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Quantification of sweat gland innervation in patients with Fabry disease: A case-control study

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ABSTRACT

Introduction: Hypohidrosis and heat intolerance, frequently reported by men and women with Fabry disease (FD), is thought to be related not only to the deposition of globotriaosylceramide (Gb3) in eccrine sweat glands, but also to reduced sweat gland sympathetic innervation.

Methods: We performed a case-control study to compare the density of sweat gland innervation between patients with FD and healthy controls by examining lower leg skin punch biopsies. We used a standardized grid of circles superimposed upon the immunofluorescent specimen to create a simple pattern of circles over the sweat gland. Nerve fibers that crossed within the circles were manually counted ("crossed circles"). Nerve fibers that touched the edge of the circle but did not enter were spared ("uncrossed circles"). The percentage of crossed circles from all circles was determined.

Results: Biopsy specimens were available of 37 FD patients (median age 44 years, 19–67; $n = 18$ men) and 16 controls (median age 48 years, 24–83, $n = 7$ men). Totally there were 153 sweat glands from FD patients and 63 from controls, in which innervation was quantified. While mean sweat gland innervation per biopsy did not differ between the entire FD cohort and controls, data stratification for the reported sweating phenotype revealed a stepwise lower innervation in women with FD and hypohidrosis (n.s.) and anhidrosis ($p < .05$) compared to women reporting normal sweating.

Conclusion: Sweat gland innervation is reduced in women with FD and anhidrosis compared to female patients without sweating impairment. Loss of sweat gland innervation may play a role in FD associated anhidrosis, at least in women.

1. Introduction

Fabry disease (FD) is a multisystem lysosomal storage disease, inherited in an X-linked manner and characterized by total or partial deficiency of the enzyme alpha-galactosidase A (a-Gal A) [1]. Autonomic symptoms, reported in up to 50% of FD patients, are related to the involvement of small unmyelinated (type C) and thinly myelinated (type Aδ) fibers of the autonomic nervous system [2]. Particularly, reduced sweating and heat intolerance is present in many men and women with FD and in most cases is also accompanied by disturbances in the production of tears and saliva [3,4]. Hypohidrosis is assumed to be not only related to the deposition of globotriaosylceramide (Gb3) in eccrine sweat glands, but also to sympathetic nerve dysfunction [5–7]. However, sweat gland innervation has never been directly quantified in FD. We therefore set out to evaluate the innervation density of sweat glands in skin biopsy specimens from FD patients in comparison to those obtained from healthy control individuals.

2. Methods

We performed a case-control study to compare the density of sweat gland innervation between patients with FD and healthy control

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subjects. Patients and healthy controls were recruited at the Department of Neurology, University of Würzburg, Germany and healthy controls at the Laboratory of Clinical Electrophysiology, Aeginition Hospital, School of Medicine, National and Kapodistrian University of Athens, Greece. The patient cohort consisted of 18 men (median age 43, range 19–62 years) and 19 women (median age 45, range 21–67 years). Seven patients (median age 48, range 39–62 years, 5 men) were followed up for 1–4 years (median 2 years) after receiving enzyme replacement therapy (ERT). The control group consisted of seven men (median age 53, range 24–83 years) and nine women (median age 45, range 25–72 years). All control subjects were healthy volunteers without history of diabetes mellitus, neuromuscular disorders, or autonomic dysfunction and without any regular medication.

All FD patients underwent complete neurological examination and were asked for symptoms of peripheral neuropathy (hypoesthesia, numbness) and sweating disturbance (hypohidrosis, anhidrosis). Additionally, we used a structured questionnaire to characterize pain symptoms (permanent pain, pain attacks, pain crises, evoked pain) [8]. All FD patients also underwent an electrophysiological evaluation including sympathetic skin response (SSR) elicited at the hand and recorded from the feet and quantitative sensory testing (QST) for cold and warm detection thresholds (CDT, WDT) and thermal sensory limen (TSL) as functions of the small caliber nerve fibers following the protocol of the German Research Network of Neuropathic Pain (DFNS) at the dorsum of the foot [9,10]; individual QST data were compared with published normative values [11].

In all subjects we performed 3-mm skin punch biopsies 10 cm above the lateral malleolus of the leg as described earlier [12]. Biopsy specimens were fixed with fresh 4% buffered paraformaldehyde, washed in phosphate buffer and subsequently stored in 10% sucrose with 0.1 M phosphate buffer and finally cryoprotected. Fifty-μm sections were immunoreacted with antibodies to the pan-neuronal marker PGP9.5 (1:800, Ultraclone, UK) and Cy3-labeled anti-rabbit secondary antibodies (1:100, Amersham, USA).

All sweat glands were digitally captured using a fluorescence microscope (Axiophot 2, Zeiss, Oberkochen, Germany) with an Axiocam MRm camera (Zeiss), with images taken at 20× magnification (Fig. 1A). All images were analyzed applying Image J software by Java, an open platform for scientific image analysis (*https://imagej.nih.gov/ij/*). All visible sweat glands available were investigated. First, an out of focus image of each sweat gland was created to aid in background visual resolution thresholding. Then the area of interest (AOI) was selected using the out of focus image of the sweat gland. All images were analyzed using the composite image with the selected AOI around the sweat gland by manual quantification. A standardized grid of circles 10 μm in diameter spaced 50 μm apart horizontally, and offset 25 μm vertically, superimposed upon the immunofluorescent specimen, created a pattern of circles over the sweat gland [13]. Nerve fibers that crossed within the circles were manually counted (crossed circles). Nerve fibers that touched the edge of the circle but did not enter were not counted (uncrossed circles) (Fig. 1B). Results were expressed as the percentage of circles intersected from the total number of circles within the sweat gland (crossed circles / all circles) [13] referred to as "percent grid intercepts".

In all biopsy specimens acquired from FD patients, we additionally assessed the intraepidermal nerve fiber density (IENFD) quantified by the number of nerve fibers per mm epidermis [14].

The study was approved by the Ethics Committee of our Departments, and all patients and controls signed informed consent before study participation.

Statistical analysis was performed using SPSS software version 24 (IBM, Ehningen, Germany). Non-normally distributed data are expressed as median and range; data are visualized as box plots giving the median, the 25% and 75% percentile and the minimum and maximum values. For group comparisons the Mann-Whitney $-U$ test was applied. Statistical significance was assumed at $p < .05$.

3. Results

3.1. Clinical characterization

The baseline clinical and laboratory data of patients with FD are presented in Table 1. The follow 24 different genetic alterations were recorded in the patient cohort: C408T > A, 368 T > C, C494A > T, Trans c.404C > T, C.1069C > T, C.1025G > T, IVS0–10 (C > T), IVS4–16 (A > G), IVS6–22C > T, Trans IVS6-10G > A, 664A > G, 845C > T, C1208delAAG, D313 Y, Del 1354 fs del 15 bp, Del c.972delG, c.756 or 757 del A fs 268×, Del c.1223delA, Del IVS5-3_2, IVS3 + 1G > A, L129P, W349X, Trans c.611G > A, Trans c.838C > T, Trans c.973G > A, IVS0–10C > T, IVS4–16A > G, IVS6–22C > T. FD patients and controls did not differ in terms of age and gender. Hypo- and anhidrosis (8/18 FD men; 8/19 FD women), pain (14/18 FD men; 13/19 FD women), and peripheral neuropathy symptoms (6/18 FD men, 2/19 FD women) were frequent in the study cohort. Six of ten treated men and six of twelve treated women reported improvement of sweating under ERT.

3.2. Sweat gland innervation – Baseline

A total of 153 sweat glands from the biopsies of FD patients (72 from men, 81 from women) and 63 from healthy controls (24 from men, 39 from women) were available. The range of sweat glands that were analyzed per patient was 1–12 with mean value 3.37 ± 2.When comparing the entire group of patients with FD with all healthy controls

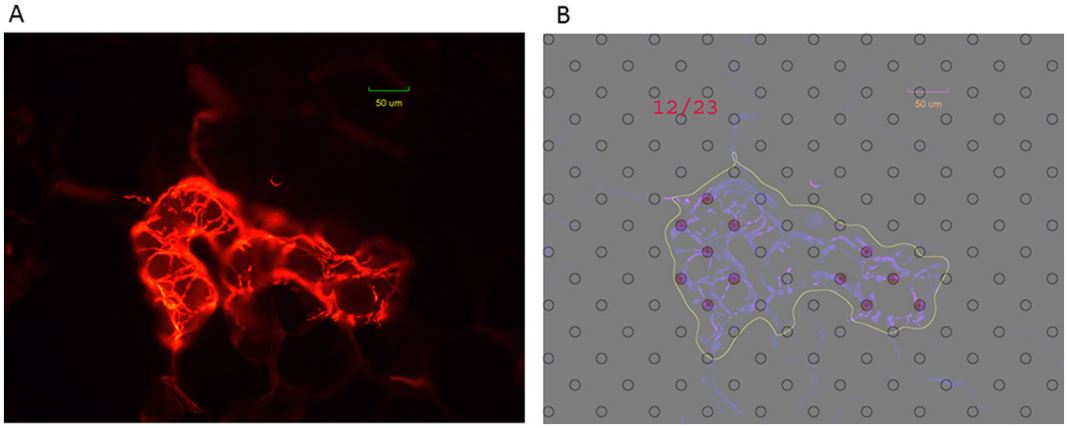

Fig. 1. A: Image of the innervation (red) of a sweat gland from a control subject. B: The same image after analysis with the grid superimposed (filled circles are counted). Scale bars = 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)