Fabry disease pain: patient and preclinical parallels

Anthony J. Burand Jr, Cheryl L. Stucky*

Abstract

Severe neuropathic pain is a hallmark of Fabry disease, a genetic disorder caused by a deficiency in lysosomal α-galactosidase A. Pain experienced by these patients significantly impacts their quality of life and ability to perform everyday tasks. Patients with Fabry disease suffer from peripheral neuropathy, sensory abnormalities, acute pain crises, and lifelong ongoing pain. Although treatment of pain through medication and enzyme replacement therapy exists, pain persists in many of these patients. Some has been learned in the past decades regarding clinical manifestations of pain in Fabry disease and the pathological effects of α-galactosidase A insufficiency in neurons. Still, it is unclear how pain and sensory abnormalities arise in patients with Fabry disease and how these can be targeted with therapeutics. Our knowledge is limited in part due to the lack of adequate preclinical models to study the disease. This review will detail the types of pain, sensory abnormalities, influence of demographics on pain, and current strategies to treat pain experienced by patients with Fabry disease. In addition, we discuss the current knowledge of Fabry pain pathogenesis and which aspects of the disease preclinical models accurately recapitulate. Understanding the commonalities and divergences between humans and preclinical models can be used to further interrogate mechanisms causing the pain and sensory abnormalities as well as advance development of the next generation of therapeutics to treat pain in patients with Fabry disease.

Key words: Fabry disease, Neuropathy, Pain, Nerve, DRG, Human, Preclinical, Animal models, Nociceptors

1. Introduction

What is Fabry disease? It is a lysosomal storage disorder caused by dysfunction or absence of α-galactosidase A (α-Gal A), a lysosomal hydrolase that catalyzes the removal of the terminal α-galactose residue from glycosylated molecules in the lysosome. Fabry disease is caused by a range of mutations in the galactosidase alpha (GLA) gene that encodes for expression of α-Gal A.[17,104] Dysfunctional α-Gal A causes accumulation of glycosylated products such as globotriaosylceramide (Gb3), lyso-globotriaosylceramide (lyso-Gb3), B glycolipid antigen, P1 antigen, and digalactosylceramide.[102,138] These products are mostly glycosphingolipids and cannot be otherwise degraded; they build up and form aggregates within the lysosomes. The exact pathogenesis of these accumulated glyco-sphingolipids remains to be elucidated. Buildup of these lipids can take the form of large electron-dense, lamellar-shaped inclusions called zebra bodies.[133] Over time, this lipid accumulation leads to loss

of sensory nerve fibers, sensory abnormalities and pain, as well as organ dysfunction including myocardial infarction, arrhythmia, renal deficiency, and strokes. These morbidities occur in both male and female patients and generally shorten their lifespan by 10 to 20 years.[174] Pain is one of the earliest symptoms that occurs in patients with Fabry disease, with onset frequently in early childhood, and pain often persists throughout life.[207] Because multiple organ systems are affected, patients with Fabry disease have significant disruptions in their normal functions including work, daily activities, and social interactions.[6,67] In addition, patients suffer high rates of depression and anxiety, which contribute to a severely diminished quality of life.[7,103] Treatment with enzyme replacement therapy (ERT) is costly and does not adequately alleviate their pain.[7,163] It has been estimated that the prevalence of Fabry disease is between 1: 40,000 to 1:117,000,[132,227] but genetic testing in recent newborn screenings suggests that the prevalence could be as high as 1: 1,400.[45,91,118] Although most mutations are private and do not occur within multiple families, some mutations do occur in multiple unrelated families and mutations may be more commonly found in exons 5 and 7.[54] Most mutations are missense, but nonsense mutations, insertions and deletions (INDELS), or mutations causing improper gene splicing also occur leading to variable degrees of enzyme activity.[3,17,29,226] The genetic prevalence of Fabry disease is considerably higher than current estimates of the clinical prevalence. This discrepancy likely occurs because not all genetic mutants result in severe disease, and other genetic and environmental factors can play a role in modulating the disease severity as evidenced by the diverse phenotypes observed for the same mutation.[3,59,200] In addition, Fabry disease has many nonspecific symptoms, which can lead to difficulty in detecting and diagnosing the disease.[46,150] Regardless, this high prevalence makes Fabry disease the most common lysosomal storage disease.

Patients with Fabry disease experience a host of pain symptoms including intense episodic pain, hypersensitivity to mechanical stimuli, gastrointestinal pain, heat and cold intolerance, chronic pain, and

other sensory abnormalities that drastically impact everyday function.[157,207] However, the high degree of heterogeneity in human patients with the same mutation in the GLA gene makes it difficult to predict which patients will manifest symptoms of pain.[207] Several groups have created preclinical models to interrogate mechanisms underlying Fabry pain. A goal is to use these models to test novel targets to determine therapeutic efficacy of novel compounds in treating pain-like behavioral outcomes before advancing the targets and compounds to clinical trials.

Preclinical models for a variety of different disorders associated with pain have been helpful in developing and testing therapeutics including development of patient-controlled analgesia, localized drug delivery system, and testing TRPV1 antagonists in humans.[32,221] However, there have been few successful novel pain therapeutics for patients with pain.[224] One reason may be that there are significant differences in the genetics, physiology, and pathology between human and preclinical models.[38,70,165] These differences may have contributed to different therapeutic outcomes between preclinical animal models and humans.[16] Therefore, it is important to understand the strengths and limitations of the models and how closely they recapitulate patient phenotypes. Successful preclinical models have been well characterized and validated to determine how accurately they represent the specific human physiology they are aiming to model. Although they often reduce the complexity of the disease, the molecular mechanisms of the condition are intact. Finally, in successful preclinical models, the pain phenotype is robust and can be replicated by multiple laboratories in rigorously controlled, blinded, well-controlled experiments.

The goal of this review is to provide an overview of pain in Fabry disease and how preclinical models replicate aspects of pain observed in patients to better aid in future mechanistic pain studies as well as the development of therapeutics. Herein, we will discuss human pain in Fabry disease, and the complexity of sensory deficiencies experienced by these patients. In addition, we will discuss how different characteristics of patients may influence their pain. Finally, we will examine similarities and differences in preclinical models and human patient pain and pathology, and discuss current limitations and strengths in preclinical models.

2. Methods

We searched the PubMed database for all articles containing the words "Fabry" and either "pain," "nerve," or "neuron" (**Fig. 1**, Supplemental File 1, available at http://links.lww.com/PAIN/B235). We used article titles and abstracts to screen for materials, excluding works that were not about Fabry disease including reviews, comments, letters to the editor, editorials, guidance documents, abstracts, and articles not in English. In addition, we excluded articles that had no abstract or full text. Subsequent screening was done using the abstract and full text, specifically looking for work that answered the following questions: (1) What types, qualities, duration, and intensity of pain are experienced by patients with Fabry disease? (2) What connection does Fabry pain have with sex, age, or ethnicity? (3) What dorsal root ganglia (DRG), nerve, or neuron pathology is observed in patients with Fabry disease? (4) What pain-related phenotypes (eg, depression, anxiety, weight gain, and exercise) are experienced by patients? (5) What preclinical models examine pain or nerve/neuron pathology in Fabry disease? (6) What types of pain or pain-depressed behaviors have been documented in preclinical models? A flowchart of the screening process we used is provided in **Figure 1**.
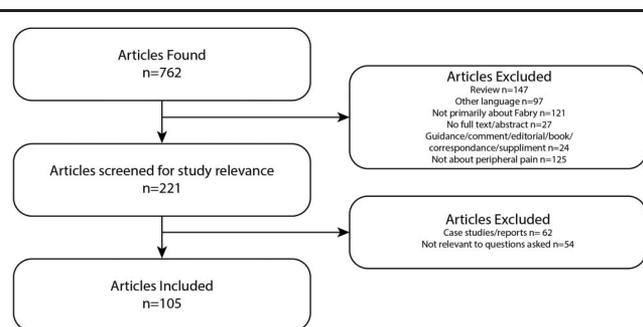

Figure 1. Screening process for relevant articles included in this review.

Data were extracted pertaining to the questions above and then synthesized into the narrative review by AB. In addition, articles familiar to either author but not indexed in PubMed were reviewed for relevance and included. A citation list of included and excluded articles is provided in the supplemental material (Supplemental Table 1, available at http://links.lww.com/PAIN/B234).

3. Results

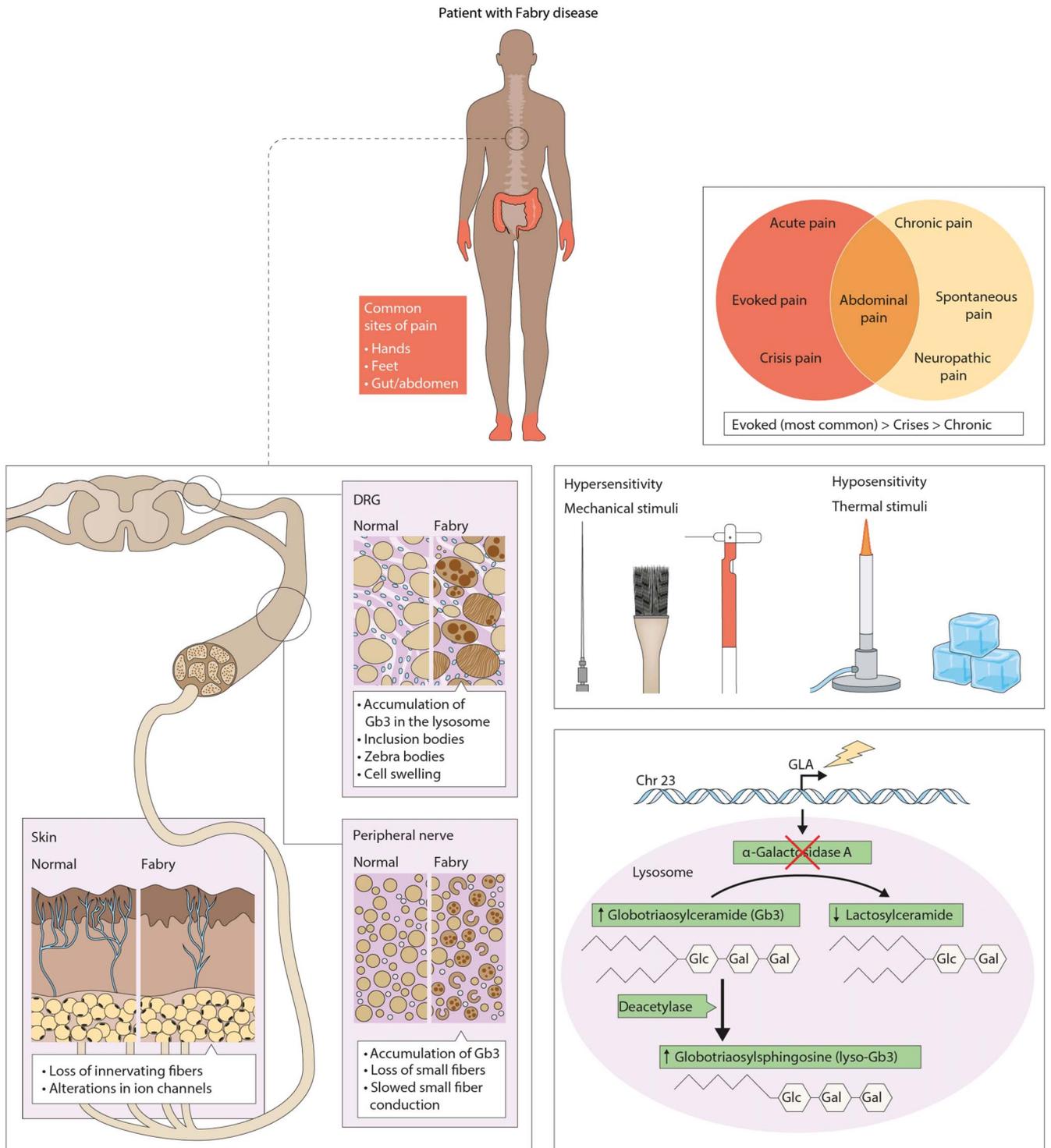### 3.1. Fabry disease in patients

3.1.1. Pathological findings in Fabry disease

3.1.1.1. Lysosomal dysfunction

Buildup of glycosylated-products originates in lysosomes of a cell and causes dysfunction of the lysosomes[31,108] (**Fig. 2**). Dysfunction of the lysosome can have profound impacts beyond simply the lack of degradation of select materials. The lysosome contains a variety of hydrolases, proteases, and lipases, and is the major degradation and recycling center of the cell.[179,225] This includes degradation of damaged or excessive cellular organelles and mitochondria through autophagy and mitophagy, respectively.[179,225] In addition, the lysosome is critical in sensing pathogenic material that enters the cell through toll-like receptors as well as presenting antigen on major histocompatibility complex molecules.[167] Lysosomes also sense nutrients, such as growth factors, amino acids, fatty acids, and cholesterol, and provide signals to regulate production of these nutrients.[11,113,167] Finally, lysosomes are responsible for processing extracellular material internalized in endosomes and contribute to the release of substances to the extracellular environment.[11,167] Therefore, dysfunction of the lysosome in Fabry disease can affect many basic cellular functions, which we hypothesize could negatively impact neuronal health and axonal regeneration leading to dying back and loss of small fibers. However, the full pathology of mechanisms underlying the pain in Fabry disease and its relationship to enlarged and dysfunctional lysosomes has not been elucidated yet.

3.1.1.2. Globotriaosylceramide accumulation in neurons and neuropathy

It is well established that Gb3 inclusions are present in DRG neurons of both patients and rodent preclinical models and these inclusions lead to cell swelling[53,148,194] (**Fig. 2**). Reports document increases in DRG neuron soma diameter and total DRG volume.[63,64,146] Although Gb3 deposits are present in Fabry disease in the brain, Gb3 content can be more than 10-times higher in the DRG than in regions of the brain including the frontal cortex, temporal lobe, parietal lobe, and hippocampus.[101,194] These high levels of Gb3 can result in DRG neuron stress and death.[81] Thus, the DRG is an

Figure 2. Human pain in Fabry disease. Patients with Fabry disease experience acute and chronic pain commonly in their hands, feet, and abdomen. Evoked pain is commonly experienced by patients, although pain can be spontaneous as well. Pain crises are intense pain episodes experienced by individuals with Fabry disease. Patients are often hyposensitive to thermal stimuli and hypersensitive to mechanical stimuli. The pathophysiology begins with dysfunction or absence of the lysosomal enzyme α-galactosidase A, which is essential for degrading the glycosphingolipid globotriaosylceramide (Gb3). Excess Gb3 is converted to globotriaosylsphingosine (lyso-Gb3). When Gb3 and lyso-Gb3 accumulate due to lack of enzyme, they builds up in the peripheral nervous system causing inclusion bodies and neuronal swelling, especially in the somata (dotted line indicates the zoomed-in image of spinal cord, DRG, and peripheral nerve). In addition, Gb3 and lyso-Gb3 build up in the blood and are transported into the DRG and nerve. Over time, many patients experience loss of small-diameter fibers from the skin and peripheral nerves. In addition, ion channels, including sodium, potassium, and transient receptor potential channels, in these nerves are dysfunctional. DRG, dorsal root ganglia.

epicenter for pathology of the disease. In addition to DRG neuron cell bodies, lipid inclusions are prominent in the axons of peripheral nerves and correlate with axon morphological abnormalities including irregular-shaped axons, enlarged axons, and nonuniform myelin.[114,148,177,194] Peripheral neuropathy affects over a quarter of patients with Fabry disease and is characterized by loss of small myelinated and nonmyelinated fibers, whereas larger fibers are largely unaffected[148,157,177,194] (**Fig. 2**). This dramatic small fiber loss is most prominent in the distal long axons of the lower extremities. There can also be a decline in the innervation of proximal sites such as the thigh, but the loss is most pronounced around the feet.[177,182,201,209] Interestingly, studies suggest that the fiber loss is substantially higher in the skin than in the peripheral nerve trunk.[177] Previous studies in patients with diabetic peripheral neuropathy have shown a good correlation between intraepidermal nerve fiber density and the presence of neuropathic pain with lower nerve fiber density correlating with higher pain.[187,198] This negative correlation between intraepidermal nerve fiber density and pain intensity is also observed in patients with chemotherapy-induced neuropathy.[23] However, in Fabry disease, it is not yet clear whether lower intraepidermal nerve fiber density is associated with increased pain because there has been limited patient sampling in current studies.[19] Although intraepidermal nerve fiber density is not perfectly predictive of pain, it is an easy clinical diagnostic tool that can be used to detect early onset of neuropathy. Although it has not been directly shown in Fabry disease, length-dependent dying back of the nerves as is seen in other small fiber neuropathies (eg, diabetic neuropathy)[196] is likely responsible for the difference in fiber densities observed between the distal vs proximal nerve ends. It remains to be determined whether the accumulation of Gb3 leads to metabolic changes through mitochondrial dysfunction, whether long-term high concentrations of Gb3 can have neurotoxic effects, or whether Gb3 accumulation can lead to activation of inflammatory states within the neuron that subsequently cause distal axon degeneration at the molecular level. It is not known when during the disease course the fiber loss begins to occur and peaks in severity, although distal fiber loss has been documented in patients in their twenties.[177]

3.1.1.3. Fiber types affected

Nerve fiber conduction can be abnormal in peripheral neuropathies and correlate with sensory deficiencies. Studies of patients with Fabry disease indicate that there is minimal impact on nerve conduction in large-diameter, myelinated Aβ-type fibers, but there are deficits in small fiber function.[65,180,182,211] The evidence for this is the finding that the maximal speed of large-diameter myelinated sensory fiber conduction is normal, whereas the minimal conduction velocities of slower unmyelinated small fibers is lower than in healthy patients.[43,122,201] There is disagreement about the small Aδ- or C-fiber abnormalities that are responsible for transduction of noxious thermal and mechanical stimuli. Some investigators have found impairment in C- fibers[141] including increased conduction velocities in mechanically and heat-sensitive C- fibers,[146] whereas others have observed decreased activation thresholds, increased hyperpolarizing currents, and axonal hyperexcitability to stimuli.[57] Pain-related evoked potential testing is a clinical diagnostic tool that measures the response of nociceptive fibers, specifically Aδ fibers, through depolarizing the superficial layers of the skin where these fibers are located.[100] The pain-related evoked potential response is believed to be indicative of Aδ-fiber function. As such, decreased amplitudes of Aδ-fiber action potentials have been found in patients with Fabry disease, suggesting an impaired function of these fibers.[182,183,209] Although these abnormalities have been found in sensory fibers, there are little to no impairments found in sympathetic fibers in the

skin, which mediate sweating reflexes.[43,76,109,122,141,146,182] Although cutaneous sympathetic responses are relatively normal for most patients, some studies have shown reduced or absent sympathetic responses in patients with Fabry disease.[65,94,222] The finding that sympathetic responses are normal despite the finding of reduced sweating in most patients suggests that there may be defects in sweat gland function in Fabry disease. Gb3 accumulation in sympathetic ganglia,[85,98] sympathetic denervation of the heart,[89,188,189,223] and reduced vascular tone[74,75,190] have all been observed in patients with Fabry disease and suggest that there are likely multiple defects in the sympathetic nervous system. In summary, it seems that there is defective function of cutaneous Aδ- and C- sensory neurons but not sympathetic fibers, in addition to the loss of small Aδ- and C-fiber sensory neurons. The reason for the heterogeneity in fiber function across patients has not yet been determined but it may be that there are genetic factors that predispose certain individuals to develop substantial small fiber dysfunction whereas other patients are resilient.

3.1.1.4. Ion channels

Ion channels regulate the activity of sensory neurons and neurons within the pain circuits. A number of ion channels have been implicated in neuropathic and chronic pain states.[15,203] Ion channels that have generally been associated with spontaneous pain include members of the voltage-gated sodium, potassium, and calcium channels as well as transient receptor potential (TRP), acid–sensing (ASIC), and hyperpolarization-activated cyclic nucleotide-gated (HCN) ion channels.[202] In diabetic peripheral neuropathy, a number of sensory neuron ion channels have been implicated in the development and maintenance of pain, including $Na_v1.7$, $Na_v1.8$, $Ca_v3.2$, TRPV1, and P2X3 channels.[199] Although there is currently no consensus on which ion channels contribute to Fabry pain in human patients, several have been implicated. One measure of nerve function is activity-dependent slowing, the slowing of action potential generation from nerve fibers as a result of repetitive electrical stimulation. Activity-dependent slowing has been associated with sodium channel function.[33,147,178] One study in patients with Fabry disease found no differences in nociceptive C-fiber activity-dependent slowing, thereby suggesting that sodium channel function and expression is normal in C- fibers from patients with Fabry disease.[146] Normal sodium channel function could indicate that the pathogenesis of pain in patients with Fabry disease is different from other small fiber neuropathies. Another study calculated the hyperpolarizing slope after subthreshold stimulation with electrical current in patient neurons; this is an indirect measure of potassium channel function. This study found increased inward rectifying potassium currents in Fabry patient nerves, and suggested that potassium channel abnormalities and increased sensory neuron depolarization correlated with pain severity.[57] Therefore, although there is some evidence of abnormal ion channel function in sensory neurons from patients with Fabry disease through in vivo recording methods; however, these data are derived from indirect measures and more experiments must interrogate ion channel deficits in Fabry disease and their contribution to pain.

3.1.2. Pain experienced in Fabry disease

Patients with Fabry disease experience both evoked and spontaneous pain which can be chronic or episodic in nature[207] (**Fig. 2**). These multiple pain phenotypes or syndromes manifest as sensitivity to thermal/mechanical stimuli, pain crises, pain after eating, and as ongoing chronic pain.[80,126,157,207] Interindividual pain heterogeneity is high, with some patients experiencing no

pain and others reporting debilitating pain requiring leave from work, school absence, and reduction in exercise.[83,103,182,207] On average, patients score a 2 to 3/10 for pain on a visual analogue scale,[7,83,126,184] and some patients describe intense pain episodes.[7] Pain initiation is primarily associated with exercise, ambient temperature changes, and fever, although pain can also be reported to be triggered by stress and shifts in weather.[157,207] By contrast, some patients have no discernible triggers for their pain.[157,207] Pain is primarily reported in the glove and stocking regions of the body, and localized in the fingers, palms, and soles.[201] However, high-intensity pain can encompass the legs, arms, abdomen, back, shoulders, joints, teeth, and head.[78,207] In addition, many patients with Fabry disease experience severe gastrointestinal pain, both nonevoked chronic pain between meals and episodic pain after eating that is associated with diarrhea, bloating, and early satiety.[80,90,126,152] Other pain experienced can include frequent headaches, priapism, and chest pain.[1,10,39,124] The initial onset of pain is between the ages of 3 and 16 years,[207] although pain can develop <1 year of age or in adulthood.[83,207] Therefore, there are both acute and chronic aspects of peripheral pain experienced by patients with Fabry disease that can be triggered by stimuli or occur spontaneously.

3.1.2.1. Neuropathic and inflammatory pain

Patients most often describe pain with neuropathic characteristics including burning, searing, squeezing, pressing, dragging, prickling, electrifying, tingling, sharp, and sore.[128,157,207,218] These characteristics together with reduced nerve fiber density[57] and the effectiveness of neuropathic pain medication[207] strongly suggest that pain is driven by neuropathy.[43,83,140] Acute pain can be managed with a variety of anti-inflammatory medications, and anti-inflammatory treatment encompasses most medication used to treat the acute Fabry pain.[57,207] Increases in proinflammatory cytokine mediators in the immune system including TNF-α, IL-1β, TLR4, and IL-6 suggest that inflammation potentially contributes to Fabry pain.[51,210] Therefore, Fabry pain may encompass both neuropathic and inflammatory components.

3.1.2.2. Evoked pain

Evoked pain can be triggered by stimuli that are not painful in healthy individuals. There are conflicting reports regarding whether evoked pain is increased or decreased in patients with Fabry disease. One study shows that between 31% and 45% of patients reported pain evoked by brushing, pressure, or cold.[182,201,207] Evoked pain was the most common pain form, and 60% of these patients reported evoked pain episodes occurring in frequency between once a month and 4 times each week.[207] By contrast, other researchers showed a decrease in hyperalgesia with application of topical capsaicin cream and subsequent mechanical stimulation.[142] Temporal summation is a signal integration phenomenon whereby subthreshold signals from a presynaptic neuron are integrated to elicit transmission in postsynaptic neurons. Therefore, pain induced by repetitive stimuli is perceived to increase. This study on a small population of patients also showed significantly reduced temporal summation of pain.[142] Together, this suggests that some patients experience decreased mechanical sensitivity, but many patients suffer from allodynia and mechanical hypersensitivity.

3.1.2.3. Pain crises

Pain crises are distinct from chronic or evoked pain because they are spontaneous or triggered by exercise, fever, exhaustion, or changes in ambient temperature and are often debilitating.[63,207] Pain crises begin anatomically in the extremities and spread to larger, proximal body regions.[207] Studies report that around 10% to 30% of patients have pain crises, with higher prevalence in male patients than females.[63,182,207] Pain crises are reported to occur between 4 times a month to once a year, lasting hours to weeks, but some patients experience several crises a week.[201,207] A small proportion (<10%) of patient pain crises have no predictable frequency of occurrence.[144,207] Therefore, although less prevalent than other forms of pain, pain crises are a major morbidity to patients with Fabry disease.

3.1.2.4. Chronic pain

Patients with Fabry disease report chronic pain (ongoing pain lasting >3 months) mostly in the hands and feet, but some have pain in the legs, shoulders, abdomen, back, head, and joints.[207] Most studies suggest that 12% to 40% of patients report chronic pain.[182,207] Regardless, chronic pain in patients with Fabry disease is not as prevalent as evoked pain or pain crises.[39,63,182,207] One study reported temporal variability in chronic pain with some patients' pain being constant and tolerable but severe a few days each week, or constant but subsiding a few days each week.[144] It is unclear whether chronic pain develops shortly after the onset of Fabry pain or whether acute pain transitions into chronic pain over time.

3.1.3. Sensory abnormalities in Fabry disease

In addition to chronic and acute pain, patients with Fabry disease can also experience abnormalities in the sensation of thermal and mechanical stimuli at both noxious and nonnoxious intensities (**Fig. 2**). Patients endure decreased tolerance to environmental heat due to poor thermoregulation and defects in sweating.[83,126,145,201] Both heat and cold can trigger pain episodes,[126,157] although heat is a more common trigger with one study reporting 62% of a 40-patient cohort indicating heat as a trigger for their pain.[157] There are conflicting reports regarding heat and cold sensitivity and pain thresholds. Several studies observed hyposensitivity to warmth in patients. An average healthy person can detect a thermal change of 1°C,[191] but patients with Fabry disease required a 2 to 5°C deviation to perceive a warmth sensation.[122,146] However, one study found that patients with Fabry disease experience hypersensitivity to warmth from mild-heat 40°C handheld heated metal rollers, suggesting patients had a lower threshold to heat.[201] Similarly, patients require a colder stimulus to sense cold compared with healthy controls,[109,122] although some patients reported cold hypersensitivity.[201] Overall, thermal hyposensitivity is more pronounced in the feet than in the hands.[122,146] Interestingly, some patients report paradoxical sensation of cold where they describe a cooling stimulus as warm or hot.[123,201,208,209] Although there are conflicting data, quantitative sensory testing data generally indicate impairment in patient sensation of warmth and cold.[193] However, when patients are exposed to hot or cold environments, severe pain can be triggered. This differential effect of temperature on normal thermal perception and pain is consistent with the observation that changes in warm and cold detection thresholds do not always correlate well with patient pain experience.[220] It is unclear if thermally evoked pain caused by environmental temperature changes or fever is caused by peripheral nerves that exhibit aberrant thermal sensitivity or is due to a completely different mechanism. Differences in patient sensitivity to thermal stimuli may be due to the sex of the patients tested and/or the number of patients using ERT because ERT may improve small fiber function.[13,79]

Several studies report hypersensitivity to mechanical stimuli including allodynia.[182,201,207–209] Wind-up is a phenomenon whereby repeated application of a painful stimulus becomes more painful over time. Mechanical pain is associated with increased wind-up, a quantitative sensory measure of central sensitization, and is reported to be significantly higher in patients with Fabry disease.[123] However, as with heat and cold sensation, some patients describe decreased sensitivity to mechanical pain, including increased touch pain thresholds, pressure pain threshold, and vibration thresholds.[121,122,182,201,209] Although primarily located in the feet and hands, increased sensitivity to mechanical stimuli has been reported over the whole body.[201] Heterogeneity in thermal and mechanical responses could arise from severity of the disease in the patient and the where the patient is in the course of the disease. Hyposensitivity to warmth, cold, and touch is consistent with small fiber neuropathy because polymodal C fibers and Aδ thinly myelinated fibers that transmit cold sensation, as well as heat and mechanically sensitive C-fibers that transduce noxious heat and gentle touch are lost.[24,125,146,151] The mechanisms underlying mechanical hypersensitivity are still to be elucidated in human patients with Fabry disease. Although hyposensitivity to thermal and mechanical stimuli arises from fiber damage and loss, hypersensitivity to mechanical and thermal stimuli could arise from abnormal glycosylation of mechanically and thermally gated ion channels in the remaining small fibers. For example, it is possible that Piezo2 mechanically-sensitive channels[36] or TRPV1 channels that sense heat[26,27] could be abnormally glycosylated. However, these hypotheses remain to be tested.

3.1.4. Pain impact on daily function

Fabry pain can directly impact patients' ability to function and perform normal tasks including eating, exercise, and learning. Fabry pain often begins in childhood and can include gastrointestinal pain, which can be associated with eating.[80,126] This pain may be one factor that can lead to changes in body weight. In addition, other symptoms such as early satiety, nausea, and vomiting can also influence eating habits.[126,144] In one study, researchers found that male children with Fabry disease decreased in average weight percentile over childhood, but female patients, by contrast, are consistently between a normal 55th to 65th percentile.[83] Pain and heat intolerance in patients with Fabry disease affect their physical activity.[22,126,201] Several studies have reported that many patients with Fabry disease have moderate to severe impairment of mobility and ability to do physical activities due to interference from pain.[7,144,201] In addition, patients report high rates of fatigue and poor sleep quality.[22,63,103,144,164] Patients with Fabry disease experienced substantially higher rates of depression (27%–57% of patients) compared with the general population (7%–27%).[7,28,49,72,103,164,204] Depression rates also correlated with pain intensity.[164] Two studies found that 9% to 16% of the patients were taking medication for depression, which is higher than the proportion estimated in the general U.S. population.[28,103,120,164] As pain intensity increases, it progressively interferes with mood and general enjoyment of life.[144] In addition, a study found that approximately 32% of patients with Fabry reported anxiety,[7] which is higher than the 8% in the general population.[204] Moreover, mild cognitive impairment has been observed in some patients with Fabry disease[57,103]; however, it is not known whether this impairment correlates with pain patients. Overall, patients with Fabry disease report that pain both directly and indirectly disrupts their daily function and severely impairs their quality of life.

3.1.5. Demographics and pain

There are 2 types of Fabry disease, classic (most prevalent) and nonclassic (or atypical) Fabry disease. In addition, there are almost 1000 identified mutations in the GLA gene resulting in varying degrees of α-Gal A activity.[35] Some mutations have occurred in multiple families and are associated with the classic form (p.R112C, p.R342Q, p.R220X, p.R227X, and p.R342X[59]) of the disease or the nonclassic form (p.A143T, p.F113L, IVS4+919 G>A, p.N215S, p.296I, p.G328R, and p.R301Q[9,34,59,171]). Minimal activity of the enzyme leads to the classic form of the disease.[8,171] The classic form is more severe with damage to multiple organ systems and pain that begins early in life.[8,58] Nonclassic Fabry disease tends to involve single-organ systems and have less severe symptoms.[19] Both pain and sensory abnormalities are less pronounced in patients with nonclassic mutations.

Age affects pain and, subsequently, the quality of life of patients with Fabry disease. The majority of patients with Fabry disease experience lifelong pain,[158,207] but patients commonly report less severe pain as they get older.[144] However, 2 studies of children younger than 18 years and adults older than 18 years found that pain at its worst was similar between the 2 age groups.[7,83] This may suggest that pain in patients with Fabry disease is often lifelong, but some patients experience decreased pain intensity as they get older.

Fabry disease is known to be inherited in an X-linked fashion, which would affect primarily homozygous females and hemizygous males. However, heterozygous females also experience neuropathic and abdominal pain in Fabry disease[55,67,201,215,219] despite having higher α-Gal activity levels.[213] Interestingly, women describe similar types of pain that can be more intense than reported in males.[7,62,103,164] However, on average, most studies have found that women report less severe pain[103,182,207] and sensory abnormalities[20] than men. There is generally a lower percentage of women who experience lifelong pain and a higher percentage who do not report any pain symptoms.[182,207] Both men and women exhibit neuropathy as well as thermal and mechanical sensation abnormalities.[182,201] However, women on average have been reported to have less severe thermal sensory impairment.[182] Therefore, sex is an important attribute that affects pain severity and the resulting quality of life in patients with Fabry disease.

Fabry disease is a pan-ethnic disease but there have been no studies comparing pain and ethnicity. A few studies reported on pain in specific ethnic groups including Caucasian,[46,83] Hispanic,[127,164] Dutch,[103,213] and German.[182,207] Pain incidence was highest in Hispanic populations from Latin America (70%) with Caucasian, Dutch, and German populations showing a prevalence of pain typically between 50% and 65% of patients.[126,164] The average pain intensity reported by patients was similar between ethnic groups.[7,83,103,207] Therefore, all types of pain combined, the majority of patients with Fabry disease experience pain regardless of their ethnicity. However, there may be ethnic pain differences, and this should be further explored because identification of populations more at risk for Fabry-related pain could provide insight into genetic and environmental factors that contribute to this pain.

3.1.6. Therapeutic alleviation of pain in Fabry disease

Fabry disease has a wide range of clinical manifestations including pain, renal deficiency, arrhythmia, myocardial infarction, and stroke, among others.[19] These broad clinical manifestations can make diagnosis difficult for physicians, and patients with

Fabry disease are diagnosed years after the onset of symptoms.[46,150] This leads to a high fraction of patients who are not treated until later in life.[46,83] Therefore, Gb3 may accumulate for decades before the start of the therapy, causing long-term damage to sensory neurons. In addition, many studies in pain may be confounded by the heterogeneity of therapeutics patients are receiving.

Many pain medication regiments are used to treat patients with Fabry disease. Neuropathic pain is treated using a variety of medications including tricyclic antidepressants, serotonin-norepinephrine reuptake inhibitors, and anticonvulsants.[78,207] These medications include carbamazepine, phenytoin, amitriptyline, gabapentin, pregabalin, duloxetine, and venlafaxine.[154,159,207] Opioids or opioid-like medications such as morphine, tramadol, and tilidate have also been used to treat acute pain, although opioid prescription frequency is low.[154,207] Acetaminophen, ibuprofen, metamizole, acetyl salicylic acid, and naproxen are more commonly used to treat acute pain.[207] The sodium channel blocker lidocaine administered intravenously was also used to treat acute pain crises.[154] Reports indicate that approximately a quarter of patients take medication to manage their pain[207]; however, 2 studies reported that a large proportion of patients also use nonpharmacological means such as avoiding pain triggers, thermal compresses, floatation-restricted environmental stimulation techniques, resting, and diet to better manage their pain.[52,84,112,207] It is unclear if patients who are not currently on medication have tried medication and experienced minimal benefit or if patients have never taken these medications. Collectively, several pharmacological and nonpharmacological treatments can reduce pain, but not all patients experience relief from pain despite having tried many treatments. Therefore, further research in pain management strategies in patients with Fabry disease is needed.

Enzyme replacement therapy has been the standard of care for patients with Fabry disease with renal and cardiovascular symptoms since the early 2000s in the United States with 30% to 40% of patients on ERT[7,207] (**Fig. 3**). Enzyme replacement therapy reduces circulating Gb3 levels, and severe neuropathic pain is a criterion for initiating treatment with ERT.[18] Although ERT has beneficial effects on the lifespan and renal disease, its efficacy in relieving pain is unclear. Some studies find small improvements in pain after treatment[13,21,40,68,79,156,168,170] and other studies do not report significant changes.[5,48,87,88,155,208,213] In one study, administration of ERT led to reduced numbers of male patients with abnormal vibratory, cold, and heat pain thresholds possibly indicating reversal of sensitization to the

sensory system, but this study lacked a placebo control.[73] In an initial safety study, both ERT and placebo decreased pain, thus indicating a large placebo effect.[47] Improvements in numerical pain rating are less than one point in several studies and about 10% of patients reported having worsening pain with the treatment, a rate similar to other studies.[47,173,207] Reports indicate that gastrointestinal symptoms, intraepidermal nerve fiber density, and quality of life are not always improved with ERT.[144,162,172] In addition, quality of life did not show correlation with duration or when in the patient's life ERT was first administered.[7] Enzyme replacement therapy is expensive. It is estimated that ERT increases time without end-organ damage by 1.5 years but costs $13 to 14.5 million over the patient's life.[162] Many patients also develop antibodies to the therapy, which preclude them from continuing treatment.[114] Although ERT may not be fully efficacious in treating neuropathic pain in all patients with Fabry disease, a subpopulation of patients such as males with the classic form of the disease may realize improvements in pain and quality of life due to ERT.[73] In addition, there may be some short-term pain relief with ERT infusion, but this does not always persist.[157] Although ERT can reduce damage to kidneys, cardiovascular system, and increase the lifespan, pain and patient quality of life are not always improved.

Galafold (migalastat) has recently been approved by the FDA for treatment of Fabry disease.[204] This drug is a small molecule chaperone that can stabilize α-Gal A in patients with residual enzyme activity.[61] Galafold has been shown to improve gastrointestinal symptoms in patients including abdominal pain.[60,169] These improvements may be more pronounced in the gut because this drug is administered orally.[204] Patient pain severity and quality of life metrics over 2 years did not significantly improve or worsen with Galafold treatment.[60] Therefore, Galafold may show promise in treating gastrointestinal symptoms, but further study is needed to determine if Galafold may improve or stabilize other pain metrics such as pain crises and chronic pain.

3.2. Preclinical models used to study pain in Fabry disease

Several preclinical models are used to study pain and test therapeutic agents. There are no naturally occurring animal models of Fabry disease as are observed with other lysosomal storage diseases such as mucopolysaccharidosis and Pompe disease.[96,185] However, researchers have used genetic manipulations, RNA manipulations, and administration of substrate to mimic human Fabry disease. Therefore, several models including mice, rat, and
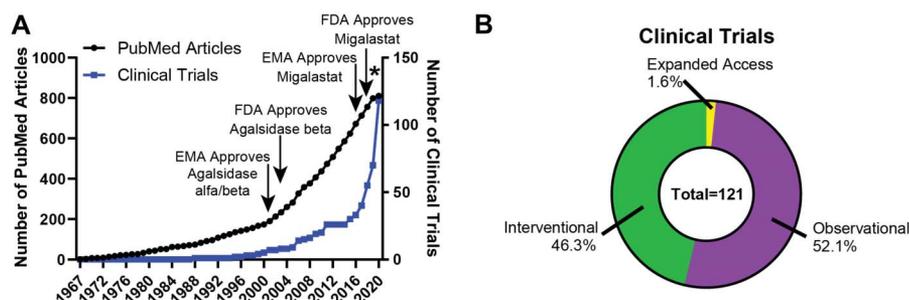
Figure 3. Research efforts: Publications and clinical trials for Fabry disease. (A) Cumulative summation of all PubMed articles (black) matching the search terms "Fabry" and "pain," "nerve," or "neuron" (not subject to screening in Fig. 1) and clinical trials listed on clinicaltrials.gov matching the "Condition or disease" "Fabry disease." Data from the PubMed search were collected on May 29, 2020, and data from clinicaltrials.gov were collected on June 22, 2020. *Indicates that data collected for the year 2020 are only for the partial year. Currently there are 3 main therapies: Agalsidase alfa (Replagal), Agalsidase beta (Fabrazyme), and Migalastat (Galafold), approved by the European Medicines Agency (EMA) and U.S. Food and Drug Administration (FDA) to treat enzyme deficiency in patients with Fabry disease. (B) Clinical trials were stratified based on classification by the clinicaltrials.gov website. Interventional trials indicate patients were given one or more treatments, and observational trials indicate patients are assessed for biomedical or heath outcomes, but patients are not given a specific treatment. Expanded access indicates a clinical trial that may allow patients with serious disease complications to participate in the clinical trial, although they are not previously recruited (compassionate use).

even in vitro cell cultures of Fabry disease has been investigated to interrogate underlying mechanisms that may cause pain in patients with Fabry disease. The most popular model is in mice due to their low cost, availability of molecular reagents, and available genetic manipulations. However, studies have shown that the mouse Fabry model does not show the severity of symptoms that occurs in human patients with Fabry disease. In addition, human DRG have different populations of CGRP and P2X3R neurons compared with mice.[181] Fabry mice are bred to produce male and female mice that are GLA null, while in the Fabry rat model,[136] heterozygote female rats can be generated better replicating the genotypes possible in human patients. Due to limitations in the mouse models' re-capitulation of the Fabry phenotype, we as well as other groups have used a rat model[136] or in vitro cell cultures to interrogate mechanisms of nociception and pain. These models and their strengths and limitations are described below.

3.2.1. Modeling Fabry disease in mice

Two mouse models have been used to study Fabry pain. One of the simplest animal models uses C57Bl/6 mice with Gb3 injected directly into the paws of mice to induce a Fabry-like phenotype.[30] To date, this mouse model has only been published once in studying possible relevance to pain. This model recapitulated the mechanical hypersensitivity reported in some patients with Fabry disease and suggested that excessive Gb3 and lyso-Gb3 in peripheral tissues could affect and sensitize neuronal function.[30] Although this model showed some similarities to Fabry disease, the model is severely limited because it uses a single acute extracellular administration of Gb3/lyso-Gb3, whereas Fabry disease involves long-term chronic exposure of neurons to glycolipids and buildup of glycolipid products inside neurons that together leads to changes in gene expression, decreased metabolic heath, and cellular adaptation.

To recapitulate the chronic presence of Gb3/lyso-Gb3 levels and exposure to patient cells in the disease, Ohshima et al. developed a Fabry mouse model. This GLA knockout mouse model abolishes α-Gal A protein levels through deletion of a 1 kb sequence in intron III and exon III, which is located on the q22.1 region of the X chromosome.[149] This animal displays buildup of Gb3/lyso-Gb3 in the plasma and various tissues, and lipid inclusion bodies occur in cells of the kidney, liver, and DRG; however, accumulation of Gb3/lyso-Gb3 is lower than reported in human patients.[149] Although there is clinically relevant pathology at the molecular level, this model lacks key patient symptoms such as renal disease. In addition, there are conflicting pain and sensory abnormalities observed between studies using this mouse, making it challenging to study Fabry-related pain.[134,146,205] Because this mouse was produced from manipulation of J1 embryonic stem cells from 129/SvJ mice and subsequent mice are crossbred from C57BL/6 mice,[149] there is variability in the genetic background of the offspring, making it impossible to obtain strain-matched healthy control mice. In an effort to improve the model, the Fabry mouse was crossbred with a Gb3 synthase-overexpressing mouse to increase the accumu-lation of Gb3 throughout the mouse.[93,195] This mouse has a greatly shortened lifespan and severe pathology unlike what is observed in patients and has not been used to study pain behaviors.[195]

3.2.2. Rat model of Fabry

Rodent models have been shown to be resistant to kidney disease but there is evidence that some rat strains may be more susceptible to kidney dysfunction. We (Miller et al.) used a dark agouti rat model with knockout of α-Gal A to study Fabry disease. This model was generated through a CRISPR-induced deletion of a 47bp region of the GLA gene in exon 2, causing a premature stop codon, thus preventing functional protein from being produced.[136] Knockout rats have elevated Gb3 and lyso-Gb3 as well as clinical manifestations of Fabry disease including ocular defects, kidney dysfunction, cardiac dysfunction, lenticular opacities, and mechanical hypersensitivity.[136–138] To date, only one study has been published regarding pain-like behaviors in this model[136]; however, several other studies demonstrate relevant renal, vascular, ocular, and auditory dysfunction in Fabry rats.[135,137,138]

3.2.3. Modeling Fabry disease in vitro

In vitro models have gained popularity for studying mechanistic function of cells due to the ability to control and manipulate the extracellular environment, study specific cell types, and test human-derived cells. However, it is not known how molecular changes in isolated cells relate to their function in vivo or behavioral responses. Although these models can provide insight into cellular function, there are substantial concerns over whether these models reflect in vivo function and therefore in vitro models must be validated to in vivo findings. Models of Fabry disease have been developed in human embryonic kidney cells HEK and human neuroblastoma cells differentiated into cholinergic-like neurons.[81,99] In both cell types, α-Gal A RNA expression was knocked down using short hairpin RNAs. Both studies observed accumulation of Gb3 deposits.[81,99] A-Gal A–deficient HEK cells exhibited an altered sodium channel function, and the neuro-blastoma cells showed differences in proliferation and release of the neurotransmitter acetylcholine.[81] These cells are relatively easy to culture and manipulate; however, both HEK and neuroblastoma cells are phenotypically different from native sensory neurons and therefore are likely limited in modeling sensitization mechanisms in sensory neurons. However, in specific well-validated situations, these cells may provide a high throughput screening tool for drug discovery.

3.3. Pain manifestations in preclinical models of Fabry disease

3.3.1. Pathological findings in Fabry disease

Similar to humans, Fabry mice and rats show pronounced lipid inclusions in DRG neurons with corresponding increases in nociceptor cell diameters.[136,146] In mice, there are lipid inclusions in approximately 30% of neurons at 3 months of age and these increase to around 65% by 17 months of age.[12] Inclusions are also observed in the trigeminal ganglia, spinal cord, and brain of the mice as well as increase in caspase 3, a marker of cell stress and death, in DRG neurons.[81] Sensory neuron somata from Fabry rats are also reported to be more fragile to mechanical forces than those from wild-type animals.[136] Fabry mouse DRG have an increase in RNAs associated with lysosomal and ceramide metabolic process.[105] There is also enrichment of retrograde transport, ion channels, and G-protein-coupled receptor signaling pathways, which could contribute to sensiti-zation of sensory neurons.[105] However, most RNA changes are relatively small, between 1.2- and 2-fold increased, making it difficult to identify specific RNA targets for further analysis. Recent work in human DRG suggest that RNA profiles are different between humans and mice, making it difficult to draw direct

comparisons between the 2 species.[38,165,216] It is unclear if other models of Fabry disease improve translation to patients. However, although similar pathology of lipid inclusions is shown in rodents and humans, the direct functional ramifications of these inclusions is not fully known.

Fabry mice have a loss of nerve fibers in sites including the sciatic nerve, skin, and intestinal mucosa.[81,111,129,146] Nerve fiber loss can occur as early as 10 weeks in mice and declines with age.[129] Similar to humans, mice exhibit less fiber loss in the nerve bundle compared with the skin.[81] Fabry mice have similar motor nerve conduction velocities to that of controls and slower conduction velocities in C fibers.[146] However, unlike human patients, mice seem to have normal Aδ-fiber conduction velocities.[146] The normal response of Aδ fibers in the Fabry mice might account for the small and inconsistent thermal phenotype observed.

Ion channel function is altered in neurons from Fabry animals. Activity of these channels is determined by translational regulation of channel expression and ion channel conductance of ions through their pores. Changes in several ion channels that regulate neuron activity and response to environmental stimuli have been documented including voltage-gated sodium channels (including $Na_v1.3$, $Na_v1.8$, and $Na_v1.9$),[81,110,146] voltage-gated potassium channels (including Kcnb2),[105,146] voltage-gated calcium channels(including Cacna1h),[30,105,146] and TRP channels in Fabry rodents (including TRPV1 and TRPA1).[105,110,136] Sodium channel current is decreased in Fabry mouse DRG and $Na_v1.7$-expressing HEK cells,[81,146] and it is unclear if RNA regulation may contribute to those changes.[81,105,110,146] Although decreased $Na_v1.7$ conductance could contribute to hyposensitivity of neurons to stimuli, it is unclear if sodium channels have a role in the development of human Fabry pain. Voltage-gated potassium channels regulate repolarization of the neuron after action potential firing. Potassium channel currents showed similar current densities between Fabry and control neurons in Fabry mice, but exhibit a higher voltage for activation.[146] Expression of the potassium channel subunits is inconclusive.[105] A higher activation voltage of potassium channels could indicate prolonged action potential duration, but this has not yet been demonstrated. Voltage-gated calcium channels display increased current density in Fabry mice, but similar activation voltages as in control mice.[30,146] Neurons from C57BL/6 mice treated with lyso-Gb3 have significantly increased calcium influx and more neurons responding compared to vehicle-treated cells.[30] This provides evidence that lyso-Gb3 may be able to modulate calcium influx, although it is currently unknown which channels are involved. However, it has not been demonstrated whether lyso-Gb3 directly acts on calcium permeable channel(s) to cause them to open or whether lyso-Gb3 merely sensitizes the calcium channel(s). Further experiments identifying what calcium permeable channels lyso-Gb3 act on could be an important step in understanding lyso-Gb3 effects on calcium channels. It is also conceivable that long-term exposure to Gb3/lyso-Gb3 both intracellularly and extracellularly can result in modifications to ion channels affecting their function. However, the relative contribution of extracellular Gb3/lyso-Gb3 and intracellular Gb3/lyso-Gb3 along with lysosomal dysfunction is unknown. More research into mechanisms of how these channels become sensitized will be critical to understanding the molecular basis of neuron dysfunction in Fabry disease.

Transient receptor potential channels mediate sensation of heat and cold, and TRPA1 can also contribute to mechanical sensation.[44,115] Fabry mouse neurons respond less than controls to temperature changes, a finding that is consistent with decreased sensation of warm stimuli in human patients.[146,160] Although TRP channels that mediate nonnoxious heat stimuli have not been investigated in Fabry disease, TRPV1, which mediates noxious heat sensation, shows similar expression levels and function in both Fabry mice and rats,[81,136] although some groups have reported increases in TRPV1 and Cdk5, a TRPV1 modulator.[105,110,111] TRPM8 mediates cold sensation and expression is decreased in Fabry mice, which could parallel the loss in cold-sensation experience by humans.[110,111] The proportion of TRPA1-sensitive neurons has been shown to increase in Fabry rats, possibly indicating sensitization of this channel in these animals.[136] In addition, treatment of rats with a TRPA1 inhibitor decreased tactile and pinprick responses in Fabry animals, indicating a functional role of TRPA1 in mechanical Fabry pain.[136] Finally, acid-sensing ion channels (ASIC) sense changes in pH and have been associated with several pain states including neuropathic and chronic pain.[186] Gb3/lyso-Gb3 treatment of HEK and mouse hippocampal and cortical neurons led to significant upregulation of ASIC1a.[25] Together, these findings indicate that sensory neuron ion channel expression and sensitization could contribute to the abnormal mechanical and thermal sensation in patients. Preclinical models provide insight into possible pathological mechanisms of Fabry disease in humans, but definitive and confirmatory studies in humans or human-derived tissues remain to be conducted (**Table 1**).

3.3.2. Pain sensation abnormalities in Fabry animals

The considerable pain and treatment heterogeneity in the Fabry patient population poses a significant complication in understanding the underlying causes of Fabry pain and how to adequately model it in preclinical models. Although thermal pain and mechanical-evoked pain have been assessed in Fabry mice and rats, it is unknown whether these animal models exhibit abdominal pain, pain crises, or chronic pain (**Table 1**). Although animal models show evoked sensitivity to mechanical stimuli, there is insufficient evidence that thermal stimuli evoke similar responses as reported in patients.

Fabry mice show conflicting results regarding responsiveness to heat. Two studies found that Fabry mice have increased sensitivity to radiant heat,[160,205] but other studies observed decreased sensitivity to heat in these mice.[146,160] In addition, a

Table 1

Summary of pain in Fabry humans, rodents, and in vitro models.

	Human	Mouse	Rat	In vitro cells
Gb3 deposits	Yes	Yes	Yes	Yes
Neuron swelling	Yes	Yes	Yes	???
Ion channel abnormalities	Yes	Yes	Yes	Yes
Abnormal small fiber conduction	Yes	C-fibers	???	???
Pain crises	Yes	???	???	N/A
Chronic pain	Yes	???	???	N/A
Abdominal pain	Yes	???	???	N/A
Heat/cold intolerance	Yes	???	???	N/A
Hyposensation of heat/cold	Yes	Unclear	No	???
Mechanical hypersensitivity	Yes	Unclear	Yes	???
Pain-depressed phenotype	Yes	No	???	N/A

???, unknown; gb3, globotriaosylceramide; N/A, not applicable.

similar ambiguity was observed in Fabry mice with some researchers finding small decreases in animal sensitivity to noxious cold, whereas others reported increased sensitivity.[110,205] Fabry rats exhibited no differences in response to heat or cold.[136] Differential responses to heat and cold indicate an inconsistent mouse phenotype. In light of these findings, further research is needed to understand if these discrepancies in thermal sensation in mice are due to heterogeneity in the population of animals tested or differences in experimental techniques between laboratories.

Hypersensitivity to mechanical stimuli has been shown in several animal models of Fabry disease. Injection of Gb3 or lyso-Gb3 into the paw of wild-type C57Bl/6 mice significantly decreased the 50% paw withdrawal threshold, indicating that a single acute peripheral exposure of sensory terminals to glycolipids/glycosphingolipids can increase the sensitivity to mechanical stimuli.[30] Therefore, in addition to intracellular changes that can result in hypersensitivity of neurons, these data suggest that circulating Gb3 and lyso-Gb3 can also result in sensitization of neurons from outside the neuron. However, the relative contributions of extracellular and intracellular glycosphingolipids to neuronal sensitization have yet to be determined. Similarly, in Fabry mice and rats, knockout animals showed lower paw withdrawal thresholds and shorter latencies to the mechanical stimuli compared with wild-type controls, suggesting that chronic mechanical sensitivity develops with the disease.[110,136,205] By contrast, some Fabry mice have been shown to exhibit hyposensitivity to von Frey stimulation.[146] This inconsistency in observation with Fabry mice along with small effect sizes observed and that von Frey stimulation is unlikely to be noxious makes it difficult to draw conclusions regarding the sensitivity of Fabry mice to evoked mechanical stimuli. Therefore, it may be necessary to characterize the responses of these mice with known noxious mechanical stimulation such as pinprick to the paw. Fabry rats showed increased nocifensive behaviors upon pinprick.[136] These data suggest that rodent models may be useful in investigating mechanical hypersensitivity to noxious mechanical stimulation, especially the Fabry rat model; however, it has not been determined whether these rodent models exhibit allodynia as is seen in human patients.

3.3.3. Motor, affective, and cognitive impairment in Fabry animals

Unlike humans, Fabry mice have not been shown to exhibit many pain-depressed behaviors (**Table 1**). Male mice display increased body weight with age, but females demonstrated no general weight change trend.[205] Male Fabry rats, however, showed a weight decline from wild-type rats, whereas female Fabry homozygotes showed an increase in weight from controls.[136] Although not completely consistent with what is reported in humans, Fabry rats more closely resembled the weight changes observed in human patients than Fabry mice. In studies of Fabry mice, no consistent differences in the voluntary treadmill running of mice have been observed, but mobility of animals in an SHIPRA behavioral screen was notably less compared to controls.[106,160,205] This could suggest that Fabry mice have decreased mobility in nonstrenuous walking as experienced by patients, but unlike humans, this does not affect exercise in mice. Studies in mice did not find depressive-like or consistent anxiety-like behaviors or abnormal learning and memory.[82] However, based on the low prevalence and degree of impairment in humans, these minor impairments may not be evident in rodent models. No studies to date have investigated sleep behaviors or evidence of fatigue in Fabry animals.

3.3.4. Age and sex effects on pain in Fabry animals

Age has a minimal effect on pain, sensation, and pain-related behaviors in Fabry mice. Mice displayed subtle increases in sensitivity to mechanical and noxious cold stimuli as well as decreases in wheel running activity as they aged, but these changes are not different from those observed in wild-type animals.[205] Stride angle widened in male knockout mice with age, but gait impairments are not reported in humans.[205] It has been established that Gb3 deposits accumulate in mice over time in neuronal tissues from an early age.[129] This has been shown to affect the cell size in a time-dependent manner in ganglia cells surrounding the intestines.[129] Interestingly, in the intestines, although there was a loss in innervating fibers, there was no progressive decline in nerve fiber density over time.[129] In Fabry rats, progressive accumulation of Gb3 in DRG has been documented.[136] Furthermore, when paw withdrawal responses to tactile stimulation were analyzed across ages, sensitivity does not develop until later in the rat's lifespan.[136] However, nocifensive responses to needle pricks exist at a relatively young age and persist.[136] This demonstrates that these animal models resemble aspects of lifelong pain that is observed in patients, but neither the rat or mouse Fabry models necessarily exhibit the decline in pain at older ages that occurs with patients.

Few studies stratify pain responses in male and female animals. One study showed similar paw withdrawal thresholds to von Frey stimulation as well as similar withdrawal latencies to noxious heat and cold in both male and female GLA knockout mice.[205] At older ages, male and female mouse treadmill running was similar.[205] In Fabry rats, both male and female rats exhibit mechanical but not thermal hypersensitivity. However, in heterozygous female rats, no mechanical or thermal phenotype was observed.[136] Heterozygous female human patients, however, do demonstrate a pain phenotype.[136] These data suggest that there are limited sex differences for pain in Fabry rodent models.

3.3.5. Therapeutic alleviation of pain in Fabry animals and in vitro models

In mice, HEK cells, and human fibroblasts, administration of ERT has been clearly shown to reduce Gb3 and lyso-Gb3 levels.[14,81,92] In mice, this occurs in many tissues including the liver, spleen, skin, kidneys, and DRG.[92] However, despite the use of the Fabry mouse model to validate retention of the enzyme and monitor its effectiveness clearing Gb3, researchers have not extensively assessed its impact on any pain or sensory abnormalities. Although in vitro models can provide information on effectiveness of Gb3 clearance and potential effects of ion channels,[81,99] these cellular models cannot predict the actual effect on pain or pain behavior. Therefore, more preclinical animal work that examines the effects of ERT and other therapies on pain in vivo is needed to better understand why ERT in patients is not as effective in alleviating pain as expected.

4. Discussion

Pain is a major factor in the life of many patients with Fabry disease and significantly impacts their daily tasks and reduces their quality of life.[144,207] Although evoked pain is common in patients, pain crises are the most debilitating acute pain experienced by patients.[207] Accumulation of Gb3 in the lysosomes of cells, including DRG neurons, causes inclusion bodies, enlargement of cells, and alterations in cellular function

and is accompanied by the development of pain.[30,63,194] Over time, this build up in glycosphingolipids leads to selective destruction of small sensory fibers in a length-dependent fashion in the patient limbs, beginning with the distal tissues.[182,201] This destruction of fibers leads to loss of sensory sensation including warmth, cold, and sometimes mechanical touch.[122,201,209] However, patients identify heat, cold, and mechanical touch as triggers of their pain.[157] In addition, patients report spontaneous pain and a smaller fraction have chronic pain over their lifetime.[207] It is unclear what cellular adaptations to the chronic accumulation of glycosylated products in DRG result in the hypersensitivity to mechanical and thermal stimuli, but it is likely that accumulation of Gb3 in the intracellular milieu and in the extracellular milieu induces alterations in ion channel function in sensory neurons.[57,146] Glycosylation patters are altered in the DRG of Fabry rats.[136] Changes in N-glycans have been shown to modulate neuron excitability and electrical properties.[71,176] Specifically, N-glycans have been shown to directly modulate sodium and potassium channel activity.[71,176] Therefore, we hypothesize that excessive glycosphingolipids can lead to aberrant glycosylation of ion channels in the Golgi and those glycosylation changes can increase ion channel activity leading to increases in pain. This hypothesis has yet to be tested.

Several other diseases associated with chronic pain also lead to loss of small fibers including diabetes, channelopathies, autoimmune disorders, exposure to chemotherapeutic agents, and viral infections.[50,66,77,116,197] Neuropathic pain, which is evident by patient descriptors of burning, tingling, and electric sensations, follows the presence of neuropathy.[50,192] Glove and stocking regions are commonly affected as in the case of diabetic neuropathy.[42] Patients with small fiber neuropathies experience episodic and spontaneous pain as well as hypersensitivity to mechanical stimuli and allodynia.[50,77] Interestingly, other small fiber neuropathies can lead to autonomic dysfunction causing temperature sensitivities and gastrointestinal symptoms that are similar to those that occur in Fabry disease.[116,197] Although the direct cause of neuropathic pain is still under investigation in several small fiber neuropathies, sodium channel alterations may contribute. Mutations in voltage-gated sodium channel subunits have been associated with increased excitability[2] of neurons, and chemotherapy-induced neuropathy shows upregulation of sodium channels.[117] However, there is limited evidence for sodium channel involvement in patients with Fabry disease. In diabetes, there is evidence of changes to both intracellular and extracellular signaling as well as lysosomal function.[42] Inflammatory mediators present in diabetic patients including TNF-α, IL-1β, and IL-6 can sensitize neurons and lead to neuron damage.[95] Neurons from diabetic patients also show lysosomal defects that interfere with normal autophagy and result in cellular stress, neurotoxicity, and apoptosis.[214] Similarly, in addition to lysosomal dysfunction,[31,108] immune cells such as peripheral blood mononuclear cells show altered signaling pathways and inflammatory molecules including TNF-α, IL-1β, IL-6, and TLR4 are found to be upregulated in patients with Fabry disease reporting pain.[166,210] Other pain syndromes including complex regional pain syndrome, fibromyalgia, and chronic pelvic pain syndrome show elevation in proinflammatory cytokines as well.[86,206,212] These similarities in inflammatory and lysosomal pathways should be explored further in Fabry disease to determine if they precipitate neuropathy and pain. Despite the similarities, diseases such as diabetes largely do not show evidence of the pain crises observed in Fabry disease. Sickle cell disease presents with vaso-occlusive events, which trigger debilitating pain crises in patients.[37] Similar to Fabry disease, patients with sickle cell disease also have chronic pain,

acute pain flare-up episodes, and neuropathic pain.[37,207] However, sickle pain crises are often located in the back or joints and patients describe them as throbbing and sharp,[37] whereas Fabry patient crises originate in the extremities and spread to other parts of the body and are usually described as burning.[207] Likely, these discrepancies in disease symptoms are due to dissimilarities in the mechanisms of pain development between diseases.

Lysosomal storage diseases including Gaucher disease, Pompe disease, Niemann–Pick disease, and Fabry disease have dysfunctions of different lysosomal proteins.[153] Although these diseases originate in the same cellular compartment, the pain presentation is diverse. Acute bone crises and chronic bone pain is common in Gaucher disease,[119,131] whereas back, leg, and shoulder pain that is characterized as exhausting, pulling, and dull is reported in Pompe disease,[69] and joint, limb, and abdominal pain is present in Niemann–Pick disease.[130] Although pain manifests differently, it significantly impacts daily activities of patients.[130,175] Gaucher disease has several similarities to Fabry disease including denervation of the skin, neuropathic pain in the hands and feet, hyposensitivity to thermal stimuli, and paradoxical thermal sensation.[41] However, a large fraction of patients with Gaucher disease have neuropathy that occurs in nerves regardless of the length of the nerve (non–length-dependent) that is not typically observed in Fabry disease.[41] Therefore, despite similarities, Fabry disease has unique manifestations including length-dependent neuropathy and pain crises in the extremities.

Preclinical models are critical to understanding pain mechanisms and circuitry and discovering and validating new therapeutic targets. However, differences in disease manifestation in these models compared with humans can impede drug development. Pathologically, mice, rats, and in vitro cell cultures show accumulation of Gb3 and inclusion bodies in the absence of α-Gal A as is observed in patients.[12,81,136] Similarly, in human, rats, and mice, these inclusions lead to swelling of DRG neurons.[53,136,146] Small fiber neuropathy is evident in both mice and humans.[81,209] However, changes at a behavioral level are not completely consistent between rodent models and humans. For instance, patients with Fabry disease show hyposensation to hot and cold stimuli,[182] but Fabry mice present with small inconsistent thermal sensation abnormalities and Fabry rats lack a thermal phenotype.[110,136,205] Fabry mice do not seem to exhibit the depression-like behaviors as reported prominently in humans.[82,164] Although there are deficiencies in rodent model behavior, some similarities exist. For example, both mice and rats have been shown to exhibit mechanical hypersensitivity and rats experience weight changes similar to patients with Fabry disease.[110,136,207] In addition, mice show similar functional defects in C-fibers.[43,146] Therefore, further characterization of rodent models and development of new models is needed to understand the pathogenesis of Fabry pain.

There are several areas that require further research both clinically and preclinically. Clinically, little is known about involvement of support and inflammatory cells such as Schwann cells, stromal cells, and glia in Fabry pain. There is evidence of lipid inclusions and cellular dysfunction in glia,[12] endothelial cells,[133,148] Schwann cells,[148,177] and skin cells,[12,157] which all can play a role in normal sensation and pain.[97,139,217] Alterations in immune cell inflammatory phenotype have been recently documented,[210] but further understanding of their involvement in acute and chronic Fabry pain is needed. In addition, sex and age have been explored as variables that modulate pain,[7] but other factors such as ethnicity and comorbidities have been largely unexplored.[103,133] The field would also greatly benefit from

transcriptional data from human DRG neurons from patients with Fabry disease to identify novel pain targets that could be vetted in preclinical Fabry models. Preclinical animal models would benefit from a better understanding of whether pain crises, abdominal pain, or chronic pain is present in current rodent models. Due to lack of sweating in rodents, it is possible that thermal triggers of pain may not be present in these models. Presence of chronic pain and pain crises would allow the field to use these animal models to assess therapeutic effectiveness on the treatment of these types of pain or pursue new preclinical models of this pain. There are several studies in Fabry mice and in vitro cell cultures that tested the effectiveness of therapeutics such as mRNA therapy,[228] substrate reduction,[14] and gene therapy[229] in the prevention of Gb3 accumulation and reduction in pathology such as kidney and heart function. However, these studies often do not assess therapeutic effects on sensory neurons and pain. Therefore, further exploration of therapeutic effects on afferent nerve fibers and neuron function would aid in assessment of these therapeutics' effects on pain.

Finally, because the mechanical phenotype in rodents is most similar to humans,[136,201,205] further examination of mechanically relevant ion channels could provide valuable information on the development of hypersensitivity in Fabry disease. There are a large host of mechanically responsive ion channels or channels that modulate mechanical responses. Specifically, in the DRG sensory neurons, Piezo1/2, TRPV1, TRPA1, ASIC1-3, and BNaC1α have been demonstrated to play a role in mechano-transduction.[56,72,107,143,161] Therefore, we hypothesize that identifying mechanically responsive channels that are sensitized in Fabry disease may provide insight into mechanisms of acute or spontaneous pain in Fabry disease. On the molecular level, this could be accomplished through measurement of ion channel expression, posttranslational glycosylation, activity, and changes in response after Gb3/lyso-Gb3 exposure. Identification of these mechanotransduction pathways would allow for design of therapeutics to target mechanically evoked pain.

In conclusion, preclinical models of Fabry have provided valuable information about gene expression changes, ion channel function, and alterations to nerve fibers and DRG neurons that are not easy or even impossible to obtain from patients. However, currently there is a basic lack of understanding regarding the basic pathogenesis of pain types in Fabry disease. These questions should be answered to develop more effective therapeutics. Although there are some translationally relevant models available for Fabry disease, these models have not been shown to comprehensively model all aspects of human Fabry pain and new preclinical models could fill in these gaps. Further development of human in vitro models of Fabry neurons and animal models are important to accurately understand the pathogenesis of the human disease. To be translationally relevant, future models should (1) ensure that the underlying characteristics of human neurons are phenotypically similar to the cells being observed in the model, (2) identify what aspects of the model recapitulate clinical findings in patients with Fabry disease or findings from Fabry patient tissues, (3) be well characterized so other researchers know the limitations of the model (negative data), (4) researchers should use multiple metrics to test the behavioral or molecular differences detected along with blinded analysis to ensure robustness of the observation, (5) when possible, researchers should have results verified by independent laboratories, and (6) researchers should not inflate the significance of positive results in preclinical models. In addition, the aforenoted characterization of the models should occur before researchers attempt to use them to test pharmacologic interventions for which pharmacokinetics and pharmacodynamics are evaluated. There can be barriers to publishing negative data or data characterization of model systems. However, we encourage researchers to do so because those results can lead to a consensus on the limitation and advantages of preclinical models as well as help increase productivity of the research community as a whole. Further examination of current models and development of new models will significantly advance our understanding of pain in this disease and aid in development of therapeutics for the patients with Fabry disease who suffer daily throughout their lives.

Conflict of interest statement

The authors have no conflicts of interest to declare.

Acknowledgements

Appendix A. Supplemental digital content

Supplemental digital content associated with this article can be found online at http://links.lww.com/PAIN/B235 and http://links.lww.com/PAIN/B234.

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