = 0.07, p = 0.549
Leg strength	n = 69; R = −0.171, p = 0.159
Vibration sensitivity	n = 75; R = −0.22, p = 0.0542
Pin sensitivity	n = 75; R = −0.252, p = **0.0304**

corresponding to 4.8 IENF/mm (16). The IENFD was significantly lower in CMT1A patients than control subjects (5.8 [0–22.24] vs 9.57 [4.04–19.29] IENF/mm, p < 0.0001). Moreover, 48% of CMT1A patients had a large reduction of IENFD below the normal lower limit.

As the IENFD varies by age and sex, comparisons were made between populations over 50 and under 50 years of age, as well as between men and women (Fig. 6). It appears that the significant decrease in IENFD in CMT1A patients is not influenced by age or sex, since the difference in IENFD between the CMT1A and control populations is still significant in the age- and matched-analysis.

Analysis of LCs

We quantified the number of LCs in 23 CMT1A patients and 23 control subjects randomly selected from the CMT1A and control populations. We observed a morphological difference between the LCs of the CMT1A and control population: LCs in CMT1A patients were smaller and had fewer extensions (Fig. 7A). This observation was common to all patients.

Quantitative analysis showed significantly fewer LCs in skin biopsies of CMT1A patients than those of the control group (CMT1A: 10.62 ± 3.87 vs control: 16.37 ± 4.72, p < 0.0001; Fig. 7B). There was no correlation between the number of LCs and IENFD of CMT1A patients (n = 23;

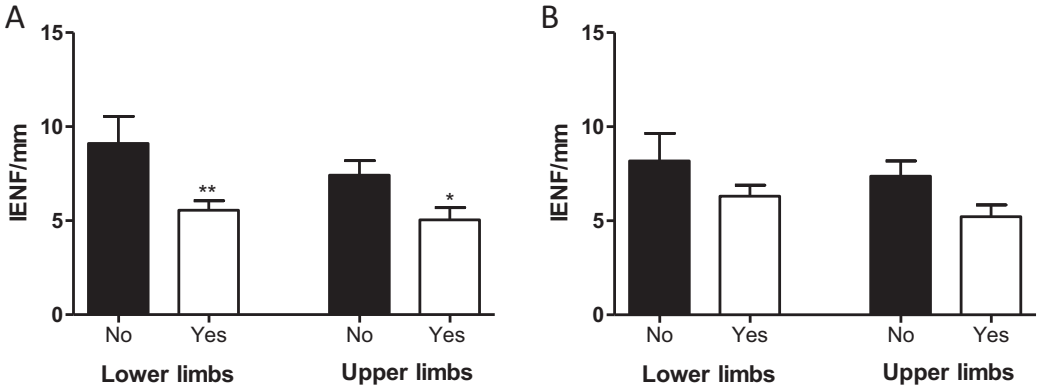

FIGURE 4. Relationship between IENFD and disorders of superficial **(A)** and deep **(B)** sensitivity of the upper and lower limbs. There was a significant difference of IENFD between patients with superficial sensitivity disorders and those without. However, there was no influence of deep sensitivity on IENFD. Bars represent the mean ± standard deviation of IENFD of patients who express sensory impairment (Yes, white bar) or not (No, black bar). Comparisons were made using the Student *t*-test (*$p < 0.05$ and **$p < 0.01$).

TABLE 3. Correlations Between IENFD and Motor Nerve Conduction Parameters in the CMT1A Population

Motor Nerve Conduction Parameters Versus IENFD		Pearson's Chi-Squared Test
Median nerve	Latency	$n = 60$; $R = 0.118$, $p = 0.370$
	Amplitude	$n = 71$; $R = -0.122$, $p = 0.307$
	Conduction velocity	$n = 69$; $R = -0.171$, $p = 0.159$
Ulnar nerve	Latency	$n = 61$; $R = -0.097$, $p = 0.453$
	Amplitude	$n = 75$; $R = -0.186$, $p = 0.11$
	Conduction velocity	$n = 70$; $R = -0.116$, $p = 0.337$

$R = 0.3317$, $p = 0.1220$) or the control population ($n = 23$; $R = 0.0006$, $p = 0.998$).

DISCUSSION

The objectives of this study were to determine the involvement of IENFs and intraepidermal LCs in CMT1A disease in a large cohort of patients who were recruited in the PXT3003-01 study.

We showed that IENFD is affected by age and weight in both CMT1A patients and controls, as previously reported (12, 13), and that women have an overall higher IENFD than men, also as previously described (14).

The median IENFD at the distal region of the leg in our control population was 9.57, similar to the results obtained by Shun et al (15), but slightly below those of most published studies (14–16). Several factors may explain this discrepancy. Our control population does not precisely correspond to normal controls as all skin biopsies were taken from patients who were initially referred for pain. However, people that had a known disorder that could be associated with polyneuropathy were excluded, although no systematic screening for classical causes of polyneuropathy was carried out. Moreover, retrospective examination of medical files of our controls (when available) showed that they did not have neuropathic pain. Another explanation of our relatively low IENFD is that we stained skin samples on slides (and not by immersion in the antibody solution) and did not use confocal microscopy. However, we used the same exact technique for controls and CMT1A patients.

We show here that small nerve fibers are affected in patients with CMT1A. These results are in accordance with those of a recent study in a far smaller cohort of patients (17). The involvement of small fibers in CMT1A has only recently been suggested. Several studies showed elevated heat response thresholds in CMT1A patients, suggesting that functionality of sensory small nerve fibers, unmyelinated C, and poor myelinated Aδ fibers, was affected in CMT1A (18). More recently, a detailed analysis of sural nerve samples from patients with CMT1A by electron microscopic examination showed alterations of nonmyelinating Schwann cells with unaffected unmyelinated axons (19). Unmyelinated C-fibers represent 90% of IENFs, thus we suggest that reduction of IENFD in CMT1A patients express the degeneration of this nociceptor population. These findings and our results suggested that axon-Schwann cells interactions are dysregulated in CMT1A, in myelinated nerve fiber and in Remack bundles too. Moreover, PMP22 is expressed by nonmyelinating Schwann cells (20). Thus, we hypothesize that overexpression of PMP22 observed in myelinated Schwann cells of CMT1A patients could equally affect nonmyelinating Schwann cells. Distal degeneration of small nerve fiber observed in skin biopsies could result from pronounced injury of nonmyelinating Schwann cells proximally. Skin biopsies can probably show abnormalities that may be missed by examination of nerve biopsy samples due to the length-dependency of axonal degeneration in CMT1A because they investigate the most distal endings of unmyelinated fibers.

The comparison of IENFD and clinical findings of CMT1A patients provides new information concerning the relationship between the severity of the disease and small fiber involvement. Among all clinical variables, only superficial sensory involvement and pinprick sensitivity correlated (as expected) with the IENFD. This suggests that the degeneration of IENFs is totally or partially independent from the course of this demyelinating neuropathy. A recent study highlight that

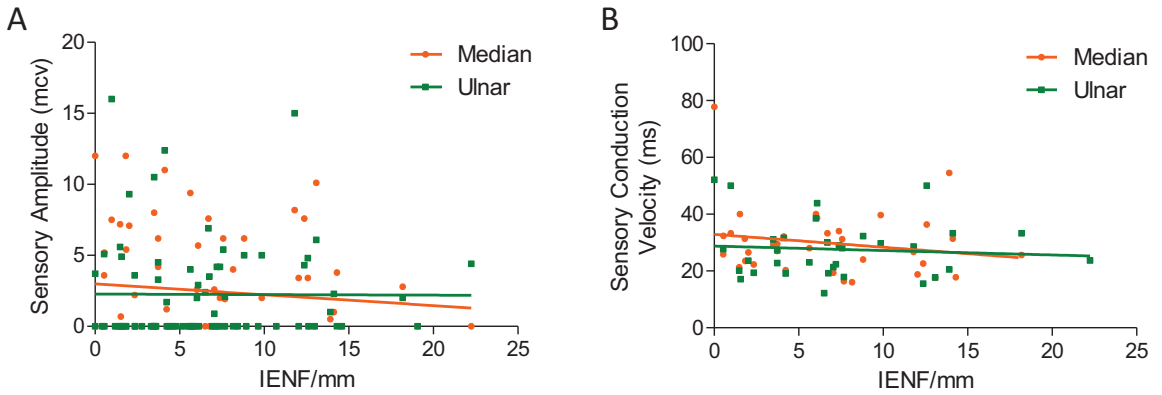

FIGURE 5. Correlation between IENFD and sensory nerve conduction parameters of the upper limbs in CMT1A population. There was no correlation between IENFD and the sensory nerve action potential amplitude **(A)** (Median nerve: n = 74; R = -0.106, p = 0.367, ulnar nerve: n = 75; R = -0.0052, p = 0.964) or nerve conduction velocity **(B)** (Median nerve: n = 35; R = -0.189, p = 0.276, ulnar nerve: n = 75; R = -0.081, p = 0.654).

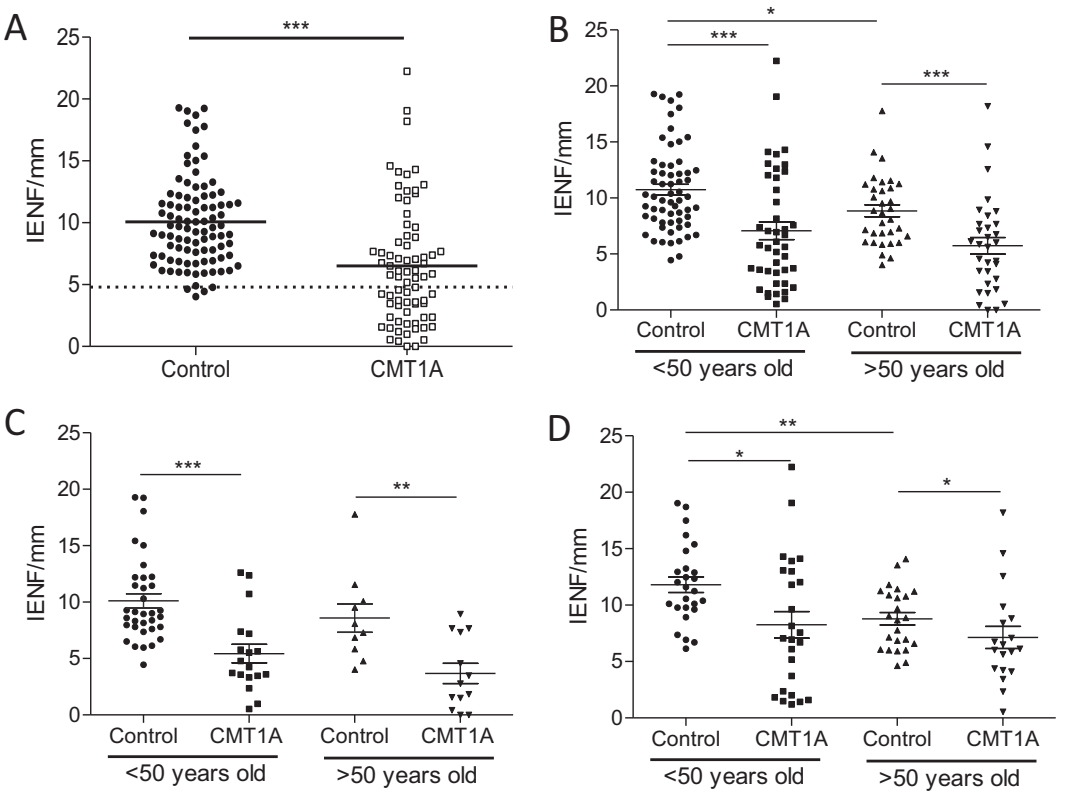

FIGURE 6. Age- and sex-matched comparison of the IENFD between CMT1A patients and the control population. **(A)** Comparison of IENFD between overall of CMT1A patients and the control population. The horizontal bar represents the average for each group. The dotted line represents the pathological lower threshold corresponding to the fifth percentile of the control population (4.8 IENF/mm). **(B)** Age-matched overall population: Men **(C)**, women **(D)**. Student *t*-test was used (*p < 0.05, **p < 0.01, ***p < 0.001).

demyelination of A-fibers lead to increased sensitivity of C-nociceptors originally from spontaneous discharges (21). Authors suggest that normal activity of A-fibers produce an inhibitory effect on C-nociceptors. In CMT1A disease, early stage of demyelination of sensory A-fibers (i.e. Aβ and Aδ fibers) could sensitize C-fibers and lead to neuropathic pain. Hyperexcitability of C-fiber could favor its degeneration, explaining reduction of IENFD in CMT1A patients.

In this study, pain, as evaluated by VAS, did not correlate with IENFD. However, the questionnaires that were used

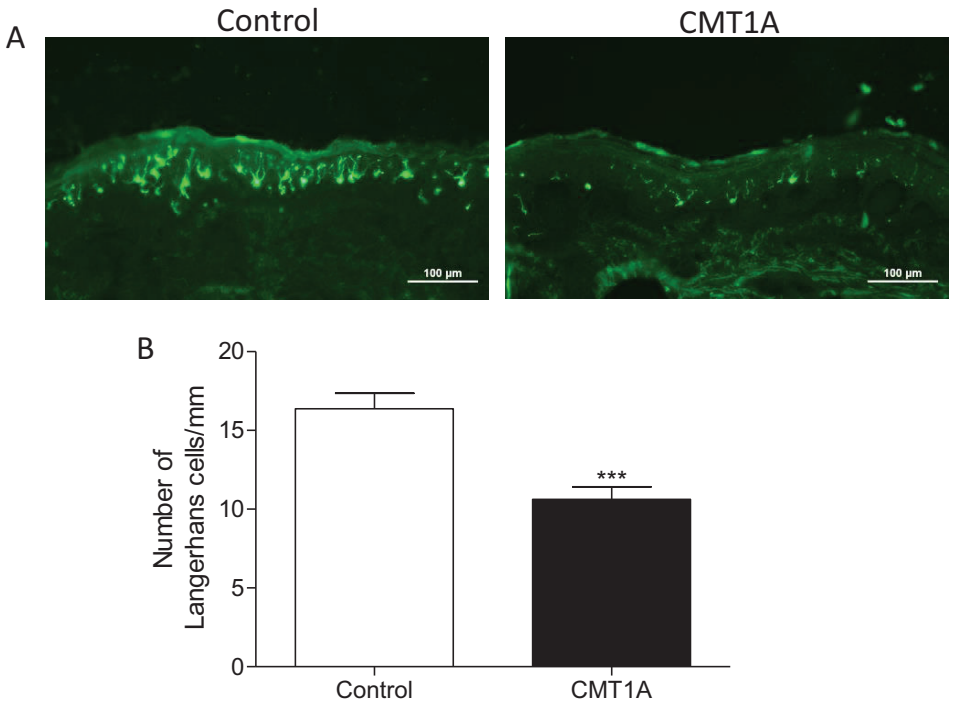

FIGURE 7. Langerhans cells (LCs) in skin biopsy stained with anti-langerine antibody. **(A)** Morphometric analyze of Langerhans cells in CMT1A and control patients. **(B)** Quantification of intraepidermal LCs in CMT1A and control population. The density of LCs was expressed as number of LCs/mm. Student *t*-test was used ($n = 23$ in each group, $^{***}p < 0.001$).

were not able to distinguish between neuropathic and nonneuropathic pain. A study on a cohort of 49 patients with CMT1A showed that most patients with this disorder had pain of musculoskeletal origin, with only 18% of them reporting neuropathic pain as assessed by the DN4 scale (3). Thus, the absence of a correlation between overall pain and IENFD was not completely surprising.

The quantification of LCs in this study also provides new information. Decreased epidermal LC density was associated with morphological abnormalities in CMT1A patients relative to controls. This is in contrast to most studies of SFN, which show an increase in LC density or their activation in diabetic patients (8), experimental sciatic nerve sectioning in rodents (5), and a mouse model of chemotherapy-induced neuropathy (7). However, an experimental study in rats showed that depletion of LCs could induce a decrease in the number of PGP9.5- and CGRP-positive skin axons associated with a reduction of neurotrophin levels (nerve growth factor and glial derived neurotrophic factor) in the epidermis (22). Moreover, a decrease in LC density in patients newly diagnosed with diabetes mellitus has been reported (23). Epidermal Langerhans' cells have been shown to be closely associated anatomically and functionally with IENFs (9). LC alterations observed in skin biopsies of CMT1A patients could be related to a dysfunction of small nerve fibers, prior to IENF degeneration. Indeed, we observed morphological abnormalities of the LCs in all 23 analyzed samples, whereas several CMT1A patients analyzed for LC density did not show a loss of IENFs. An alternative explanation could be a primary involvement of LCs in CMT1A, which remains to be investigated. Prospective studies would be useful for determining whether a reduction in LC density could be an early marker of small fiber involvement in CMT1A and the link between overexpression of PMP22, Schwann cell alterations, and small fiber loss in CMT1A.

REFERENCES

1. Ribiere C, Bernardin M, Sacconi S, et al. Pain assessment in Charcot–Marie–Tooth (CMT) disease. Ann Phys Rehabil Med 2012;55:160–73
2. Pazzaglia C, Vollono C, Ferraro D, et al. Mechanisms of neuropathic pain in patients with Charcot–Marie–Tooth 1 A: A laser-evoked potential study. Pain 2010;149:379–85
3. Laurà M, Hutton EJ, Blake J, et al. Pain and small fiber function in Charcot–Marie–Tooth disease type 1A. Muscle Nerve 2014;50:366–71
4. Tavakoli M, Marshall A, Banka S, et al. Corneal confocal microscopy detects small-fiber neuropathy in Charcot–Marie–Tooth disease type 1A patients. Muscle Nerve 2012;46:698–704
5. Hsieh ST, Choi S, Lin WM, et al. Epidermal denervation and its effects on keratinocytes and Langerhans cells. J Neurocytol 1996;25:513–24
6. Lindenlaub T, Sommer C. Epidermal innervation density after partial sciatic nerve lesion and pain-related behavior in the rat. Acta Neuropathol 2002;104:137–43
7. Siau C, Xiao W, Bennett GJ. Paclitaxel- and vincristine-evoked painful peripheral neuropathies: Loss of epidermal innervation and activation of Langerhans cells. Exp Neurol 2006;201:507–14
8. Casanova-Molla J, Morales M, Planas-Rigol E, et al. Epidermal Langerhans cells in small fiber neuropathies. Pain 2012;153:982–9
9. Gaudillere A, Misery L, Souchier C, et al. Intimate associations between PGP9.5-positive nerve fibres and Langerhans cells. Br J Dermatol 1996; 135:343–4
10. Attarian S, Vallat J-M, Magy L, et al. An exploratory randomised double-blind and placebo-controlled phase 2 study of a combination of baclofen, naltrexone and sorbitol (PXT3003) in patients with Charcot–Marie–Tooth disease type 1A. Orphanet J Rare Dis 2014;9:199

11. Lauria G, Cornblath DR, Johansson O, et al. EFNS guidelines on the use of skin biopsy in the diagnosis of peripheral neuropathy. Eur J Neurol 2005;12:747–58
12. Herman RM, Brower JB, Stoddard DG, et al. Prevalence of somatic small fiber neuropathy in obesity. Int J Obes (Lond) 2007;31:226–35
13. Verdú E, Ceballos D, Vilches JJ, et al. Influence of aging on peripheral nerve function and regeneration. J Peripher Nerv Syst 2000;5:191–208
14. Gøransson LG, Mellgren SI, Lindal S, et al. The effect of age and gender on epidermal nerve fiber density. Neurology 2004;62:774–7
15. Shun C-T, Chang Y-C, Wu H-P, et al. Skin denervation in type 2 diabetes: Correlations with diabetic duration and functional impairments. Brain 2004;127:1593–605
16. McArthur JC, Stocks EA, Hauer P, et al. Epidermal nerve fiber density: Normative reference range and diagnostic efficiency. Arch Neurol 1998; 55:1513–20
17. Nolano M, Manganelli F, Provitera V, et al. Small nerve fiber involvement in CMT1A. Neurology 2015;84:407–14
18. Zambelis T. Small fiber neuropathy in Charcot–Marie–Tooth disease. Acta Neurol Belg 2009;109:294–7
19. Koike H, Iijima M, Mori K, et al. Nonmyelinating Schwann cell involvement with well-preserved unmyelinated axons in Charcot–Marie–Tooth disease type 1A. J Neuropathol Exp Neurol 2007;66: 1027–36
20. Haney C, Snipes GJ, Shooter EM, et al. Ultrastructural distribution of PMP22 in Charcot–Marie–Tooth disease type 1A. J Neuropathol Exp Neurol 1996;55:290–9
21. Duan W-R, Xie Y-K, Modulation of C-Nociceptive Activities by Inputs from Myelinated Fibers. Dordrecht: Springer 2016:33–40
22. Doss ALN, Smith PG. Langerhans cells regulate cutaneous innervation density and mechanical sensitivity in mouse footpad. Neurosci Lett 2014;578:55–60
23. Strom A, Brüggemann J, Ziegler I, et al. Pronounced reduction of cutaneous Langerhans cell density in recently diagnosed type 2 diabetes. Diabetes 2014;63:1148–53