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Neuropathy and pain in Fabry disease

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Abstract

Fabry disease (FD) is a multiorgan lysosomal storage disorder caused by mutations in the *alfa-galactosidase A* (*GLA*) gene. Pathogenic *GLA* mutations lead to impaired or even lost enzyme activity, which causes the accumulation of sphingolipids, e.g., globotriaosylceramide, in cells and tissues. The majority of FD patients experience triggerable pain, mainly acral and burning, which often begins in early childhood. While small fiber pathology is assumed to be the basis of FD pain, the underlying molecular mechanisms are not well understood. This review summarizes the clinical characteristics of neuropathy and neuropathic pain in FD, presents current treatment options, and gives an overview of the latest findings from experimental and human model systems on the pathomechanisms contributing to small fiber pathology and FD-associated pain.

Keywords: Fabry disease, small fiber neuropathy, Fabry-associated pain

INTRODUCTION

Fabry disease (FD) is an X-linked hereditary lysosomal storage disorder with an estimated prevalence of 1 in 1,368 to 1 in 8,882[1]. FD is caused by genetic alterations in the gene encoding alfa-galactosidase A (GLA), which is located on chromosome Xq21 and consists of five exons. To date, over 1000 GLA mutations[2] have been identified, which may lead to an impairment or complete loss of GLA enzyme activity. The degree of GLA impairment is defined by the pathogenicity of the respective genetic mutation, which may range from apathogenic to pathogenic[3]. Since GLA is a key enzyme involved in the cleavage of sphingolipids, impairment in its function leads to the accumulation of Gb3 in lysosomes within cells of different tissues.

An increase in cellular Gb3 load is pathognomonic for FD; however, the role of Gb3 in disease development remains unclear. Current concepts highlight lysosomal dysfunction as the main driver of FD pathophysiology rather than the accumulation of Gb3[4]. Despite X-chromosomal inheritance, women are also affected by FD and may reach all levels of disease severity[5,6]. Intravenous enzyme replacement therapy (ERT) via recombinant agalsidase alfa or agalsidase beta has been available for FD-specific treatment since 2001[7,8] and the chaperone migalastat as the first oral treatment since 2016[9]. Pain is one of the earliest FD symptoms and can begin as early as childhood[10,11]. While the exact mechanisms of pain pathophysiology in FD are incompletely understood, impairment of the small caliber thinly-myelinated A-delta and unmyelinated C nerve fibers is a key factor[12,13]. In this review, we summarize current knowledge on the clinical phenotype, pathophysiology, and treatment options of FD-associated neuropathy and pain, and provide an outlook into novel approaches to improve scientific insight and patient management.

NEUROPATHY IN FD

In FD, mainly the thinly-myelinated A-delta and unmyelinated C nerve fibers, i.e. the small nerve fibers, are affected[14,15]. Neuropathy of the large nerve fibers in terms of polyneuropathy with a sensorimotor clinical phenotype is rare and rather occurs as a complication of advanced nephropathy in FD patients suffering from chronic kidney disease and uremia[16,17]. In FD patients with small fiber neuropathy (SFN), the clinical phenotype is characterized by thermal hypoesthesia. Sensory profiles of FD patients, e.g., established using quantitative sensory testing (QST), mainly show an impairment in cold sensation[15,18,19]. In a long-term follow-up study of over 5 years, treatment-independent progress of thermal hypoesthesia was observed using QST in men with FD[15]. Another aspect of FD-associated SFN is the autonomic dysfunction regularly observed in FD[17,20-22]. Here, hypo- to anhidrosis is the main symptom that may also trigger and enhance heat intolerance which is frequent in FD. FD patients may further suffer from gastrointestinal complaints due to autonomic dysfunction of small caliber nerve fibers, such as dysmotility, diarrhea, and dyspepsia[23]. Autonomic dysfunction may further include cardiac dysautonomia, which may be relevant when deciding on medication with cardiac effects.

FD-ASSOCIATED PAIN

Pain is one of the earliest, most frequent symptoms in FD that may start in childhood[24] and compromises patients' health-related quality of life[25,26] up to the development of depressive symptoms[26,27]. FD-associated pain is mostly episodic and triggerable by fever, heat, and physical activity. Patients describe it as a burning pain, primarily located at soles, toes, palms, and fingertips; however, it can affect any part of the body, including causing abdominal pain[28]. FD pain can be categorized into four pain subgroups[29]: (1) Pain attacks are the most frequent variant, with sudden and intensive pain upon triggers. Pain attacks remain during a trigger and typically resolve upon trigger removal; (2) Pain crises are severe pain attacks that spread over the body and may be so intense that patients need to be hospitalized. Pain crises may occur with and without triggers and are challenging to treat; (3) Evoked pain is often reported by FD patients in the extremities upon contact with hot or cold objects; and (4) Chronic pain, i.e., persistent pain, is rarely experienced by FD patients. As reported in a previous study, FD-associated pain may resolve over time independent of FD-specific treatment[29].

Pain in FD is mostly categorized as neuropathic pain due to its clinical characteristics (burning) and the impairment of small nerve fibers (QST, skin punch biopsy). It is, however, intriguing that FD patients report analgesic effects using non-steroidal anti-inflammatory drugs (NSAID). While NSAID are classically inefficient in neuropathic pain, they are the first-line treatment of nociceptive pain. Together with findings pointing to a systemic pro-inflammatory profile of FD patients[30-32], these data support the presence of a

nociceptive component in FD pain. Further factors, such as spontaneous ectopic firing or central nervous system sensitization, are discussed.

Lyso-Gb3 and FD-associated pain

Globotriaosylsphingosine (lyso-Gb3) is a deacetylated form of Gb3 and its levels may be elevated in the plasma of FD patients[33]. Injection of lyso-Gb3 into the hind paws of mice caused acute pain-associated behavior upon mechanical stimulation, which completely resolved after 24 h. Additionally, mouse DRG showed an increase in intracellular calcium levels together with an increase in voltage-gated calcium channel current densities upon treatment with lyso-Gb3. These findings suggest that lyso-Gb3 may be involved in FD-associated pain by increasing intracellular calcium levels and causing hyperexcitation of nociceptors[34]. If lyso-Gb3 has a pathophysiological role in FD-associated pain is yet to be elucidated. Data are scarce and contradictory. While increased levels of lyso-Gb3 at baseline may serve as a risk factor for adverse outcomes in FD[35] and in one study, baseline plasma lyso-Gb3 levels correlated with baseline pain in male FD patients, no correlation was found between changes in plasma lyso-Gb3 levels and pain scores after treatment with migalastat[36].

DIFFERENTIAL DIAGNOSES OF FD-ASSOCIATED PAIN

The main distinguishing characteristic of FD-associated pain is its manifestation in early childhood as pain triggerable by stimuli such as fever, heat, or physical activity. Moreover, its location, mainly at soles and palms, is an important hallmark in patients' medical history. One differential diagnosis is erythromelalgia (hereditary or secondary), which may manifest as triggerable pain in single extremities accompanied by local redness, swelling, and severe allodynia. Patients with SFN of other origin (e.g., diabetic, genetic, autoimmune) or idiopathic SFN may also present with acral burning pain. In patients with SFN, the lack of a family history and multiorgan involvement typical of FD can be informative. It is not recommended to conduct unselected genetic testing for FD in all patients with SFN unless typical hallmarks are present[37].

DIAGNOSING PATIENTS WITH FD NEUROPATHY AND PAIN

The main pillar of the diagnostic workup in patients with FD-associated pain is the detailed interview focusing on triggerable and spontaneous pain. Pain location, triggers, and characteristics are crucial information to be collected. Additionally, factors alleviating pain (e.g., drugs, non-pharmacological measures) need to be recorded. The use of FD-pain questionnaires is recommended to systematically cover all pain aspects that are important in FD[38,39]. The clinical assessment should contain a complete neurological examination with a focus on the sensory system. A sensory profile should be recorded, e.g., using QST, which may show thermal hyposensitivity as a finding typical for FD. Nerve conduction studies will help to detect hints of large nerve fiber impairment, if present. For the morphological investigation of the small nerve fibers, a skin punch biopsy is recommended to determine the intraepidermal nerve fiber density (IENFD). The IENFD is typically reduced early in FD. Skin punch biopsy should be performed at the baseline visit and can be repeated at follow-up if the patient reports new sensory symptoms and/or pain[40].

FD-SPECIFIC TREATMENT AND PAIN

It is crucial not to miss the FD diagnosis, since causative treatment is available. While there is currently no cure for FD, ERT or chaperone therapy are efficient symptomatic treatment options that may slow down the progress of FD when treatment is initiated early in the disease course[41,42]. The effect of ERT or chaperone is presented inconsistently in literature. Some studies have documented a relevant reduction in patients' pain ratings[7,18,43-45], while others have found no such effect[46-48]. The personal experience of the authors (N.Ü.) regularly seeing patients at a large FD center is that only a subgroup of patients experience sufficient and sustained analgesia under FD-specific treatment and that symptomatic treatment is usually

necessary. ERT is the most common treatment for FD patients with classic phenotypes. Since FD patients have low levels of GLA enzyme, replacement of this enzyme with recombinant protein shows beneficial effects. Two forms of the recombinant, agalsidase alfa and agalsidase beta, are commercially available. Treatment with both is clinically safe and efficient for FD patients. Agalsidase alfa is a glycosylated protein and structurally identical to GLA. It binds to the mannose-6-receptor on the cell surface and is then eventually transported to lysosomes, where it cleaves Gb3. Agalsidase beta also exhibits similar biochemical properties as agalsidase alfa; however, agalsidase beta shows higher glycosylation than agalsidase alfa[49].

Agalsidase alfa

Agalsidase alfa is administered intravenously every two weeks and the recommended dosage is 0.2 mg/kg body weight. The first clinical trial with agalsidase alfa showed sustained reduction in neuropathic pain and improvement in health-related quality of life of FD patients[8]. Subsequent long-term follow-up studies also supported this notion[50] and also showed improvement in cold and warm thresholds[18]. However, results from other long-term studies and Fabry Outcome Surveys reported no improvement in pain in FD after treatment with agalsidase alfa[51,52]. In a Japanese study, both male and female patients showed improvement in pain scores after treatment with agalsidase alfa and health-related quality of life remained stable during the course of treatment[53]. However, the non-interventional observational post-marketing surveillance from Japan revealed that there was no improvement in pain or health-related quality of life in patients who received ERT. Still, some patients experienced a reduction in pain at times, but they also received additional analgesics during ERT. Hence, it remains inconclusive if the reduction in pain was an effect of agalsidase alfa or the analgesics[46].

Agalsidase beta

Agalsidase beta is administered intravenously every two weeks and the recommended dosage is 1 mg/kg body weight. In early clinical trials, FD patients showed improvement in pain after treatment with agalsidase beta[7]. Treatment with agalsidase beta also showed improvement in the function of C-, Aδ-, and Aβ-nerve fibers in FD patients[45]. When young male and female FD patients ≤ 30 years were treated with agalsidase beta, male patients exhibited a reduction in both chronic peripheral pain and acute pain crises, but not abdominal pain after short-term follow-up (≥ 0.5 year). In contrast, women experienced a reduction solely in acute pain crises. Upon long-term follow-up (≥ 2.5 years), men with FD demonstrated a reduction in all types of pain, whereas women showed improvement only in abdominal pain[44].

Migalastat

Migalastat is the first oral drug for the treatment of FD[54]. Chemically, it is 1-deoxygalactonojirimycin and is an analog of the terminal galactose of Gb3. Oral therapy with migalastat is possible only in patients with so-called "amenable" GLA mutations, i.e., in cases where the rest of GLA activity is still preserved. The majority of these mutations are missense mutations, where the GLA enzyme is misfolded and thus migalastat functions as a chaperone by reversibly binding to the active site of GLA and improving its transportation to lysosomes[9]. Migalastat is administered orally once every other day at a dosage of 123 mg/d. In a recent multicenter study, FD patients were treated with migalastat orally every other day for 2 years. After 12 months of treatment, the pain was reduced and the health-related quality of life in patients also improved[43]. However, other clinical studies show that migalastat has a neutral effect on pain experiences and pain severity remains mostly stable[47,48,57].

SYMPTOMATIC ANALGESIC TREATMENT OF FD PAIN

Symptomatic analgesic treatment is necessary for the majority of FD patients experiencing pain, since FD-specific treatment often does not sufficiently lead to FD pain alleviation. With FD being a rare disease, no randomized and controlled trials are available on pain treatment in FD patients. Hence, clinical

management of FD pain follows national and international treatment guidelines for neuropathic pain[56] and expert opinion recommendations[57]. FD pain treatment covers the management of acute episodic FD-associated pain and the long-term analgesic treatment in patients with permanent FD pain, or as prophylaxis to reduce episodic FD pain[29]. As first non-pharmacological measures, FD patients regularly try to avoid any known triggers. Many patients also report pain relief upon cooling the aching extremities. If pain persists, often NSAIDS and non-opioid analgesics are used by FD patients. This underscores a potential inflammatory pain component. One major challenge is the cardiac and renal comorbidity of many patients, which makes careful selection and dosing mandatory. Since FD pain is often local, topically applicable analgesics such as lidocaine or capsaicin may be further options. In patients with extreme pain intensities such as in FD crises, opioid treatment may be indicated[57]. Furthermore, the application of earlier-generation anti-convulsant drugs such as phenytoin or carbamazepine may be beneficial in individual cases[57]. In extreme cases of FD crises, hospitalization for intravenous analgesic pain management may become necessary. In patients experiencing persistent FD-associated pain and aiming to reduce episodic FD pain frequency and intensity, prophylactic use of analgesics recommended as first-line treatment for neuropathic pain is also the current approach in FD patients. As an example, gabapentinoids and tricyclic antidepressants may be used, however, with caution for renal and cardiac contraindications[57].

INSIGHTS FROM FABRY ANIMAL MODELS

Rodent models of FD

To elucidate the pathophysiology of FD neuropathy, extensive work has been done in animal models. In mice, *GLA* knockout models (KO mice) are frequently used to understand the impact of Gb3 accumulation on different organs[58]. This mouse model shows age-dependent accumulation of Gb3 in different tissues including the dorsal root ganglia (DRG)[59]. Further studies have shown that KO mice, particularly males, display pain-associated behavior such as mechanical and thermal hypersensitivity starting at a very young age and turning to warm and cold hyposensitivity[59].

When investigating potential mechanisms that may contribute to neuropathy and pain in FD, Gb3-dependent alterations in mouse DRG neuron expression and functioning of voltage-gated sodium channels were found[59] in KO mice. Additionally, it was shown that altered expression of immune-related genes may lead to changes in the expression of pain-associated ion channels, contributing to pain in FD[60]. Further, macrophages in the DRG of KO mice have an increased expression of CD68 and CD163[61], which may contribute to pain in FD. GLA KO mice also show elevated expression of transient receptor potential cation channel subfamily V member 1 (TRPV1) in skin and DRG, potentially leading to thermal sensitivity[62] in contrast to thermal hyposensitivity seen in FD patients.

The impact of Gb3 accumulation on the density of peripheral nerve fibers was also investigated in *GLA* KO rats[63]. The findings revealed a decrease in myelinated nerve fiber density in the saphenous and distal tibial nerves, while no notable difference was observed in the proximal tibial nerves. Additionally, a reduction in unmyelinated nerve fiber density was seen in the saphenous nerve, but not in the femoral motor branches of *GLA* KO rats. Consistent with findings in KO rat DRG, larger axon diameters were noted in the proximal, but not distal parts of the tibial nerve in KO rats. Furthermore, prominent osmophilic accumulations were found in the myelinated axons of the proximal tibial nerve in KO rats. To determine the nature of these accumulations, immunofluorescence staining was performed using antibodies targeting lysosomes or Gb3 deposits. The results revealed that 2% of myelinated nerves in KO rats exhibited lysosomal accumulation of Gb3, a percentage still higher than that in WT rats. However, a significant amount of Gb3 staining did not colocalize with the axonal marker. Further, it is predicted that these accumulations could be a result of immune cell infiltration or Gb3 accumulation in non-neuronal cells such as Schwann cells.

KO rats displayed increased mechanical sensitivity which progressed with age. Further, their mechanical sensitivity was linked to the sensitization of the transient receptor potential ankyrin 1 (TRPA1) ion channel[64].

Various types of non-neuronal cells, such as keratinocytes and Schwann cells, play pivotal roles in transmitting noxious stimuli to sensory neurons[65-67]. Moreover, these cells may contribute to the manifestation of pain phenotypes observed in patients with FD. A recent study has revealed that cultured DRG from KO rats exhibits hyperexcitability when stimulated with cultured media from Schwann cells[68]. Furthermore, it has been demonstrated that Schwann cells from KO rats secrete p11, a protein that induces spontaneous activity and hyperexcitability in cultured DRG. Notably, upon removal of p11, the observed hyperexcitability was attenuated.

Since there is evidence from human FD studies that FD patients have systemic pro-inflammatory profiles[31,69], a potential immune-mediated component contributing to neuropathy and pain in FD was investigated. In line with results from FD patients, it was also observed that, the KO rats showed elevated levels of blood pro-inflammatory cytokines[70].

Mitochondrial dysfunction has been associated with inflammation, sensory disturbances, and pain in different animal models. Mitochondrial protein, ATP synthase c subunit lysine N-methyltransferase (ATPSc-KMT) acts as a switch for transforming pain from acute to chronic in mice models of chronic pain[71]. It was also found that macrophages transfer mitochondria to sensory neurons in order to resolve pain[72]. Furthermore, the expression of the ATPSc-KMT gene was also reduced in subchondral bone in patients suffering from osteoarthritis[73]. Although these findings seem to be exciting, their role in mitigating pain in FD has not yet been investigated.

Defects in autophagy and autophagosome maturation have been reported in different cells from FD patients, such as fibroblasts[74], peripheral blood mononuclear cells (PBMCs)[75], and conjunctival epithelia[76]. Additionally, renal biopsies from FD patients show impairment in autophagy with the presence of vacuoles in podocytes and mesangial cells[74]. Labeling for autophagy marker microtubule-associated protein 1A/1B-light chain 3 (LC3) in these biopsies revealed that the vacuoles are autophagic in nature. In mouse models of FD, impairment in the autophagy-lysosomal pathway was found in different brain regions[77]. Studies in animal models of peripheral nerve injury have shown a context-dependent role of autophagy in either suppressing[78,79] or enhancing[80,81] neuropathic pain. However, the role of autophagy in sensory neurons, especially small fiber degeneration, and its role in conferring pain in FD is yet to be understood.

Drosophila model of FD

Drosophila (*Drosophila melanogaster*) is an invertebrate and non-traditional model for studying nociception. Flies consist of sensory neurons[82] and respond to noxious stimuli by showing pain-like behavior[83]. Due to a short life cycle and ease of developing several mutant lines, drosophila can be employed to generate FD-relevant transgenic lines and to investigate nociception in FD. A transgenic fly line expressing human TRPV1 was recently developed to study pain mechanisms and effects of analgesic treatment in drosophila[84]. A drosophila model was also developed to study FD pathophysiology[85]. Transgenic fly lines with two different mutations in the *GLA* gene were generated: A156V and A285D. In these flies, the GLA enzyme is misfolded and remains trapped in the endoplasmic reticulum. The study also followed the lifespan of flies expressing the two different *GLA* mutations. The mutant *GLA* mutations resulted in premature death compared to wild-type *GLA* flies. Further, treatment with migalastat prolonged

the lifespan of flies expressing the A156V mutation and showed little effect on flies expressing the A285D mutation.

Zebrafish model of FD

Zebrafish (*Dania rerio*) is a highly utilized model for pharmacological and toxicological studies[86]. Due to their genetic homology to humans, exploitable genetics and low maintenance costs, zebrafish present as an attractive model for studying nociception in FD. They respond to a variety of noxious stimuli like temperature[87] and pharmacological agents such as allyl-isothiocyanate[88], formalin[89], and acetic acid[90]. Since zebrafish lack the Gb3 synthase, they cannot produce Gb3, and hence, this model is very useful for studying FD pathomechanisms independent of Gb3 accumulation. They also exhibit robust nocifensive behavior, which is restored upon treatment with analgesics. Most recently, a zebrafish model was described that exhibited reduced GLA activity and impairment in glomerular filtration, as seen in human FD patients[91].

NOVEL PERSPECTIVES

iPSC- and CRISPR/Cas9-derived sensory neurons

Recognizing the limitations of animal models to fully recapitulate human disease, attention has turned toward induced pluripotent stem cell (iPSC)-based cell culture models, which hold promise as attractive alternatives for FD research[92]. With FD caused by pathogenic mutations in the *GLA* gene, developing animal models for all mutations is not feasible. iPSC-derived cell lines, originating from patient biomaterial, present an opportunity to closely mirror phenotypic characteristics observed in FD patients[92].

In two separate studies, the *GLA* gene was edited using CRISPR/CAS9 technology: once in an immortalized rat DRG cell line[93] and once in human embryonic stem cells[94] to develop sensory neurons relevant to FD. In the rat DRG cell line, the edited cells showed a reduction in GLA levels and activity and an increase in Gb3 accumulation, consistent with the FD phenotype. In human embryonic stem cells, the introduction of FD-related genetic mutations also resulted in decreased levels and activity of GLA and increased Gb3 accumulation. However, no quantification of GLA or Gb3 was performed in iPSC-derived sensory neurons. Both the rat DRG cell line and iPSC-derived sensory neurons showed expression of ion channels such as TRPV1, NaV 1.7, and NaV 1.8, indicating nociceptive properties. However, the functional characteristics of these sensory neurons were not investigated in either of the studies.

To study pain mechanisms in FD, iPSC lines were generated from skin fibroblasts of patients with FD suffering from pain. These cell lines were then differentiated into sensory neurons (SN) using a small molecule-based method. Interestingly, these SN showed an accumulation of Gb3 in both the soma and the neurites. Furthermore, increased calcium activity upon thermal stimulation, a common pain trigger in FD patients, was observed in these SN. In addition, the study showed that these SN exhibited reduced fiber diameter and impaired mitochondrial trafficking, which may be related to pain in FD[95].

In another study, SN were generated from iPSC using fibroblasts derived from a male subject harboring an S126G mutation in the *GLA* gene, which is classified as a variant of unknown significance (VUS)[96]. No difference in GLA activity was found in these cells, and there were also no Gb3 accumulations. In addition, electrophysiological properties such as rheobase and threshold potential were comparable to those of the control group. This study underscores the importance of recognizing different *GLA* mutations and determining their pathogenicity, since not all *GLA* mutations lead to clinically relevant GLA impairment.

iPSC-derived endothelial cells

Endothelial cell (EC) dysfunction is closely associated with FD. ECs are involved in vascular angiogenesis and the application of tube formation assays is one of the quantitative and sensitive methods used to determine angiogenesis[97]. It has been consistently reported that iPSC-derived ECs from FD patients show reduced tube formation ability compared to ECs from healthy individuals[98,99], indicating impaired angiogenesis. Apart from Gb3 accumulation hypoxia and mitochondrial dysfunction[99], increase in expression of thrombospondin 1[98], oxidative stress[100], increased release of heparanases and pro-inflammatory cytokines[101], and degradation of calcium-activated potassium channel KCa3.1[102] may contribute to EC dysfunction. Impairment in EC function leads to hypovascularization, which may also contribute to the pathophysiology of pain in FD.

Outlook

High-throughput screening of drugs using automated electrophysiological methods provides an opportunity to discover new therapeutic compounds using cell cultures[103]. iPSC-based cell cultures may serve as perfectly relevant model for developing new and repurposing existing analgesics to treat pain in FD. Recent studies have shown that this approach could be one future way of discovering novel analgesics[104].

However, it should be noted that these drug discovery methods rely on strong phenotypic readouts such as electrophysiological output. Additionally, iPSC- derived cell lines come with some caveats such as batch variability, clone variability, and heterogenous populations. Further research to identify reliable readouts and establish screening platforms is crucial for treating pain, not only in FD.

CONCLUSION

Although extensive clinical data on pain characteristics in FD is available, the correlation between genotype and pain phenotype is not yet clear. Animal models have provided some clues about pathological manifestations in FD. Understanding these mechanisms using patient-specific models will be crucial for treating pain and also developing pain-alleviating therapeutics for FD patients. With new treatment perspectives such as gene therapy and substrate reduction therapy for FD, hopes also remain high for more efficient analgesia in patients suffering from FD-associated pain.

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Authors' contributions

Composed and edited the manuscript, literature research on the topic: Medala VK, Üçeyler N

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