Cover Letter

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Research Plan

Background

Small-fiber polyneuropathy, or SFPN, is the medical term for whole-body dysfunction and/or degeneration of small-diameter nerve fibers that transmit pain and control the body's involuntary (autonomic) functions. Symptoms of SFPN include wide-spread chronic pain, low blood pressure and/or rapid heart rate, and gastrointestinal distress. Abnormal sweating has also been associated with SFPN [1,2].
One of the first clinical manifestations of Fabry disease include acroparesthesias (pain), sweating impairment, and gastrointestinal dysmotility. Up to 70% of children and adolescents with classical Fabry disease develop pain in hands and feet at the average age of 9 years for boys or 16 years for girls [3]. These are the most common complaints by Fabry patients and the ones that impact their quality of life the most; long before kidney, heart and central nervous system involvement becomes apparent. These symptoms are thought to be caused by SFPN and improved with enzyme replacement therapy (ERT)[4]. Major efforts have attempted to raise awareness of Fabry disease and to screen high risk populations presenting with symptoms consistent with SFPN to promptly initiate ERT.

The pathophysiology of SFPN in Fabry disease is still not fully elucidated but there is consensus that the dysfunction involves: 1) Hypofunction of the nerve Aδ fibers preferentially which is different from SFPN in diabetes and amyloidosis where both C fibers and Aδ fibers are equally affected[5,6]. This is likely related to accumulation of glycolipids and loss of cell bodies in the dorsal root ganglia[7,8]. These deposits may disrupt ion channels and/or lead to nerve tissue cytotoxicity which could explain why certain anti-epileptic drugs targeting ion channels work so well to relieve acroparesthesias in Fabry patients[9]. 2) Chronic nerve ischemia secondary to glycolipids accumulation within endothelial cells of the blood vessels supplying nerve fibers[10].

Being able to properly diagnosis and monitor SPFN is therefore not only important for patients with idiopathic SFPN to have adequate, earl diagnoses for proper treatment and counseling, but also for patients with established Fabry disease to have good monitoring of SFPN progression and assessment of ERT efficacy and adjunct therapy for pain management.

Differentiating Fabry-induced SFPN from other causes (diabetes, amyloidosis, hereditary neuropathies and other multisystemic genetics and inflammatory/auto-immune disorders) can be clinically challenging early in the disease presentation when only symptoms of SFPN are apparent. Neuropathic pain with an onset in childhood or adolescence and/or chronic pains in the hands and feet exacerbated by changes in outside or body temperature, and decreased cold sensation on examination are strongly suggestive of Fabry disease. This data might however be elusive for years leading to considerable delay in diagnosis[11].

In general SFPN is diagnosed through a combination of symptoms, signs and confirmatory diagnostic testing. Nerve conduction studies are not sensitive enough in most of the cases leaving the ankle skin punch biopsy with measurement of intraepidermal nerve fiber density (IENFD) as the main gold standard diagnostic tool. Despite its utility and reproducibility, skin biopsy is invasive, expensive and requires a central laboratory for processing and interpretation.

Sophisticated autonomic function test (AFT) equipment, which includes a quantitative sudomotor axon reflex testing (QSART), is already in use at major medical centers, including MGH, as one of several methods recommended by the American Academy of Neurology for diagnosing SFPN[12]. However, full AFT testing takes an hour to complete and requires rigorous preparation to undergo the test.

A new device, Sudoscan™ (Impeto Medical, Paris, France) has been cleared by the FDA as a non-invasive galvanic skin response method to measure sweat gland function. There are no preparations required and the measurement takes about 2 minutes. The Sudoscan device consists of four metal plates on which patients place their hands and feet while standing. A small, low-voltage DC current is applied to the plates, which attracts chloride ions from the sweat glands on the palms of the hands and soles of the feet. The resulting electrochemical skin conductance (ESC) is measured and related to the subject's capacity to sweat. Normative scales of adult sweat function are preloaded in the device and compared to actual measurements to determine if a subject's sweat response is reduced, which is associated with neuropathy. Thus, Sudoscan is marketed as a rapid screening test for SFPN. Several researchers have shown Sudoscan to be useful for detecting early SFPN in diabetic adults, the largest group at risk of SFPN[13-15], chemotherapy-induced SFPN as well as sudomotor changes in cystic fibrosis (unpublished data). Because much of the focus has been on diabetes, there are no studies to-date of the feasibility of using Sudoscan for screening for other causes of SFPN.

Sudoscan™ is a promising modality to test SFPN in Fabry disease. Defective sweating is one of the hallmarks of Fabry disease and is thought to originate not only from a decrease in nerve fiber density of sweat gland innervation, but also from storage of glycolipids in the sweat glands themselves causing sweat gland dysfunction [16] which might be unique to Fabry disease and contributing to a specific pattern of ESC. Data by the Sudoscan™ manufacturer suggests increased sensitivity with the ability of detecting abnormalities very early in the disease process even when only mild fiber density changes are seen on skin biopsy and long before any significant change can be detected by AFT (unpublished data). This modality; if proven useful in detecting SFPN in Fabry disease could be used not only as a diagnostic tool but also as monitoring tool to evaluate the effect of ERT and other adjunct therapy.

In the Neuro-genetics unit at the Massachusetts General Hospital (MGH), we also follow hundreds of patients with rare diseases such as mitochondrial disease and connective tissue disorders. Despite the variable pathophysiology of these disorders SFPN seems to be a common pathogenic pathway shared with Fabry disease and leading to the same multiple complaints from the patients. In mitochondrial disease, SFPN is thought to be the result of lack of energy production and abnormal nerve metabolism, whereas in connective tissue disease, the abnormal structural proteins within the tissues are likely the cause. Because of these very different mechanisms and the fact that Sudoscan may be more sensitive to subtle SFPN changes, one might speculate that there are possibly discriminating ESC parameters (intensity, slope and pattern of conductance on the hands and feet) that might be able to distinguish SFPN within these 3 disease groups.

Thus we seek to find faster, less-invasive ways to diagnose and monitor small-fiber polyneuropathy in Fabry disease as well as identify a signature pattern of Fabry-induced SFPN in comparison to other rare diseases. Since Sudoscan measurement is non-invasive and takes only 2 minutes, it is an attractive potential add-on to the routine clinical care of our patients.

<u>Aims:</u>

The aims of this preliminary study are to:

Aim 1.
Analyze electrochemical skin conductance and compare data to SFPN standard of care diagnostic testing (QSRT and skin biopsy) in patients with Fabry disease and evaluate the reliability and sensitivity of SUDOSCAN™. as a new method to asses sweat gland function for the screening of Fabry disease.

Aim 2.
Determine whether there is a specific signature pattern of the ESC (intensity, slope and pattern of conductance on the hands and feet) that distinguishes Fabry disease from other disorders causing SFPN such as mitochondrial disease and connective tissue disorders.

Methods

Study population

Adults and children ages 4 to 65 years recruited at MGH Neurogentics clinic. If local recruitment is incomplete, advertisement will be made on patient support groups for broader national patients' enrollment. Informed consent will be obtained from all participants prior to the measurement with SUDOSCAN™. Young participants age 17 and below will provide Assent in addition to Consent from their parents.

Submission to the Institutional Review Board (IRB) for this protocol will be initiated once this proposal has been approved.

A. Study groups - Inclusion criteria

-Patients (males and females) with confirmed Fabry disease (by GLA enzymes and/or DNA testing) naïve and on ERT, mitochondrial diseases (electron transport chain and/or DNA testing) and connective tissue diseases (clinical criteria and/or DNA testing when available)

-Consenting adults and children who are old enough to stand with hands and feet on the Sudoscan plates for at least 2 minutes.

B. Subject population - Exclusion criteria

- In general we exclude potential subjects with cognitive, psychiatric, or other problems that preclude informed consent.
- Patients with history of glucose intolerance or diabetes.
- Patient on chemotherapy
- Other exclusion criteria are:

- People with any open or bleeding wounds at any sensor plate contact surface location
- People with any type of implantable device
- People with missing hand(s) and/or leg(s)
- Pregnant women or women who are uncertain about a possible pregnancy

Study Design

Aim 1.
Analyze electrochemical skin conductance and compare data to SFPN standard of care diagnostic testing (QSRT and skin biopsy) in patients with Fabry disease and evaluate the reliability and sensitivity of SUDOSCAN™. as a new method to asses sweat gland function for the screening of Fabry disease.

- Thirty patients with Fabry disease will be enrolled. Emphasis will be given to treatment-naïve patients but we expect the majority of the patients to be on ERT. We will proactively seek patients who have been on ERT for a short period of time (less than 1 year) and patients who are on ERT with persistent acroparesthesia to maximize the yield of SFPN findings.

- Patients' charts will be reviewed for pertinent medical history, inclusion/exclusion criteria assessment and demographic data (age, gender, body mass index (BMI)). Medications intake will also be reviewed to eliminate patients who are on neurotoxic drug that might cause neuropathies.

- All subjects will receive a complete neurological examination comprising: the neurological symptom score, cranial nerve function, and an evaluation of muscle weakness, muscular atrophy, bilateral reflexes, and sensory function of upper and lower extremities.

-SUDOSCAN™ will be used on all patients at baseline to collect ESC. A subset of these patients (15) will also undergo standard of care diagnostic testing for SFPN: QSART at the MGH neuromuscular clinic' AFT suit as well as skin biopsy interpreted in Dr. Anne Louise Oaklander's laboratory. Dr. Oaklander is the director of the neuropathology laboratory and has tremendous expertise in SFPN with normative data for both children and adults[17].

Results of QSRT, skin biopsy and ESC output from SUDOSCAN™ will be compared at baseline.

Aim 2.
Determine whether there is a specific signature pattern of the ESC (intensity, slope and pattern of conductance on the hands and feet) that distinguishes Fabry disease from other disorders causing SFPN such as mitochondrial disease and connective tissue disorders.

- Twenty patients with mitochondrial disease and 15 with connective tissue disease will be enrolled. Patients' charts will be reviewed for pertinent medical history, inclusion/exclusion criteria assessment and demographic data (age, gender, body mass index (BMI)). Medications intake will also be reviewed to eliminate patients who are on neurotoxic drug that might cause neuropathies.

- All subjects will receive a complete neurological examination comprising: the neurological symptom score, cranial nerve function, and an evaluation of muscle weakness, muscular atrophy, bilateral reflexes, and sensory function of upper and lower extremities.

-SUDOSCAN™ will be used on all patients at baseline to collect ESC. A subset of these patients (8 to 10) will also undergo standard of care diagnostic testing for SFPN: QSART at the MGH neuromuscular clinic' AFT suit as well as skin biopsy interpreted in Dr. Anne Louise Oaklander's laboratory. Results of QSRT, skin biopsy and ESC output from SUDOSCAN™ from each group will be compared to the Fabry patients group to assess for any signature pattern of ESC in Fabry disease.

Study Procedure

1) SUDOSCAN™

Subjects will be studied at MGH at a time convenient to them. No special preparations are required. This study is accomplished in a single session lasting less than 30 minutes including paperwork. Study staff will explain the study and gather informed consent. Subjects will then remove their shoes and socks and stand on two metal plates on the floor while placing the palms of their hands on two metal plates on a table. The plates are connected to the SUDOSCAN™ device. The subject stands still for two minutes while skin conductance data are gathered. There is no perceptible sensation. Results are expressed immediately as Electrochemical Skin Conductance (ESC, µS) representing the ratio between the current generated and the constant DC stimulus (lower than 4 V) applied on the electrodes. Sudomotor dysfunction is evaluated according to the ESC measured on the limbs: > 60 lS = no dysfunction; 60–40 lS = moderate dysfunction; and < 40 lS = severe dysfunction (normative database on >300 subjects).
The SUDOSCAN™ apparatus is small and can be transported in a small case. This will be available at each clinic and will be used to screen every patient seen in the MGH neurogenetics clinic during their regular clinic visit. Fabry patients who are not regular MGH patients and enrolled for the purpose of this study will be assessed in our research center after full examination and comprehensive medical history is obtained.

There are no risks or discomforts associated with sweat measurement with SUDOSCAN™ . The procedure is similar to standing on a digital scale for 2 minutes. The applied voltage is less than or equal to 4V with a current of about 0.2 mA. At this level there is no sensation from contact with the electrode plates. There is no radiation associated with the SUDOSCAN™ device.

2) QSRT

Quantitative sudomotor axon reflex test measures axon reflex-mediated sudomotor responses quantitatively and evaluates postganglionic sudomotor function. The recording is typically performed on the forearm and three lower extremity skin sites to evaluate the distribution of postganglionic deficits. The test has a high sensitivity, specificity and reproducibility. The test is straightforward in established laboratories such as the MGH neuromuscular AFT suite[18]. There is no specific risk associated with this procedure.

3) Skin biopsy for intraepidermal nerve fiber density (IENFD)

IENFD and sweat gland nerve fiber (SGNF) density evaluations uses a skin biopsy and immunostaining of the tissue to identify and count the intraepidermal and sudomotor nerve fibers. Assessment of nerve fiber density typically involves a 3-mm punch biopsy of skin from the leg (10 cm above the external malleolus). After sectioning by microtome, the tissue is immunostained with anti-protein-gene-product 9.5 (PGP 9.5) antibodies and examined with immunohistochemical or immunofluorescent methods.

Skin biopsy is a well-tolerated procedure and complications which are very rare include: pain, infection, bleeding and delayed healing with scaring.

Primary endpoints
ESC output from SUDOSCAN™. Normative ESC scales previously obtained by Sudoscan ™. are provided by the manufacturer for comparison.

Secondary points
1) Specific Fabry disease ESC signature pattern on SUDOSCAN™ testing comparison to other rare diseases with SFPN.

2) ESC characteristics within the Fabry group with assessment of specific phenotypic features of Fabry disease such as disease severity, kidney and cardiac involvement to evaluate any meaningful correlation between the degree of ESC derangement to the disease severity/progression.

Statistical analysis
For a p value <1% at 99.5 power a sample size of 20 patients is sufficient (taking into account standard deviations and data variation from diabetes studies). Multivariate analysis will be used to uncover any ESC variation correlating with the severity of Fabry disease and the different phenotypic variability of the disease.

Anticipated results
We expect SUDOSCAN™ results to be in accordance with the decrease in total sweat response measured by QSART and skin biopsy small fiber neuropathy low density. We expect to see an improvement of ESC in patients on ERT. We expect to see a specific ESC pattern in patients with Fabry disease as compared to other diseases with SFPN.

Potential benefits to society
We anticipate adding to the body of knowledge about SFPN. This method may in time develop into a clinical diagnostic tool with the potential to supplement or replace more arduous tools such as skin biopsy or AFT.

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