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Direktor: Professor Dr. med. Klaus V. Toyka

Small fiber involvement in Fabry's disease

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Lan He

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Referentin:	Prof. Dr. med. C. Sommer
Korreferentin:	Prof. Dr. med. E. Broecker
Dekan:	Prof. Dr. med. M. Frosch

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List of Abbreviations

FD	Fabry's disease
α-GAL	α-galactosidase A
GL- 3	Globotrioacylceramide
ERT	Enzyme replacement therapy
NPSI	Neuropathic Pain Symptom Inventory
GCPS	Graded Chronic Pain Scale
CES-D	Center for Epidemiologic Studies Depression Scale
QST	Quantitative sensory testing
CDT	Cold detection threshold
WDT	Warm detection threshold
TSL	Thermal sensory limen
PHS	Paradoxical heat sensations
CPT	Cold pain threshold
HPT	Heat pain threshold
MDT	Mechanical detection threshold
MPT	Mechanical pain threshold
MPS	Mechanical pain sensitivity
DMA	Dynamic mechanical allodynia
WUR	Wind-up ratio
VDT	Vibration detection threshold
PPT	Pressure pain threshold
SNAP	Sensory nerve action potential
NCS	Nerve conduction studies
SSR	Sympathetic skin response
IENFD	Intraepidermal nerve fiber density

1. Introduction

1.1 Definition and prevalence

Fabry's disease (FD) is a rare inherited X-linked lysosomal storage disease caused by deficient or absent activity of the enzyme α-galactosidase A (alpha-D-galactoside galactohydrolase (α-GAL); EC 3.2.1.22) due to mutations in the GLA-gene. The enzymatic defect leads to the systemic accumulation of glycosphingolipids, mainly globotrioacylceramide (GL-3) in a wide variety of tissues including vascular endothelium, renal glomeruli and tubules, dorsal root ganglia, cardiac myocytes, conducting tissue and valves, cornea, and skin [1].

The German dermatologist, Johannes Fabry and the English dermatologist, William Anderson, independently described the first patients with FD in 1898 [2,3], and therefore the disease is also known as "Mobus Anderson-Fabry".

FD is a X-linked rare hereditary disorder which affects more males than females: It is estimated that 1 in 40,000 [4] males has FD, whereas the estimated prevalence in the general population is 1 in 117,000 people [5]. In recent years, more researches have revealed that this disorder is probably underdiagnosed. In a screen of 37,104 newborns for α-galactosidase A, the incidence of FD was found to be 1:4600 [6]. In urinary screenings of patients who were undergoing hemodialysis to treat end-stage renal disease, 1.2% were shown to have FD [7]. With patients who had cryptogenic stroke or hypertrophic cardiomyopathy, the prevelance was also relatively high [8-10].

1.3 Heredity and Mechanisms

The GLA-gene is located on the X chromosome, which means a male patient will only pass his X chromosome on to his daughters. His daughters may not have FD but be only Fabry carriers, because the daughters' other X chromosome will likely carry a healthy gene that is capable of making α-GAL. On the other hand, if a mother carries the Fabry gene, there is a 50% chance that she will pass the gene on to her sons or daughters. Her sons who inherit the gene will have FD. Her daughters who inherit the gene will be carriers, but may also be affected by FD, see below.

The 12-kilobase long GLA-gene is located at Xq22 on the long arm of the X chromosome; it has seven exons [11], and encodes a 55-kDa precursor glycoprotein which is then proteolytically cleaved to the mature 51-kDa α-GAL [12]. α-GAL is a homodimeric glycoprotein consisting of 2 identical 49-kDa subunits; each monomer is composed of two domains (Fig 1.), and domain 1 contains the active site. After binding a galactosylated substrate (primarily globotriaosylceramide), α-GAL cleaves the glycosidic linkage and removes the galactose from the glycolipids during the catabolism of macromolecules [13].

Mutations in the GLA-gene leads to absent or deficient synthesis of α-GAL with two corresponding general phenotypes. The classic (or severe) phenotype shows no α-GAL activity detectable in the tissues and has symptoms affecting multiple organ systems, and the mild phenotype shows some residual α-GAL activity and has symptoms generally restricted to cardiac or renal anomalies[14].

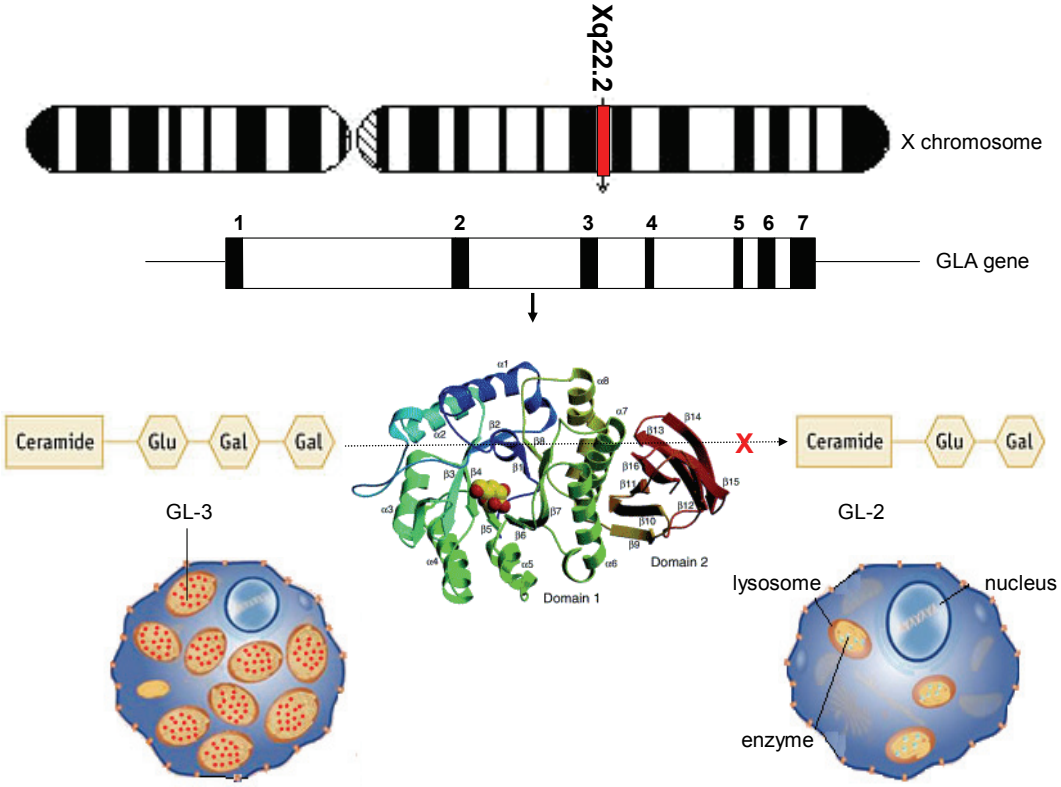


Fig. 1. Mechanism of the FD, modified from [11,12]

Due to the lack of α-GAL activity, deposition of GL-3 occurs in plasma and in the tissue lysosomes, increasing the risk for characteristic acroparesthesia of the hands and feet, angiokeratoma, strokes, hearing loss, myocardial microvascular ischemia and infarctions, arrhythmias, hypertrophic cardiomyopathy, valvular insufficiency, gastrointestinal symptoms, hypohidrosis, temperature and exercise intolerance, dysregulation of vascular tone and autonomic functions, obstructive lung disease, and chronic kidney disease leading to kidney failure.

To date, more than 400 different mutations of GLA-gene have been reported [15], but the genotype/ phenotype correlations are only partially established since most patients have “private mutations” (i.e., confined to a single Fabry pedigree), with the exception of N215S, R227Q, R227X, R342Q, and R342X,

which were each found in several unrelated families from different ethnic backgrounds [16]. N215S was a common mutation among atypical hemizygotes who were asymptomatic or had mild disease manifestations; while R227Q and R227X were the most common mutations causing the classical phenotype [17]. Recently a direct correlation was reported between the levels of urinary excretion of GL-3/creatinine and the types of mutations; and patients with the A143P mutation suffered more severe symptoms [18].

1.3 Clinical manifestations

1.3.1 General clinical manifestations

FD is a multisystem disorder with a wide range of symptoms which can affect both males and females. Virtually all males with the Fabry gene develop the disease and are likely to express some or many of the classic Fabry symptoms. In women however, symptoms can range from none (in asymptomatic carriers) to very serious manifestations similar to those seen in males.

The Fabry Registry is a global ongoing, observational database that tracks natural history and outcomes of patients with FD. According to its latest survey based on 2236 patients (1159 male, 1077 female), male patients were generally more severely affected than female patients, and the symptoms developed about 5~10 years later with female than male patients [19]. The age when first Fabry symptoms were recognized was 13.5 +/-12.1 years with male and 19.9 +/-15.7 years with female patients, and their Fabry diagnosis was made with male at 26.3+/- 15.5 years and with female 32.1+/- 17.6 years. There was no significant difference of the time from symptom onset to diagnosis between male (14.2 +/-13.0 years) and female (15.7+/-14.8 years) patients.

Males with FD present early in life (4–8 years of age) with burning pain in their hands and feet, paresthesias (spontaneous or evoked abnormal sensations that are not unpleasant) and hypohidrosis [20,21]. Angiokeratoma, a characteristic skin disorder of flat dark red to blue colored lesions, is found primarily in the bathing trunk area and the mucosal membranes from adolescence. Corneal and lenticular opacities appear around the same time. Fatigue, vertigo, and dizziness occur later in life. Life-threatening symptoms, including cerebrovascular events, cardiovascular symptoms and events (hypertrophic cardiomyopathy, valve disease, arrhythmias and myocardial infarction) and kidney disease, are among the latest occurring manifestations, and would cause death in the third to fifth decade in the absence of interventions such as hemodialysis, kidney transplantation, and heart protective measures [22].

1.3.2 Women and children

Formerly, females were considered only rarely to be as severely affected as males due to "X chromosome inactivation" [23,24]. However, now it is acknowledged that female Fabry carriers can experience all symptoms of FD, although the onset of symptoms is not as early as is typically observed in males, and there is considerable variation between individuals.

Cardiac involvement was the most common serious manifestation of FD among the females. LVH and major cardiac events occurred nearly 10 years later in life among females than in males. Cardiac involvement in children with Fabry is also frequent and may progress even at young age [25]. Strokes occurred in 4.2% of the females at a median age of 43.5 years. This was about 5 years later in occurrence than in males. Gastrointestinal and renal symptoms are very common among males and females, although the age of onset is later among females. End-stage renal disease is less common in females with FD than in

males and develops at approximately the same age (median of 38 years) for both male and female patients.

The most frequent early clinical manifestations of FD with children are also neurological (acroparesthesia, altered temperature sensitivity) symptoms. Recurrent abdominal cramps and diarrhea are the second most common presenting symptoms [26]. Tinnitus, vertigo, fatigue and angiokeratoma were noted in early childhood and occurred with similar frequency in boys and girls, although the onset of symptoms was 2-5 years later in girls than in boys. There was an approximately 3-year delay from onset of symptoms to diagnosis with children [27].

1.4 Diagnosis

Typical signs like angiokerotomata of the skin of the trunk area and cornea verticillata are clues leading to the diagnosis. But since FD is rare, and the patients may have only a few of the typical symptoms, genetic diagnosis is essential. Because FD is an X-linked disorder and most cases result from inherited mutations rather than new mutations, identification of affected males is relatively easy, by using a combination of pedigree analysis and measurement of α-GAL activity in plasma or leukocytes. The identification of carrier females is more difficult because many have normal levels of α-GAL. The presence of the characteristic cornea verticillata or the demonstration of increased concentrations of GL-3 in urine sediment is highly suggestive of the diagnosis. However, the only way to make a definitive diagnosis is to show that the female carries the same GLA-gene mutation as her affected male relative. In symptomatic women who do not have an affected male relative with a GLA-gene mutation, identification of a disease-causing mutation is often difficult and time consuming [28].

1.5 Treatment

1.5.1 Enzyme Replacement Therapy (ERT)

The first case of FD was reported in 1898; and in 1970, α-GAL was identified. Over the hundred of years, the therapy was merely confined to symptomatic treatment (e.g., analgesics, dialysis, and renal transplantation); until 2001, the first medication for Enzyme Replacement Therapy (ERT) was approved in Europe.

Two pharmaceutical companies have developed enzyme replacement therapy in FD: agalsidase alfa and agalsidase beta. Both are versions of human α-GAL that are produced in genetically engineered cell lines by different techniques. The recommended doses of agalsidase alfa and agalsidase beta are 0.2 mg/kg and 1.0 mg/kg biweekly, respectively. Only agalsidase beta is approved for treatment for FD in the United States, although both agents are approved for clinical use in other countries. Enzyme replacement therapy with either drug is very expensive, costing approximately $250 000 per year for the average adult with the disease.

ERT has a primary role in the treatment of patients with FD. Numerous patients have now been treated using this approach, and randomized, placebo-controlled clinical trials and longer-term, open-label extension studies have shown that ERT could reduce the GL-3 levels in plasma and urine sediment; and accumulation of GL-3 in capillary endothelial cells, renal glomerular cells, and tubular epithelial cells as well [14,29].

Relief of gastrointestinal symptoms is one of the earliest and most consistently beneficial effects of ERT [30]. Positive effects on hypohidrosis, neuropathic pain, and small-fiber function were reported [31]. Many reports have described

stabilization and even significant improvement of renal function and cardiomyopathy, and ERT may be able to slow down the natural decline in renal function in patients with moderate reduction in glomerular filtration rate. Although patients with normal or only mildly abnormal renal function seem to remain stable while receiving ERT, renal function deteriorates in those with glomerular filtration (GFR) rates less than 55 ml/min per 1.73 m^2, although perhaps at a slower rate [32]. Cerebrovascular attacks have occurred in some patients despite ERT, but the rate of progression was slowed [33]. In conclusion, among patients with FD, a subpopulation with impaired renal function (reduced glomerular filtration rate, proteinuria, glomerulosclerosis) at baseline has a less favorable outcome and may develop renal progression despite treatment with ERT [34]. Advanced baseline cardiac involvement (late enhancement) also appears to predict a less favorable cardiac course of disease [35].

1.5.2 Other treatments

Besides of ERT, other possible treatment options have also been developed, although they have not yet been implemented due to the requirement of further research to show whether they are feasible for use in patients.

Enzyme enhancement (chaperone) therapy: In FD, premature degradation of α-GAL was demonstrated to occur within the endoplasmic reticulum-associated degradation as a result of the misfolding of mutant proteins. Strategies directed at preventing premature degradation by pharmacologic stabilizing of the mutant protein have been shown to substantially increase residual α-GAL activity [36]. Because the level of enzyme activity necessary to prevent the disease is relatively low (<10%), even a modest increase in chaperone-induced enzyme activity might be expected to arrest the progression of FD. 1-Deoxygalactonojirimycin is one of the most

potent inhibitors of alpha-galactosidase A. It has also been shown to be the most effective active-site-specific chaperone at increasing residual enzyme activity in cultured fibroblasts and lymphoblasts established from Fabry patients with a variety of missense mutations. Such active-site-specific chaperone approach using functional small molecules may be broadly applicable to FD and other lysosomal storage disorders [37].

Restriction of GL-3 synthesis (substrate reduction therapy) and gene therapy are other approaches that are being investigated [38,39].

1.6 Small fiber involvement in FD and its reaction to ERT

1.6.1 Neuropathic pain

In FD, the accumulation of glycosphingolipids, mostly GL-3 in the central and peripheral nervous systems induces a predominantly small nerve fiber dysfunction.

Two types of pain are described in patients with FD: the constant burning and lancinating neuropathic pain, and Fabry crises in which aggravated pain is observed in attacks induced by exercise, stress, and temperature changes, including fever. Fabry crises can last for a few minutes to several days, and are so debilitating that many patients are confined to bed. The pain is located largely in the hands and feet, but might also occur in joints, and is often associated with dysesthesias.

Strong neuropathic pain affects about 65% male and 43% female patients [19,40]. Acute pain crisis with acroparesthesia of prickling and burning character was typically the earliest symptom of FD, with a mean age at onset of 9.4 years in

boys and 16.9 years in girls [19,20]. According to the International Association for the Study of Pain (IASP), acroparesthesia is defined as “numbness, tingling, or other abnormal sensations in one or more of the extremities”. In FD, the term is used for the burning pain and discomfort in the palms of the hands and the soles of the feet that occurs with fever and exercise. Although the term is a misnomer, it will be retained here, because it is so widely used in the FD-literature [1]. Chronic pain syndromes become manifest later, at a mean age of 19.4±1.2 years. In FD, chronic neuropathic pain is thought to be in part caused by GL-3 deposition in dorsal root ganglia and sympathetic ganglia [41,42]. Such deposits are likely to damage the neurons and nerve fibers and thus may lead to neuropathic pain in FD. In general, the level of pain appears to be stable during aging, although individual patients may have persistent pain, diminishing pain with age, or onset of pain later in life [19]. Both acute pain attacks and chronic background pain have been reported as major causes of disability and psychiatric symptoms both in children and adults [43].

ERT has been reported to significantly reduce pain and improve quality of life [44,45]. Patients who complained of severe pain at baseline showed the greatest improvement; and no patient who was pain free at baseline shifted to severe pain after 2 or 3 years of ERT [40]. It is conceivable that clearance of nervous tissue from GL-3 may account for the improvements, but this hypothesis still needs confirmation by histological investigations.

1.6.2 Small fiber neuropathy

Neuropathic pain in Fabry patients may also be caused by the small fiber neuropathy. Sural nerve biopsies show a pronounced reduction of small diameter, thinly myelinated A-delta nerve fibers and unmyelinated C-nerve fibers. Large diameter, thickly myelinated fibers are less vulnerable and

therefore have rather well-preserved function. In accordance with the biopsy studies, quantitative sensory testing (QST) in Fabry patients showed significant Aβ-, Aδ-, and C-fiber dysfunction characterized by vibratory (VDT), cold (CDT), and heat-pain (HPT) detection threshold [46], while assessment of large, thickly myelinated fibers by means of nerve conduction studies (NCS) showed only mild impairment . Similarly, recordings of sympathetic skin responses (SSR) did not show significant changes in the Fabry patients, although hyperhidrosis is a frequent and early symptom [47].

Early ERT steadily improves small nerve fiber function and sweat function [48], but such improvement can only be seen over 18 months consequent therapy, and ERT cannot improve nerve fiber function in patients with severe dysfunction of thermal perception such as loss of cold sensation [31].

1.6.3 Intraepidermal nerve fiber density (IENFD)

As the earliest symptom of FD, the neuropathic pain can occur in patients as young as 5 years of age. Although the pain may be severe, routine physical examination fails to detect any neurologic abnormality. Moreover, in patients who have not yet developed renal insufficiency, electrophysiologic studies and sural nerve biopsy detect no abnormality [46]. Interest in studying cutaneous innervation has been kindled by the ability to visualize intraepidermal axons using antibodies to a panaxonal molecule, PGP 9.5. By counting the number of free nerve endings visualized in the epidermis, intraepidermal nerve fiber density (IENFD) can be determined [49]. The most important value of skin biopsy is the ability to perform multiple biopsies over time. Serial biopsies could prove useful in detecting and quantitating increases in IENFD resulting from specific therapies such as ERT.

In recent years, significantly reduced IENFD in the distal leg was reported in FD patients [50,51,52], and patients demonstrated a greater proportional loss of innervation at the distal biopsy site than at the proximal biopsy site. Although patients with a small-fiber neuropathy showed significant improvement in thresholds for warm and cold temperatures in the hands and feet as well as a reduction in neuropathic pain by 3 years of ERT, no evidence of reinnervation was seen [53]. While the IENFD in patients with stable normal kidney function was maintained over a period of 1 to 3 years [52], a continued decline of IENFD occurred in patients with severely impaired renal function [53].

1.7 Aim of the study

After the initiation of the enzyme replacement therapy (ERT) in Fabry patients, investigations concerning FD related peripheral neuropathy and its reaction to ERT suggest that ERT reduces neuropathic pain, improves sweating and peripheral nerve function in the hands and feet [31,48]. However, distal epidermal nerve fiber regeneration could not be shown after 12-18 months of ERT [53]. In addition, patients with impaired renal function at baseline have less favorable outcomes and may develop final stage events with kidney and heart despite treatment of ERT [35,54]. Furthermore, ERT cannot improve nerve fiber function in patients with severe dysfunction of thermal perception such as loss of cold sensation [31].

We therefore investigated the neurological function in patients with Fabry's disease and its response to enzyme replacement therapy (ERT). Moreover, we looked into the role renal function plays on small fiber function in FD and its influence on ERT. We also tested whether ERT can induce proximal epidermal nerve fiber regeneration.

2. Methods

2.1 Subjects

To evaluate the neurological, especially small fiber involvement, in FD, we examined a cohort of 76 Fabry patients including 39 males (13.9-63 years) and 37 females (8.9-68.8 years). The diagnosis was confirmed by family history, mutation and enzyme activity analysis. 37 patients (26 male, 11 female) were on ERT. The study was approved by the local ethics committee and all subjects had given written informed consent. All patients filled in the pain questionnaires NPSI and GCPS, and the depression questionnaire CES-D. All patients had neurological examination, quantitative sensory testing (QST), extra- and transcranial Doppler sonography and clinical neurophysiology including sural nerve conduction studies and the sympathetic skin response. 41 (22/39 male, 19/37 female) patients agreed to have a skin biopsy, and 11 of them had repeated biopsy after one year follow up. 32 healthy volunteers served as control subjects for QST (mean age 43.5+/- 11.4 years, 40.6% men). They were age- and gender- matched to the patients groups.

2.2 Pain and depression questionnaires

Neuropathic Pain Symptom Inventory (NPSI) [55], a new self-questionnaire specifically designed to evaluate the different symptoms of neuropathic pain was used to help justify the neuropathic pain experienced by patients. NPSI includes 12 items: 10 descriptors reflecting spontaneous ongoing or paroxysmal pain, evoked pain (i.e. mechanical and thermal allodynia/hyperalgesia) and dysesthesia/paresthesia. The mean intensity of each of these items during the last 24 h had to be reported on a 0–10 numerical scale in which 0 was ‘no pain’ and 10 was ‘the most intense pain imaginable’.

Two additional categorical items evaluated the temporal sequence of spontaneous ongoing pain (i.e. number of hours during the last 24 h) and paroxysmal pain (i.e. number of paroxysms during the last 24 h). The German version of the NPSI is presented in Appendix A.

The Graded Chronic Pain Scale (GCPS) [56] includes a measure of pain intensity and the extent to which pain is psychosocially disabling. The average of three pain intensity items is used to measure characteristic pain intensity. Disability days and ratings of interference with social, occupational, and recreational activities result in a disability score and a grade of severity of chronic pain status. Graded chronic pain categories correspond to the following: grade 0, pain free; grade I, low intensity pain and low interference; grade II, high-intensity pain, low interference; grade III, moderate interference; grade IV, high interference. Grades I and II correspond to a pain patient who is functioning adaptively to his pain, whereas grades III and IV correspond to dysfunctional adaptation. In this study, we used the total score of the three pain intensity items as an indicator of pain severity, and the total score of the three items rating interference with social, occupational, and recreational activities as disability score. The German version of the GCPS is presented in Appendix B.

The CES-D (Center for Epidemiologic Studies Depression Scale) [57] is a short self-report scale designed to measure depressive symptomatology in the general population. This 20 item questionnaire includes six components: depressed mood; feelings of guilt and worthlessness; feelings of helplessness and hopelessness; psychomotor retardation; loss of appetite; and sleep disturbance. Respondents indicate how often within the last week they experienced the symptoms, responding: “rarely or none of the time” (0); “some or little of the time” (1); “occasionally or a moderate amount of time” (2); and “most or all of the time” (3). The scores for the 20 items are added, resulting in a range of possible total scores from 0 to 60. A total score higher than 16 is taken

as a indicator of depression. The German version of the CES-D is presented in Appendix C.

2.3 Quantitative Sensory Testing (QST)

2.3.1 Thermal detection, thermal pain thresholds and paradoxical heat sensations

The tests for thermal sensation were performed using a Thermotest Type 1 (Somedic AB, Sweden) thermal sensory testing device. Cold detection threshold (CDT) and warm detection threshold (WDT) were measured first. The number of paradoxical heat sensations (PHS) was determined during the thermal sensory limen procedure (TSL, the difference limen for alternating cold and warm stimuli), followed by cold pain threshold (CPT), and heat pain threshold (HPT). The mean threshold temperature of three consecutive measurements was calculated. All thresholds were obtained with ramped stimuli (1 °C/s) that were terminated when the subject pressed a button. Cut-off temperatures were 10 and 50 °C. The baseline temperature was 32 °C (center of neutral range) and the contact area of the thermode was 7.84 cm^2. During the experiment, the subjects were not able to watch the computer screen. All thermal tests were first performed over cheek as a self control and then tested over dorsal foot.

2.3.2 Mechanical detection threshold for modified von Frey filaments

Mechanical detection threshold (MDT) was measured with a standardized set of modified von Frey hairs (Optihair2-Set, Marstock Nervtest, Germany) that

exert forces between 0.25 and 512 mN. The contact area of the von Frey hairs with the skin was of uniform size and shape (rounded tip, 0.5 mm in diameter) to avoid sharp edges that would facilitate nociceptor activation. The final threshold was the geometric mean of five series of ascending and descending stimulus intensities.

2.3.3 Mechanical pain threshold for pinprick stimuli

Mechanical pain threshold (MPT) was measured using a set of seven custom-made weighted pinprick stimulators (flat contact area of 0.2 mm diameter) that exert forces between 8 and 512 mN. Again using the "method of limits", the final threshold was the geometric mean of five series of ascending and descending stimulus intensities.

2.3.4 Stimulus–response-functions: mechanical pain sensitivity for pinprick stimuli and dynamic mechanical allodynia for stroking light touch

Mechanical pain sensitivity (MPS) was tested using the same weighted pinprick stimuli as for MPT. To obtain a stimulus–response-function, these seven pinprick stimuli were applied in a balanced order, five times each, and the subject was asked to give a pain rating for each stimulus on a 0–100 numerical rating scale ('0' indicating "no pain", and '100' indicating "most intense pain imaginable").

Stimulus–response-functions for dynamic mechanical allodynia (DMA) were determined using a set of three light tactile stimulators: a cotton wisp exerting a force of ~3 mN, a cotton wool tip fixed to an elastic strip exerting a force of ~100

mN, and a standardized brush (Somedic, Sweden) exerting a force of 200~400 mN. The three tactile stimuli were applied five times each with a single stroke of approximately 1–2 cm in length over the skin. They were intermingled with the pinprick stimuli in balanced order and subjects were asked to give a rating on the same scale as for pinprick stimuli.

2.3.5 Wind-up ratio – the perceptual correlate of temporal pain summation for repetitive pinprick stimuli

In this test of temporal summation, the perceived magnitude of a single pinprick stimulus was compared with that of a train of 10 pinprick stimuli of the same force repeated at a 1/s rate (128 mN, when tested over face, and 256 mN, when tested over hand and foot). The train of pinprick stimuli was given within a small area of 1 cm^2 and the subject was asked to give a pain rating representing the pain at the end of the train using a numerical rating scale. In contrast to the more sophisticated technique of VAS-ratings at a 1/s rate this method is likely more appropriate for clinical routine assessment. Single pinprick stimuli were alternated with a train of 10 stimuli until both were done five times at five different skin sites within the same body region. The mean pain rating of trains divided by the mean pain rating to single stimuli was calculated as wind-up ratio (WUR).

2.3.6 Vibration detection threshold

Vibration detection threshold (VDT) test was performed with a Rydel–Seiffer tuning fork (64 Hz, 8/8 scale) that was placed over a bony prominence (cheek, processus styloideus ulnae, malleolus medialis). Vibration threshold was determined with three series of descending stimulus intensities.

2.3.7 Pressure pain threshold

The final test in the protocol was performed with a pressure gauge device (FDN200, Wagner Instruments, USA) with a probe area of 1 cm^2 (probe diameter of 1.1 cm) that exerts pressure up to 20 kg/cm^2/~200 N/cm^2/~2000 kPa. The pressure pain threshold (PPT) is determined with three series of ascending stimulus intensities, each applied as a slowly increasing ramp of 50 kPa/s.

2.3.8 Statistics

Two-sample t-tests were performed to analyze for differences between patients and controls for data with a normal distribution. In case of non-normality, Wilcoxon's signed rank test was applied. All statistical calculations were performed by using the SPSS software 11.5 for Windows (SPSS Inc., USA). Log-data of thresholds were retransformed to linear values representing the original unit of each test.

To compare the patients' QST data profile with control data, the data were *Z*-transformed [58] for each single parameter by using the following expression:

$$\mathbf{Z\text{ - score}} = (\mathbf{X}_{\text{single patient}} - \mathbf{Mean}_{\text{controls}}) / \mathbf{SD}_{\text{controls}}$$

This procedure results in a QST profile where all parameters are presented as standard normal distributions (zero mean, unit variance). *Z*-values above "0" indicate a gain of function when the patient is more sensitive to the tested stimuli compared with controls, while *Z*-scores below "0" indicate a loss of function referring to a lower sensitivity of the patient. Thus, elevations of threshold (CDT, WDT, TSL, HPT, CPT, MDT, MPT, VDT, PPT) resulted in negative *Z*-scores, whereas increases in ratings (MPS, ALL, WUR) resulted in positive *Z*-scores. Paradoxical heat sensations (PHS) were interpreted as a

loss of thermodiscriminative function resulting in negative *Z*-scores.

2.4 Neurological examination

Detailed family history, pain complaints, analgesic treatment and possible pain triggers of the patients were recorded. Standard neurological examination was performed prior to skin biopsy. Renal status was based on medical records and creatinin-clearance.

2.5 Clinical electrophysiological examination

2.5.1 Sural nerve conduction studies

72 patients underwent nerve conduction studies. With the patient reclined, the right foot was placed on a firm pillow. An antidromic sensory nerve conduction study was performed on the right sural nerve, using standard nerve technique. The sensory nerve action potential (SNAP) was recorded at the lateral malleolus with surface electrodes close to the nerve, amplified, digitized and 2 recordings were averaged for off-line analysis (Duoliner, Toennies GmbH, Höchberg, Germany; filter settings: low cut 2 Hz, high cut 2 kHz). The SNAP was started by stimuli (1 ms in duration) at the midcalf, with surface electrodes (Toennies), using a distance from the recording electrode of more than 10 cm. Supramaximal stimulation was achieved by increasing the stimulation current until the amplitude of the SNAP did not further increase. For measurements of minimal SNAP latency and amplitude, 2 responses were averaged and the distance between the stimulating and recording electrode was measured.

Measurements of sensory nerve conduction velocity and of SNAP amplitude

were compared to normal values of the laboratory. In all measurements, the temperature of the skin at both recording and stimulation sites was controlled using a thermometer, securing a temperature above 34°C. In case of low skin temperatures, the leg was warmed in a footbath.

2.5.2 Sympathetic skin response

SSR was recorded from the foot. The recording surface electrode was placed on the sole of the foot, while the reference electrode was placed on the dorsal side. The stimulus was electrical stimulation of the ipsilateral median nerve with the patient unaware of the stimulation time point. The tests were done using standard techniques with a Toennies Duoliner and analyzed with Toennies Duoliner software vers. 2.0 (Toennies). If no responses were recorded from two or more tests, the test result was considered abnormal.

2.6 Extra- and transcranial Doppler sonography

The subjects were studied in a supine resting state with their eyes closed. TCD examination was carried out by an experienced sonographer with ultrasound equipment (Model: PIONEER 2021, Inc.: NICOLET). The temporal window was used for studying middle cerebral artery (MCA), anterior cerebral artery (ACA) and posterior cerebral arteries (PCA) with a 2-MHz pulse-wave probe. The CCA, extra-cranial segment of internal carotid artery (ICA), vertebral artery (VA) and subclavian artery were insonated bilaterally by 4-MHz continuous wave Doppler probe.

2.7 Skin biopsy

Intraepidermal nerve fiber density (IENFD) was assessed on five-mm punch biopsies (Disposable biopsy punch, WPI, USA) taken under sterile conditions, after topical anesthesia with 10 % lidocaine [49]. Specimens were taken on the distal leg 10 cm proximal to the medial malleolus and on the back at Th12 level and 10 cm lateral to the spinal cord. These locations were chosen because it was clinically acceptable; the lengths of the axons at the distal leg are similar to those innervating the foot where quantitative sensory testing was done, and the IENFD at Th12 level on the back is much higher than that of distal leg and therefore can serve as good control. For the repeated biopsy, the site was 1 cm distal from the scar where last biopsy performed; when the scar was not obvious, the biopsy site was empirically determined by the operator.

Specimens were fixed in fresh 4% buffered paraformaldehyde for 2 to 4 hours, washed in phosphate buffer and subsequently stored in 10% sucrose with 0,1M phosphate buffer until analysis. Fifty-µm cryostat sections were immunoreacted with the pan-neuronal marker PGP9.5 (1:800, Ultraclone, UK) and visualized with Cy3-labelled anti-rabbit antibodies (1:100, Amersham, USA).

IENFD quantitation: Three immunofluorescence-stained biopsy sections per site were viewed with a Zeiss Axiophot 2 microscope (Wetzlar, Germany). Single unmyelinated sensory nerves were counted only if they crossed the basement membrane of the epidermis as previously described [59]. Fibers that branched within the epidermis after crossing the basement membrane were counted as a single unit. Nerves that branched in or below the basement membrane and then traveled separately into the epidermis were counted as multiple units depending upon the number of branch points. Fragments in the epidermis that did not cross the basement membrane were not counted. The lengths of epidermis were digitized using a Image Pro Plus 4.0 software (Media

Cybernetics, Leiden, The Netherlands). The total number of fibers was divided by the total length of the counted sections and expressed as intraepidermal nerve fiber number per mm (IENFD, f/mm). Each section analyzed was counted by an observer blinded to the source of the specimens.

The density of the subepidermal plexes was judged visually as “normal”, “slightly decreased”, “decreased”, or “depleted”. Our lab’s standard for distal IENFD (female: 9.5 +/- 3.7 f/mm, male: 10.4 +/ 2.5 f/mm) from 68 healthy volunteers (mean age 49.4 +/- 15.3; 42.6% men) was used as normal value for skin biopsies. We have not collected enough objects to draw a standard for proximal IENFD, and the corresponding comparison in this study was only performed within the patients.

Statistics

Two-sample t-tests were performed to analyze for differences between patients and controls for data with a normal distribution. In case of non-normality, Wilcoxons signed rank test were applied. Pearson and Spearman correlation coefficients were used to evaluate the relationship between clinical, QST, neurophysiologic and neuropathologic variables.
$P < 0.05$ was considered significant.

3. Results

3.1 Patients demographics and clinical characteristics

As of May 6, 2008, a total of 76 patients were enrolled in the neurological screening of the Fabry Centre of Würzburg; 39 (51.3%) male and 37 (48.7%) female. Most patients had a positive family history of FD. 51 patients (67.1%) were detected via family screen, 9 patients (11.8%) through renal problems, 5 patients (6.6%) through ophtalmological examination, 4 patients (5.3%) through cardiac examination, 3 (3.9%) through cerebral events, 2 (2.6%) through angiokeratoma, and two patients (2.6%) (mother and son) noticed their symptoms through the media and then turned to our center. All the patients were screened and followed up after one year. 21 patients were followed up for two years. We took patients younger than eighteen years as a subgroup and divided the adult patients into two subgroups based on their age (<40 and ≥40years). Demographic and clinical characteristics are indicated in Table 1.

Generally, male patients experienced higher frequencies of symptoms with kidney, heart and CNS than females. These differences were not large in patients older than 40 years, but in this group there were ten percent fewer female patients receiving ERT. The median age at diagnosis in females (38.1 +/-16.4 years) was older than that in males (36.6+/-13 years), although with no significant difference.

Table1. Demographics and clinical characteristics in all Fabry patients

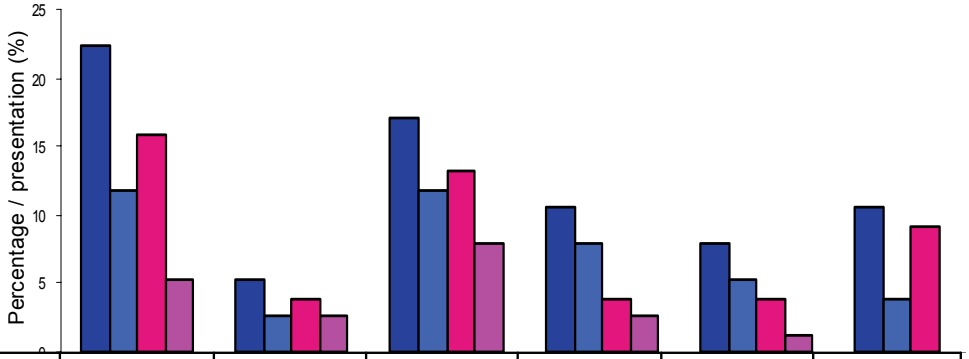
Parameter		Number of male patients	Number of female patients
Total number of patients	n	39	37
current age (years)	Median (range)	39.3 (13.9, 63)	39.8 (8.9, 68.8)
Family members diagnosed with FD	n (%)	37 (94.8%)	34 (91.9%)
Age category (years)			
0–<18	n (%)	3 (7.7%)	5 (13.5%)
≥18–<40	n (%)	15 (38.5%)	10 (27%)
≥40	n (%)	21 (53.8%)	22 (59.5%)
Age at FD diagnosis (years)	Mean (SD)	36.6 (13.0)	38.1(16.4)
Patients with renal problems	n (%)	22 (56.4%)	12 (33.3%)
Age category (years)			
0–<18	n (%)	0	0
≥18–<40	n (%)	5 (12.8%)	1 (2.7%)
≥40	n (%)	17 (43.6%)	11 (30.6%)
Patients with cardiac problems	n (%)	23 (59.0%)	17 (47.2%)
Age category (years)			
0–<18	n (%)	0	0
≥18–<40	n (%)	6 (15.4%)	3 (8.3%)
≥40	n (%)	17 (43.6%)	14 (38.9%)
Patients with CNS problems	n (%)	10 (25.6%)	4 (11.1%)
Age category (years)			
0–<18	n (%)	0	0
≥18–<40	n (%)	4 (10.3%)	1 (2.7%)
≥40	n (%)	6 (15.4%)	3 (8.3%)
Patients with hypohidrosis	n (%)	17 (43.6%)	5 (13.5%)
Age category (years)			
0–<18	n (%)	0	0
≥18–<40	n (%)	6 (15.4%)	2 (5.4%)
≥40	n (%)	11 (28.2%)	3 (8.1%)
Patients with auditory impairment	n (%)	14 (35.9%)	7 (18.9%)
Age category (years)			
0–<18	n (%)	0	0
≥18–<40	n (%)	3 (7.7%)	0
≥40	n (%)	11 (28.2%)	7 (18.9%)
Patients with α-GAL activity < 0.4 nmol/min/mg protein	n (%)	34 (100%)	24 (77.4%)

Patients on ERT	n (%)	26 (66.7%)	11 (30.6%)
Age category (years)			
0–<18	n (%)	0	0
≥18–<40	n (%)	8 (20.5%)	1 (2.7%)
≥40	n (%)	18 (46.2%)	10 (27.0%)

3.2 General Neurological Symptoms and Findings

The symptom most frequently reported in our cohort was pain, which affected 30 of 39 male and 24 of 37 female patients (Fig. 2). 15 patients (13 m, 2 f) had constant burning pain in the limbs with GCPS pain score of 12+/-4, and 10 patients (6 m, 4 f) also complained of numbness or tingling in this region. Nine patients (5 m, 4 f) reported fatigue. Hypohidrosis and auditory impairment were more prevalent in the male than in female group. Patients older than forty years had a higher incidence of hearing loss and tinnitus in both male and female groups. 14 patients had a history of cerebral events, but only two suffered from neurological sequelae. 15 patients (13 m, 2 f) had constant burning pain in the limbs, and 10 patients (6 m, 4 female) also complained of numbness or tingling in this region.

On neurological and electrophysiological examination, 47 patients were normal. In 29 patients, minor abnormalities were found. These were: decreased tendon reflexes (n=12), minor sensory deficits (n=10); reduced amplitude of the sensory nerve action potential (SNAP) (n=13), and reduced or absent sympathetic skin response (SSR) (n=10). Extra- and transcranial Doppler sonography gave normal results in all patients.



	Neuro pain	Headache	Other pain	Hypohidro.	Cerebro.	Audio imp.
Male ⩾40 (%)	22.4	5.3	17.1	10.5	7.9	10.5
Male < 40 (%)	11.8	2.6	11.8	7.9	5.3	3.9
Female⩾40 (%)	15.8	3.9	13.2	3.9	3.9	9.2
Female <40 (%)	5.3	2.6	7.9	2.6	1.3	0.0

Fig. 2. Neurological symptoms in Fabry patients.

Neuro pain= neuropathic pain, Hypohidro= hypohidrosis, Cerebro= cerebral events, Audio imp= auditory impairment

3.3 Pain and Depressive Symptoms

Pain was reported by 30 of 39 male and 24 of 37 female patients. Together with the confirmation of the NPSI questionnaire (NPSI score ⩾ 1/10) and patients' description of 'acroparesthesia', neuropathic pain was reported in 34.2% of male and 21.1 % female patients (Fig. 2).

Graded Chronic Pain Scale (GCPS) questionnaire and depression screening questionnaire (CES-D) data were available on 36 male and 35 female patients. The severity of pain (items 1-3 of GCPS) and pain related disability (items 4-6 of GCPS) and depression were analyzed by comparing the mean scores of males and females with three age categories, as shown in Table 2.

A CES-D score higher than 16 is thought to be an indicator of depression. In eight children we screened, five complained of pain, though with low severity

and little effect on daily life. None of the children showed an indication of depression. In adult patients, females younger than 40 years showed less pain related daily disability than patients older than 40 years (p= 0.046). There was no such difference between age groups in male patients. When comparing female with male patients, females younger than 40 years showed less pain related disability (p= 0.031) and also less depression (p= 0.034) than males younger than 40 years. Females older than 40 years suffered pain, pain related disability and depression as severely as male patients.

Table 2. Severity of pain, pain related disability and depression

		Male patients			**Female patients**		
Age category (years)		Pain score	Disability score	CES-D score	Pain score	Disability score	CES-D score
0–<18	Pt. n (%)	2 (2.9%)			3 (4.3%)		
	Mean	1.0	1.0	10.0	3.3	1.0	10.3
⩾18–<40	Pt. n (%)	14 (20%)			10 (14.3%)		
	Mean (SD)	9.2 (6.6)	8.2 (6.9)	18.8 (6.3)	5.1 (5.6)	2.1 (3.4)	10.3 (5.9)
⩾40	Pt. n (%)	22 (31.4%)			22 (31.4%)		
	Mean (SD)	7.7 (5.6)	6.4 (6.2)	15.9 (10.4)	7.1 (7.7)	7.7 (9.6)	17.3 (14.0)

Pt.= patient

3.4 Quantitative sensory testing

All 68 adult patients had QST and abnormalities were found in 63 (34/36male, 29/32 female) of them (Fig.3). Male patients had more frequent abnormal QST profiles and significantly increased CDT (p<0.001), WDT (p<0.001) and TSL

(p<0.001) than female patients, which revealed that male patients had more severe thermal sensory loss than female patients.

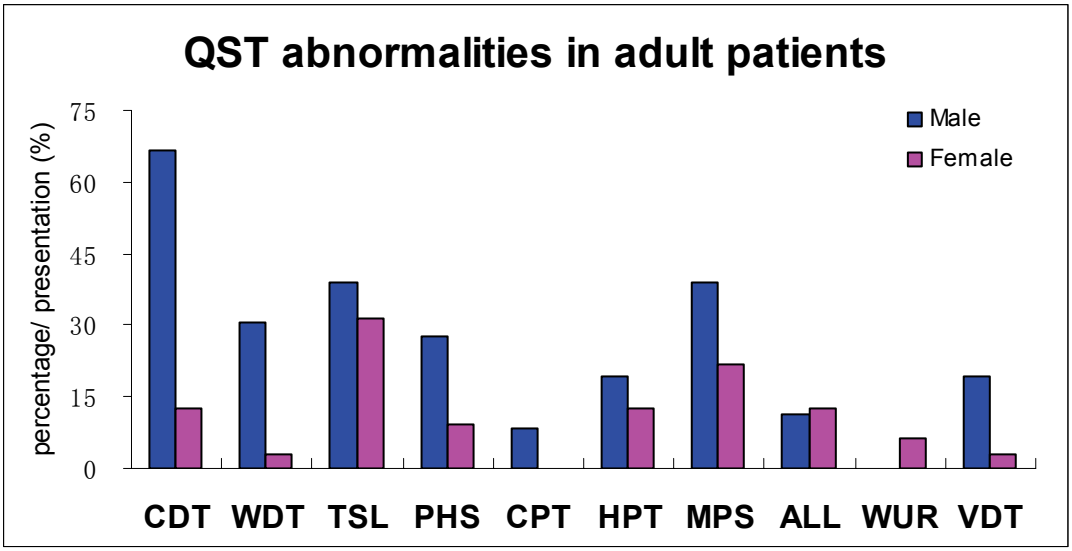


Fig. 3. QST abnormalities in adult patients.

3.4.1 Male patients

A Z-score was calculated to describe the QST profile of the patients. As shown in Fig. 4a and Fig. 4b, compared with age and gender matched healthy controls, cold detection threshold (CDT) was significantly decreased in both patients older (n=22, p<0.001) and younger than 40 years (n=14, p<0.001). The older patients had also increased paradoxical heat sensations (PHS), which indicates the loss of sensory function. In patients younger than 40 years, wind-up ratio (WUR) was increased as a sign for sensitized Aδ- fibers, while the thermal sensory limen (TSL) and vibration detection threshold (VDT) were decreased, which are signs indicating the loss of sensory function of C- and Aβ- fibers respectively. Compared to patients with relatively normal renal function (n=26), patients with pathological renal function (n=10) had more decreased PHS and VDT, although without significant difference (Fig. 4c).

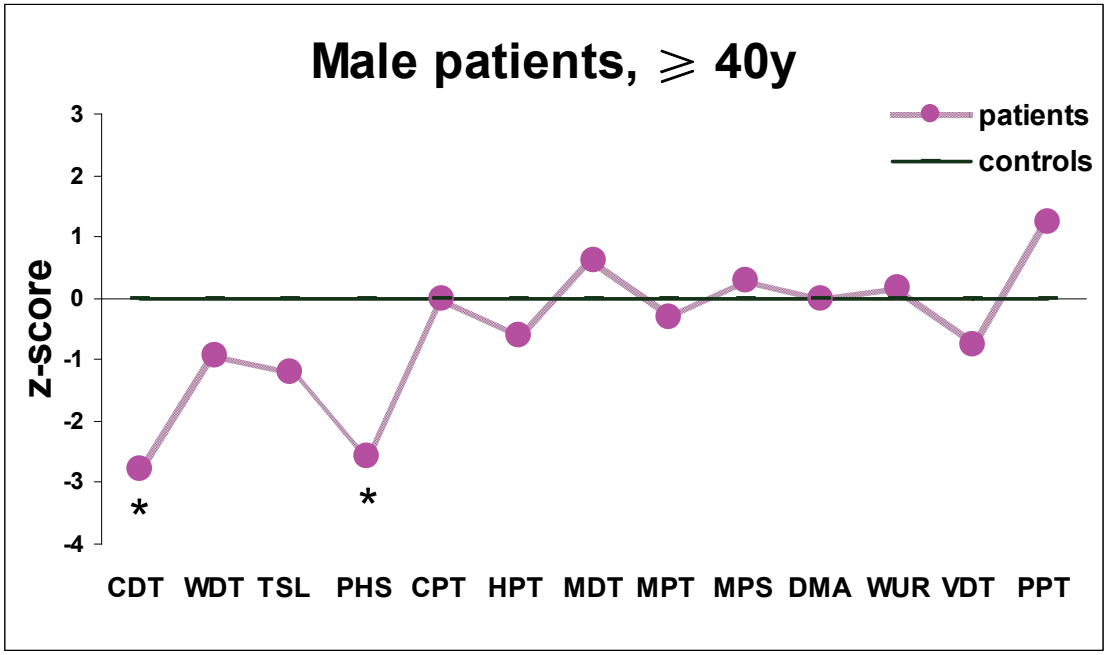


Fig. 4a. Z-score of male patients older than 40 years.

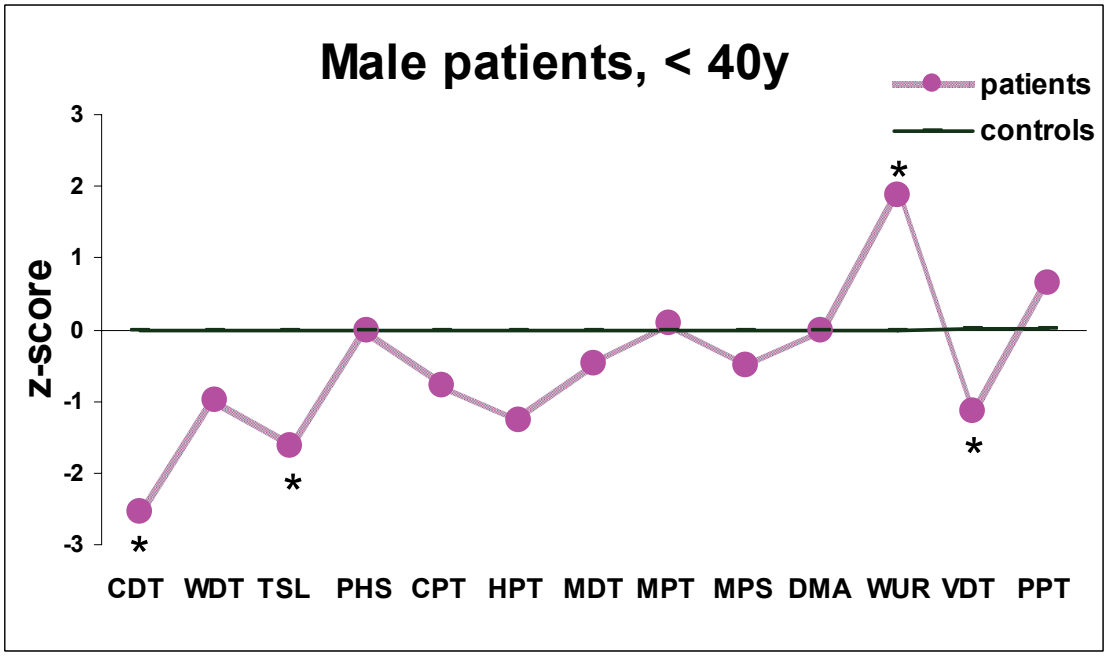


Fig. 4b. Z-score of male patients younger than 40 years.

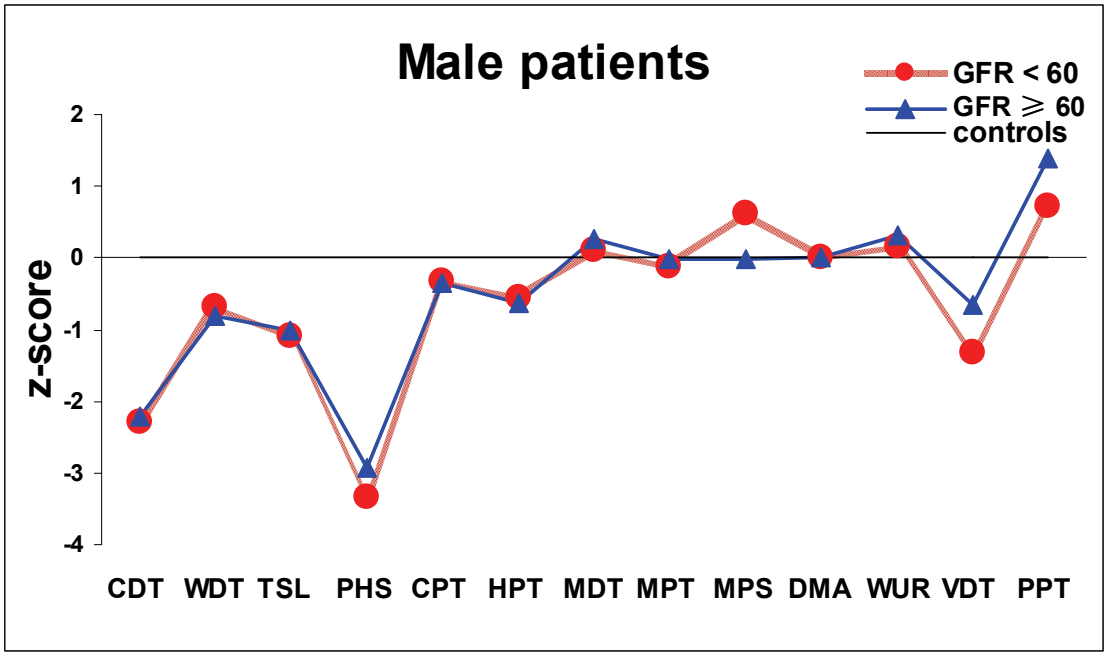


Fig. 4c. Z-score of male patients with normal and pathological GFR.

3.4.2 Female patients

In 32 adult female patients, 29 had abnormal QST values, among them four had reduced CDT, nine had increased TSL, and 11 had abnormal PHS. However, as whole groups, no significant difference between patients and healthy controls was found in any item observed except for DMA in patients older than 40 years (Fig. 5a and 5b). Patients with pathological renal function (n=5) had increased dynamic mechanical allodynia (DMA) indicating a peripheral and central sensitization, and decreased thermal detection threshold compared to patients with normal renal function, although not statistically significant (Fig. 5c).

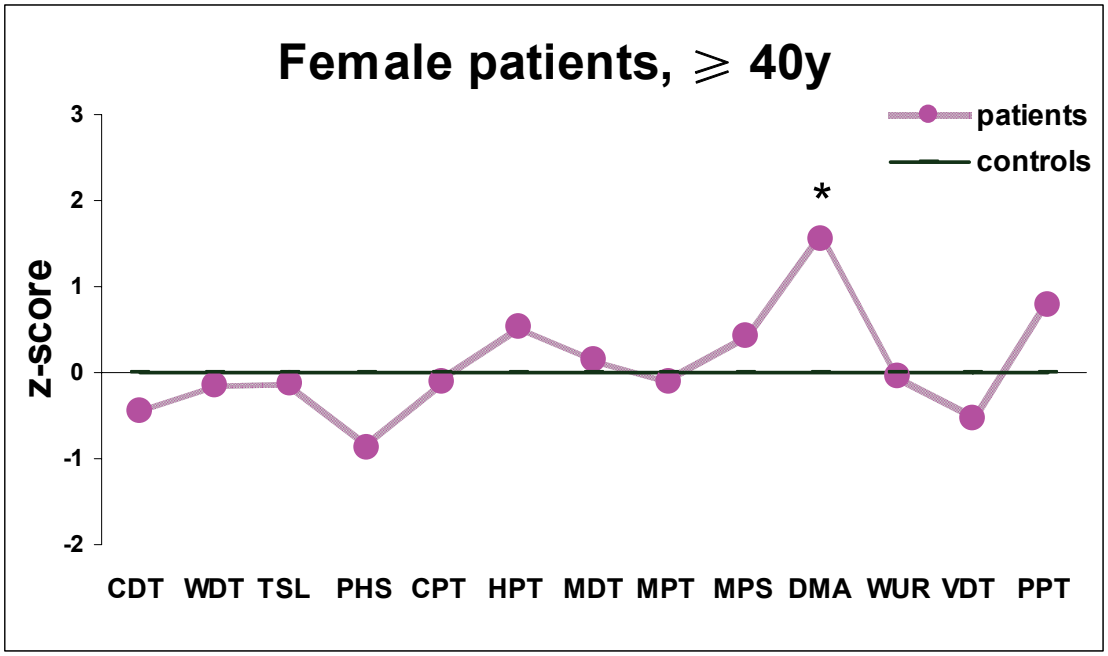


Fig. 5a. Z-score of female patients older than 40 years.

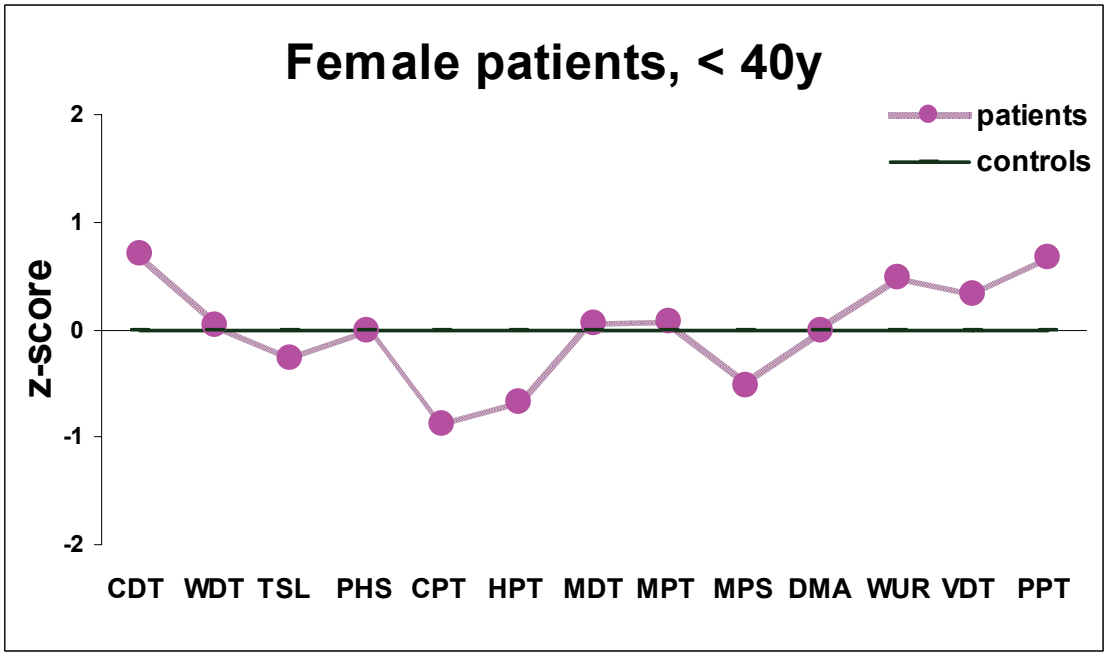


Fig. 5b. Z-score of female patients younger than 40 years.

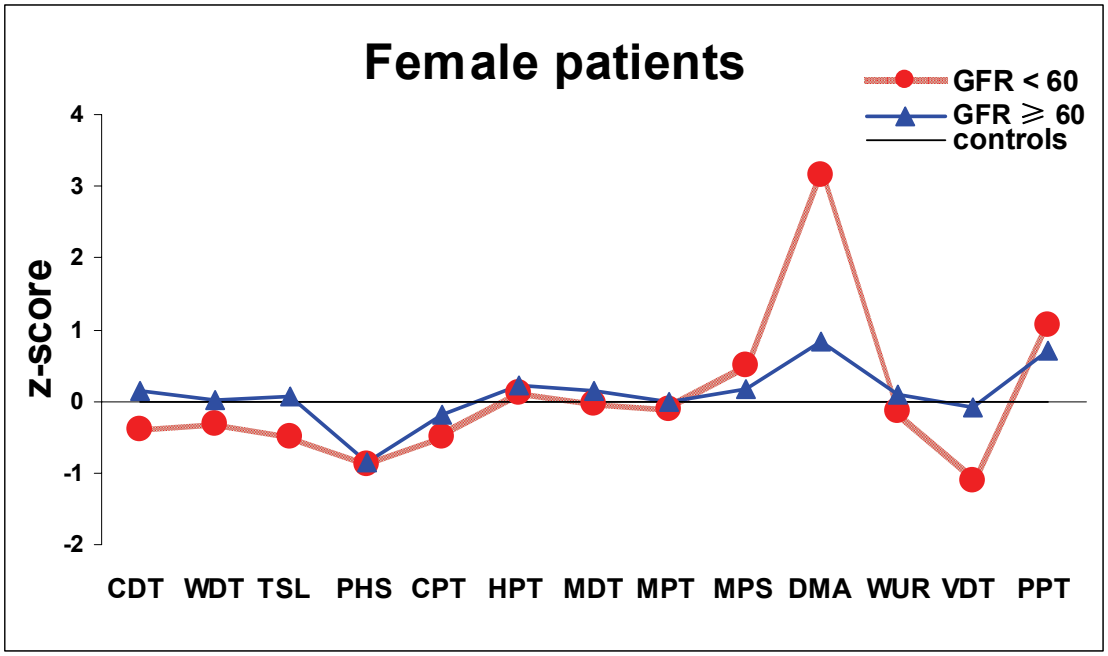


Fig. 5c. Z-score of female patients with normal and pathological GFR.

3.5 Skin innervation (IENFD)

Skin biopsies from the dermatome Th12 and from the lower leg about 10 cm above the lateral malleolus were analyzed in 41 (22/39 male, 19/37 female) patients in whom these biopsies were taken for diagnostic reasons. Skin samples were processed for immunohistochemistry and the intra- and subepidermal innervation was quantified (Fig. 6). Compared to our laboratory's normal values for Th12 and the lower leg, the intraepidermal innervation density was significantly reduced in male patients (18.0 +/- 8.8 fibers/mm proximally and 2.2+/-2.8 fibers/mm distally, Fig. 7a and 7b), and in most patients the density of the subepidermal nerve plexus was also reduced. In the 19 female patients, we found less reduced mean intraepidermal innervation density (26.2 +/- 9.9 fibers/mm proximally and 6.3 +/- 4.3 fibers/mm distally), six had a reduced subepidermal plexus density.

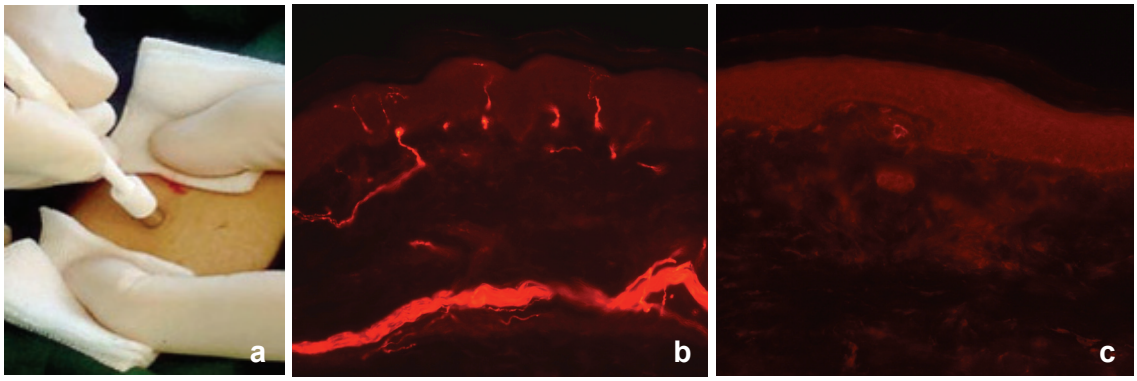


Fig. 6. (a) Skin punch biopsy in a patient with FD. (b, c) Immunofluorescent stain of nerve fibers in the epidermis and subepidermis of a healthy control (b) and a Fabry patient (c).

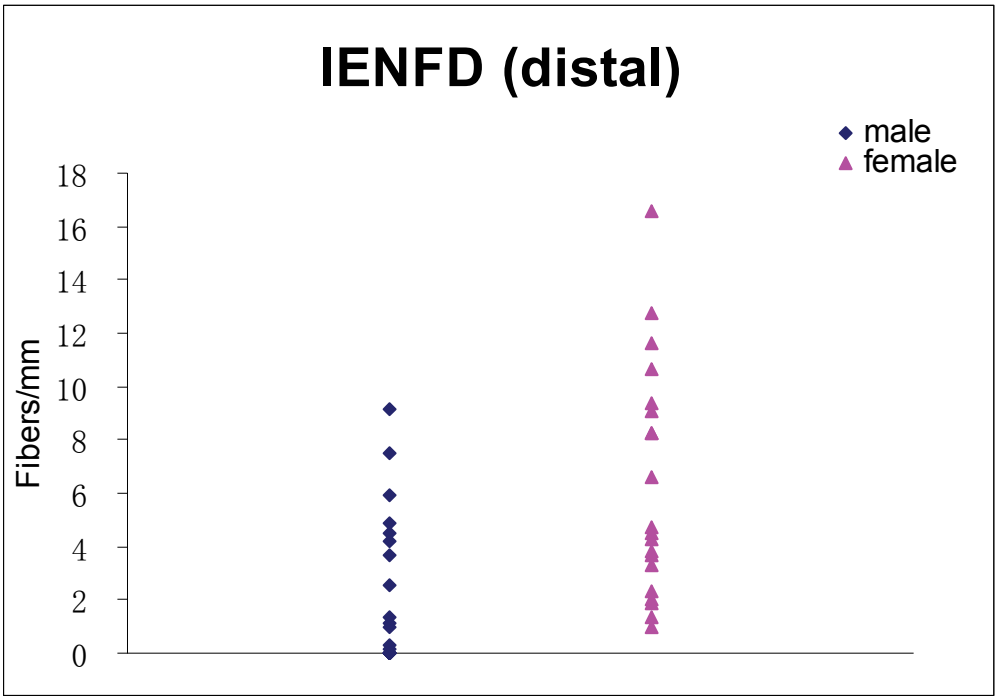


Fig.7a. Distal IENFD in male and female patients.

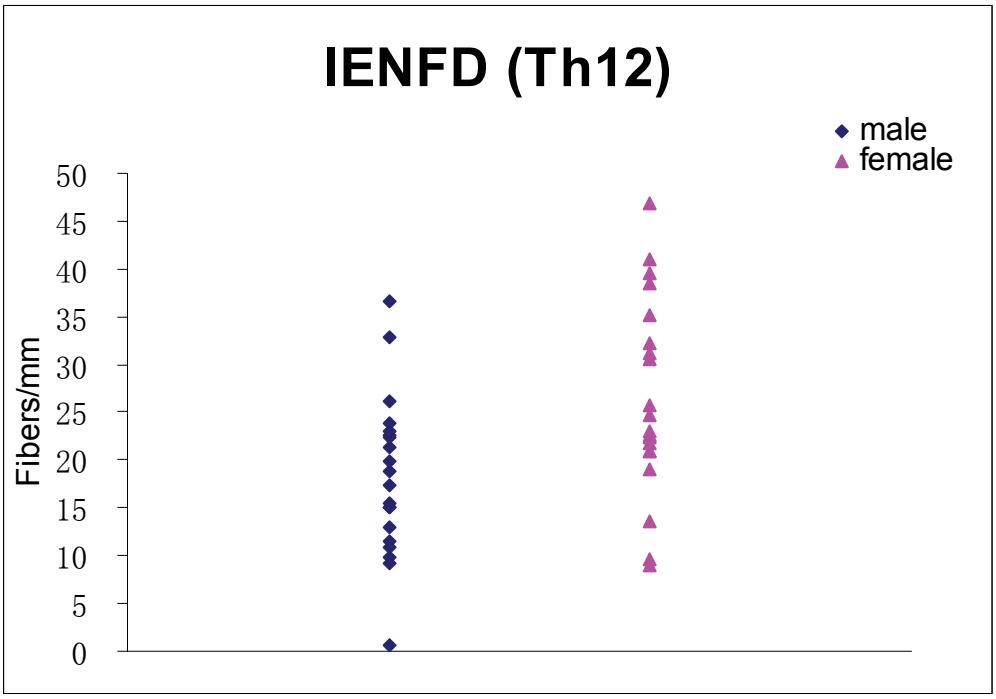


Fig.7b. Proximal IENFD in male and female patients

3.6 Follow up study

Renal function has been reported to play an important role in the ERT effect on vital organs like kidney and heart [34,60]. To look into its role in small fiber function, 21 patients (17 male and 4 female) were divided into subgroups according to their renal function for the follow up study. 17 male and two of female patients were on ERT, two female patients with mild symptoms were taken as controls. Seven patients were screened for the third time and the other fifteen patients for the second time. All the follow up patients had electrophysiological examinations and completed the questionnaires on severity of pain (items 1-3 of GCPS), pain related disability (items 4-6 of GCPS) and depression symptoms (CES-D). Data are displayed in Table 3.

Table 3. Neurological complaints and findings in the follow-up patients

Pt	V	Age	Time of ERT (year)	GFR (ml/min/1.73 m^2)	Neuropathic pain complaints	Pain score	Disability score	CES-D score	Hypohidrosis	Sural nerve NCS	
										SNAP (μV)	NCV (m/s)
Male patients with GFR < 60											
FA	V1	52.8	0.1	15	Y	12	16	25	Y	38	2.97
	V2	*53.9*	*1.1*	*14*	*Y*	*14*	*9*	*32*	*Y*	*36*	*1.5*
	V3	*54.9*	*2.1*	*15*	*Y*	*9*	*5*	*10*	*Y*	*41*	*3.81*
KJ	V1	40.4	3.2	10	Y	12	6	3	Y	40	9.6
	V2	*41.2*	*4.0*	*93*	*Y*	*12*	*6*	*5*	*N*	*46*	*6.2*
	V3	*42.2*	*5.0*	*78*	*Y*	*11*	*6*	*4*	*N*	*49*	*13.7*
RW	V1	48.2	5.0	23	Y	10	7	19	Y b		19.6
	V2	*49.2*	*6.0*	*5*	*Y*	*13*	*11*	*31*		*43*	*18.8*
FO	V1	41.1	2.9	15	Y	10	13	2	Y	41	4.2
	V2	*42.2*	*4.1*	*13*	*Y*	*9*	*6*	*2*	*Y*	*40*	*2.75*
KR	V1	40.8	5.3	11	N	13	5	10	N	37	5.2
	V2	*41.8*	*6.3*		*N*	*6*	*1*	*16*	*N*	*37*	*5.6*
BD	V1	28.8	0	84	Y	14	0	19	Y	46	13.3
	V2	*29.7*	*1.1*	*58*	*Y*	*14*	*16*	*30*	*Y b*	*43*	*14.9*
	V3	*30.7*	*2.0*	*58*	*Y*	*16*	*21*	*24*	*Y b*	*44*	*12.8*
Male patients with GFR ≥ 60											
RA	V1	48.4	0.1	88	Y	0	0	11	Y	43	5.8
	V2	*49.4*	*1.1*	*68*	*N*	*0*	*0*	*9*	Y	*47*	*9.08*
LM	V1	45.1	2.2	130	Y	0	10	26	Y	45	19
	V2	*46.1*	3.0	*111*	*Y*	*0*	*0*	*6*	Y	*44*	*18.2*
	V3	*47.1*	*4.0*	*96*	*N*	*0*	*0*	*23*	Y	*43*	*18.3*
LS	V1	41.1	3.2	95	Y	11	7	20	N	44	18.3
	V2	*42.1*	*4.2*	*90*	*Y*	*8*	*11*	*30*	*N*	*42*	*17*
	V3	*43.2*	*5.3*	*98*	*Y*	*6*	*2*	*31*		*45*	*23.6*
LK	V1	45.3	0.1	112	Y	9	3	11	N	46	23
	V2	*46.3*	*1.1*	*103*	*Y*	*12*	*6*	*17*	*N*	*46*	*16.4*
	V3	*47.3*	*2.1*	*111*		*12*	*6*	*16*	*N*	*44*	*16.5*
KR	V1	41.3	3.5	100	Y	14	13	17	N	41	16.5
	V2	*42.1*	*4.3*	*131*	*Y*	*17*	*15*	*35*	*N*	*44*	*11*
RA	V1	40.4	3.0	106	Y	16	27	10	N	50	18.4
	V2	*41.4*	4.0	*195*	*Y b*	*9*	*5*	*23*	*N*	*47*	*17.8*
GS	V1	37.3	0.2	102	Y	4	2	21	N	41	28.9
	V2	*38.2*	*1.2*	*102*	*Y b*	*7*	*3*	*20*	*N*	*45*	*32*
SW	V1	38.8	4.1	119	Y	17	16	18	N		
	V2	*39.8*	*5.1*	*72*	*Y b*	*16*	*10*	*18*	*N*	*40*	*5.6*
	V3	*40.9*	*6.1*	*71*	*Y w*	*22*	*16*	*25*	*N*	*42*	*2.3*

OT	V1	22.2	0.1	150	Y	6	3	16	N	47	30.3
	V2	*23.3*	*1.2*	*107*	*Y b*	*10*	*9*	*16*	*Y b*	*49*	*30.5*
KD	V1	19.2	0.0	132	Y	12	15	18	Y	50	30.9
	V2	*20.2*	*1.1*	*131*	*Y*	*11*	*16*	*22*	*Y*		
RG	V1	36.4	2.1	71	Y	5	3	20	Y	43	11.9
	V2	*37.2*	*3.0*	*75*	*Y b*	*7*	*4*	*22*	*Y*	*43*	*13*
Female patients with GFR < 60											
GR	V1	44.8	0.1	50	N	13	6	34	N	43	6.5
	V2	*45.8*	*1.1*	*44*	*Y*	*21*	*23*	*25*	*N*	*52*	*11.7*
	V3	*46.8*	*2.1*	*56*	*N*	*24*	*24*	*35*	*N*	*56*	*35.4*
TL	V1	38.2	0	43	N	4	0	7	N	57	19.4
	V2	*39.2*	*1.0*	*48*	*N*	*4*	*0*	*10*	*N*	*54*	*26.5*
Female patients with GFR ≥ 60 (control)											
SS	V1	41.7		98	Y	0	0	4	N	40	21.1
	V2	*43.7*		*125*	*Y*	*0*	*0*	*3*	*N*	*49*	*15.8*
PP	V1	33.8		102	N	0	0	9	Y	45	25
	V2	*34.9*		*135*	*N*	*0*	*0*	*6*	*N*	*46*	*33.1*

Pt: patient; V: visit; V1: 1st visit; V2: 2nd visit after one year; V3: 3rd visit after two years;

Red: deteriorated; Green: improved; Y: yes; N: no; b: better; w: worse

3.6.1 Neurological Symptoms and Findings

As shown in the Table 3, in 19 follow up patients on ERT, seven patients with relatively normal renal function reported less severe acroparesthesia. Four patients reported better perspiration, including two patients with impaired renal function. One patient described that the improvement of pain and perspiration in the third year was not as obvious as during the first two years of the ERT initiation. Eight patients showed improved sural nerve NCS, while other thirteen patients had relatively stable values. No patient had a CNS event or worsening of cerebral blood flow parameters during the follow up observation.

3.6.2 Pain and Depressive Symptoms

As shown in Table 3, in 19 follow up patients on ERT, seven patients with relatively normal renal function reported less severe acroparesthesia, three patients with pathological renal function also showed improved pain and pain related disability scores; eight patients had stable pain severity, only one patient complained of more pain. However the pain scores increased in 11 patients and the pain related disability scores also increased in 11 patients. The CES-D scores also increased in 15 patients, indicating more severe depression. Even in three patients whose pain and pain related disability scores obviously decreased, CES-D still increased.

3.6.3 QST

All 21 follow up patients had repeated QST, and the Z-score was used to describe their response to ERT.

3.6.3.1 One year follow up

Of 19 patients (17male, 2 female) who were on ERT, an improved CDT was found in four patients, improved WDT and CPT was found in four and one patient respectively. Impressive improvement was found in one patient (Fig. 8) with normal renal function. As shown in Fig. 9a and 9b, 12 of 18 male patients with relatively normal renal function showed a generally stable QST profile, while the 6 patients with severely impaired renal function had a deteriorated profile despite of ERT (Fig. 9c). Similar results were also found in the four female patients, among whom two with pathological renal function were on ERT (data not shown).

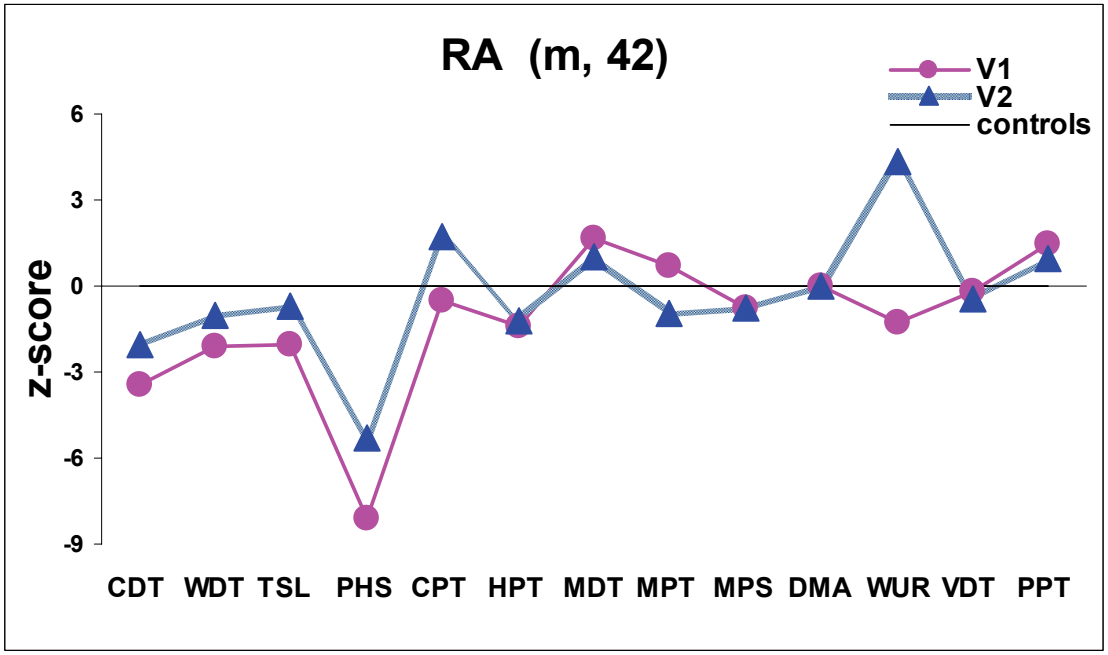


Fig. 8. Improved QST profile in a male patient with normal renal function.

V1: First QST V2: Second QST after one year

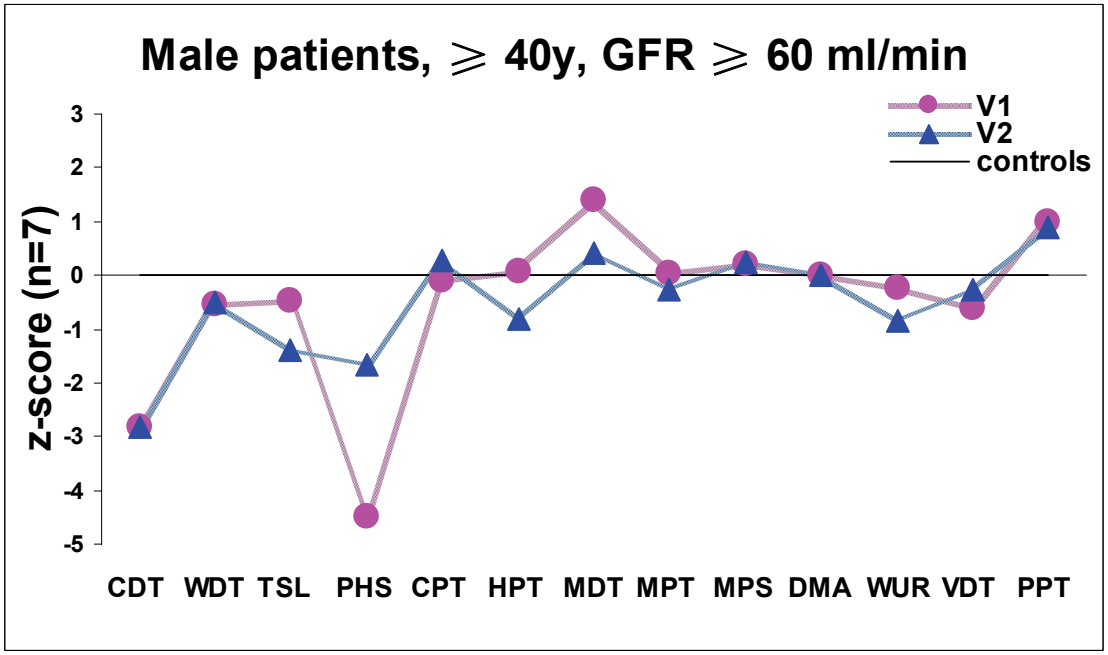


Fig. 9a. QST profile of follow up male patients older than 40y on ERT.

V1: First QST V2: Second QST after one year

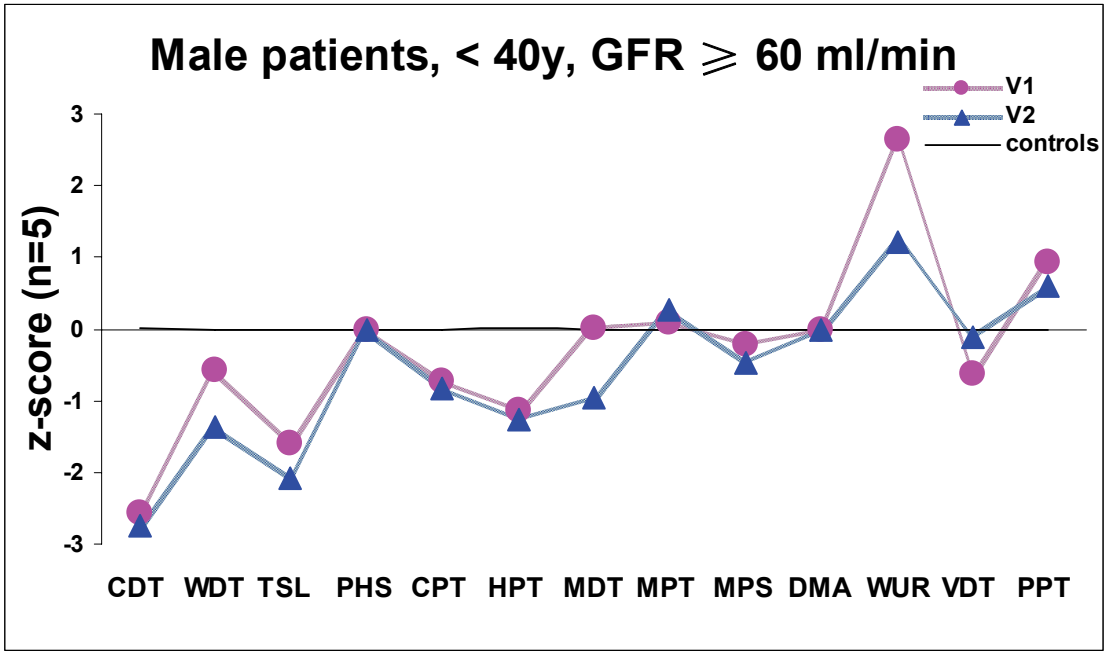


Fig. 9b. QST profile of follow up male patients younger than 40y on ERT.

V1: First QST V2: Second QST after one year

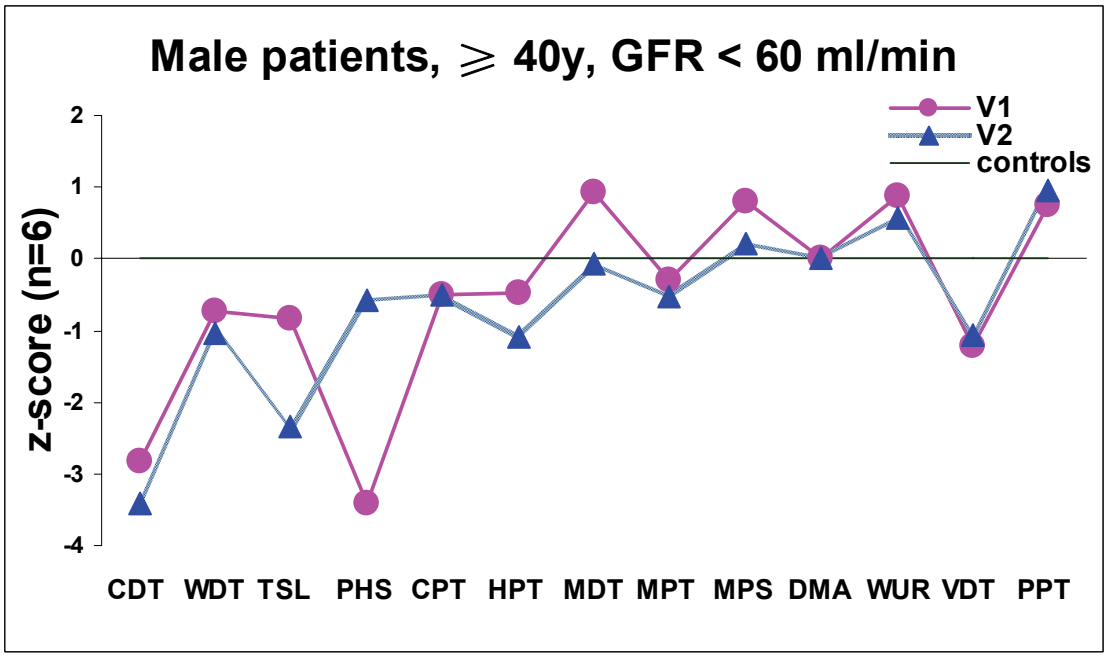


Fig. 9c. Renal function and QST profile of follow up male patients on ERT.

V1: First QST V2: Second QST after one year

3.6.3.2 Follow up after two years

Seven male patients have had the third time QST (Table. 4), and three of them had improved TSL, one had improved CPT and DMA. Interestingly, two of these three patients had pathological renal function. As shown in Fig. 10, although these patients were on ERT, their QST profile still showed slightly deteriorated thermal sensory function.

Table 4. 2-year follow up QST values in seven patients on ERT.

Pt	V	Age	GFR	CDT	WDT	TSL	PHS	CPT	HPT	MDT	MPT	MPS	DMA	WUR	VDT	PPT
FA	V1	52	15	-5.57	3.43	5.9	3	10	45.57	0.35	17.15	7.48	0.19	2.63	5.67	530
	V2	*53*	*14*	*-22*	*17.1*	*40*	*0*	*10*	*50*	*10.56*	*34.3*	*24.26*	*0*	*1*	*4.67*	*540*
	V3	*54*	*15*	*-22*	*16.07*	*27.03*	*0*	*10*	*50*	*2.14*	*6.5*	*1.86*	*0.59*	*2.31*	*5.33*	*389*
KJ	V1	40	10	-15.17	4.23	16.43	1	10	43.57	3.25	45.25	6.67	0.83	2.15	5.5	500
	V2	*41*	*93*	*-22*	*3.8*	*33.13*	*0*	*10*	*47.33*	*0.87*	*90.51*	*2.59*	*0*	*1.93*	*6.33*	*379*
	V3	*42*	*78*	*-5.07*	*7.37*	*18.57*	*0*	*19.73*	*47.17*	*1.41*	*90.51*	*2.34*	*0*	*1.80*	*5.00*	*419*
LM	V1	45	130	-22	6.13	35.1	0	10	45.37	0.87	181.02	0.16	0	1.18	5.83	883
	V2	*46*	*111*	*-22*	*18*	*40*	*0*	*10*	*50*	*13*	*21.11*	*0.36*	*0*	*1.14*	*6.83*	*543*
	V3	*47*	*96*	*-22*	*17.27*	*40*	*0*	*10*	*50*	*9.19*	*97.01*	*1.27*	*0*	*1.91*	*6.17*	*729*
LS	V1	41	95	-10.63	11.53	19.47	2	11.5	44	0.54	274.37	0.41	0	2.67	6.33	510
	V2	*42*	*90*	*-20.33*	*17.63*	*40*	*3*	*10*	*50*	*0.81*	*137.19*	*0.44*	*0*	*2.17*	*7*	*386*
	V3	*43*	*98*	*-21.07*	*16.33*	*34.4*	*2*	*10*	*48.8*	*0.71*	*238.86*	*0.52*	*0*	*2.1*	*6.33*	*487*
LK	V1	44	112	-18.73	12	12.23	2	10	43.17	1.87	8	8.59	0	3.38	6	481
	V2	*45*	*103*	*-22*	*18*	*40*	*0*	*12.7*	*50*	*6.96*	*97.01*	*16.62*	*0*	*1.42*	*7.5*	*576*
	V3	*46*	*111*	*-22*	*17*	*40*	*0*	*10*	*50*	*1.87*	*111.43*	*8.11*	*0*	*2.45*	*6.83*	*647*
GD	V1	29	84	-19.43	2.6	22.9	0	10	44.47	1.07	48.5	7.43	0	1.56	6	428
	V2	*30*	*58*	*-22*	*3.87*	*27.93*	*0*	*10*	*38.43*	*1.74*	*90.51*	*2.82*	*0*	*2.26*	*5.83*	*549*
	V3	*31*	*58*	*-22*	*4.43*	*29.27*	*0*	*10*	*42.17*	*2.14*	*84.45*	*1.78*	*0*	*6.67*	*5.67*	*399*
SW	V1	39	119	-20.37	1.93	28.07	0	14.87	46.2	1.52	14.93	9.6	0.03	2.5	5.83	458
	V2	*40*	*72*	*-22*	*18*	*40*	*0*	*10.23*	*49.2*	*3.25*	*5.66*	*2.74*	*0*	*1*	*5.83*	*419*
	V3	*41*	*71*	*-19.67*	*16.53*	*30.53*	*0*	*12.43*	*50*	*2.83*	*24.25*	*4.66*	*0*	*1.39*	*4.5*	*474*

Pt: patient; V: visit; V1: 1st visit; V2: 2nd visit after one year; V3: 3rd visit after two years; Red: deteriorated; Green: improved

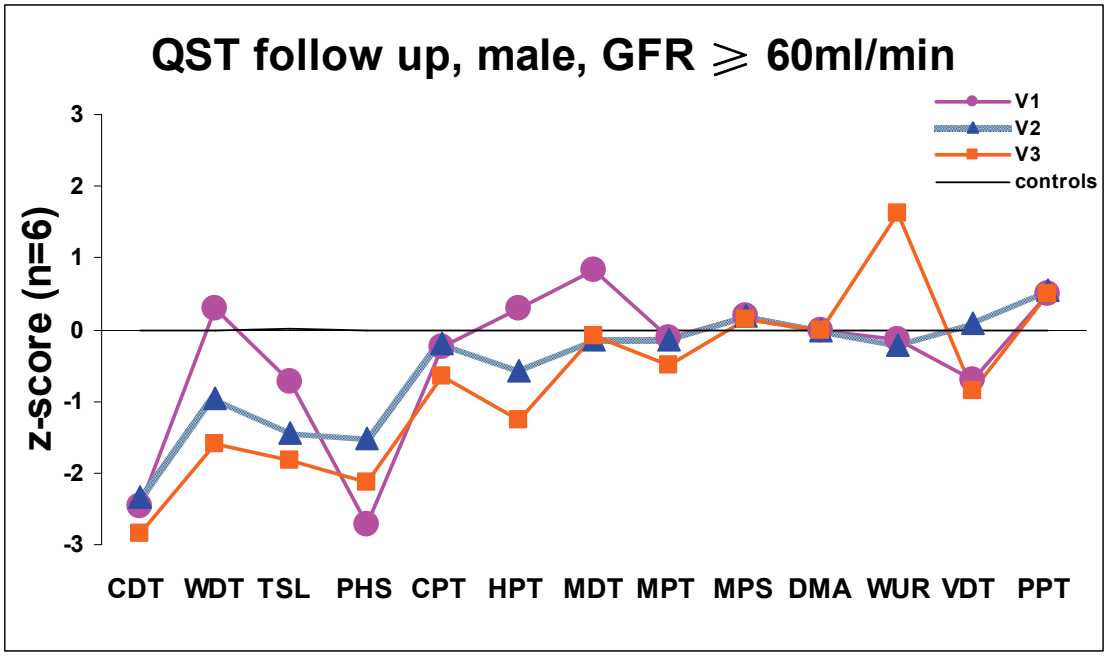


Fig. 10. QST profile in 7 two-year follow up patients. V1: First QST V2: Second QST after one year V3: Third QST after two years

3.6.4 Skin innervation (IENFD)

Of 19 follow up patients under ERT, 11 had repeated skin biopsies, Table 5.

Proximal IENFD: Four patients had increased IENFD from 11.3 +/- 2.3 f/mm to 21.1 +/- 1.9 f/mm, and also improved peripheral neurological profiles; all of them had relatively normal renal function, including one patient with twice renal transplantation. Four patients had reduced IENFD, and all of them had severe renal impairment and worsened QST or NCS values. Three patients with stable IENFD also had stable peripheral neurological profiles; two of them had severely impaired renal function.

Distal IENFD: No increase of IENFD was shown in any of the 11 patients. In four patients with increased proximal IENFD, two still showed reduction of distal IENFD. In two of the three patients with stable proximal IENFD, the distal value also reduced. Six patients had no distal innervation and one had a stable IENFD around 3 f/mm.

Table 5. Skin innervation and peripheral neurological profile in follow up patients.

Pt		Age	GFR	Neuropathic pain complaints	Hypohidrosis	Sural nerve NCS		QST			IENFD	
						SNAP (μV)	NCV (m/s)	CDT	WDT	TSL	Distal	Proximal
OT	V1	22.2	150	Y	N	47	30.3	ND	ND	ND	7.49	13.91
	V2	*23.3*	*107*	*Y b*	*Y b*	*49*	*30.5*	*-13.6*	*9.97*	*12.93*	*5*	*22.46*
LK	V1	46.3	103	Y	N	46	16.4	-22	18	40	0	8.07
	V2	*47.3*	*111*	*Y*	*N*	*44*	*16.5*	*-22*	*17*	*40*	*0*	*19.95*
RA	V1	40.4	106	Y	N	50	18.4	-22	18	32.73	1.13	12.9
	V2	*41.4*	*195*	*Yb*	*N*	*47*	*17.8*	*-9.1*	*10.97*	*18.4*	*0.48*	*22.99*
KJ	V1	40.4	10	Y	Y	40	9.6	-15.17	4.23	16.43	0	10.45
	V2	*41.2*	*93*	*Y*	*N*	*46*	*6.2*	*-22*	*3.8*	*33.13*	*0*	*11.44*
	V3	*42*	*78*	*Y*	*N*	*49*	*13.7*	*-5.07*	*7.37*	*18.57*	*0*	*18.97*
FA	V1	53.9	14	Y	Y	36	1.5	-22	17.1	40	0	10.8
	V2	*54.9*	*15*	*Y*	*Y*	*41*	*3.81*	*-22*	*16.07*	*27.03*	*0*	*4.76*
KR	V1	40.8	11	Y	N	37	5.2	-11.87	18	11.63	0	21.29
	V2	*42*	*10*	*Y*	*N*	*37*	*5.6*	*-22*	*16.7*	*38.47*	*0.58*	*13.64*
BD	V1	29.7	58	Y	Y	43	14.9	-22	3.87	27.93	2.58	37.2
	V2	*30.7*	*58*	*Y*	*Y*	*44*	*12.8*	*-22*	*4.43*	*29.27*	*2.65*	*23.85*
KR *	V1	41.3	100	Y	N	41	16.5	-17.17	5.1	16.67	0	22.29
	V2	*42.1*	*131*	*Y*	*N*	*44*	*11*	*-12.2*	*9*	*36.57*	*0.17*	*9.18*
GR	V1	45.8	44	Y	N	52	11.7	-0.63	1.3	4.07	9.34	28.81
	V2	*46.8*	*56*	*Y*	*N*	*56*	*35.4*	*-5.47*	*7.6*	*25.7*	*3.67*	*30.59*
TL	V1	38.2	43	N	N	57	19.4	-1.47	5.63	19.57	9.07	28.82
	V2	*39*	*48*	*N*	*N*	*54*	*26.5*	*-3.87*	*4.63*	*19.83*	*5.23*	*30.59*
RG	V1	36.4	71	Y	Y	43	11.9	-7.43	12.97	13.17	0.95	14.99
	V2	*37.2*	*75*	*Y b*	*Y*	*43*	*13*	*-15.6*	*5.13*	*15.9*	*1.64*	*16.41*

Red: reduced; Green: increased; Blue: stable; ND: not done

* Note: this patient had proteinnuria 2000 mg/d.

3.7 Subgroup analysis

3.7.1 Patients with normal and patients with impaired renal function

All the patients were divided into subgroups (Table. 6) based on their renal function (GFR ≥ 60 or < 60ml/min per 1.73 m^2) and age (≥40 or <40 years). We

took patients younger than eighteen years as children.

All the children (3 boys and 5 girls) had normal renal function. None of them had vital organ impairments, sign of depression, nor concomitant diseases. Skin biopsy was not performed with them, and no one received ERT.

For patients under forty years, only two male and one female had severely impaired renal function. Both of these two male patients had cardiac problems and cerebral events and they showed indications of depression. The female patient displayed mild symptoms with a reduction of distal IENFD. All the three patients were on ERT.

8 (10.5%) male and 6 (7.9%) female patients older than forty years had severely impaired renal function. Most of them had vital organ impairments and neuropathic pain. The female group showed a higher ECS-D score (23.7 +/- 11.2 f/mm) than that of the male group (14.5 +/- 12.5 f/mm), though without statistic significance. Both the male ($P<0.05$) and female ($P<0.05$) groups had reduced distal IENFD, while the female group had a milder loss of innervation than male group ($P<0.05$). 7/8 male and 3/6 female patients were on ERT.

In the group of patients with relatively normal renal function, 14 male and 16 female patients were older than forty years. A majority of them had vital organ impairments. 9/14 male and 9/16 female patients complained of neuropathic pain, and both groups had ECS-D scores close to the diagnostic limit. The female group had less reduction of IENFD both proximally (27.8 +/- 8.1 f/mm) and distally (5.3 +/- 3.4 f/mm) than male patients (20.1 +/- 5.1 f/mm and 0.9 +/- 1.0 f/mm respectively). 3/14 male and 8/16 female patients had concomitant diseases, while 10/14 male and 7/16 female patients were on ERT.

10 male and 8 female patients younger than forty years had relatively normal renal

function. Approximately half of them had vital organ involvement. 9/10 male and 4/8 female patients complained of neuropathic pain, and the male group showed indications of depression. The female group had a relatively normal innervation both proximally and distally, while the male group had a mild reduction of distal IENFD (4.8 +/- 2.7 f/mm). 2/10 male and 1/8 female patients had concomitant diseases. None of the female patients and eight of ten male patients were on ERT.

Table 6. Patients with normal and impaired renal function.

Parameters		**Male patients (n=39)**						**Female patients (n=37)**					
		GFR<60 (ml/min/1.73 m^2)			**GFR≥60 (ml/min/1.73 m^2)**			**GFR<60 (ml/min/1.73 m^2)**			**GFR≥60 (ml/min/1.73 m^2)**		
Age category (years)		<18	18-40	≥40	<18	18-40	≥40	<18	18-40	≥40	<18	18-40	≥40
Patients	n	0	2	8	3	12	14	0	1	6	5	9	16
	(%)		(2.6)	(10.5)	(3.9)	(15.8)	(18.4)		(1.3)	(7.9)	(6.6)	(11.8)	(21.1)
Cardinal problem	n	0	2	7	0	4	9	0	0	5	0	3	9
	(%)		(2.6)	(9.2)		(5.3)	(11.8)			(6.6)		(3.9)	(11.8)
Cerebral events	n	0	2	2	0	1	1	0	0	1	0	0	1
	(%)		(2.6)	(2.6)		(1.3)	(1.3)			(1.3)			(1.3)
Neuropathic pain	n	0	1	6	2	9	9	0	0	4	0	4	7
	(%)		(1.3)	(7.9)	(2.6)	(11.8)	(11.8)			(5.3)		(5.3)	(9.2)
Pain score	M		0	10.1	1	8.9	6.5		4	9.0	4.3	5.3	5.1
	(SD)			(4.3)		(7.1)	(6.1)			(6.7)		(6.0)	(7.4)
Disability score	M		7.5	6.4	1	8.3	6.0		0	11.5	0.8	2.4	5.3
	(SD)			(3.6)		(7.5)	(7.8)			(10.1)		(3.5)	(8.9)
CES-D score	M		18.5	14.5	11	16.7	15.9		10	23.7	13	10.8	13.1
	(SD)			(12.5)		(6.2)	(9.6)			(11.2)		(6.1)	(14.3)
Proximal IENFD (f/mm)	M		36.6	7.6		21.2	20.1		30.6	19.9		29.4	27.8
	(SD)			(8.7)		(7.4)	(5.1)						(8.1)
Distal IENFD (f/ mm)	M		5.9	0.1		4.8	0.9		5.2	3.3		9.4	5.3
	(SD)			(0.1)		(2.7)	(1.0)						(3.4)
α-GAL activity <0.4 nmol / min/mg protein	n	0	2	8	3	12	14	0	1	4	3	7	14
	(%)		(2.6)	(10.5)		(15.8)	(18.4)		(1.3)	(5.3)	(3.9)	(9.2)	(18.4)
Concomitant diseases	n	0	1	2	0	2	3	0	0	6	0	1	8
	(%)		(1.3)	(2.6)		(2.6)	(3.9)			(7.9)		(1.3)	(10.5)
On ERT	n	0	2	7	0	8	10	0	1	3	0	0	7
	(%)		(2.6)	(9.2)		(10.5)	(13.1)		(1.3)	(3.9)			(9.2)

3.7.2 Female patients

FD causes significant morbidity and mortality in affected males. As recently as 2001, most FD females were thought to be asymptomatic throughout a normal life span or to develop only minor manifestations of the disease. As shown in Table 7 above, females with Fabry disease experienced intense neuropathic pain (n= 16); they also suffered from headache (n=5) and other pain (n=16), and pain produced comparable distress (n=11).

Moreover, compared with the normal population, a much higher incidence of concomitant diseases was found in female patients older than 40 years. In this subgroup of 22 patients, 14 had diabetes, severe genitourinary system problems, thyroid disease, and other disorders. Among the 9 younger female patients under 40 years, only one had a concomitant disease. Such concomitant diseases also occurred in male patients, but with a far lower incidence (Fig. 12). Besides, of these 22 female patients older than 40 years, 6 had pathological renal function and 14 had cardiac involvement. However, compared with the ratio of 27/36 in male adult patients, only 11/32 female patients were under ERT.

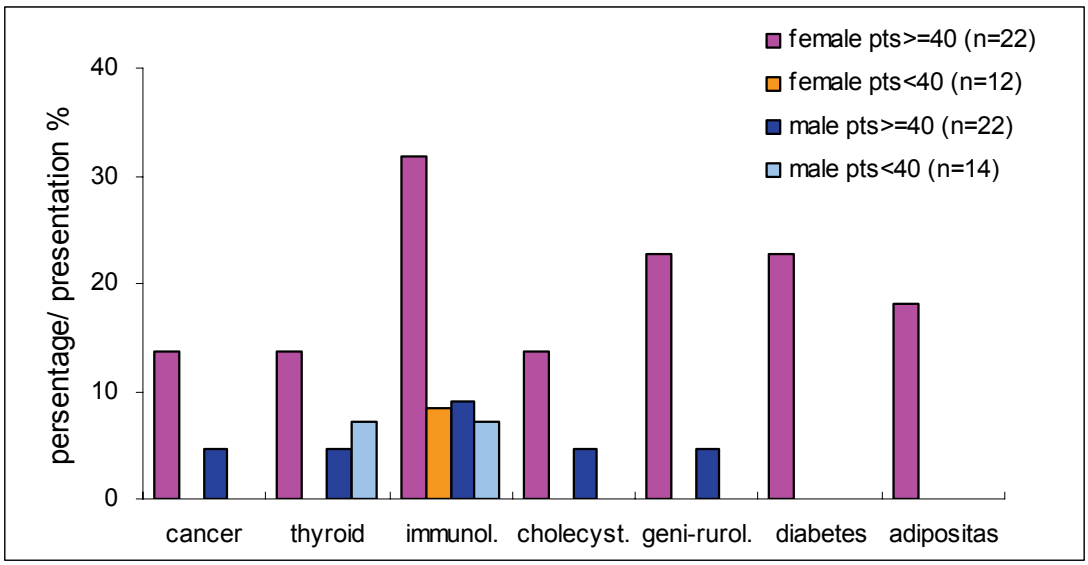


Figure 12. Perccentage of patients with concomitant diseases
Thyroid: thyroid disease. Immunol: immunological disease.
Chole: cholecystolithiasis. Genito-urol: genitourological disorder

3.7.3 Patients free from neurological complaints

Eleven patients (4 male, 7 female) were free from any neurological complaints. Among them QST abnormalities were found in eight patients, in seven patients who had skin biopsies, six showed abnormalities (Table 7). Combined QST and skin biopsy revealed abnormalities in all these eleven patients.

Table 7. QST and skin biopsy abnormalities in patients free from neurological complains.

Pt	Sex/ age	QST abnormalities	Skin biopsy abnormalities
RD	m/63	CDT↓, CPT↑, HPT↓	Not done
IH	m/63	Normal	distal IENFD ↓
HP	m/60	CDT↓, MPS↑	distal IENFD↓
MH	m/46	CDT↓	Not done
BT	m/22	Normal	Subepidermal innervation ↓
BH	f/46	PHS↑	distal IENFD↓
TU	f/44	CDT↓, TSL↑	distal IENFD↓
KL	f/63	Normal	distal and proximal IENFD↓
BA	f/51	WUR↑	Normal
NH	f/49	MPS↑	Not done
PP	f/34	MPS↑	Not done

3.7.4 Children with FD

All the children (3 boys and 5 girls) had normal renal function, and none of them had vital organ impairments. Two of the three boys complained of acroparesthesia, two of the five girls had headache, the other patients were free from neuropathic pain. Healthy controls are under enrollment, and the QST analysis for children will be done in the near future.

3.8 Correlations

GFR- Pain, disability and depression: In the female group, there was a correlation between GFR and the disability severity ($p=0.031$, $r=-0.332$), and depression symptoms ($p=0.042$, $r=-0.305$) but not between GFR and pain severity. There was no such correlation in the male group.

GFR- IENFD: there was a strong correlation between GFR and distal IENFD in male patients ($p<0.001$, $r=0.798$), but not between GFR and proximal IENFD, neither in female patients.

Age-IENFD: there was a strong correlation between age and distal IENFD in male patients ($p<0.001$, $r=-0.726$) and female patients ($p=0.001$, $r=-0.688$), but not between age and proximal IENFD, neither in female patients.

CDT- IENFD: correlations between cold detection threshold (CDT) and distal IENFD were found in both the male ($p=0.042$, $r= 0.329$) and the female group ($p=0.041$, $r= 0.413$). No other correlation between QST and IENFD was found.

4. Discussion

Our study achieved four main findings: First, for the first time, we found nerve regeneration in truncal skin may occur under ERT. Thus, the proximal IENFD might serve as a marker for the disease progression and its response to ERT in Fabry patients. Second, renal function plays an important role in small fiber function and its response to ERT. Third, such involvement of the peripheral nervous system (neuropathic pain, sensory function and IENFD) is age related. Fourth, female patients were less affected than male patients concerning small fiber involvement, but taking into account the involvement of other systems, the older female patients suffered from the disease as severely as male patients.

Fabry disease is an X linked recessive inheritance with age related clinical features, among which neurological ones are the earliest to develop [20,61].To assess the small nerve fiber function, QST is currently widely used, and data analysis revealed that most thermal and mechanical thresholds increased with age [58]. In addition, clinical trial results emphasize that baseline characteristics can substantially influence the outcome of ERT [34,35,54]. We therefore divided our cohort into subgroups according to their age and GFR. GFR was chosen to reflect the renal and thus the overall condition of the patients，because the kidney is one of the most affected vital organs in Fabry patients and GFR is the most reliable and quantitative item which can serve as a baseline marker. We defined in this study the renal function as pathological when GFR was lower than 60ml/min per 1.73 m^2, which is the borderline of Stage III chronic kidney disease [62].

4.1 Small fiber involvement and the role of renal function

4.1.1 Neuropathic pain, pain related disability, and depression

Peripheral neuropathy in patients with Fabry disease is axon length-dependent and characterized by selective involvement of small fibers [31,46,52], and neuropathic pain is the earliest symptom appeared in most Fabry patients. Although we screened only a limited number of children, there were no differences in the prevalence of neuropathic pain between children and adults, which was also reported by another group [40]. Compared with adults, the children had less pain and none of them showed indication of depression. In adult patients, no difference of pain severity and pain related disability was found between older and younger male patients. While female patients younger than 40 years showed less pain, pain related daily disability, and depression than the corresponding male patients and female patients older than 40 years; such differences disappeared in older female patients. Contrary to the traditional view of females as carriers, female patients experienced intense disease-related pain and pain produced comparable distress, and seemed to be age-related, although such correlation was not statistically established. Although male patients had higher pain score, the interference of pain with daily life and mood was more obvious in female patients, which is corresponding with other reports [40, 63].

GFR did not significantly affect pain in male patients. In women, correlations between GFR and the disability severity ($p=0.031$, $r=-0.332$), and depression symptoms ($p=0.042$, $r=-0.305$) were found, which indicate that renal function may play a role of pain related depression in female patients. Although pain perception is dependent on small fiber involvement, our study could not detect any correlation between pain, QST profile, and IENFD in any group of patients.

4.1.2 QST and small fiber function

A significantly increased cold detection threshold (CDT, indicating loss of sensory function for cold stimuli) was found in male patients older and younger than 40 years, while unmyelinated C-fiber -mediated warm detection thresholds were often within the normal range. That cold perception is more frequently and severely impaired than warm perception in male patients was also reported previously [31,64,65]. We also found correlations between CDT and distal IENFD both in male (p=0.04, r= 0.329) and female patients (p=0.04, r= 0.413). No other correlation between QST and IENFD was found. Based on these findings, there might be a more pronounced loss of Aδ-fibers than C-fibers in Fabry disease. In our study, PGP 9.5 antibody was used. It is a polyclonal panaxonal marker that stains both Aδ- and C-fibers, and currently the most commonly used marker for IENFD studies. Although CGRP and Substance P are specific antigens for C-fibers [66,67], the fact that many patients had only very low IENFD or even no innervation prevented us from specifying the fiber losses. We speculate that the GL-3 deposits in the cell bodies and axons of Aδ fibers and in the adaxonal cytoplasm of the surrounding Schwann cells might lead to more severe functional loss than the unmyelinated axons of C fibers [46,68].

Besides increased CDT, significantly increased thermal sensory limen (TSL) and decreased vibration detection threshold (VDT) were found in male patients younger than 40 years, which were also signs indicating loss of sensory function of C- and Aß- fibers respectively. This finding indicates that sensory function disturbance developed when patients were still young and maybe due to the fact that most thermal and mechanical thresholds increased with age [58], the differences of sensory function and the corresponding QST values might not be obvious at an older age.

In male groups, compared to patients with relatively normal renal function,

patients with pathological renal function had increased dynamic mechanical allodynia (DMA) indicating a peripheral and central sensitization. Dynamic mechanical allodynia is the pain due to a light moving mechanical stimulus, and occurs in subgroups of patients with peripheral or central neuropathic pain. Both peripheral and central nervous system mechanisms have been implicated in the generation of DMA. In subgroups of patients with post-herpetic neuralgia peripheral sensitization of nociceptive afferents have been suggested as a basis for dynamic mechanical allodynia [69], while Baron et al. argue that this symptom may be due to secondary changes in the central nervous system processing that might strengthen the synaptic ties between A beta-fibers and central nociceptive pathways and not necessarily related to ongoing nociceptive C-fiber input [70]. In our study, there was more severe small fiber loss in patients with pathological renal function. We hypothesize that more severely damaged small fibers may indicate more disturbed function or damage of the neurons both peripherally and centrally, so besides the small fiber dysfunction, the secondary central hyperexcitability might contribute to the DMA in patients with pathological renal function.

Of 32 adult female patients, 29 had abnormal QST values, but only 4 patients had reduced CDT. A recent study reported that 25% of female patients had abnormal CDT in the foot [51], the small sample (n=12) of their study may count for this discrepancy. Generally, although female patients also suffer from severe neuropathic pain, especially in older group, the small nerve fibers are less affected, which supports our finding that there is a lack of correlation between pain and QST profile in Fabry patients. Patients with pathological renal function seemed to have decreased thermal detection threshold compared to patients with normal renal function, although not statistically significant, which also in accord with our finding that female patients are less affected concerning small fiber function.

4.1.3 Skin innervation (IENFD) in FD

Since standard nerve-conduction studies and large nerve biopsy results are typically normal in small-fiber neuropathy, the investigation of intraepidermal nerve fibers is widely used for the diagnosis of small-fiber neuropathy including FD related neuropathy [53,71,72]. Compared to our laboratory's normal values for Th12 and the lower leg, the intraepidermal innervation density was significantly reduced in male patients (18.0 +/- 8.8 fibers/mm proximally and 2.2+/-2.8 fibers/mm distally), and mildly reduced in female patients (26.2 +/- 9.9 fibers/mm proximally and 6.3 +/- 4.3 fibers/mm distally). In male patients a severe loss of intraepidermal innervation at the ankle but less severe at the distal thigh has been described [52,53,64], which is in line with our findings. The reduced density of the subepidermal nerve plexus was also more frequent in male patients. Unlike the other vital organ involvement which affected females as severely as males in the groups older than forty years, the small fiber loss is more severe in males than in females in all age groups. Although female patients also suffer from severe neuropathic pain, especially in the older group, the loss of small nerve fibers was less severe, which is consistent with our findings in the QST profile and the lack of correlation between pain and IENFD in Fabry patients. However, strong correlations between age and distal but not proximal IENFD were found both in male ($p=0.000$, $r=-0.726$) and female patients ($p=0.001$, $r=-0.688$), which is also the case with healthy people [73,74] and is consistent with the fact that most thermal and mechanical thresholds increased with age [58].

In male patients there was a strong correlation between GFR and distal IENFD ($p=0.000$, $r=0.798$). Such a correlation was not found in the female group, the possible reason is that in the 19 female patients who had the skin biopsies, only three had pathological renal function. Clinical trials have shown that patients with impaired renal function at baseline have a less favorable outcome and may

develop renal progression despite treatment with ERT [34]. Guidelines for the treatment of Fabry disease are being developed but currently not uniformly applied [75], and the initiation of ERT is based mainly on the clinical course of the disease, in particular the vital organ events [76]. Our findings indicate that combined together, GFR and IENFD may serve as useful items in the decision when to start ERT and other comprehensive treatment to prevent the irreversible nerve damage.

Strong correlations between age and distal IENFD were found both in male ($p<0.001$, $r=-0.726$) and female patients ($p<0.001$, $r=-0.688$). In healthy people, most thermal and mechanical thresholds increased with age [58], while the distal IENFD decreased with age [73,74]. Such trends were also found in our Fabry patients.

4.2 Hypohidrosis, auditory impairment, and CNS symptoms

Hypohidrosis was present in 18.4% male and 6.5% female patients in our cohort. The incidence of such a classic symptom of FD has been previously reported with high variances from 52% to 93% in male and from 1% to 32.8% in female patients [77-79].

Audiovestibular symptoms were reported to be present in 57-80% of male and 11-85% female patients [20,21,77,80], such a high incidence was not found with our patients, but the more precise instruments and examination applied in the published reports may account for this discrepancy. Hypohidrosis and audiovestibular symptoms are more frequent in male patients than female patients, which is in accord with our own findings. Another finding in our subgroups was that patients older than 40 years had a higher incidence of auditory impairment in both the male and female groups. For CNS, 18.4%

patients had a history of cerebral events, but only 2 suffered from neurological sequelae. Since we did not focus on the CNS, only routine extra- and transcranial Doppler sonography was applied and it gave normal results in all patients.

The failure of Doppler to detect the possible abnormality of CNS in Fabry patients indicates that the deposit of GL-3 in the CNS may not necessarily lead to pathological global and local cerebral blood flows (CBF) which also reported in an animal study [81]. Probably, more precise methods are needed to measure subtle abnormalities.

4.3 Response of peripheral nervous system to ERT and the role of renal function

Before 2001, only symptomatic treatments (e.g., analgesics, dialysis, and kidney transplantation) were available to patients with FD. Since then, the development of ERT represents a major therapeutic breakthrough. The results of initial clinical trials of agalsidase beta showed clearance of GL-3 in multiple dermal cell types, less pain, and functional improvement of different nerve fibers [29,31,45]. However, a better understanding of the long-term neurologicall benefits that can be expected from ERT remains essential.

In our cohort, 21 patients (17 male and 4 female) were divided into subgroups according to their renal function for the follow up study. 17 male and 2 of female patients were on ERT, 2 female patients with mild symptoms were taken as untreated controls.

4.3.1 Skin innervation (IENFD) and proximal regeneration

In recent years, serial punch skin biopsy and IENFD analysis has been proved useful to document disease-modifying treatment effects in peripheral neuropathy [53,82,83]. Many Fabry patients, especially males have very low IENFD or even no innervation in the distal leg. Moreover, none of our 11 follow up patients who had repeated skin biopsies showed increased distal IENFD, which was also reported by Schiffmann's group [53]. We therefore looked into a proximal site with a higher baseline of IENFD, and intriguingly we found proximal regeneration in four patients. Previous studies on regeneration did not give consistent results. Trials using local denervation showed regeneration in both healthy people and patients with neuropathy; while the rate of regenerative sprouting was significantly lower in patients with neuropathy and significantly associated with the baseline IENFD [83-85]. On the other side, serial skin biopsies from Fabry patients under ERT revealed no regeneration even after long term treatment [53]. Although in cases when the scar from previous biopsy was not obvious, the repeated biopsy site was empirically decided by the operator and this may likely cause a systematic error, our finding is reliable since good reproducibility of biopsies taken from adjacent sites has been shown in normal controls and patients with neuropathy [84,86]. We hypothesize that regeneration was apt to occur proximally but not distally in Fabry patients. The possible reason may be that at the Th12 level, there are more intact nerve fibers and high baseline IENFD, and thus higher levels of trophic support. Moreover, in this length dependent neuropathy of FD, the accumulation of GL-3 in the dorsal root ganglia [41,87], although it may get somehow cleared under ERT, might still result in a limited ability to maintain the metabolic demands of proximal regeneration but fail to keep up with the necessary demands of distal regeneration. Functional disturbance in Fabry patients not being reversed despite clearance of GL-3 was reported in other vital organ involvements [28,60].

In this study we tested the hypothesis that proximal IENFD can serve as a marker for the response of the peripheral nervous system to ERT in Fabry patients. Studies of other vital organs involvement in Fabry patients have shown that patients with initially impaired renal function have continued deterioration in renal function and had 12 clinical events over the course of treatment despite adequate substrate clearance [34,60]. A study of the ERT effect on cardiac morphology, function, and late enhancement (LE) showed that only patients without LE at baseline had significant reductions in left ventricular mass during ERT and had an improvement of regional myocardial function [35]. In our study, of the 11 patients who had the repeated biopsy, the four who had increased proximal IENFD from 11.3 f/mm to 21.1 f/mm also had improvement in acroparesthesia and other pain, regained the ability to perspire and had better tolerance of heat. They also had improved values in QST and neurophysiological examination. Four patients had reduced IENFD, and also worsened QST or NCS values. Three patients with stable IENFD had relatively stable peripheral neurological profiles. These results support our hypothesis that proximal IENFD can serve as a marker for the peripheral nervous systems' response to ERT. Another important finding is that all the four patients with increased IENFD had relatively normal renal function, including one patient with twice renal transplantation. Four patients with reduced IENFD and two of the three patients with stable IENFD had severe renal impairment. Such role of renal function on distal IENFD in Fabry patients has also been reported by other groups [52,53]. Although the number is relatively small, these cases proved that proximal IENFD can serve as a marker for the progression of Fabry disease and its response to ERT.

Moreover, renal function is an important factor that influences the ERT effect on the peripheral nervous system. Another thing need to be mentioned is that patients with severe renal impairment can still benefit from ERT if they have a relatively stable proximal IENFD.

4.3.2 QST follow up

Of 19 patients (17male, 2 female) who were on ERT, improved values of CDT were found in 4 patients, improved WDT and CPT was found in 4 and 1 patient respectively. Although considerably overall thermal sensory improvement was found in one patient (Fig. 8), patients with relatively normal renal function showed a generally stable QST profile. Initial clinical trials applying QST have shown more obvious improved function of C-, Aδ-, and Aß-nerve fibers and intradermal vibration receptors [31]. Compared with our patients (40.4 ± 8.0 years), the fact that the patients they enrolled were much younger (27.9 ± 8.0 years) and received ERT at earlier age may attribute to this difference. Another trial showed that prolonged ERT in Fabry disease leads to a modest improvement of cold and warm sensation in the foot [48], which is corresponding to our findings.

For the first time we reported the effect of renal function on the sensory function in Fabry patients. Six patients with severely impaired renal function showed a deteriorated QST profile despite of ERT. In a long term ERT study [54], all 12 clinical events occurred in patients with initially impaired renal function in whom renal function continued to deteriorate and whose left ventricular posterior wall thickness did not improve over the course of treatment. An 18-months trial of ERT showed decreased IENFD at distal thigh, and that renal function and uremic neuropathy likely contributed to the decline [53]. Interestingly, three of the seven male patients who had QST for the third time (Table 5) showed improved TSL, and two of these three patients had pathological renal function. One had a GFR only around 15ml/min per 1.73 m^2, and the other has had a second renal transplantation but with an improved GFR from 10 to 78 ml/min per 1.73 m^2. That the overall QST profile of the six two-year follow up patients showed some deterioration might indicate the importance of a early start of ERT. Our study revealed that renal function plays important role on the sensory function and its

response to ERT in Fabry patients, which is consistent with its role on IENFD. Although impaired renal function did not preclude sensory improvement under ERT, it made it less successful.

Among the patients with improved thermal detection, some of them had reduced distal IENFD or even no innervation. There might be several explanations for this discrepancy. First, it has been shown in various studies that the thermal QST results do not correlate well with IENFD in Fabry patients [51,53]. Second, the skin biopsy site is not identical with the QST site. Although the IENFD at distal leg is reliable to reflect the innervation at the dorsal side of foot where the QST is performed, the area of the QST site is much larger than that of the punch biopsy site and therefore may address more fibers. Third, besides the cutaneous nerve fiber endings, the fibers in the dermis and even deeper layers may also contribute to the thermal perception.

4.3.3 Neuropathic pain and other manifestations

In our 19 follow up patients on ERT, ten patients reported less severe acroparesthesia or showed improved pain and pain related inability score, including three patients with pathological renal function. However, the pain and pain related disability scores increased in 11 patients. The CES-D scores also increased in 15 patients, indicating more severe depression. Even in three patients whose pain and pain related disability scores obviously decreased, CES-D still increased. This may be due to psychological factors: knowing that the disease is a life-long process, fear of pain onset, ERT infusion burden, etc. Patients may need more psychological help than hitherto thought.

Four patients reported better perspiration, including two patients with impaired renal function. Seven patients showed improved sural nerve NCS, and no

patient had a CNS event or worsening of cerebral blood flow parameters during the follow up observation. Involvement of the peripheral nervous system affected mainly small Aδ- and C- fibers, and hypohidrosis and other abnormalities attributed to autonomic nervous system dysfunction. Improvement in pain, sweating and small fiber function has also been noted following ERT by other groups [31,45,48].

4.4 QST and skin biopsy as early diagnostic methods

Although the characteristic pain may be first noted in childhood or adolescence, patients may not be diagnosed until adulthood, because physicians often do not attribute these signs and symptoms to FD, but misdiagnose them as other more common disorders, such as rheumatoid or juvenile arthritis, rheumatic fever, “growing pains” and etc [88]. In our 68 adult Fabry patients, most had peripheral nerve symptoms, and QST abnormalities were found in all of them. 11 patients were totally free from neurological complains, but if QST was combined with skin biopsy, abnormalities were revealed in all of them. Fabry disease is a progressive disorder; therefore it is important that patients should be identified as early as possible. While the clinical diagnosis of small-fiber neuropathy is difficult, the diagnostic yield can be increased using a combination of thermal QST and IENFD measurements, which was also reported by another group [51].

4.5 Female patients and concomitant diseases

Contrary to the traditional view of females as carriers, females with Fabry disease experienced intense disease-related acroparesthesia and other pain, also pain produced considerable distress, which was also shown in other surveys [63,89]. In our cohort, female patients younger than 40 years showed less

pain, pain related daily disability and depression than the corresponding male patients and female patients older than 40 years; while such differences disappeared in older female patients. Moreover, in patients older than 40 years, the frequency of impairment of vital organs is similar between males and females. Furthermore, female patients older than 40 years suffered from a high number of comorbidities. Fewer than half of these patients were receiving ERT. Such comorbidities contribute considerably to their loss of quality of life. Deegan et al [61] reported age related clinical features in female Fabry patients, and we hypothesize that there is also an intimate relation between age and concomitant disease, especially in female Fabry patients. We conclude that more attention and early intervention should be given to female patients to prevent the onset of the later manifestations of the disorder.

4.6 Conclusion

Both male and female patients with FD suffer from severe neuropathic pain. The small Aδ- and C- fiber loss visualized by skin biopsy and the sensory function impairment manifested by QST profile is more severe in male than female patients. Renal function plays an important role on the disease progression and its response to ERT. Patients with normal renal function have a better chance to benefit from ERT. Proximal IENFD can serve as a marker for ERT effect on peripheral nervous system in Fabry patients. Patients with severely impaired renal function can still benefit neurologically from ERT when they had a stable proximal IENFD. Despite of the reduction in neuropathic pain and the improvement in thermal sensation and sweating following ERT in some patients, taking all follow up patients as a whole group, ERT did not normalize the function of the peripheral nervous system. Consequently, it appears logical to consider ERT as a preventive treatment that should be started as early as possible before irreversible damage has occurred.

Probably due to psychological factors, some patients showed indications of more depression although they reported less pain with ERT. More psychological help should be given. While the clinical diagnosis of small-fiber neuropathy is difficult, the diagnostic yield can be increased using a combination of thermal QST and IENFD measurements. Besides pain and vital organ involvement, female patients older than 40 years suffered from a high number of comorbidities. Therefore more attention and early intervention should be given to female patients. In addition to ERT, comprehensive management of the disease is mandatory.

It is mandatory to collect further information in our future study to verify the role proximal IENFD plays as a mark for the disease progression and the ERT effect. Peripheral neural function needs to be looked into in more pediatric patients. Since some patients with similar baseline characters showed different response to ERT, the relation between genotype and phenotype should be studied.

Summary

Fabry's disease (FD) is a rare inherited X-linked lysosomal storage disease caused by deficient or absent activity of the enzyme α-galactosidase A (α-GAL) due to mutations in the GLA-gene. This leads to the systemic accumulation of glycosphingolipids (mainly GL- 3) in multiple organs and tissues, including the central and peripheral nervous system. Fabry patients often suffer from small-fiber neuropathy, pain attacks, and from burning pain of the hands and feet. Previously, a reduction in pain, an increase in small nerve fiber function and an improvement in sweat gland function, but not an increase in skin innervation have been shown under enzyme replacement therapy (ERT).

The aim of this study was to longitudinally investigate the neurological function in Fabry patients, with special emphasis on the peripheral nervous system. A cohort of 76 patients (39 men and 37 women) with FD was prospectively recruited and studied by clinical neurological examination, neurophysiology, quantitative sensory testing (QST), Doppler sonography, and skin punch biopsy from the lower leg and lower back with quantification of the intraepidermal innervation. Median follow up was 1.1 years (range 0-2.8 years).

Our study achieved four main findings: First, for the first time, we could show that ERT induced epidermal nerve regeneration in proximal skin. Thus, proximal intraepidermal nerve fiber density (IENFD) might serve as a marker for the disease progression and its response to ERT.

Second, renal function played an important role in small fiber function and its response to ERT. Correlations between the glomerular filtration rate (GFR) and pain were found in female patients, and a correlation between GFR and distal IENFD was found in male patients. In the follow-up study, patients with normal

renal function had a better chance to increase their IENFD under ERT.

Third, abnormal QST values and an IENFD reduction were present in some otherwise asymptomatic patients. Small fiber involvement (neuropathic pain, sensory function and IENFD) in FD was age related.

Fourth, female patients were less affected than male patients concerning small fiber involvement, but vital involvement and comorbidities increased with age in female patients. Thus, this patient group deserves special attention.

In conclusion, a reduction in skin innervation is a very early sign of nervous system involvement in patients with FD and should be carefully monitored. Peripheral nerve regeneration is possible in patients with FD under ERT, and is best in patients with normal renal function. The mechanisms of the typical pain suffered by Fabry patients are as yet unclear and deserve further studies.

Appendix

A. NPSI

FRAGEBOGEN NEUROPATHISCHE SCHMERZEN

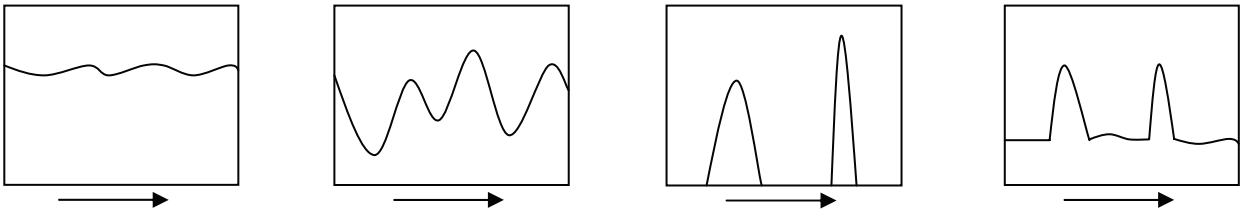
Datum: ……..

Vorname: …… Name: …….

Geschlecht: ……..

Alter: ……

Sie leiden an Schmerzen.
Möglicherweise haben Sie so genannte Spontanschmerzen, die ohne einen erkennbaren Auslöser auftreten. Diese sind entweder dauerhaft, d.h. ohne Unterbrechung ständig vorhanden, treten mit Unterbrechungen auf und/oder schwanken in ihrer Stärke. Zum besseren Verständnis haben wir versucht, dies anhand der folgenden Bilder deutlich zu machen:



Dauerschmerzen mit leichten Schwankungen	Dauerschmerzen mit starken Schwankungen	Schmerzattacken (unterbrochener Schmerz) dazwischen schmerzfrei	Schmerzattacken (unterbrochener Schmerz) auch dazwischen Schmerzen

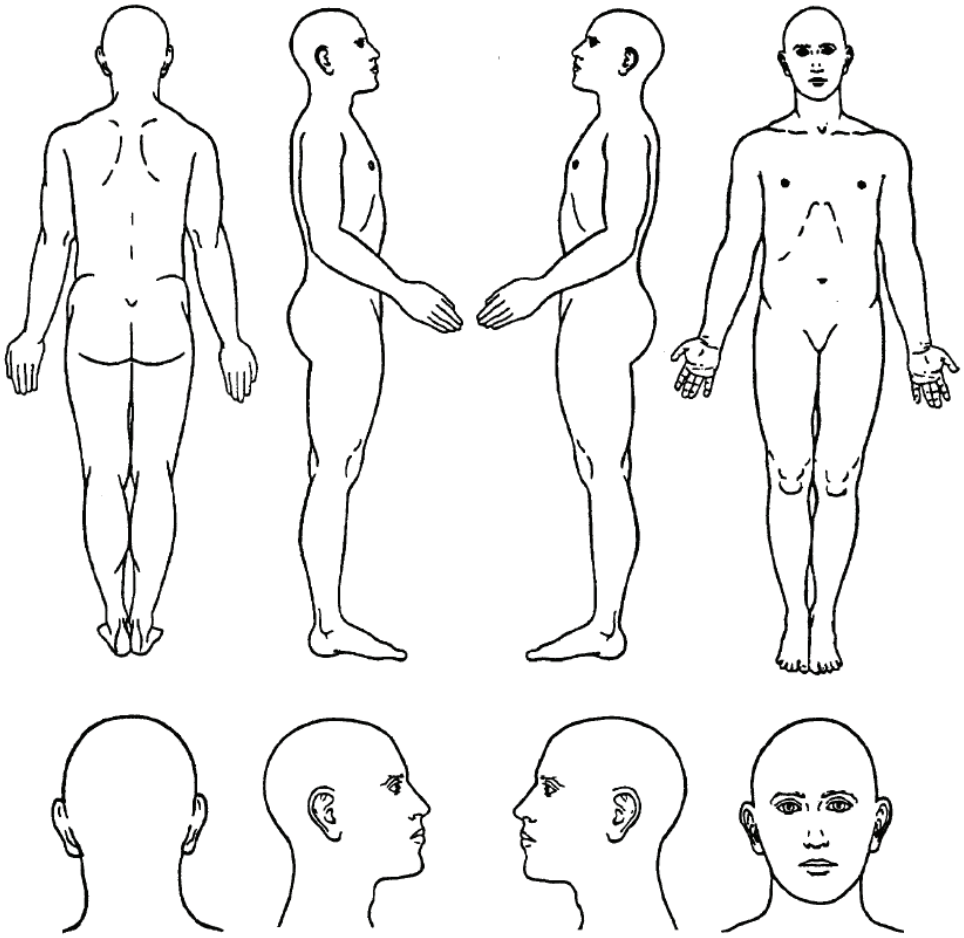
Vielleicht haben Sie auch Schmerzen, die durch bestimmte äußere Auslöser (Berührung, Druck, Kälte) hervorgerufen werden können.
Bei einem Patienten können gleichzeitig mehrere Arten von Schmerzen bestehen.

Schmerzen sind für Patienten oft schwer zu schildern und Ärzte haben häufig Schwierigkeiten diese richtig zu verstehen. Deswegen werden Fragebögen entwickelt, die den Ärzten helfen sollen, die Schmerzen ihrer Patienten auch im Einzelnen zu erfassen. Dieses ist ein neuartiger Fragebogen, dessen Nützlichkeit wir testen wollen. Dazu benötigen wir Ihre Hilfe.

Wo haben Sie Schmerzen ?

Vorab möchten wir Sie fragen, wo überall Sie unter Schmerzen leiden.

Malen Sie bitte in den nachfolgenden Körperschemata ein, wo Sie überall Schmerzen haben.
Bitte kennzeichnen Sie das ganze Schmerzgebiet (durch Schraffierung mit Bleistift oder Kugelschreiber), damit wir wirklich wissen, wo Sie überall Schmerzen haben.



Nehmen Sie Medikamente?

Haben Sie in den letzten 24 Stunden Medikamente gegen Ihre Schmerzen genommen?

☐ ja ☐ nein

Welche Medikamente haben Sie **in den letzten 24 Stunden** gegen Ihre Schmerzen genommen?

Name des Medikaments	**Wie oft haben Sie das Medikament genommen?**	**Wie viele Tabletten/ Tropfen /Zäpfchen haben Sie genommen?**
z.B. Paracetamol 500 mg	2 mal	jeweils 2 Tabletten

Sie werden, wie Sie es vielleicht schon aus anderen Fragebogen kennen, nach der Stärke ihrer Beschwerden gefragt. Da man die Stärke von Beschwerden nicht einfach messen kann, verwenden wir hier eine Skala von 0 bis 10. 0 bedeutet dabei immer, dass Sie die entsprechenden Beschwerden nicht haben. 10 bedeutet, dass Sie die Beschwerden in der für Sie schlimmsten vorstellbaren Stärke haben.

Spontanschmerzen

Die ersten Fragen beziehen sich nur auf Spontanschmerzen, d.h. solche Schmerzen die ohne äußere Auslöser auftreten.
Haben Sie **Spontanschmerzen**, d. h. Schmerzen, die ohne äußeren Auslöser auftreten?

Bitte kreuzen Sie für jede der folgenden Fragen die Ziffer an, die am besten der **Stärke Ihrer Spontanschmerzen im Mittel über die letzten 24 Stunden entspricht**. Kreuzen Sie „0“ an, wenn Sie diese Art Schmerz nicht verspürt haben. (kreuzen Sie bitte immer nur eine Ziffer an)

Q1. Ist Ihr Schmerz brennend?

kein Brennen	0	1	2	3	4	5	6	7	8	9	10	schlimmstes vorstellbares Brennen

Q2. Fühlt sich Ihr Schmerz an wie eingeschnürt oder wie in einem Schraubstock eingeklemmt zu sein?

kein Einschnüren	0	1	2	3	4	5	6	7	8	9	10	schlimmstes vorstellbares Einschnüren

Q3. Fühlt sich Ihr Schmerz wie ein Druck an?

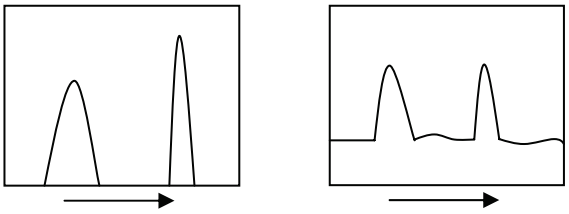
kein Druck	0	1	2	3	4	5	6	7	8	9	10	schlimmster vorstellbarer Druck

Q4. Wie lange dauerten Ihre Spontanschmerzen **in den letzten 24 Stunden**?
Kreuzen Sie die Antwort an, die der Dauer am besten entspricht:

dauerhaft (mehr als 12 Stunden)	☐
zwischen 8 und 12 Stunden	☐
zwischen 4 und 7 Stunden	☐
zwischen 1 und 3 Stunden	☐
weniger als 1 Stunde	☐

Schmerzattacken / unterbrochener Schmerz

Beschreibt eines der beiden Bilder die Schmerzen, wie Sie sie verspüren?



Schmerzattacken (unterbrochener Schmerz) dazwischen schmerzfrei	Schmerzattacken (unterbrochener Schmerz) auch dazwischen Schmerzen

Für jede der folgenden Fragen kreuzen Sie bitte die Ziffer an, die **am besten die mittlere Stärke Ihrer Schmerzattacken während der letzten 24 Stunden** angibt. Kreuzen Sie „0“ an, wenn Sie einen solchen Schmerz nicht verspürt haben. (kreuzen Sie bitte immer nur eine Ziffer an)

Q5. Empfinden Sie Ihre Schmerzattacken wie elektrische Schläge?

überhaupt nicht	0	1	2	3	4	5	6	7	8	9	10	schlimmste vorstellbare elektrische Schläge

Q6. Fühlt sich Ihr Schmerz stechend an?

kein Stechen	0	1	2	3	4	5	6	7	8	9	10	schlimmstes vorstellbares Stechen

Q7. Wie viele dieser Schmerzattacken hatten Sie **in den letzten 24 Stunden**?
Wählen Sie die Antwort, die am ehesten zutrifft:

- mehr als 20 ☐
- zwischen 11 und 20 ☐
- zwischen 6 und 10 ☐
- zwischen 1 und 5 ☐
- keine Schmerzattacken ☐

Schmerzen, die durch bestimmte Auslöser hervorgerufen oder verschlimmert werden

Haben Sie Schmerzen, die durch bestimmte Auslöser hervorgerufen oder verschlimmert werden, z. B. durch Reiben, Druck, oder Kontakt mit kalten Gegenständen im schmerzhaften Bereich?

Für jede der folgenden Fragen kreuzen Sie bitte die Ziffer an, die am besten der Stärke der Schmerzen, die durch Auslöser hervorgerufenen oder verschlimmerten entspricht, die Sie **im Mittel in den letzten 24 Stunden** hatten. Kreuzen Sie „0“ an, wenn Sie diesen Typ Schmerz nicht verspürt haben. (kreuzen Sie bitte immer nur eine Ziffer an)

Q8. Haben Sie im schmerzhaften Bereich Schmerzen, die durch Reiben hervorgerufen oder verschlimmert werden?

kein Schmerz	0	1	2	3	4	5	6	7	8	9	10	maximal vorstellbarer Schmerz

Q9. Haben Sie Schmerzen, die durch Druck auf den schmerzhaften Bereich hervorgerufen werden?

kein Schmerz	0	1	2	3	4	5	6	7	8	9	10	maximal vorstellbarer Schmerz

Q10. Haben Sie Schmerzen, die durch Kontakt mit einem kalten Gegenstand im schmerzhaften Bereich hervorgerufen oder verschlimmert werden?

kein Schmerz	0	1	2	3	4	5	6	7	8	9	10	maximal vorstellbarer Schmerz

Gefühlsstörungen

Haben Sie **im schmerzhaften Bereich** ungewöhnliche Gefühlsstörungen?

Für jede der folgenden Fragen kreuzen Sie bitte die Ziffer an, die **am besten der Stärke Ihrer ungewöhnlichen Gefühlsstörungen** entspricht, die Sie **durchschnittlich in den letzten 24 Stunden** hatten. Kreuzen Sie „0“ an, wenn Sie dieses Gefühl nicht hatten (kreuzen Sie immer nur eine Ziffer an).

Q11. Empfinden Sie ein Kribbeln?

kein Kribbeln	0	1	2	3	4	5	6	7	8	9	10	maximal vorstellbares Kribbeln

Q12. Empfinden Sie etwas, das sich anfühlt wie Ameisenlaufen?

kein Ameisenlaufen	0	1	2	3	4	5	6	7	8	9	10	maximal vorstellbares Ameisenlaufen

B. GCPS

Schmerzfragebogen (GCPS)

Wie würden Sie Ihren Schmerz **jetzt im Augenblick** auf einer Skala einschätzen, wenn *0 = kein Schmerz* und *10 = stärkster vorstellbarer Schmerz* bedeuten? **Bitte markieren Sie die für Sie zutreffende Zahl:**

0	— 1	— 2	— 3	— 4	— 5	— 6	— 7	— 8	— 9	— 10
kein Schmerz									stärkster vorstell-barer Schmerz	

Wie stark war der **stärkste** Schmerz **in den letzten 4 Wochen**, wenn *0 = kein Schmerz* und *10 = stärkster vorstellbarer Schmerz* bedeuten?

0	— 1	— 2	— 3	— 4	— 5	— 6	— 7	— 8	— 9	— 10
kein Schmerz									stärkster vorstell-barer Schmerz	

Wie stark war der Schmerz **in den letzten 4 Wochen im Durchschnitt**, wenn *0 = kein Schmerz* und *10 = stärkster vorstellbarer Schmerz* bedeuten?

0	— 1	— 2	— 3	— 4	— 5	— 6	— 7	— 8	— 9	— 10
kein Schmerz									stärkster vorstell-barer Schmerz	

An wie vielen Tagen der letzten 4 Wochen konnten Sie aufgrund der Schmerzen nicht Ihren **üblichen Aktivitäten** nachgehen (z.B. Arbeit, Schule, Haushalt, Hobby)?

an ☐ Tagen

In welchem Maße haben Schmerzen Ihre **alltäglichen Aktivitäten (Ankleiden, Waschen, Essen, Einkaufen etc.)** in den letzten 4 Wochen beeinträchtigt, wenn *0 = keine Beeinträchtigung* und *10 = keine Aktivitäten mehr möglich* bedeuten?

0	— 1	— 2	— 3	— 4	— 5	— 6	— 7	— 8	— 9	— 10
keine Beeinträchtigung									keine Aktivität mehr möglich	

Wie sehr haben Ihre Schmerzen während der letzten 4 Wochen Ihre **Freizeitaktivitäten** oder **Unternehmungen** im Familien - und Freundeskreis beeinträchtigt, wenn *0 = keine Beeinträchtigung* und *10 = keine Aktivitäten mehr möglich* bedeuten?

0	— 1	— 2	— 3	— 4	— 5	— 6	— 7	— 8	— 9	— 10
keine Beeinträchtigung									keine Aktivität mehr möglich	

In welchem Maße haben Ihre Schmerzen während der letzten 4 Wochen Ihre **Arbeitsfähigkeit (einschließlich Hausarbeit)** beeinträchtigt, wenn *0 = keine Beeinträchtigung* und *10 = keine Aktivitäten mehr möglich* bedeuten?

0	— 1	— 2	— 3	— 4	— 5	— 6	— 7	— 8	— 9	— 10
keine Beeinträchtigung									keine Aktivität mehr möglich	

C. ADS

Bitte kreuzen Sie bei den folgenden Aussagen die Antwort an, die Ihrem **Befinden während der letzten Woche** am besten entspricht / entsprochen hat.

Antworten:	selten	= weniger als 1 Tag oder überhaupt nicht
manchmal		= 1 bis 2 Tage lang
öfters		= 3 bis 4 Tage lang
meistens		= die ganze Zeit (5 bis 7 Tage lang)

Während der letzten Woche ...	selten	manchmal	öfters	meistens
1. ... haben mich Dinge beunruhigt, die mir sonst nichts ausmachen.	.....	.....	.....	.....
2. ... hatte ich kaum Appetit.	.....	.....	.....	.....
3. ... konnte ich meine trübsinnige Laune nicht loswerden, obwohl mich meine Freunde/Familie versuchten aufzumuntern.....	.....	.....	.....	.....
4. ... kam ich mir genauso gut vor wie andere.....	.....	.....	.....	.....
5. ... hatte ich Mühe, mich zu konzentrieren.....	.....	.....	.....	.....
6. ... war ich deprimiert / niedergeschlagen.....	.....	.....	.....	.....
7. ... war alles anstrengend für mich.	.....	.....	.....	.....
8. ... dachte ich voller Hoffnung an die Zukunft.....	.....	.....	.....	.....
9. ... dachte ich, mein Leben ist ein einziger Fehlschlag.	.....	.....	.....	.....
10. ... hatte ich Angst.....	.....	.....	.....	.....
11. ... habe ich schlecht geschlafen.	.....	.....	.....	.....
12. ... war ich fröhlich gestimmt.	.....	.....	.....	.....
13. ... habe ich weniger geredet als sonst.	.....	.....	.....	.....
14. ... fühlte ich mich einsam.....	.....	.....	.....	.....
15. ... waren die Leute unfreundlich zu mir.	.....	.....	.....	.....
16. ... habe ich das Leben genossen.....	.....	.....	.....	.....
17. ... mußte ich weinen.	.....	.....	.....	.....
18. ... war ich traurig.....	.....	.....	.....	.....
19. ... hatte ich das Gefühl, daß die Leute mich nicht leiden können.....	.....	.....	.....	.....
20. ... konnte ich mich zu nichts aufraffen.....	.....	.....	.....	.....

Bitte prüfen Sie, ob Sie alle Feststellungen beantwortet haben!

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CURRICULUM VITAE

PERSONAL DATA

Name	Lan He
Date of birth	06.03.1977
Nationality	Chinese
Status	Single

EDUCATION

10.2005 - 2008 Doctoral student
University of Würzburg, Würzburg, Germany
Thesis:
1) Small nerve fiber involvement in patients with Fabry's disease;
2) Methylprednisolone prevents the nerve-injury induced hyperalgesia in the NEP ko mouse

9.2002 - 7.2005 Master Degree (Clinical pathology)
Tianjin Medical University, Tianjin, China
Thesis: Lanthanide inhibits proliferation and suppresses MMP-9 expression in osteosarcoma in vitro.

9.1995 - 7.2000 Bachelor Degree (Medicine)
Medical College of Armed Police, Tianjin, China