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Dyshidrosis is associated with reduced amplitudes in electrically evoked pain-related potentials in women with Fabry disease

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HIGHLIGHTS

- Pain-related evoked potential (PREP) recording allows the objective assessment of A delta-fibers.
- Symptoms of small fiber impairment including dyshidrosis are frequent in female Fabry disease (FD) patients.
- PREP amplitudes are reduced in female FD patients with dyshidrosis already in patients with mild to moderate FD.

ABSTRACT

Objective: To investigate A-delta fiber conduction in mild to moderate Fabry disease (FD) patients using pain-related evoked potentials (PREP).
Methods: In this case-control study we prospectively investigated 58 patients with mild to moderate FD and compared data with those of healthy controls. Small fiber function (quantitative sensory testing, QST and sympathetic skin response, SSR), morphology (intraepidermal nerve fiber density, IENFD), and electrical conduction (PREP) were assessed and correlated with sweating as major autonomic function disturbed in FD. Patients were further stratified for gender, disease severity as reflected by renal and cardiac function, and genetics.
Results: An- or hypohidrosis (i.e. dyshidrosis) was reported by 7/32 (22%) women and 15/26 (58%) men with FD ($p < 0.01$). QST showed small fiber impairment in female and male patients regardless of clinical symptoms, while SSR was obtained in all patients except one man with hypohidrosis. IENFD was reduced in 50% of FD patients, with no differences between groups with and without autonomic symptoms. However, PREP amplitudes were reduced independent of the stimulation site only in female patients with dyshidrosis ($p < 0.01$). Genetics had no influence on PREP parameters.
Conclusion: A-delta fiber conduction investigated using PREP is impaired in mild to moderately affected female FD patients with clinical signs of hypohidrosis.
Significance: Small fiber assessment in FD is of diagnostic value already in mild to moderate stages of disease.

1. Introduction

Fabry disease (FD) is an inborn lysosomal storage disorder with X-linked inheritance. Mutations in the gene encoding the α-galactosidase A (α-GAL) result in a reduction or a complete loss of enzymatic function during cleavage of glycoconjugates. Consecutively, globotriaosylceramide (Gb3) accumulates in diverse tissues and leads to multiorgan impairment (Schiffmann et al., 2009).

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FD affects the peripheral nervous system in terms of a small fiber pathology, which is frequently associated with neuropathic pain and autonomic dysfunction (Laaksonen et al., 2008; Torvin Moller et al., 2009; Liguori et al., 2010; Üçeyler et al., 2011). Accordingly, many patients suffer from gastrointestinal dysmotility and hypo- or anhidrosis, which in turn enhances intolerance to heat and physical exercise (Burlina et al., 2011; Verrecchia et al., 2016).

The exact mechanism underlying dyshidrosis in FD is unknown. Gb3 deposits congesting sweat glands (Lao et al., 1998), potential cytotoxic effects of Gb3 on small nerve fibers (Liguori et al., 2017), and gland denervation (Dütsch and Hilz, 2010; Burlina et al., 2011) have been suggested to be of pathophysiological relevance. However, obtaining direct evidence via functional tests and histological studies of sweat glands remains a challenge.

Electrically evoked pain-related potentials (PREP) elicited by concentric surface electrodes allow the objective investigation of A-delta pathway integrity (Katsarava et al., 2006; Obermann et al., 2008; Yoon et al., 2011). In a previous study, PREP amplitudes decreased with FD severity (Üçeyler et al., 2013). Furthermore, impaired sweating was associated with small fiber neuropathy in women with FD and was independent of the degree of efferent eccrine C-fiber loss (Møller et al., 2009). C-fibers mainly mediate afferent heat sensation and supply efferent innervation of sweat glands. Studies using quantitative sensory testing (QST) show a predominant A-delta dysfunction with cold hypoesthesia particularly in male FD patients (Üçeyler et al., 2013). This is in contrast to the frequency of dyshidrosis present in female and young FD patients (Üçeyler et al., 2014), indicating an early damage of C-fibers. Thus, a shared mechanism of A-delta and C-fiber impairment leading to FD associated pain, disturbed thermal perception, and dyshidrosis may be assumed.

In contrast to the majority of studies examining male FD patients in advanced disease stages and without correlating data with clinical symptoms of small nerve fiber impairment, we focused on patients with mild to moderate FD and on the potential impact of autonomic symptoms clinically manifesting as dyshidrosis on PREP parameters.

2. Patients and methods

2.1. Subjects

Our study was approved by the Würzburg Medical Faculty Ethics Committee and written informed consent was obtained from all study participants before study inclusion. We enrolled 58 patients (32 women and 26 men) with a confirmed diagnosis of FD (by α-GAL activity measurement in leucocytes and genetic testing), including patients with no or mild cardiac involvement (i.e. no reduced left ventricular ejection fraction or relevant arrhythmia) and no end-stage renal failure. Hence, we investigated patients who had mild to moderate FD as determined by heart and kidney function. Patients were prospectively recruited (2012–2013) at the Würzburg Fabry Center for Interdisciplinary Therapy (FAZIT), University of Würzburg, Germany and were investigated at the Department of Neurology, University of Würzburg. Inclusion criteria were: age ≥18 years, no neurological symptoms of other cause than neuropathy in FD, no pacemaker or implantable cardioverter defibrillator, no epilepsy.

We compared our data with those of 31 age- and gender-matched healthy controls (16 women and 15 men) recruited mainly among friends and family members of the patients. Additional inclusion criteria for healthy controls were: no FD, no neuropathy, neuropathic pain or other sources of pain, normal sural nerve conduction study results.

2.2. Clinical examination and laboratory assessment

FD patients were examined neurologically, and disease history was recorded with particular focus on symptoms due to small fiber impairment including autonomic symptoms. Patients were asked to categorize their ability to sweat (hyperhidrosis, hypohidrosis, anhidrosis, euhidrosis), their heat tolerance, the presence of heat- or exercise induced pain, and gastrointestinal symptoms.

Large fiber neuropathy was excluded by additional nerve conduction studies of the sural nerve following a standard procedure (Kimura, 2001). Data were compared with normative values of our department.

Laboratory tests included glomerular filtration rate (GFR). Renal function was considered normal with a GFR >60 ml/min/1.73 m^2. Cardiac function was evaluated using the cardiovascular score of the "Mainz Severity Score Index" (MSSI) including clinical data of arterial hypertension, electrocardiography, echocardiography, implantation of a pacemaker or cardioverter defibrillator (Whybra et al., 2004). Examinations were performed during the routine work-up of patients at FAZIT, and the MSSI was calculated using these data documented in patients medical records.

Genotypes of FD patients were stratified according to the type of mutation likely leading to a classical phenotype (i.e. the mutation is known to be associated with typical symptoms and signs of FD) and likely leading to a non-classical phenotype (i.e. the mutation is associated with late onset or predominant involvement of one organ) (van der Tol et al., 2016). Individual genotypes were also cross-checked using https://lih16.u.hpc.mssm.edu/pipeline/js/dbFabry/Mutation.html#.

2.3. Quantitative sensory testing (QST)

QST was performed with a calibrated device (Somedic, Hörby, Sweden) and following a standardized procedure as previously described (Rolke et al., 2006; Üçeyler et al., 2013). Tests were performed at the left dorsal foot of all subjects. Based on the log transformed raw values for each QST item, a z-score sensory profile was calculated as follows: z-score = (value of the subject − mean value of controls)/standard deviation of controls. Negative z-scores indicate loss of sensation, positive z-scores indicate gain of sensation. We assessed the following parameters: cold and heat detection thresholds (CDT, HDT), the ability to detect temperature changes (thermal sensory limen, TSL), and paradoxical heat sensation (PHS, i.e. reporting heat after a cold stimulus), heat and cold pain thresholds (HPT and CPT), pressure pain threshold (PPT), and mechanical pain sensitivity (MPS). Large fiber function was assessed using the vibration detection threshold (VDT). Data of patients were compared with those of healthy controls as detailed above.

2.4. Sympathetic skin response (SSR)

SSR were recorded following a standard procedure (Vetrugno et al., 2003). Stimulation was performed using superficial electrodes at the hand, while recordings were performed at the feet. SSR was considered normal if a potential was obtained.

2.5. Pain-related evoked potentials (PREP)

PREP were recorded as previously described (Üçeyler et al., 2013). Potentials were elicited using superficial planar concentric electrodes (Inomed Medizintechnik GmbH, Lübeck, Germany) and a stimulator (Digitimer DS7A, Welwyn Garden City, UK). Defined body sites were determined for electrical stimulation in patients and controls: bilateral face (above eyebrow), hands (medial phalanx second and third digit), and feet (center dorsum).

Twenty triple pulses were applied with an intensity of two-fold of the individual pain threshold, a duration of 0.5 ms, and a random inter-stimulus interval of 15–17 s. The individual pain threshold was determined by stimulation of the area of interest twice with increasing and decreasing current intensities until the subject reported a pin-prick sensation, resulting in an average individual pain threshold. PREP potentials were recorded from Cz by a subcutaneously placed needle electrode referred to linked earlobes (A1–A2) of the international 10–20 system using Signal Software (version 2-16; Cambridge Electronic Design, Lt., UK). Potentials were recorded using the following setting: gain: ×5000, bandwidth: 1 Hz–1 kHz, sweep length: 400 ms, digitalization sampling rate: 2.5 kHz. Two sets of averaged curves (from n = 10 single sweeps each) were investigated for reproducible N1- (i.e. first negative peak), P1- (i.e. subsequent positive peak) latencies and peak-to-peak amplitudes (PPA) using MATLAB software (version 7.7.0.471, The MathWorks, Ismaning, Germany). All records were individually evaluated to exclude technical or biological artifacts by the same investigator who was blinded for the subject. Alterations of skin conduction due to local cutaneous influences were prevented by standardized cleaning of the skin prior to stimulation.

2.6. Skin biopsy

The intraepidermal nerve fiber density (IENFD) was investigated on 5-mm skin punch biopsies (punch device by Stiefel, Offenbach, Germany) taken from the lower leg (10 cm above the lateral malleolus) and the back (at th5 level). Skin samples were processed as described previously (Üçeyler et al., 2010). Samples were immunoreacted with antibodies against protein-gene product (PGP) 9.5 (Ultraclone, UK, 1:800; primary antibody) with goat anti-rabbit IgG labeled with cyanine 3.18 fluorescent probe (Amersham, USA, 1:100; Cy3, secondary antibody), and IENFD was quantified by an observer blinded to the identity of the specimen following published rules (Lauria et al., 2005). The results were compared with our laboratories normative data base: lower leg: n = 110 (63 females, 47 males), median age: 50 years (range 20–84), median IENFD 7 fibers/mm, range 1–15 fibers/mm; back: n = 42 (23 female, 19 males), median age: 50, range 20–81, median IENFD 22 fibers/mm, range 6–40 fibers/mm.

2.7. Statistical analysis

We used IBM SPSS Statistics version 24.0 (IBM, Ehningen, Germany) for statistical analysis and graph design. All data were non-normally distributed as shown with the Kolmogorov-Smirnov-test. For group comparisons the non-parametric Mann-Whitney test and Spearman rank analysis were used. Results are

Table 1
Distribution of mutations in the study group.

	Female FD patients	Male FD patients
Classical mutations	22/32 (69%)	16/26 (62%)
Nonsense mutation (ns)	2/32 (6%)	8/26 (31%)
Missense mutation (ms)	10/32 (31%)	6/26 (23%)
Consensus splice mutation	5/32 (16%)	2/26 (8%)
Small deletion	5/32 (16%)	0
Non-classical mutations	10/32 (31%)	10/26 (38%)
Later onset phenotype (ms mutations)	4/32 (13%)	2/26 (8%)
Likely benign variants (ms mutations)	3/32 (9%)	2/26 (8%)
Mutations of unknown relevance	3/32 (9%)	6/26 (23%)
ns mutations	0	2/26 (8%)
ms mutations	3/32 (9%)	3/26 (12%)
Deletion	0	1/26 (4%)

Abbreviations: FD: Fabry disease, ns: nonsense, ms: missense.

Table 2
Characterization of symptoms of small fiber pathology in the study cohort.

	Female FD patients	Male FD patients
Impaired sweating	7/32 (22%)	15/26 (58%)
Hypohidrosis	5/32 (16%)	9/26 (35%)
Anhidrosis	2/32 (6%)	6/26 (23%)
Gastrointestinal symptoms	4/32 (13%)	7/26 (27%)
Diarrhea and meteorism	3/32 (9%)	3/26 (12%)
Abdominal pain	2/32 (6%)	4/26 (15%)
Fabry associated pain	16/32 (50%)	20/26 (77%)
Permanent pain	3/32 (9%)	7/26 (27%)
Pain attacks	10/32 (31%)	14/26 (54%)
Evoked pain	8/32 (25%)	10/26 (39%)
Pain crises	0	6/26 (23%)

Abbreviations: FD: Fabry disease.

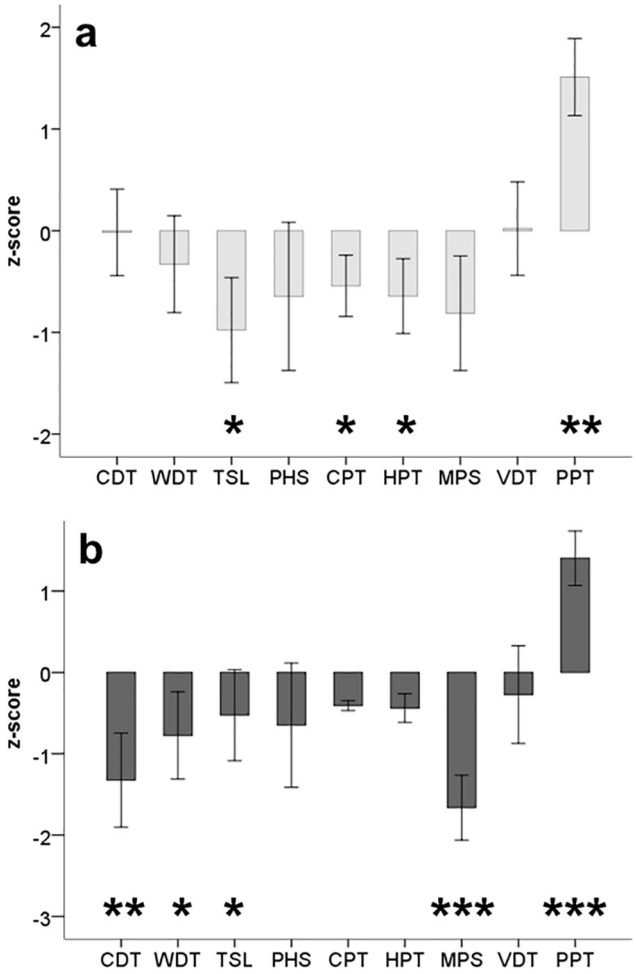

Fig. 1. QST profiles of patients with Fabry disease compared to healthy controls. Bar graphs show the z-score sensory profiles of quantitative sensory testing (QST) at the left dorsal foot in patients with Fabry disease (FD) compared to healthy controls. Healthy controls are represented by the black zero line. Z-scores < 0 indicate loss of function, z-scores > 0 indicate gain of function. (a) female FD patients have impaired TSL ($p < 0.01$), CPT ($p < 0.05$), HPT ($p < 0.05$), MPS ($p < 0.001$), and PPT ($p < 0.001$). (b) male FD patients show impaired CDT ($p < 0.01$), WDT ($p < 0.05$), TSL ($p < 0.05$), HPT ($p < 0.05$), MPS ($p < 0.001$) and PPT ($p < 0.001$). Abbreviations: CDT: cold detection threshold, CPT: cold pain threshold, HPT: heat pain threshold, MPS: mechanical pain, PHS: paradox heat sensation, PPT: pain pressure threshold sensitivity, TSL: thermal sensory limen, VDT: vibration detection, WDT: warm detection threshold. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

presented as median and range values and are illustrated as boxplots. The horizontal lines in the boxplots represent median values; the boxes end with the 25th and 75th quartile; the whiskers indicate the highest and lowest value. P-values <0.05 were considered significant.

3. Results

3.1. Clinical data and laboratory findings

Fifty-eight FD patients (32 women, 26 men) with a median age of 43 (19–75) years participated in our study. As detailed in Table 1, 22/32 (69%) of women and 16/26 (62%) of men carried a classical FD mutation. Neurological examination was normal in 43/58 (74%) of FD patients. The most common findings were hyporeflexia (5/58, 9%), hyperalgesia (4/58, 7%), hypoesthesia (4/58, 7%), focal paresis due to nerve entrapment syndrome or lumbar disc prolapse (2/58, 3%), and hypoacusis (2/58, 3%). Sural nerve conduction studies were normal in all patients.

23/58 (40%) patients were on ERT (7 female, 16 male) with a median treatment duration of six years (0.01–11). Reduced renal function was found in 8/58 (14%) of FD patients (3 women, 5 men), none of the patients was on dialysis. The median MSSI subscore for cardiovascular impairment was 4.0 (0–11), reflecting mild

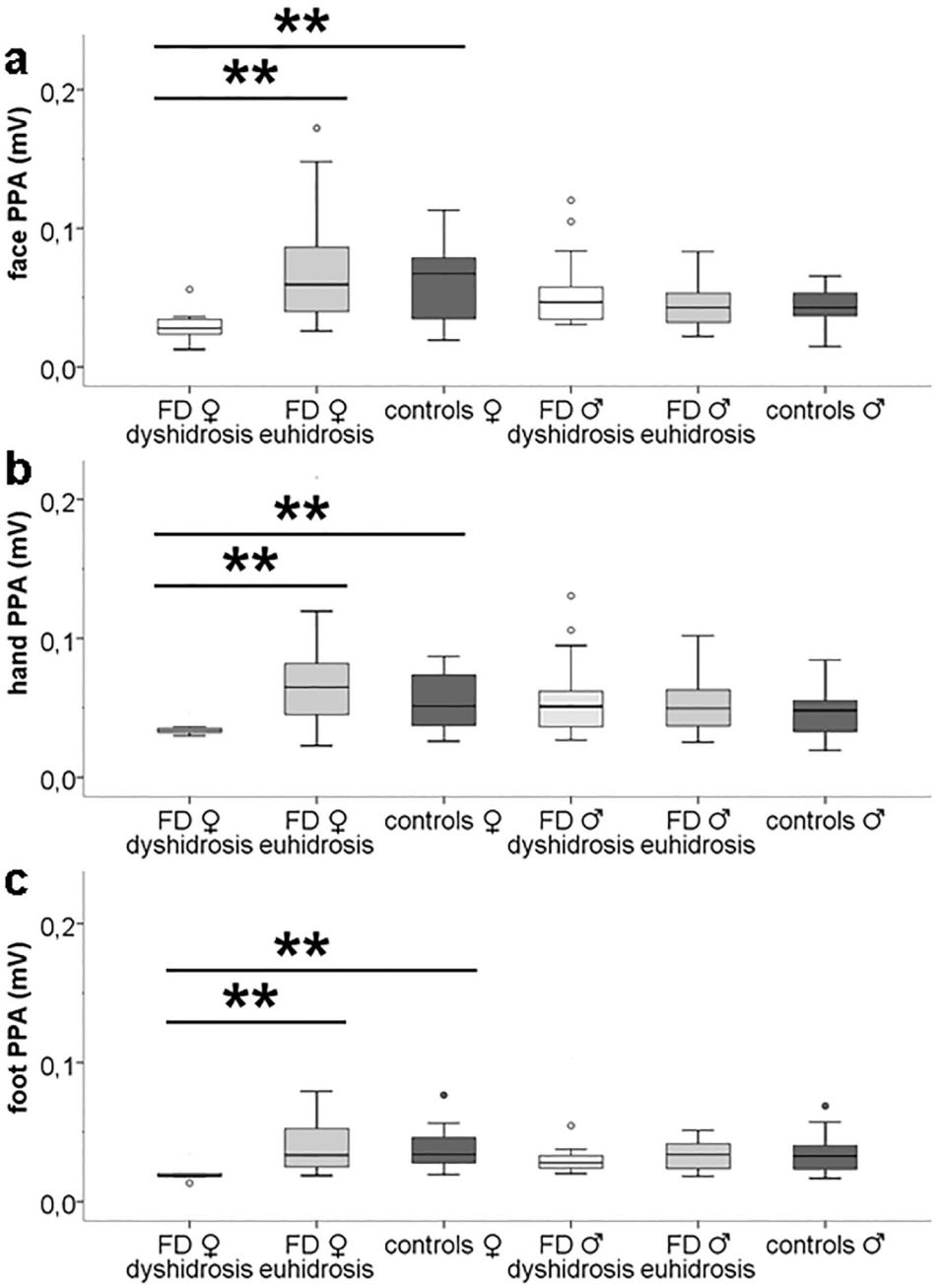

Fig. 2. PREP PPA of female and male FD patients with impaired and normal sweating compared to healthy controls. Peak-to-peak amplitudes (PPA) of pain-related evoked potentials (PREP) in patients with Fabry disease (FD) stratified according to gender and dyshidrosis vs. euhidrosis compared to female and male controls after stimulation at (a) face, (b) hand, and (c) foot. Female FD patients with impaired sweating (n: 7) show reduced PPA compared to female FD patients with euhidrosis (n: 25) and female controls (n: 16). Male FD patients with impaired sweating (n: 15) showed no difference in PPA when compared to male FD patients with euhidrosis (n: 21) and male controls (n: 17). $^{**}p < 0.01$.

cardiac symptoms (Whybra et al., 2004). In 18/58 (31%) patients no signs of cardiac FD manifestations were found.

Symptoms of small fiber pathology as stated by the patients in the current medical report are summarized in Table 2. 7/32 (22%) of FD women and 15/26 (58%) of FD men suffered from dyshidrosis ($p < 0.01$). Of all 22/58 (38%) patients reporting impaired sweating, 8/58 (14%) suffered from anhidrosis. Impaired sweating affected the whole body and did not show the typical distally symmetric distribution of FD associated pain. Non-painful heat intolerance independent of sweat gland function was present in 3/32 (9%) of women and in no men. Common gastrointestinal symptoms were diarrhea and meteorism in 6/58 (10%) of patients, and abdominal pain in 6/58 (10%) of patients. The majority of patients suffered from pain, commonly triggered by heat, fever, physical exercise, and cold. At the time of examination, 17/58 (29%) of patients (9/26 (35%) men and 8/32 (25%) women) suffered from pain, with a median current pain intensity of 2 (1–6) on numeric rating scale. Regular pain medication was taken by 8/58 (14%) of patients. 6/58 (10%) of patients had not been able to attend sports in school because of heat- or exercise associated pain, 3/60 (50%) of those patients also suffered from impaired sweating.

3.2. FD patients have impaired small fiber function in QST independent of autonomic symptoms

QST profiles of female and male FD patients with hypo- and anhidrosis did not differ from FD patients reporting normal sweating (data not shown). Women with FD showed normal small fiber function in QST as reflected by CDT and WDT, but impaired TSL ($p < 0.05$), CPT ($p < 0.01$), and HPT ($p < 0.01$). MPS ($p < 0.001$) and PPT ($p < 0.001$), as determinants of pain sensitivity, were lower compared to controls (Fig. 1a). Interestingly, female FD patients carrying a classical FD mutation only differed in WDT from healthy controls ($p < 0.05$). This was also true when comparing data of FD patients with non-classical mutations ($p < 0.05$; Supplementary Table 1).

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.clinph.2019.01.008.

In line with previous data and clinical description of patients (Üçeyler et al., 2013), men with FD showed impaired thermal perception in QST (CDT: $p < 0.01$, WDT: $p < 0.05$, TSL: $p < 0.05$) compared to healthy controls, as well as a reduction of MPS ($p < 0.001$) and lower PPT ($p < 0.001$; Fig. 1b).

Male FD patients carrying a classical FD mutation also had impaired small fiber function and pain sensitivity in QST parameters as mentioned above when compared to healthy controls (CDT: $p < 0.001$, WDT: $p < 0.05$, TSL: $p < 0.01$, MPS: $p < 0.001$, PPT: $p < 0.001$). Male FD patients carrying a classical FD mutation had higher CDT and lower PPT than patients with non-classical mutations (see Supplementary Table 1). VDT reflecting large fiber function was normal in all women and men with FD (Fig. 1).

3.3. SSR does not reflect autonomic symptoms in FD patients

SSR was examined in 49/58 (85%) of patients (28/32 women; 21/26 men). A SSR was obtained in all female patients, including all seven women who reported an- or hypohidrosis. SSR was absent in only one man reporting hypohidrosis, while ten men with subjective an- or hypohidrosis had normal SSR as well as all men with normal sweating (data not shown).

3.4. Female FD patients with hypo- or anhidrosis have reduced PREP amplitudes

PREP were elicitable after stimulation at all three body sites in 52/58 (90%) patients (one recording missing after stimulation at the hand and five after stimulation at the foot) and in 29/32 (91%) controls (one missing recording after stimulation at the hand and three after stimulation at the foot). Since recordings obtained after stimulation on the left and the right body side were not different, data were pooled.

Women with FD reporting hypo- or anhidrosis had reduced PREP PPA after stimulation at face, hands, and feet compared to female FD patients reporting euhidrosis ($p < 0.01$ each) and healthy controls ($p < 0.01$ each; Fig. 2). An example of a normal and reduced PPA after stimulation at the foot is shown in Fig. 3. No such association was found in male patients (Fig. 2). There was also no relevant difference in PREP N1- and P1 latencies comparing female and male FD patients with impaired sweating with female and male FD patients with euhidrosis and healthy controls (data not shown). Stratification for genotype did not reveal intergroup differences ($n = 4$ and $n = 3$ female patients in the respective group of classical and non-classical mutation carriers; data not shown).

Male and female FD patients with gastrointestinal autonomic symptoms did not differ in PREP parameters compared to healthy controls and FD patients without gastrointestinal symptoms (Supplementary Fig. 1); also, genotype did not have an influence on PREP parameters (Supplementary Table 2).

3.5. PREP findings of FD patients in early disease stages do not differ from healthy controls

We previously showed that male FD patients in advanced stages of disease as reflected by impaired renal function have reduced

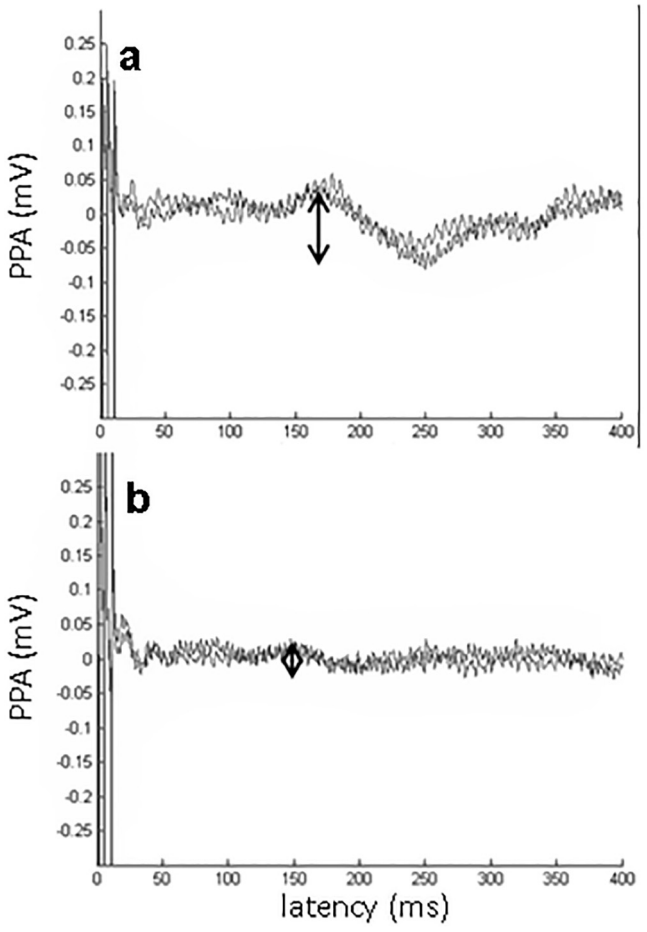

Fig. 3. Example of a normal and pathological PREP. Recordings of a normal (a) and pathological (b) PREP after stimulation at the foot. Recordings are reproducible, as the averaged first ten recordings (light grey) and the second ten recordings (dark grey) match. The double-arrow shows the peak to peak amplitude (PPA), indicating a reduction of PPA in (b).

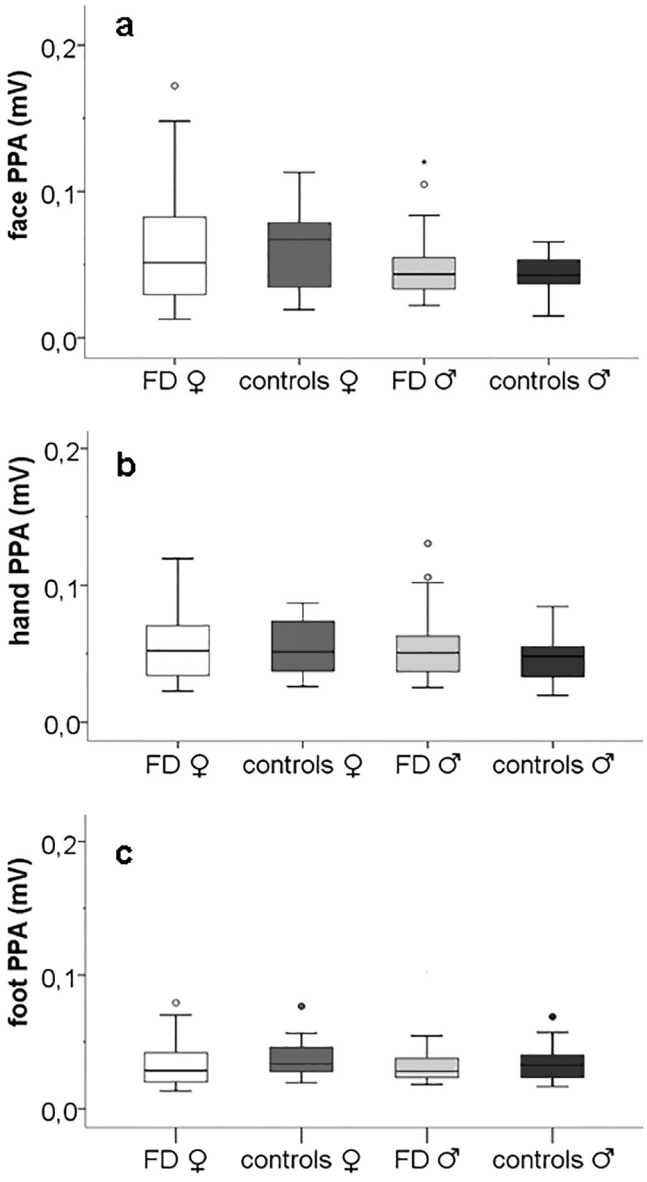

Fig. 4. PREP PPA of female and male FD patients compared to healthy controls. Pain-related evoked potentials (PREP) parameters of female and male Fabry disease (FD) patients do not differ from healthy controls. Peak-to-peak amplitude (PPA) after stimulation at the face (a), at the hand (b), and at the foot (c).

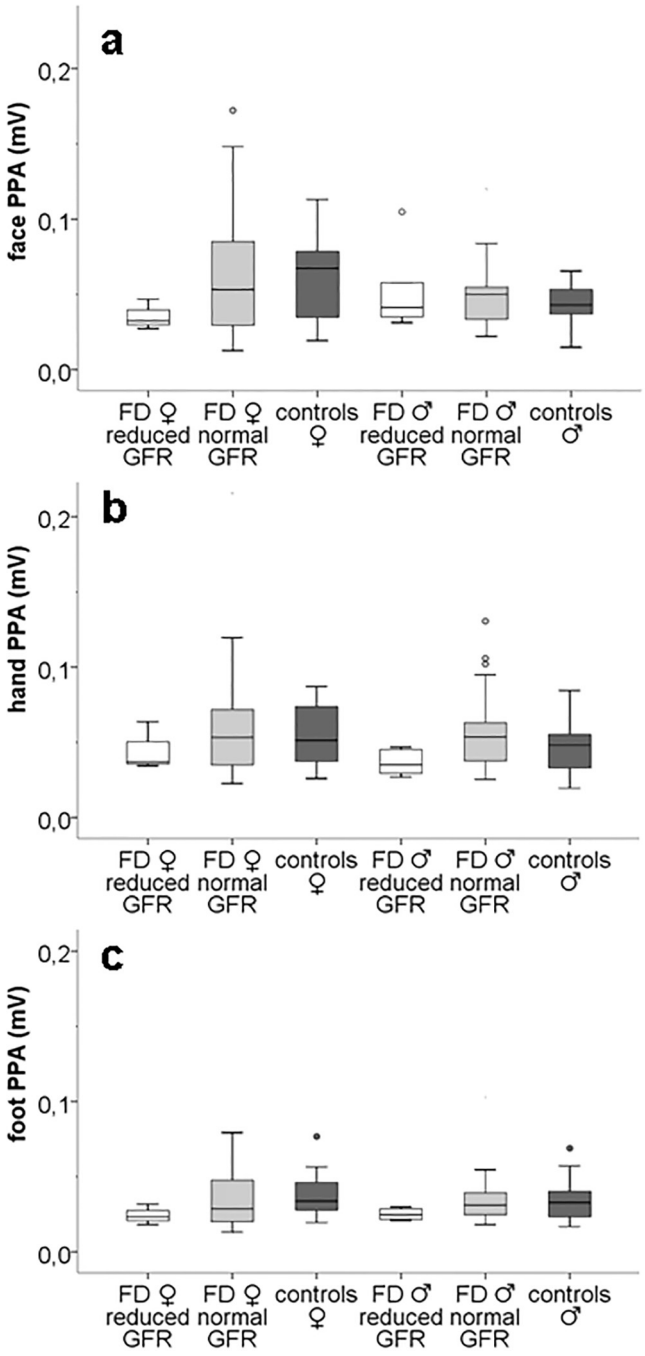

Fig. 5. PREP PPA of female and male FD patients stratified for renal function compared to healthy controls. Peak-to-peak amplitudes (PPA) of pain-related evoked potentials (PREP) in patients with Fabry disease (FD) grouped according to a reduced glomerular filtration rate (GFR) < 60 ml/min/1.73 m^2 and gender versus normal GFR > 60 ml/min/1.73 m^2 and female and male controls after stimulation at the (a) face, (b) hand, and (c) foot. Female FD patients with impaired renal function (n: 3) did not differ from female FD patients with normal renal function (n: 29) and female controls (n: 16), as well as male FD patients with impaired renal function (n: 5) did not differ from male FD patients with normal renal function (n: 21) and male controls (n: 17).

PREP amplitudes (Üçeyler et al., 2013). Investigating a clinically less affected cohort of FD patients, we did not find a relevant difference in PREP parameters of female and male FD patients compared to healthy controls, when looking at the whole patient cohort without stratification for clinical symptoms (Fig. 4). However, when stratifying our data for renal function (only n = 3 women and n = 5 men with impaired renal function), a trend was found towards reduced PREP amplitudes in male patients with impaired renal function (n.s.; Fig. 5).

3.6. IENFD in FD patients is independent of autonomic symptoms

Skin biopsies were obtained from 19/32 (60%) women and 15/26 (58%) men with FD. In female FD patients the median IENFD at the lower leg was 5.2 fibers/mm (1.8–13) and at the back 24.6 fibers/mm (1.7–41.2); in male FD patients the median IENFD was 4.5 fibers/mm (0–13) at the lower leg and 22.1 fibers/mm (8.5–27.3) at the back (Fig. 6a). Compared with our normative values, distal IENFD was reduced in 8/19 (42%) of women and 9/15 (60%) of men, proximal IENFD was reduced in 3/19 (16%) of women and 2/26 (8%) of men. IENFD of female and male patients with classical FD mutations did not differ from those with non-classical mutations (data not shown). Skin innervation did not differ in female and male patients with impaired and normal sweating

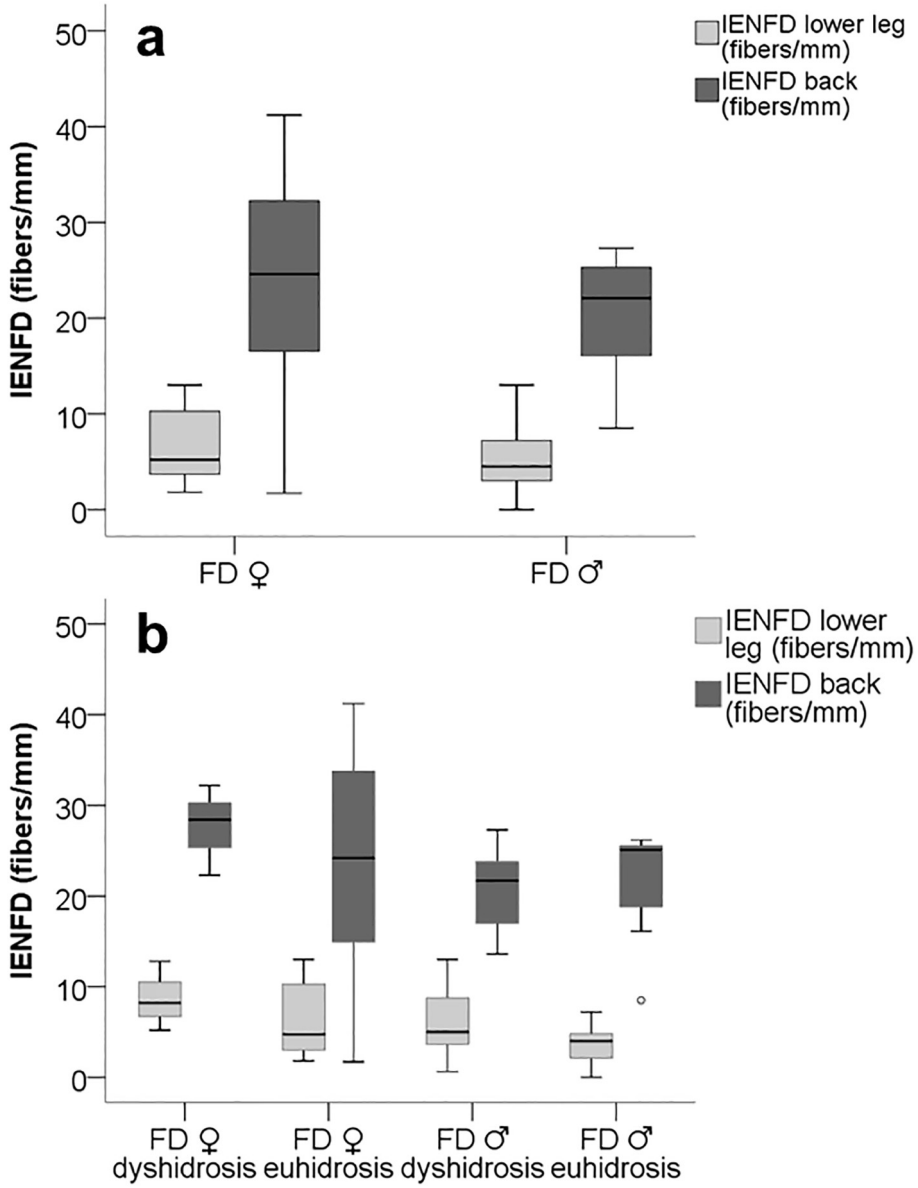

Fig. 6. IENFD of female and male FD patients at the distal and proximal biopsy site. (a) Intraepidermal nerve fiber density (IENFD) at the lower leg (distal biopsy site) and back (proximal biopsy site) of female and male Fabry disease (FD) patients assessed with the pan-axonal marker protein gene product-9.5 (PGP 9.5). Male patients showed reduced IENFD compared to females, not reaching significance. (b) IENFD at the lower leg and back of FD patients, stratified for gender and autonomic symptoms. Patients with dyshidrosis did not show a reduction of IENFD.

(Fig. 6b) and in patients with and without gastrointestinal autonomic symptoms (data not shown).

4. Discussion

We prospectively examined A-delta pathways using PREP in a cohort of FD patients with mild to moderate symptoms and report on a reduction of PPA in women with hypo- or anhidrosis.

Symptoms of small fiber pathology are frequent in FD (Burlina et al., 2011; Üçeyler et al., 2014). While in the majority of studies, FD patients in advanced disease stages have been investigated (Hilz et al., 2004; Üçeyler et al., 2011), we focused on mild to moderate FD with less organ dysfunction as defined by renal and cardiac parameters. In line with previous studies examining FD patients with similarly mild disease burden (MacDermot et al., 2001; Ries et al., 2003), we confirm the high frequency of autonomic symptoms, also in clinically less affected female heterozygotes. In contrast to previous studies on hereditary autonomic neuropathies (Hilz et al., 1999), SSR failed to reflect autonomic symptoms in mild to moderate FD regardless of their high frequency in our cohort.

Despite the mostly acral FD associated pain, dyshidrosis is reported to affect the whole body (Lidove et al., 2006). Accordingly, PREP PPA were reduced independent of the body sites stimulated in female patients with impaired sweating, nicely linking clinical phenotype with objectively measurable small fiber impairment.

In contrast to patients with autonomic dysfunction leading to dyshidrosis, patients suffering from gastrointestinal symptoms did not show a reduction of PREP PPA. First, this might be due to the small sample size. Second, although gastrointestinal symptoms are mainly assumed to be of autonomic origin, we cannot rule out other potential reasons such as Gb3 associated endothelial dysfunction or vasculopathy (Zar-Kessler et al., 2016). Third, there is

also no data available linking gastrointestinal symptoms in FD to dyshidrosis and FD pain. Hence, mechanisms of small fiber pathology in skin and the gastrointestinal endothelium may differ.

The mechanisms underlying small fiber pathology in FD are unknown, yet a cytotoxic effect of Gb3 either on the peripheral nerve fibers or the dorsal root ganglion neurons is assumed (Dütsch and Hilz, 2010; Burlina et al., 2011; Liguori et al., 2017). So far, it was not possible to find an association of clinical symptoms of small fiber dysfunction with commonly discussed pathogenic mechanisms such as Gb3 plasma levels, vascular endothelial deposits or IENFD, especially in female patients (Gupta et al., 2005). Our data support the notion that small fiber impairment clinically presenting as pain and dyshidrosis are common symptoms of early FD (Ries et al., 2003; Üçeyler et al., 2014). A shared mechanism of small nerve fiber damage leading to impaired A-delta fiber pathway and autonomic symptoms in mild to moderate FD is assumed, pointing towards high vulnerability of small caliber nerve fibers. This is supported by reduced PREP amplitudes, and also by the recent finding that cutaneous sweat gland innervation is reduced in women with FD suffering from anhidrosis (Kokotis et al., 2018) as well as previous data on early changes in small fiber morphology and function in female FD patients (Møller et al., 2009).

Apparently, this finding is not reflected by QST and IENFD. We assume that A-delta fiber pathways examined via PREP are impaired independent of histological findings of reduced IENFD, because altered conduction properties may occur prior to histopathological changes of loss of small nerve fibers. This differentiation of impaired pathways versus altered morphology is probably only possible in early disease stages, when compensatory processes are not yet established. Interpretation of QST data is challenging, as it is known to reflect small fiber damage in male FD patients typically in terms of elevated CDT (Luciano et al., 2002; Üçeyler et al., 2013). However, these findings were independent of clinical symptoms and were not prominent in female patients. Dyshidrosis may also affect heat conduction of the thermal electrode and interpretation of heat and heat induced pain of the patients, which may mask the underlying pathology. It is of note that QST, IENFD, and PREP address three different modalities of small nerve fibers, and thus eventually divergent findings are not surprising. Although, we cannot rule out additional or compensatory processes in the central nervous system influencing our results, but regarding presentation of altered PREP in small fiber neuropathies of different origins in the literature, we believe them to be of minor impact. This also highlights the diagnostic value of small fiber assessment in patients with FD.

The major limitation of our study is the small sample size in patient subgroups, which hindered analysis regarding different genotypes and effects of ERT. An influence of the regularly taken drugs on sweating cannot be ruled out, as medication was not paused. We also did not perform a second autonomic test besides SSR, e.g. for cardiac autonomic symptoms, and analysis of sweat gland innervation was not done. Further investigations of mechanisms underlying small fiber damage in FD are needed, as symptoms have relevant impact on patients' well-being and established therapeutic options are of limited clinical benefit only.

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Declaration of interest

GS, AK, CW have nothing to declare. FW has received research grants from Genzyme and Shire and speaker honoraria from Amicus, Genzyme, and Shire. CS has given educational talks for Shire and Sanofi-Genzyme. NÜ has received research grants and speaker honoraria from Genzyme and Shire.

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