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Ion channels and pain in Fabry disease

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Abstract

Fabry disease (FD) is a progressive, X-linked inherited disorder of glycosphingolipid metabolism due to deficient or absent lysosomal α-galactosidase A (α-Gal A) activity which results in progressive accumulation of globotriaosylceramide (Gb3) and related metabolites. One prominent feature of Fabry disease is neuropathic pain. Accumulation of Gb3 has been documented in dorsal root ganglia (DRG) as well as other neurons, and has lately been associated with the mechanism of pain though the pathophysiology is still unclear. Small fiber (SF) neuropathy in FD differs from other entities in several aspects related to the perception of pain, alteration of fibers as well as drug therapies used in the practice with patients, with therapies far from satisfying. In order to develop better treatments, more information on the underlying mechanisms of pain is needed. Research in neuropathy has gained momentum from the development of preclinical models where different aspects of pain can be modelled and further analyzed. This review aims at describing the different in vitro and FD animal models that have been used so far, as well as some of the insights gained from their use. We focus especially in recent findings associated with ion channel alterations -that apart from the vascular alterations-, could provide targets for improved therapies in pain.

Keywords

Fabry disease (FD), ion channels, neuropathic pain

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Methods and aim

The articles included in this review were searched at the PubMed database. We aimed at detecting articles describing the use of preclinical models for the study of pain in FD to give an overview of the models available, and the information obtained from each, particularly concerning ion channels. To organize it, we structured the information in tables (dividing cell models from animal ones, Tables 1 and 2), and additional ones with information on specific channels, and parameters obtained for pain on tests performed (Tables 3 and 4). This work describes FD in relation to pain, and describes the tables to assess and compare the information on preclinical models between different publications, the advantages and limitations, and how the different studies aim at resembling some of the tests performed on patients.

An overview of Fabry disease

Fabry disease (OMIM 301500) is a very rare disorder. The prevalence of FD was previously estimated to be between 1:40 000 and 170 000, however, pilot newborn screening studies showed a higher prevalence of 1 in 3600.[1] FD belongs to the group of lysosomal storage disorders, i.e., inborn errors of metabolism characterized by the accumulation of undegraded macromolecules in lysosomes due to a deficiency of one of the lysosomal enzymes. In Fabry disease, there is a deficient or decreased activity of the enzyme α-galactosidase-A (AGAL-A; EC 3.2.1.22; abbreviated α-Gal A), as a result of a mutation in the GLA gene located on the X-chromosome (Xq22.1).[2] A large number of mutations

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have been reported so far. A tool constructed to help in this classification is the fabry-database.org that aims at covering all possible amino-acid mutations, updated manually as soon as enough data become available[3] FD is classified from early-onset severe 'classic' form to atypical, late-onset mild 'variant'.[4] Decreased or absent activity of α-Gal A leads to the accumulation of glycosphingolipids, mainly globotriaosylceramide (Gb3), in lysosomes of various cell types: endothelial and vascular smooth muscle cells, cardiomyocytes, kidney cells[5] and sensory and autonomic ganglia of the peripheral nervous system.[6,7] In addition, the deacylated form of Gb3, globotriaosylsphingosine (LysoGb3), is dramatically increased in plasma of "classically" affected male Fabry patients and plasma and tissues of Fabry mice.[8] Also, gender differences have been referred to in relation to pain in FD, and while the traditional view assumed females as carriers (ie, asymptomatic or mildly symptomatic), a more recent position suggests that the onset of Fabry disease symptoms in females may be comparable with that of males.[9]

Neuropathic pain, defined as that arising from a lesion or disease of the spinal cord and/or brain[10] is a key feature of the disease.[11] In this review, we will focus on the preclinical models, behavioral tests, and ion channel alterations that have been described in relation to pain in FD.

Pain in Fabry disease (FD)

Pain is one of the earliest clinical symptoms in Fabry disease (FD) reported by children and young adults, and even though some improvement may be obtained through enzyme replacement therapy (ERT), pain may still be present and require the use of adjunctive medication.[12] Two types of pain are generally described in FD: the episodic painful crises, also known as ''Fabry crises,'' characterized by agonizing burning pain starting in the extremities and radiating centripetally that may be precipitated by fever, exercise, fatigue, stress, or rapid temperature changes; and the second type is chronic pain characterized by burning and shooting pain in the hands or feet.[13] The definition of the type of pain is highly relevant to classify and decide on the therapeutic approach (see Politei et al.[12] and Schuller et al.[4] for pain treatment). Üçeyler et al. classified FD pain more precisely into 4 types: evoked pain, pain attacks, permanent pain, and pain crises as reported by patients.[14]

Pain in FD is assumed to be mainly neuropathic and involving the small fibers (SF) as patients have a predominantly length-dependent reduction in the density of small, thinly myelinated Aδ, and unmyelinated C-fibers. Fiber hypofunction with a preference for Aδ fibers is unique when compared with other small fiber neuropathies, as in diabetes, amyloidosis, and other diseases known to cause small fiber neuropathy. In the latter, C fibers and Aδ fibers are equally affected.[11] Studies have shown accumulation of glycolipids and loss of cell bodies in dorsal root ganglia.[11] General recognized mechanisms for neuropathic pain in Fabry disease are the patterns of spontaneous pain (shooting and burning) that indicate increased excitability of axons; degeneration of C-fibers and exaggeration of pain due to heat, that suggests peripheral sensitization; and burning pain after cold exposure that supports the nociceptive disinhibition by degeneration of Aδ fibers,[15] i.e., reduction of the effects of the descending inhibitory pathway.[16] Impairment of small fiber conduction increases heat and cold pain perception. In general, spontaneous types of pain in patients with FD may be explained by hyperexcitability of peripheral nociceptive neurons.[17] Other mechanisms that may be responsible for pain in patients with FD include spontaneous ectopic firing, altered pain modulation, or central nervous system sensitization.[12] Repeated bouts of peripheral neuropathic pain may sensitize the central neural pain matrix, such that all pain in the body becomes amplified.[12] Additionally, an inflammatory component in FD pain has been suggested by several reports[18] as well as the contribution of the endothelium in pain pathogenesis mechanisms.[19]

Preclinical models of neuropathic pain in FD

Different in vitro cell cultures and preclinical animal models have been used in the field of pain. We will give a summary of models available and used in the field of FD in in vitro cell models (Table 1) and animal models (Table 2) in general, to continue on the ones that have been predominantly used in pain in FD, described in detail (Tables 3 and 4).

The development of *in vitro* models may contribute to the discovery of promising drug targets that can be tested in future clinical trials. In addition, *in vitro* testing can reduce the duration and costs of translation to clinical trials by helping to identify the mechanism of action together with any associated risks.[20] The development of in vitro human disease models hinges on the availability of tissue- and organ-specific cell types that accurately recapitulate disease phenotypes. To date, most tissue engineering strategies rely on established cell lines (often transformed cell lines) or primary cells derived from patients. Human-induced pluripotent stem cells (iPSCs), which are derived from somatic cells by overexpression of a few transcription factors, can be generated from patients with or without a specific disease, and the resulting pluripotent cells can self-renew indefinitely or be differentiated into other specialized cells. Human

Table 1. Overview of preclinical in vitro models in FD.

Model	Description/ feature recapitulated from FD	Main findings	Reference
Mouse and human endothelial cells (IMEF: immortalized endothelial Fabry cell line)	Human FD vein endothelial cells and newborn's umbilical cord veins.	Receptor-mediated lipoprotein uptake: Gb3 accumulation in lysosomes.	Johnson and Desnick[23]
	Endothelial cell line from the umbilical vein of an aborted FD male fetus.	Deficient α-Gal A. endothelial cells as an alternative to fibroblasts in vitro.	Hasholt and Sørensen[24]
	Human umbilical venous endothelial cells transformed with a virus 40, tsA640.	Reduction of α-Gal A activity, without cell injury, and glycosphingolipid storage.	Inagaki et al.[25]
	Primary cultures of aortic endothelial cells from wild-type and Glako mice.	High globo-series glycosphingolipids in lysosomes; extended lifespan.	Shu et al.[26]
	Human telomerase reverse transcriptase introduced in FD hemizygote endothelial cells.	Reduced activity of α-Gal A and accumulation of Gb3 in lysosomes (IMEF1).	Shen et al.[27]
	Endothelial cells from skin biopsy from Fabry patients incubated with Gb3.	Oxidative stress and up-regulation of cellular adhesion molecules.	Shen et al.[28]
	IMFE1 cell line transfected with a plasmid which encodes α-Gal A.	Increase in α-Gal A activity up to 4-fold vs non-treated IMFE1 cells.	Ruiz De Garibay et al.[29]
Mouse and human bone marrow (BM)	FD Human BM CD341-enriched cells transduced with α-Gal A retrovirus	Increased α-Gal A activity, secretion and correction of lipid accumulation.	Takenaka et al.[30]
	FD mouse BM cells transduced with α-Gal A and human IL-2Ra chain (retroviral vector).	Multilineage corrected hematopoietic cells in transplanted animals.	Qin et al.[31]
Fibroblasts cell lines	FD Skin fibroblasts infected with human α-Gal A cDNA retroviral vector.	Secreted enzyme observed.	Medin et al.[32]
	Fabry fibroblasts with R301Q mutation.	DGJ (as inhibitor for α-Gal A) used: increased enzyme activity.	Jenkinson et al.[33]
	FD fibroblasts treated with recombinant α-Gal A and DGJ (as chaperone).	Synergistic effect between ERT and pharmacological chaperone therapy.	Porto et al.[34]
	FD fibroblasts. Comparative analysis of volume regulated anion channels (VRAC).	LRRC8A protein (constituent of VRAC) levels increased in plasma membrane of FD fibroblasts; other chloride channel levels unchanged.	Lakomá et al.[35]
	FD Fibroblasts treated with lucerastat (inhibitor of glucosylceramide synthase).	Lucerastat dose dependently reduced Gb3 in all cell lines.	Welford et al.[36]
	FD Fibroblasts from hemizygous male and heterozygous female patients.	KCa3.1 mRNA expression and currents impaired.	Oliván-Viguera et al.[37]
Fibroblast like (COS)	COS-7 and COS-1 cells transfected with an α-Gal A mutant plasmid.	In silico method to predict missense mutations in gene for α-Gal A.	Andreotti et al.[38]
Lymphoblasts	FD lymphoblasts treated with DGJ.	Molecular therapy 'chemical chaperons', DGJ (at subinhibitory concentration).	Fan et al. [39]
	Lymphoblasts from FD patients with 77 different mutations.	DGJ responses comparable to cultured fibroblasts with the same mutations.	Benjamin et al.[40]
Insect cells	Sf9 insect cells baculo-virus- transfected for the α-Gal A mutants (Q279E or R301Q).	thermostability decreased, with normal specific activities α-Gal A mutants.	Kase et al.[41]
Chinese hamster ovary (CHO)	CHO expressing α-N-acetylgalactosaminidase with α-Gal A like substrate specificity.	New enzyme for ERT with low possibility of allergic reaction.	Tajima et al.[42]
	Gene engineering screen in Chinese hamster ovary cells.	CHO cell lines enable systematic studies towards improving α-Gal A therapy.	Tian et al.[43]

(continued)

Table 1. Continued.

Model	Description/ feature recapitulated from FD	Main findings	Reference
Human embryonic kidney cells (HEK-293T)	CRISPR/Cas9-mediated GLA-knockout HEK-293T cells.	α-Gal A activity restored by MG132 proteasome inhibitor and α-Gal A.	Song et al.[44]
	HEK-293 cells with six hundred Fabry disease–causing mutations.	Clinically validated method to test migalastat treatment (as chaperone).	Benjamin et al.[45]
	HEK-293 cells treated with lyso-Gb3.	DNA damage of oxidative origin in purines and pyrimidines.	Biancini et al.[46]
	HEK293 cells treated with Gb3, LysoGb3 and DGJ (to inhibit α-Gal A).	Gb3 accumulation triggered ERK pathway via ASIC1a channels upregulation.	Salinas et al.[47]
Podocyte Cell Culture	A human podocyte cell line with knockdown of α-Gal A gene.	Immortalized cell line with α-Gal A activity reduction and Gb3 accumulation.	Liebau et al.[48]
	Immortalized human Fabry podocytes with α-Gal A gene edited by CRISPR/Cas9.	Low α-Gal A activity and decreased levels of Gb3.	Pereira et al.[49]
	Fabry podocytes treated with α-Gal A.	High Gb3 clearance, but deregulated signaling pathways unchanged.	Braun et al.[50]
Tobacco cells	Plant cell culture expressing Pegunigalsidase-alfa, a chemically modified stabilized version of the recombinant α-Gal A.	Reduced clearance and increased stability of α-Gal A (modified).	Kizhner et al.[51]
Renal epithelial cells	kidney tubular epithelial cell line with knocked down of α-Gal A.	Increased Gb3 levels, enlarged lysosomes, and accumulating zebra bodies.	Labilloy et al.[52]
	Urine-derived primary cells of FD patients.	Decreased activity and concomitant Gb3 accumulation.	Slaats et al.[53]
	Immortalized primary urinary cells from FD patients.	chaperone therapy not sufficient to all FD patients with low α-Gal A activity.	Lenders et al.[54]
Induced pluripotent stem cells (iPSC)	FD fibroblasts differentiated into cardiomyocytes treated with a glucosylceramide synthase inhibitor.	Prevented accumulation and increased clearance of lysoGb3 in cardiomyocytes. Expression profile of cardiomyocytes.	Itier et al.[55]Birket et al.[56]
	FD patients' peripheral blood cells differentiated into vascular endothelial-like cells expressing CD31, VE-cadherin, and von WF.	Excess Gb3 suppressed SOD2 (superoxide dismutase 2) expression, increased ROS production, enhanced AMPK activation, causing vascular endothelial dysfunction.	Tseng et al.[57]
	FD peripheral blood mononuclear cells differentiated into cardiomyocytes.	Low α-Gal A, cellular hypertrophy, GB3 accumulation, contractility impaired.	Chou et al.[58]
	Human ES cells differentiated into cardiomyocytes CRISPR/Cas9 GLA knocked out.	FD model that accumulated Gb3 with an increase in cell surface area.	Song et al.[59]
	FD fibroblasts differentiated into endothelial cells; GLA mutation corrected via CRISPR-Cas9 and Thrombospondin-1 deletion.	FD vascular endothelial cells dysfunction associated with overexpression of Thrombospondin-1 secondary to Gb3 accumulation.	Do et al.[60]
Neuronal	knockdown of α-Gal A in the human LA-N-2 cell line cholinergic cell line.	Specific reduction of α-Gal A activity and Gb3 and acetylcholine release.	Kaneski et al.[61]
	Mouse neurons (brain cortex and hippocampus) treated with Gb3, lysoGb3, and DGJ.	Gb3 accumulation triggered ERK pathway via ASIC1a channels upregulation.	Castellanos et al.[62]

Table 2. Overview of FD animal models.

Model	Description/ feature recapitulated from FD	Main findings	Reference
Caenorhabditis elegans	GANA-1 is a single C. elegans ortholog of both human α-GAL A and α-NAGA. Phylogenetic, homology modeling.	GANA-1 produced protein has dual enzymatic activity and is localized in an acidic cellular compartment.	Hujová et al.[68]
Rodents			
GLAko mouse	Disruption of the GLA gene by homologous recombination to obtain the GLAko mouse.	Mice are clinically normal at 10 weeks of age, although the kidneys exhibit similar lipid inclusions to those seen in FD patients.	Ohshima et al.[69]
NOD/SCID/Fabry (NSF) mouse congenic non-obese diabetic (NOD)/ severe combined immunodeficiency (SCID)	Obtained by backcrossing heterozygous GLAko Fabry females with NOD/SCID males.	Mice are deficient in α-Gal A enzyme, with absence of mature T and B cells. Reversed by transplantation of human hematopoietic cells (cDNA α-Gal A). Metabolic correction in spleen, lung, and liver. Significant increase in plasma α-Gal A activity and Gb3 reduction in the heart and kidney.	Pacienza et al.[70]
G3Stg/GLAko mouse	Crossbreeding GLAko mouse with transgenic mice expressing human Gb3 synthase.	Deficient α-Gal A activity and high Gb3 levels in major organs and serum. Gb3 level at 5–25 weeks higher than that in GLAko mice. Progressive renal impairment, with albuminuria at 3 weeks of age, decreased urine osmolality at 5 weeks, polyuria at 10 weeks, and increased blood urea nitrogen at 15 weeks.	Taguchi et al.[71]
Rats GLAko rat	GLA gene (disrupted exon 2) knocked out via CRISPR/Cas9 technology in Dark Agouti (DA) strain.	From 13 weeks, Gb3 storage in serum, brain, and dorsal root ganglia (DRG), neuropathic pain symptoms. Kidney and heart accumulate Gb3 and lysoGb3. Renal tubule dysfunction and mitral valve thickening. Corneal and lenticular opacities: ocular phenotypes to be analyzed as potential noninvasive indicators of therapeutic efficacy.	Miller et al.[72] Miller et al.[73] Miller et al.[74]
Non-human primates (NHPs)	Monkeys with intravenous administration systemic messenger RNA (mRNA) encoding human α-Gal A.	Production of a functional human α-Gal A in liver, secreted into the circulation, taken up by distal tissues (kidney, heart, spleen and targeted to the lysosomes via endocytosis). No anti human-α-Gal A antibodies after repeated administration.	Zhu et al.[75]

Table 3. Overview of Ion channels associated with FD.

Ion channel gene (protein)	Biological model	Main findings	Effect on pain behaviour	References
SCN9A (Nav1.7)	DRG neurons from WT or Glako mice and HEK293 cells	Reduced Nav1.7 current densities, with no differences in mRNA or protein levels, in old Glako mice DRG. Marked decrease in Nav1.7 currents in α-Gal A-shRNA-treated HEK cells; this recovered after α-Gal A incubation.	Protection from heat and mechanical hypersensitivity after intraplantar injection of complete Freund's adjuvant (CFA) in old Glako mice. Young (3 months) and old (≤12 months).	Hofmann et al.[6]
SCN10A (Nav 1.8)	Epidermis of frontal paw glabrous skin from WT or Glako male mice	Nav1.8 protein levels increased.	Mechanical hypersensitivity (Von Frey filaments test in 8 to 12 weeks old mice.	Lakoma et al.[100]
Na_V Tetrodotoxin sensitive*	DRG neurons from WT or Glako mice (from >18 weeks)	Conductance of TTX-sensitive currents decreased, mRNA levels unchanged.	Heat and mechanical hyposensitivity from altered excitability (Von Frey filaments and Hargreaves and hot plate test) in 20–24 week old mice.	Namer et al.[17]
K_V	DRG neurons from WT or Glako mice (from >18 weeks)	A-type and delayed rectifier currents decreased.	Heat and mechanical hyposensitivity from altered excitability (Von Frey filaments and Hargreaves and hot plate test) in 20–24 week old mice.	Namer et al.[17]
TRPV1 (TrpV1)	DRG neurons from WT or Glako mice. Young (3 months) and old (≤12 months)	TRPV1 protein (mainly observed in small-diameter neurons) increased in young and old Glako mice DRG neurons. No difference in TRPV1 gene expression between genotypes and age groups.	Heat hypersensitivity after intraplantar injection of capsaicin in old Glako mice (Based on the previous finding on heat hypersensitivity in young GLAko turning to hyposensitivity with aging; Üçeyler et al.[101]	Hofmann et al.[6]
	Epidermis of frontal paw glabrous skin from Glako male mice	TRPV1 protein levels increased.	Heat hypersensitivity to noxious hot thermal stimulation in Gla KO male mice (8 to 12 weeks).	Lakoma et al.[100]
	Primary cultures of DRG neurons from Glako male mice	TRPV1 protein levels increased.		
		In small type-C nociceptors, enhancement of the capsaicin-activated currents.	Heat hypersensitivity to noxious hot thermal stimulation in Glako male mice (8 to 12 weeks).	Lakoma et al.[35]
TRPM8 (TrpM8)	Epidermis of frontal paw glabrous skin from Glako male mice	TRPM8 protein levels decreased.	Glako males mice, (8 to 12 weeks age) less sensitive to cold stimulation (acetone application)	Lakoma et al.[100]
TRPA1 (TrpA1)	DRG neurons from Glako rat	Neurons were more responsive (sensitized) to mustard oil (channel agonist).	Mechanical hypersensitivity to supra-threshold stimuli and noxious force (von Frey filament and needle tests). Recovered with intraplantar injection of a channel antagonist	Miller et al.[72]

(continued)

Table 3. Continued.

Ion channel gene (protein)	Biological model	Main findings	Effect on pain behaviour	References
VGCC* (voltage-gated Ca2+ channels)	Primary cultures of DRG neurons from WT mice Incubated with LysoGb3	Lyso-Gb3 in clinical concentrations increased Ca2+ levels in capsaicin-sensitive small-diameter peptidergic neurons.	Gb3 or lyso-Gb3 administration induces mechanical allodynia in healthy 7-8 weeks mice (von Frey filament test).	Choi et al.[102]
	DRG neurons from WT or Glako mice (from >18 weeks)	Conductance of VGCC reduced (both, low and high voltage).	Heat and mechanical hyposensitivity from altered excitability (Von Frey filaments and Hargreaves and hot plate test) in 20-24 week old mice.	Namer et al.[17]
HCN2 (Hcn2)	DRG neurons from WT or Glako mice	Hyperpolarization-activated (Ih) current densities were reduced in DRG neurons from old GLAko mice compared with old WT mice. No difference in HCN2 mRNA levels.	Chronic constriction injury (CCI) at the right sciatic nerve of GLAko and WT littermates (≤12 months); old GLAko spared from heat hypersensitivity and mechanical withdrawal threshold.	Hofmann et al.[6]
ACCN2a (Asic1a)	Primary cell culture neurons and HEK293 cells incubated with Gb3, lyso-GB3 and DGJ (inhibiting α-Gal A)	Increased ASIC1a mRNA and protein levels. ERK ½ pathway activated, and prevented by blocking ASIC1a channels (Psalmotoxin-1).	In vitro model only	Castellanos et al.[62]
KCNMA1 (Kca 1.1)	Fibroblasts, primary cultures from skin punch biopsy of FD patients	Increased KCa1.1 mRNA and protein levels with lower current densities; incubation with α-Gal A increased KCa1.1 activity.	In vitro model only	Rickert et al.[103]
KCNN4 (Kca 3.1)	Mouse aortic endothelial cells from aged-Glako mice (MAECs).	Reduced KCa3.1 mRNA levels and current density in Gb3-treated and aged Glako MAECs by inhibiting the ERK/AP-1 pathway, up-regulating REST, and decreasing intracellular PI (3)P.	In vitro model only	Park et al.[104]
	Primary cultures of mouse aortic and human umbilical vein endothelial cells from aged-Glako mice (MAECs and HUVECs)	Exogenous Gb3 decreased the level of plasma membrane KCa3.1 via clathrin-dependent and EEA1-enriched endosome-mediated lysosomal degradation.	In vitro model only	Choi et al.[105]
	Cell culture Fibroblasts (NIH-3T3)	Decreased KCa3.1 mRNA level and current density by exogenous lyso-Gb3. This contributed to reduced myofibroblast differentiation and collagen expression.	In vitro model only	Choi et al.[106]

(continued)

Table 3. Continued.

Ion channel gene (protein)	Biological model	Main findings	Effect on pain behaviour	References
		Lyso-Gb3 inhibited KCa3.1 channel synthesis and surface expression by increasing intracellular cAMP, which inhibits ERK 1/2 phosphorylation through the PKA pathway, and by decreasing intracellular levels of PI(3)P.	In vitro model only	Choi et al.[107]
	Dermal fibroblast primary cultures from punch biopsies of FD patients	Impaired KCa3.1 gene expression and function in fibroblasts from hemizygous male FD patients.	In vitro model only	Oliván-Viguera et al.[37]

iPSCs potentially offer an unlimited supply of cells for tissue engineering, therapeutic discovery, and modeling of diseases that affect almost all human tissues or organs.[21] This is particularly interesting to understand the underlying disease mechanism and provide a cellular and molecular platform for developing novel treatment strategies.[22]

In vitro cell models (Table 1), initially, used cells obtained from FD patients especially of target organs in FD: endothelial cells[23–27,29] kidney cells[48–50,52–54] to determine glycosphingolipids accumulation and α-Gal A activity, and obtain lines for further research. Fibroblast[32,34] and bone marrow cells[30,31] were subjected to different techniques to incorporate an α-Gal A for enzyme expression, as well as for the analysis of different mutations present in FD patients. These cultures are also amenable to analyze the response to treatment, as illustrated with the use of DGJ[33,39,40] (an α-Gal A inhibitor which at submolar concentrations works as a chaperone for the enzyme), or to test inhibitors of glucosylceramide synthase (lucerastat)[36] an approach based not on the replacement of the defective glycosidase, but rather on the inhibition of an earlier step in the synthesis of the accumulating glycosphingolipid.[63]

Different established cell lines have also been used to analyze α-Gal A properties of mutant variants[24,41] as well as the mechanisms of action of glycosphingolipids accumulation.[27,46,47] Cell lines have also contributed to the development of improved enzymes.[42,43,51] Reprogramming technology is being applied to derive patient-specific iPSCs lines, which carry identical genetic information as their patient donor cells. The field of FD research has also benefited from the advent of iPSCs[55–58] and the CRISPR/CAS9-mediated genome engineering technology.[59,60] This technique facilitates site-specific DNA deletions, insertions, inversions, and replacements and thus shows therapeutic potential, and is an invaluable tool in establishing the causal relationship between genes and stem cell behavior.[64]

However, as the pain experience results from integrated pathways, all the way from sensory transduction in the periphery to perception in the brain, it is critical to study the system with each level of processing intact.[65] The use of intact animals enables the researcher to precisely manipulate physiological and pharmacological variables that allow examination of a pathway or circuit.[66]

Flies have contributed greatly to the field of genetic screening in association with pain. Transient Receptor Potential (TRP) channels were first identified phenotypically in flies and cloned in that organism. Acute nociception can be assessed in flies since the somatosensory system displays properties of sensitization. The model shows also a neuropathic-like state after an injury that involves permanent central disinhibition, as a model to

Table 4. Overview of behavioral tests in rodent models of FD.

Model	Behavioural test	Main findings	Others	References
Fabry KO (GLAko) mice (produced by disruption of the mouse **α-Gal A gene** (Ohshima et al.[69]*). α-Gal A **males**. **24 weeks and 48 weeks of age.**	**Hot Plate test** **SHIRPA** — protocol for phenotype assessment	**Thermal hypoalgesia**, Hind paw withdrawal was increased by 4 and 5 °C in GLAko mice compared with control mice of the 24 and 48-week oldgroup, respectively. Fabry mice (24 weeks of age) had **reduced locomotor activity** and time hanging on the wire (wire manoeuvre test). At 48 weeks of age, GLAko mice showed a significant effect on motor behaviour (locomotor activity), reflex and sensory function (pinna reflex), and neuropsychiatric state (transfer arousal, touch escape, and vocalization).	Histological analysis in GLAko mice: – sciatic nerve mean cross-sectional area increase accompanied by a decrease in the density of non-myelinated fibers. – trend for a decreased number of small myelinated fibers. relative preservation of large myelinated fibers and nerve conduction velocity.	Rodrigues et al.[124]
Fabry KO (GLAko) mice (*Ohshima) Heterozygous female mice, and wild-type (WT) (control) male α-Gal A (+/+). To obtain α-Gal A (-/0) hemizygous male mice, α-Gal A (+/-) female and α-Gal A (-/0) male mice were crossed. **Ages 8–12 weeks.**	**Hot Plate test** **Cold Sensitivity Acetone Test** **0° Cold Plate Assay** **Dry Ice-Cold Plantar Assay** **Von Frey up-down test**	**Thermal hyperalgesia**. Reduction in latency time in GLAko males. **Hyposensitivity to cold stimulation** of GLAko male (increase in latency time after an acetone application). The data from the cold plate experiments confirmed the **decreased sensitivity of GLAko males** to cold stimulus caused by 0°C when compared with control males. GLAko males showed a **decrease in cold thermal sensitivity compared to control mice.** **Decrease in withdrawal threshold** in FD (reaction time and force) to a mechanical stimulus (could be due to a hyperalgesic state).	GLAko mice showed a decreased and scattered pattern of neuronal terminations, consistent with the reduction in neuronal terminations observed in skin biopsies of patients with small fiber neuropathies. At the molecular level, GLAko animals showed increased expression of TRPV1 and Nav1.8 and decreased expression of TRPM8.	Lakomá et al.[100]
Fabry KO (GLAko) mice (*Ohshima) on **129/Sv** background. Males and females GLAko.	**Hot Plate test**	**Thermal hypoalgesia** GLAko mice had clear **deficits in thermosensation**, (higher latency periods). Progressive, age-dependent prolongation of the latency period. Mean latency period in GLAko mice greater than 60 seconds (12 months).	Lysosomal Gb3 inclusions increased with age in renal epithelial, intestinal, and vascular smooth muscle cells, and neurons in trigeminal and dorsal root ganglia); GLAko mice resemble type 2 later-onset FD. Gb3 accumulation in small intestine and sensory ganglia of GLAko mice: model for enteropathy and neuropathy in FD.	Bangari et al.[125]

(continued)

Table 4. Continued.

Model	Behavioural test	Main findings	Others	References
Fabry KO (GLAko) mice (*Ohshima) (89 male, 126 female), and 126 (80 male, 46 female) inbred naïve wild-type (WT) littermates of **C57BL/6J** background. Ages: 2 months up to their maximum life span of 27 months.	**Von Frey up-down test**	**Young and old GLAko mice were hypersensitive to tactile stimulation**. This hypersensitivity was independent of gender and age; thus, data was pooled.	GLAko mice with spontaneous pain behaviour: sitting on a wire mesh, and upon stimulation with a von-Frey filament, mice shifted their paws to the inner walls of the covering Plexiglas boxes and preferred keeping them on the glass surface. Also, mice tended to hold up their paws and toes while seated or while standing on their hind paws during exploratory behaviour; GLAko mice developed orofacial dysmorphism with aging, bearing similarity with patients with FD.	Üçeyler et al.[101]
	Dry Ice-Cold Plantar Assay	2-months old male, and young and old female GLAko mice were **hyposensitive to cold stimulation** compared with age-matched control littermates.		
	Hargreaves test (paw withdrawal)	**Heat hypersensitivity in young vs hyposensitive in old** GLAko **mice**		
	Gait analysis	Impaired gait with aging. Old male GLAko mice had a larger stride angle than young GLAko mice and control littermates. No differences in female mice.		
	Physical activity and body weight	Fabry mice were physically equally to less active but lost more weight during a one-week treadmill experiment. Young male and female GLAko showed similar physical performance as control littermates. Fabry mice ($\geq$18 months), fewer rounds per day. Male GLAko lost more weight with equal to lower chow intake; less weight loss in female GLAko mice at $\geq$18 months.		
Fabry KO (GLAko) mice (*Ohshima) Only males were used, **aged 8–12 weeks.**	**Hot plate test**	**Hypersensitivity to heat** (reduction in latency time) No change after administration of DD04107 peptide (inhibitor of neuronal exocytosis, thus inhibiting membrane recruitment of TRPV1).	Thermal hyperalgesia associated with an increased protein expression of TRPV1 in DRG nociceptors. TRPM8, and Nav1.8 expression unchanged.	Lakomá et al.[35]
Fabry KO (GLAko) mice (*Ohshima), **aged 20–24 weeks. Control used:** C57BL/6J.	**Von Frey test**	**Mechanical hyposensitivity**; in ex vivo skin–nerve preparation in mice, increased mechanical threshold for **Aδ fibers**; no differences in C fibers.	C-Fibers, especially heat-responsive, display higher conductance velocities. Mechanical Hyposensitivity of Aδ Fibers. Reduced excitability of cultured DRG neurons,	Namer et al.[17]

(continued)

Table 4. Continued.

Model	Behavioural test	Main findings	Others	References
	Hargreaves test (paw withdrawal)	Hyposensitivity to heat.	reduced conductance of Na_V, VGCC currents, with activation of K_V currents was at more depolarized potentials.	
	Hot plate test	Hyposensitivity to heat.		
Fabry KO (GLAko) mice (*Ohshima) inbred wild type (WT) C57BL/6N littermates age-groups of **young (3 months)** and **old (≥18 months)**. Also, CFA injected to assess inflammatory pain.	**The elevated plus maze (EPM)**	Longer time spent in open arms by young control mice compared to young GLAko mice. CFA injection induced anxiety-like behaviour in the EPM in GLAko and control mice.	The notion of a major genetic influence on neuropsychological symptoms cannot be supported by the study.	Hofmann et al.[132]
	Light-dark box (LDB)	No difference in the LDB, time spent and entries in the light and dark box between genotypes, age- and treatments; except for young control mice compared to CFA.		
	Open field test (OF)	Difference in time spent in the central zone of the OF only between young animals.		
	Forced swim test (FST)	GLAko and control mice spent similar times floating in the water basin.		
	Morris water maze (MWM)	Time spent until finding the hidden platform decreased from training day 1 to 4; the latency until first entry into the target zone was shorter for young vs old mice.		
Fabry KO (GLAko) mice (*Ohshima) on C57BL/6 background**. Young (3 months) and old (≥12 months)**; old mice reached 24 months. Injection of CFA to induce inflammatory pain.	**Von Frey up-down test**	All mice developed **mechanical hypersensitivity** 1 hour after **CFA** injection compared to baseline; **less pronounced in old GLAko mice**; and all mice remained mechanically hypersensitive until day seven after CFA injection, (GLAko and control).	Increased TRPV1 protein in DRG neurons and heat hypersensitivity upon i.pl. Capsaicin in old GLAko mice. In turn, GLAko mice are protected from heat and mechanical hypersensitivity; reduced neuronal Ih and Nav1.7 currents. In vitro α-GAL A silencing increases intracellular Gb3 accumulation paralleled by loss of Nav1.7 currents; reversed by incubation with agalsidase-A and lucerastat.	Hofmann et al.[6]
	Hargreaves test (paw withdrawal)	**Old GLAko mice showed heat hypersensitivity compared to baseline 24hr after capsaicin (**TRPV1 channel activator**). Intraplantar injection of CFA led to heat hypersensitivity in all mice groups except for old GLAko mice** in which heat withdrawal latencies did not change from baseline for the entire study period of seven days.		
Fabry KO (GLAko) mice (*Ohshima) on C57BL/6	**Hot plate test (conventional and incremental**	**Hyposensitivity to heat** in both strains of Fabry mice. The delayed response in Fabry-B6/129 appeared at as early as 1 month of	Different severity of disease phenotype; Fabry-B6/129 mice have earlier onset, more prominent	Jabbarzadeh-Tabrizi et al.[126]

(continued)

Table 4. Continued.

Model	Behavioural test	Main findings	Others	References
background and on sv129 background compared.		age In contrast, delayed heat response in Fabry-B6, at age 7 months. More severe in Fabry-B6/129. Also, different thresholds (46.3°C in WT-B6/129 ; 48.1°C in WT-B6).	cardiac and renal hypertrophy, and greater thermosensation deficit.	
Female and male **Dark Agouti rats** α-Gal A-deficient rats. Using CRISPR/Cas9 technology, Tests performed in **male and female rats 4 to 51 weeks,** at 7 different time points until 1 year of age.	**Von Frey up-down test**	**Decreased withdrawal thresholds** in GLAko males and females, highest at 51 weeks. Mechanical and noxious behavioural experiments with TRPA1 antagonist (HC-030031) to rescue mechanical hypersensitivity experienced by the control animals	Substantial serum and tissue accumulation of α-galactosyl glycosphingolipids and pronounced mechanical pain behaviour in GLAko rats. GLAko rat DRGs show global N-glycan alterations, sensory neurons with inclusions, and sensory neuron somata exhibited prominent sensitization to mechanical force associated with TRPA1 channels.	Miller et al.[72]
	Sharp noxious force (needle test)	Rats at 4 and 6 weeks: **No difference in response** (e.g., paw stomping, lifting, or licking). Aged GLAko males and females showed increased response frequencies to the needle test (at 10, 25, and 51 weeks in males; at 12, 38, and 51 weeks in females). A TRPA1 antagonist decreased the sensitization in GLAko rats compared with vehicle.		
	Dry Ice-Cold Plantar Assay	**No differences in dry ice reaction latency** (4–51 weeks), trend to cold hyposensitivity in 51-week-old GLAko male rats. No impaired thermal perception in less than 1-year GLAko rats.		
	Hargreaves test (paw withdrawal)	**No behavioural differences at all-time points**, including aged rats (52 weeks).		

analyze possible aspects of chronic neuropathy.[65] Worm and fish have been particularly instrumental for possessing a neural circuitry capable of nociception, behavioral avoidance, and signal modulation. Overexpression of gain-of-function mutations of $Na_V1.7$ in zebrafish sensory neurons led to decreased small fiber density and increased sensitivity to temperature changes[67] recapitulating hallmarks of small fiber neuropathy in patients.

Also, the introduction of mutations on nematodes has helped in the analysis of pain mechanisms using the noxious heat response of the organism.[65]

Since in humans, neuropathy commonly presents with a complex combination of different sensory signs and symptoms with the presence of multiple comorbidities, including anxiety, depression, and sleep disorders, these aspects are amenable for analysis using other animal models like mice, rats, and monkeys.

As far as preclinical animal models in FD are concerned, different models have been used (Table 2). The Drosophila model has been used in Gaucher disease, another lysosomal storage disease, with deficits of the lysosomal enzyme glucocerebrosidase. Flies generated by knocking the enzyme ortholog have been used to recapitulate the disease, and autophagosome deficits have been documented.[76] This model has not been used in FD so far.

In FD, studies using *C. elegans* revealed a single gene with homology to both human genes (α-galactosidase and α-N-acetylgalactosaminidase), and further analysis of the protein product detected a pattern of distribution compatible with lysosomal compartments.[68]

Different rodent models have also been developed. In 1997, disruption of the GLA gene by homologous recombination generated the GLAko mouse by Ohshima et al.[69] This mouse was crossed to other mice to generate a mouse line deficient in α-Gal A enzyme, with an absence of mature T and B cells to study transplantation of human hematopoietic cells expressing a cDNA for α-Gal A.[70] The GLAko mouse was also crossed to mice expressing human Gb3 synthase to obtain the G3Stg/GLAko.[71] This mouse generated higher amounts of Gb3 both in serum and the main target organs compared to the GLAko mouse and was thus proposed as phenotypically closer to classical FD. Recently, a GLAko rat model was generated using the CRISPR/CAS9 technology.[72] Non- human primates have also been used to test different therapies for the administration of systemic messenger RNA (mRNA) encoding human α-Gal A.[75]

From human to animal pain

Peripheral neuropathy in patients with FD is mainly of the SF type and is associated with impaired temperature sensation, heat intolerance, and heat-induced pain, as well as abnormal cold detection threshold and thermal sensory limen at the upper and lower limb.[77] Patients refer thermal hyposensitivity and pain, with cold and warm perception reduced over time reflected by an increase in thermal perception threshold and paralleled by a loss of intraepidermal innervation, which is length-dependent.[78] Pain in FD patients has been assayed through different tests to examine fibers functionally and structurally with quantitative sensory testing (QST), and intraepidermal nerve fiber density (IENFD), via punch biopsies, respectively. QST measures sensory thresholds for pain, touch, vibration, and hot and cold temperature sensations. Commercially available devices range from hand-held tools to sophisticated computerized equipment with complicated testing algorithms, standardization of stimulation and recording procedures, and comparisons with age- and gender-matched control values. With this technology, specific fiber functions can be assessed: Aδ-fibers with cold, cold-pain, and mechanical pain detection thresholds; C-fibers with heat and heat-pain detection thresholds; and large fiber (Aαβ-) functions with vibration detection thresholds; and mechanical detection thresholds with von Frey hairs. Elevated sensory thresholds correlate with sensory loss; lowered thresholds occur in allodynia and hyperalgesia.[79] Different parameters can be obtained: cold and heat detection thresholds (CDT, HDT); the ability to detect temperature changes (thermal sensory limen, TSL), as a read-out of small fiber function. Also, paradoxical heat sensation (PHS) (if the subject experiences cold as heat), and vibration detection threshold (VDT) can be analyzed.[80] In addition, examination of small fibers can be performed specifically by other methods: laser evoked potentials, microneurography, and pain-related evoked potential.[80] For further characterization of pain in FD, different studies have used questionnaires applied to other neuropathic pain conditions, to assess the intensity, and associated depressive symptoms.[14] Other characteristics such as localization, duration, and triggers of pain have been suggested as additional parameters to be assessed in FD as FD patients reveal a distinct pain phenotype.[14]

The assessment of neuropathic pain in preclinical models is associated with significant challenges given the need for indirect behavioral readouts as a surrogate of the pain experience. To study the mechanisms of persistent pain, animal models of inflammatory hyperalgesia that mimic human clinical pain conditions have been developed by the injection of inflammatory agents into the rat or mouse hind paw. These models attempt to mimic human clinical conditions. The presence of pain in the inflammation models is inferred by an increased response to a noxious stimulus (hyperalgesia) or a nocifensive behavior in response to an innocuous stimulus

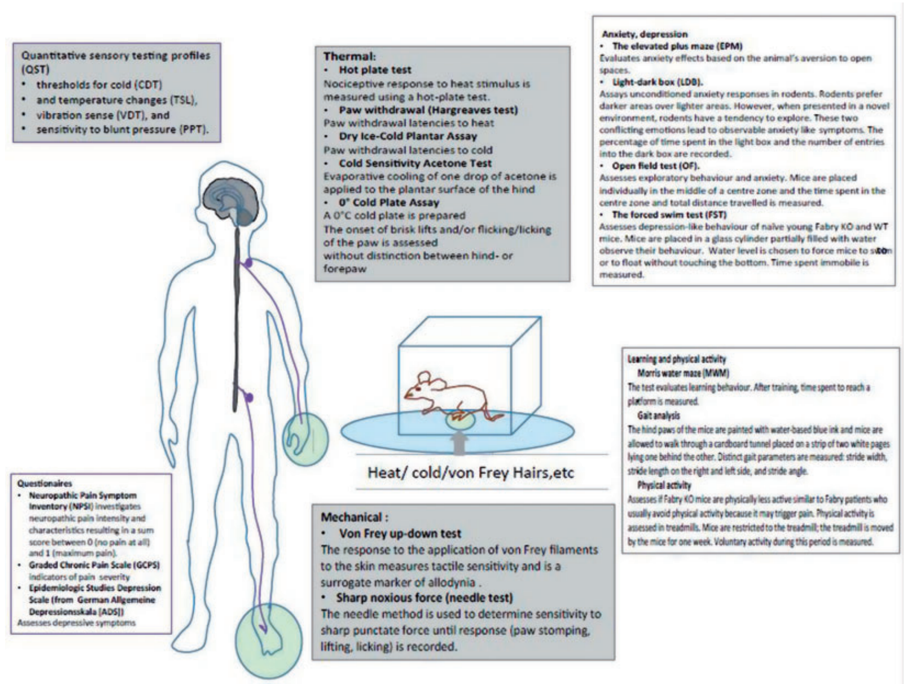

Figure 1. Pain assessment: Comparison of tests performed on human patients and animal models to assess pain. Abbreviations: QST quantitative sensory testing; description of mouse tests according to Refs.[86–91]

normally not perceived as painful (allodynia).[81] As an example, the use of inflammatory pain models help discriminate an initial phase of "nociceptive behavior" (direct effect on nociceptors), or acute peripheral pain, from a second phase considered to reflect central sensitization, due to ongoing inflammation.[82,83] These models in turn can be compared to other models of neuropathic pain used to simulate chronic pain states.[16,84,85]

For practical reasons, the most commonly assessed behavioral outcomes are reflex withdrawal thresholds evoked by thermal or mechanical stimuli (some of the tests used in FD preclinical animal models are shown in Figure 1 compared to tests carried out in patients). These tests have been useful as most animals develop marked levels of pain-like behavior to mechanical or thermal (hot or cold) stimulation; however, there are some concerns that such models neither fully mimic traumatic nerve injury, or reflect all aspects of nerve injury seen in the clinic. Nevertheless, these have given the opportunity of testing behavior as will be further described.

Ion channels and pain in FD

Preclinical models have been instrumental in dissecting molecular mechanisms and pathways involved in FD and revealing the importance of different channels in pain.

The biophysical properties of ion channels can determine nociceptor responses to noxious stimuli and ultimately the level of nociception experienced. Ion channels, in particular sodium, calcium, and potassium channels, that regulate action potential and excitability of neurons via rapid, voltage-gated changes in ion permeability, have been proposed to hold a critical role in the transition from acute to chronic pain and contributing to neuropathic pain chronicization.[92] Sensitization and hyperexcitability of sensory neurons increase neurotransmission and excitotoxic signals.

Altered channel mRNA transcript levels, altered expression of the channel proteins, changes in their functional activity, in the cell bodies of sensory or CNS neurons, as well as alteration in channel trafficking in damaged nerves, seem to be key factors in pathological pain states.[93–96] For example, the calcium channel accessory subunit $\alpha2\delta1$ is upregulated 20-fold in damaged peripheral neurons and is the site of action of the analgesic drugs gabapentin and pregabalin.[96] This subunit facilitates trafficking of $Ca_V2.1$ channels to the cell membrane, thus the analgesic action of gabapentin in patients with neuropathic pain could relate to the lowered calcium currents.[97] Additionally, acute effects of pregabalin, a related drug, has also been shown as reducing calcium currents.[98,99]

Biophysical properties of ion channels can determine nociceptor excitability, and hence abnormal channel function or expression that could lead to chronic or neuropathic pain. As an example, a central role of ion channels in chronic pain and diseases, such as epilepsy is underscored by the utility of similar drugs, such as carbamazepine, for both pathologies.[96]

Many of these ion channels have also been investigated in the field of FD. As depicted in Table 3, Voltage-gated sodium channels ($Na_V1.7$ and $Na_V1.8$), Transient receptor potential (TRP) channels (TRPV1, TRPM8, TRPA1), voltage-gated calcium channels (VGCC); Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated ion channel 2 (HCN2), voltage-gated potassium channels (K_V), calcium-activated potassium channels (Kca1.1, Kca3.1), and the acid-sensing ion channels (ASIC1a) have been implicated in FD.

Even though few studies have been carried out using animal models and patients simultaneously, Namer et al. analyzed changes in ionic conductance of nociceptors in a small number of patients as well as in a GLAko model. The study described sensory abnormalities both in patients and in animals, and detected a decrease conductance of Na_V and VGCC channels as well as activation of K_V channels at more depolarized potentials.[108]

Changes in protein levels, mRNA expression, and electrophysiological properties have been analyzed in relation to the accumulation of glycosphingolipids (Gb3 and LysoGb3) in FD. Hofmann et al. documented alteration of HCN2 and the sodium channel $Na_V1.7$, in the GLAko (C57BL/6 backcrossed) mouse compared to control animals.[6] Even though other ion channels might also be involved, this demonstrated that increased amounts of Gb3 can lead to pathological, physiological, and behavioral signs of neuropathy.[109]

Neuron hyperexcitability in DRGs soma has been analyzed in relation to Na_v channels. Local anesthetics, block pain via nonselective inhibition of Nav channels in primary afferent nerve fibers of the somatosensory nervous system.[110,111] These channels are responsible for the depolarizing phase of the action potential (for a detailed description of Na_V channels in pain, see Bennett et al.[93] For instance, ablation of Nav1.7 and subsequent behavioral analysis has made it very clear that Nav1.7 is vital for acute pain sensation and also contributes to sensitization in a number of persistent pain models.[93] Loss of Nav1.7 function leads to congenital insensitivity to pain, whereas gain-of-function mutations in the SCN9A gene that encodes Nav1.7 cause painful neuropathies.[93] As described by Vicario et al., reduction of $Na_V1.8$ channels in nociceptors has been associated with neuropathy, while ablation of $Na_V1.8$ channels was associated with the development of mechanical allodynia and thermal hyperalgesia.[92] In a rat model of bone cancer pain, pharmacological blockade of Nav1.8 alleviated mechanical allodynia and thermal hyperalgesia.[112] Both channels were analyzed in association with FD[6,100] (see Table 3). Namer et al. analyzed conduction velocities (CV) of C fibers in FD patients and a mouse GLAko model. They showed an increase in CV in FD compared to controls and related this result to heat hyposensitivity. Since CV depend on slow inactivation of voltage-gated sodium channels and intracellular sodium accumulation, these channels were analyzed. Tetrodotoxin-sensitive Na_V currents decreased in GLAko DRGs, with a decrease in conductance that could contribute to the hyposensitivity in mice and FD patients to noxious stimuli[17] Consistent with this finding, Hofmann et al. determined a reduction of $Na_V1.7$ currents in aged GLAko mice, as well as HEK cells in which α-Gal A had been silenced.[6] In addition, they show an increase in $Na_V1.8$ protein levels in younger GLAko mice.

Transient receptor potential vanilloid 1 (TRPV1), known as the capsaicin receptor, is a ligand-gated ion channel. TRPV1 channels are activated by multiple pain stimuli such as acid, heat, capsaicin, protons, lipids, and spider toxins. Gene deletion and pharmacological studies have shown that TRPV1 channels have central roles in inflammatory and neuropathic pain.[113] Alteration in thermal perception was also studied in association with the TRPV1 channels in the GLAko mouse and related to heat hypersensitivity. Hofmann et al. analyzed these channels, and showed an increase in protein levels by immunofluorescence in DRG cultures of old GLAko mice.[6] Frontal paw skin as well as primary cultures of DRG neurons from Glako male mice also showed increased TRPV1 levels as shown by Lakoma et al.[35,100]

Transient receptor potential ankyrin 1 (TRPA1), known as a noxious cold-activated ion channel, is a nonselective cation channel mainly expressed in nociceptive primary afferent sensory neurons. TRPA1 channels contribute to transmitting harmful stimuli, whereas at central terminals in the spinal dorsal horn, these channels regulate excitatory synaptic transmission to interneurons in the spinal cord.[113] Mechanical sensitivity alterations were associated with TRPA1 channels in FD rats.[72]

The TRPM8 channel is expressed by subsets of sensory neurons in the dorsal root and trigeminal ganglia and is activated by cold or ligands, such as menthol, that trigger cold sensation. In a rat model of neuropathic pain, mild cooling of the skin, peripheral or central application of icing produced marked analgesic effects, inhibiting sensitization of dorsal-horn neurons and facilitation of behavioral reflexes.[114] This channel was associated with a decreased sensitivity to cold stimulation in a GLAko mouse.[100]

Activation of voltage-gated calcium channels increases neurotransmitter release and enhances excitatory synaptic transmission in the nociceptive circuits.[92] N- and P-type VGCCs are predominantly expressed in neuronal tissue in the brain, and influx through these channels is essential for depolarization-induced transmitter release. Antagonists for N- and P-type VGCCs showed antinociceptive effects in animal models of inflammation.[113] These channels have also been studied in FD –though not fully characterized- in animals injected with glycosphingolipids.[102] Namer et al. also showed decreased conductance of VGCCs both in high and low voltage-activated channels.[17]

Hyperpolarizing activated cyclic nucleotide-gated (HCN) channels emerged as key players controlling and facilitating neuron excitability. The Na^+/K^+ inward current flowing during HCN opening, I_h, appears to contribute to spontaneous or ectopic firing in several tissues, including the central nervous system and peripheral ganglia and nerves. Evidence support the over-expression and/or gain of function of HCN in animal models of chronic, neuropathic pain.[115] Geevasinga et al. performed axonal excitability studies on the median motor and sensory nerves of FD patients and documented an upregulation of Ih which was suggested to be associated with the development of FD neuropathy and possibly neuropathic pain.[116] In turn, Hofmann et al. reported hyperpolarization-activated (Ih) current densities were reduced in DRG neurons from old GLAko mice.[6]

K_{Ca} channels are major determinants of firing adaptation because they speed the repolarization of the action potential and generate the after-hyperpolarization of the plasma membrane. K_{Ca} currents are important modulators of inflammatory and neuropathic pain. They are downregulated by nerve injury and inflammation, which induces nociceptive neuron hyperexcitability, ectopic firing, and spontaneous pain.[117] These channels are altered in different in vitro FD models,[37,103,105–107,118] especially analyzed in fibroblasts and altering different signaling pathways (see Table 3).

The use of non-steroidal anti-inflammatory drugs (NSAIDs) in Fabry as a non-typical treatment for neuropathic pain has been referred to by Politei et al. as part of the recommended analgesic drugs for supportive treatment of acute pain in Fabry disease.[12] Interestingly, one effect that has been documented in the field of pain is that of NSAIDs on ASIC channels. NSAIDs are major drugs used in the treatment of inflammation and pain in a wide variety of disorders.[119] A thorough study by Volley et al. pointed to the fact that apart from the best-known mechanism of action of NSAIDs, i.e., the inhibition of prostaglandin synthesis secondary to their action on cyclooxygenases (COX), NSAIDs also act on other targets to counteract pain, such as ASIC channels.[120] In fact, they showed that NSAIDs modulated the channels in two ways: with a COX-independent, fast, and reversible direct inhibition of their activity (blocking the channel); and by preventing the large inflammation-induced increase in channel expression.[121] We have recently documented ASIC1a upregulation in an in vitro model of FD.[47] The increase in ASIC1a channels, protein as well as mRNA levels, was associated with the activation of the ERK kinase, a kinase involved in the pain pathway.[122,123]

Involvement of channelopathies in human pain conditions has been highlighted by evidence from analysis of pain phenotypes in transgenic animal models, and different behavior in animal models could be associated with particular ion channels as assessed by pharmacological tools (Table 3, underlined, and Table 4). The behavioral tests described in Figure 1 were used to assess the GLAko mouse and the GLAko rat. As previously mentioned, the GLAko mouse has been used backcrossed to different strains (the sv129 or the BL6).

The most frequently evaluated behavioral tests were those for thermal sensitivity to heat assessed by the hot plate and Hargreaves's tests (Table 4) and mechanical sensitivity with Von Frey's test.

Comparing the behavior obtained in the different tests, thermal sensitivity was assessed as hypoalgesia by Rodrigues et al.[124] and Bangari et al.[125] while as hyperalgesia by Lakomá et al.[100] in the GLAko mouse. Recently, Jabbarzadeh-Tabrizi et al. compared GLAko mice backcrossed to either strain (sv129 or BL6), and found hyposensitivity to heat in both with different temperature thresholds.[126] No differences in this parameter was found for the GLAko rat by Miller et al.[72] Üçeyler et al.[101] subclassified the GLAko mouse in young and old groups and showed heat hypersensitivity in the young group (until 3 months) and hyposensitivity in the old group (more than 9 months). In the case of cold sensitivity, the GLAko mouse-regardless of the strain it was backcrossed to, hyposensitivity has been documented, while the GLAko rat has shown no difference to the control counterparts.

Therefore, thermal sensitivity alterations in the GLAko mouse might be a helpful model reflecting some aspects of the alterations detected in FD patients. In fact, changes in warm and cold detection thresholds do not always correlate well with patient pain experience.[127]

In the case of the mechanical tests using von Frey filaments, GLAko mice and rats show a decreased threshold to the mechanical force applied (see Table 4, and in bold letters).

The effect of sex has not been explored consistently by the different authors. Most of the studies analyzed the behavior of male animals or pooled the results of male and female animals. Üçeyler et al.[101] and Miller et al.[72] explored the effect of sex on behavior, finding no significant differences. These results contrast with the effect of sex observed in FD patients.[9] We believe that this difference observed between the animal models and FD patients warrants further studies on the effect of sex on pain behavior in FD animal models.

As stated by Mogil et al., the entire existing preclinical pain literature may be male-biased as the subjects of experiments have overwhelmingly been male.[128] Sex differences in brain region activation in response to thermal and electrical stimuli have been reported.[129] Animal studies have demonstrated effects of estrogens on dendritic growth and synaptic density; shifts in estradiol levels that accompany the menstrual cycle affect patterns and levels of neuronal activity.[130] In the case of FD, variability of the phenotype in females could be contributed by X-chromosome inactivation (XCI), as well as highly skewed XCI favoring the expression of the mutant allele, and has been proposed as a mechanism to explain the occasional development of clinical symptoms.[131]

FD patients report frequent symptoms of anxiety, and most FD patients experience episodic and chronic pain that limit their physical and everyday life activities. Their overall quality of life is reduced which induces depressive symptoms and impaired cognitive function (concentration and mental endurance). Hofmann et al. set out to explore whether these behaviors could be evidenced in animal models of FD. However, no significant differences were observed between GLAko mice and their control counterparts when anxiety, depression, and learning behavior were explored.[132]

An important point to highlight, when using rodent animal models, is the fact that neuropathic models, sometimes used in FD, were initially developed in rats, so attention should be paid due to anatomical differences, as nerves are configured differently and the constriction of a lumbar spine nerve at one level might be different in either rat, mouse, or even in different strains.[133] Also, when considering the behavior of mouse models, there is an inherent limitation in the way the transgenic animal has been generated: most lines started with a gene knocked out in a strain stem cell line (normally the "129" strain) different from the blastocyst embryo stage where it is introduced to (normally the C57BL/6).[134] Controls will also differ according to the strain used for the backcrossing. This point was thoroughly described by Gerlai in 1996[134] who claimed how phenotypical abnormalities attributed to the null mutation in several molecular neurobiological studies could simply result from the effects of background genes.[135] Even if backcrossing for several generations is performed, as described by Gerlai, eliminating the confounding effects of background genes would be a considerable undertaking and not an optimal solution.[134] Thus, control strains used are very important and perhaps the reason why as Üçeyler et al.[101] described, there are different heat withdrawal latency times for different control animals used (of different strains and whether littermate is used), leading to conclude for a hyper or hyposensitivity accordingly[6] (see Table 4, in bold letters). These results are not surprising since Mogil et al. in 1999 tested a large number of inbred mouse strains for responses in a broad range of murine assays of nociception. The main result of the work being that genotype significantly affects performance in all of the nociceptive measures: behavioral traits have a significant heritable component in mice, and among the different strains, the C57BL/6 is one of the most sensitive and genetically distinct.[136]

These factors have to be considered when interpreting results and verifying with proper controls, -that might be silencing RNA techniques or with pharmacological inhibitors- to confirm and discard the possibility that the result might be a consequence of a "hitchhiking donor gene confound" effect.[135]

As shown in Table 2, the CRISPR/CAS9 technology offers also new possibilities of analyzing animals with a gene deletion without the mentioned problems. However, the experimenter might decide on a model according to how well it reflects features of pain as present in FD patients.

To sum up, the different models described can be used to recapitulate particular aspects of pain that reflect FD characteristics. This can help analyze mechanisms and potential therapeutic targets in pain in FD, as long as the experimenter bears in mind all the limitations of the models to confirm with the proper tools. In FD research, these tools are just starting to be used and can thus benefit greatly from all previous work done in the field of pain.

Final remarks

As discussed throughout this review, pain in FD shows differences from other SF neuropathies. The use of different preclinical models has been instrumental in dissecting different players involved in the mechanism of pain. In addition, most of the documented work in FD has focused on peripheral and spinal levels, while supraspinal structures should also be taken into account, especially since Gb3 accumulation has been also detected in different areas of the brain.[137]

Another aspect that should be considered is the effect on kidney function and its contribution to pain which is not consistently analyzed.

Apart from ion channels, a significant portion of studies have also indicated a dysfunctional endothelial metabolism characteristic of sensory profiles in Fabry disease. This could indicate a dysfunctional release of endothelial nitric oxide (NO) underlying pathomechanisms in FD that may rather implicate a central disinhibition pain mechanism due to a reduced A-δ fiber input.[19]

In addition, studies published over the last decade have elucidated the role of CNS resident glial cells in many aspects of pathological neuronal functioning, occurring in neuropathic pain, with phenomenon like cell-to-extracellular communication mediated by hemichannels and cell coupling (gap junctions-GJs).[92]

A comprehensive revision of past studies, and a closer analysis on the similarities and differences between patients and animal models would help achieve translatability of preclinical models.

All these factors will have to be considered and thoroughly analyzed to aim at better therapies in FD pain.

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References

1. Rozenfeld PA, Ceci R, Roa N, Kisinovsky I. The continuous challenge of diagnosing patients with Fabry disease in Argentina: genotype, experiences, anecdotes, and new learnings. *J Inborn Errors Metab Screen* 2015; 3. doi: 10.1177/2326409815613806
2. Winchester B, Young E. Biochemical and genetic diagnosis of Fabry disease. In: Mehta A, Beck M and Sunder-Plassmann G (eds) *Fabry Disease: Perspectives from 5 Years of FOS*. Oxford: Oxford PharmaGenesis, 2006. Chapter 18. PMID: 21290697. https://pubmed.ncbi.nlm.nih.gov/21290697/
3. Saito S, Ohno K, Sakuraba H. Fabry-database.org: database of the clinical phenotypes, genotypes and mutant α-galactosidase a structures in Fabry disease. *J Hum Genet* 2011; 56: 467–468.
4. Schuller Y, Linthorst GE, Hollak CEM, Van Schaik IN, Biegstraaten M. Pain management strategies for neuropathic pain in Fabry disease – a systematic review. *BMC Neurol* 2016; 16: 1–10.
5. Askari H, Kaneski CR, Semino-Mora C, Desai P, Ang A, Kleiner DE, Perlee LT, Quezado M, Spollen LE, Wustman BA, Schiffmann R. Cellular and tissue localization of globotriaosylceramide in Fabry disease. *Virchows Arch* 2007; 451: 823–834.
6. Hofmann L, Hose D, Grießhammer A, Blum R, Döring F, Dib-Hajj S, Waxman S, Sommer C, Wischmeyer E, Üçeyler N. Characterization of small fiber pathology in a mouse model of Fabry disease. *Elife* 2018; 7: 1–21.
7. Kaye EM, Kolodny EH, Logigian EL, Ullman MD. Nervous system involvement in Fabry's disease: clinicopathological and biochemical correlation. *Ann Neurol* 1988; 23: 505–509.
8. Aerts JM, Groener JE, Kuiper S, Donker-Koopman WE, Strijland A, Ottenhoff R, van Roomen C, Mirzaian M, Wijburg FA, Linthorst GE, Vedder AC, Rombach SM, Cox-Brinkman J, Somerharju P, Boot RG, Hollak CE, Brady RO, Poorthuis BJ. Elevated globotriaosylsphingosine is a hallmark of Fabry disease. *Proc Natl Acad Sci U S A* 2008; 105: 2812–2817.
9. Gibas AL, Klatt R, Johnson J, Clarke JTRR, Katz J. A survey of the pain experienced by males and females with Fabry disease. *Pain Res Manag* 2006; 11: 828964.
10. Colloca L, Ludman T, Bouhassira D, Baron R, Dickenson AH, Yarnitsky D, Freeman R, Truini A, Attal N, Finnerup NB, Eccleston C. Neuropathic pain. *Nat Rev Dis Prim* 2017; 3: 17002.
11. Biegstraaten M, Linthorst GE, Van Schaik IN, Hollak CEM. Fabry disease: a rare cause of neuropathic pain. *Curr Pain Headache Rep* 2013; 17: 365.
12. Politei JM, Bouhassira D, Germain DP, Goizet C, Guerrero-Sola A, Hilz MJ, Hutton EJ, Karaa A, Liguori R, Üçeyler N, Zeltzer LK, Burlina A. Pain in Fabry disease: practical recommendations for diagnosis and treatment. *CNS Neurosci Ther* 2016; 22: 568–576.
13. Politei JM, Durand C, Schenone AB. Small fiber neuropathy in Fabry disease: a review of pathophysiology and treatment. *J Inborn Errors Metab Screen* 2016; 4. doi: 10.1177/2326409816661351
14. Üçeyler N, Ganendiran S, Kramer D, Sommer C. Characterization of pain in Fabry disease. *Clin J Pain* 2014; 30: 915–920.
15. Birklein F. Mechanisms of neuropathic pain and their importance in Fabry disease. *Acta Paediatr Suppl* 2002; 91: 34–37.
16. Campbell JN, Meyer RA. Mechanisms of neuropathic pain. *Neuron* 2006; 52: 77–92.
17. Namer B, Ørstavik K, Schmidt R, Mair N, Petter I. Changes in ionic conductance signature of nociceptive neurons underlying Fabry disease phenotype. *Front Neurol* 2017; 8: 1–18.
18. Rozenfeld P, Feriozzi S. Contribution of inflammatory pathways to Fabry disease pathogenesis. *Mol Genet Metab* 2017; 122: 19–27.
19. Forstenpointner J, Sendel M, Moeller P, Reimer M, Canaan-Kühl S, Gaedeke J, Rehm S, Hüllemann P, Gierthmühlen J, Baron R. Bridging the gap between vessels and nerves in Fabry disease. *Front Neurosci* 2020; 14: 448.
20. Slanzi A, Iannoto G, Rossi B, Zenaro E, Constantin G. In vitro models of neurodegenerative diseases. *Front Cell Dev Biol* 2020; 8: 328.
21. Benam KH, Dauth S, Hassell B, Herland A, Jain A, Jang K-J, Karalis K, Kim HJ, MacQueen L, Mahmoodian R, Musah S, Torisawa Y-s, van der Meer AD, Villenave R, Yadid M, Parker KK, Ingber DE. Engineered in vitro disease models. *Annu Rev Pathol* 2015; 10: 195–262.

22. Ye L, Swingen C, Zhang J. Induced pluripotent stem cells and their potential for basic and clinical sciences. *Curr Cardiol Rev* 2013; 9: 63–72.
23. Johnson DL, Desnick RJ. Molecular pathology of Fabry's disease physical and kinetic properties of α-galactosidase a in cultured human endothelial cells. *BBA Gen Subj* 1978; 538: 195–204.
24. Hasholt L, Sørensen SA. Lysosomal α-galactosidase in endothelial cell cultures established from a Fabry hemizygous and normal umbilical veins. *Hum Genet* 1986; 72: 72–76.
25. Inagaki M, Katsumoto T, Nanba E, Ohno K, Suehiro S, Takeshita K. Lysosomal glycosphingolipid storage in chloroquine-induced α-galactosidase-deficient human endothelial cells with transformation by simian virus 40: in vitro model of Fabry disease. *Acta Neuropathol* 1993; 85: 272–279.
26. Shu L, Murphy HS, Cooling L, Shayman JA. An in vitro model of Fabry disease. *J Am Soc Nephrol* 2005; 16: 2636–2645.
27. Shen JS, Meng XL, Schiffmann R, Brady RO, Kaneski CR. Establishment and characterization of Fabry disease endothelial cells with an extended lifespan. *Mol Genet Metab* 2007; 92: 137–144.
28. Shen JS, Meng XL, Schiffmann R, Brady RO, Kaneski CR. Establishment and characterization of Fabry disease endothelial cells with an extended lifespan. *Mol Genet Metab* 2007; 92: 137–144.
29. Ruiz De Garibay AP, Solinís MA, Del Pozo-Rodríguez A, Apaolaza PS, Shen JS, Rodríguez-Gascón A. Solid lipid nanoparticles as non-viral vectors for gene transfection in a cell model of Fabry disease. *J Biomed Nanotechnol* 2015; 11: 500–511.
30. Takenaka T, Hendrickson CS, Tworek DM, Tudor M, Schiffmann R, Brady RO, Medin JA. Enzymatic and functional correction along with long-term enzyme secretion from transduced bone marrow hematopoietic stem/progenitor and stromal cells derived from patients with Fabry disease. *Exp Hematol* 1999; 27: 1149–1159.
31. Qin G, Takenaka T, Telsch K, Kelley L, Howard T, Levade T, Deans R, Howard BH, Malech HL, Brady RO, Medin JA. Preselective gene therapy for Fabry disease. *Proc Natl Acad Sci U S A* 2001; 98: 3428–3433.
32. Medin JA, Tudor M, Simovitch R, Quirk JM, Jacobson S, Murray GJ, Brady RO. Correction in trans for Fabry disease: expression, secretion, and uptake of a-galactosidase a in patient-derived cells driven by a high-titer recombinant retroviral vector. *Proc Natl Acad Sci USA* 1996; 93: 7917–7922.
33. Jenkinson SF, Fleet GWJ, Nash RJ, Koike Y, Adachi I, Yoshihara A, Morimoto K, Izumori K, Kato A. Looking-glass synergistic pharmacological chaperones: DGJ and L-DGJ from the enantiomers of tagatose. *Org Lett* 2011; 13: 4064–4067.
34. Porto C, Pisani A, Rosa M, Acampora E, Avolio V, Tuzzi MR, Visciano B, Gagliardo C, Materazzi S, la Marca G, Andria G, Parenti G. Synergy between the pharmacological chaperone 1-deoxygalactonojirimycin and the human recombinant alpha-galactosidase a in cultured fibroblasts from patients with Fabry disease. *J Inherit Metab Dis* 2012; 35: 513–520.
35. Lakomá J, Rimondini R, Montiel AF, Donadio V, Liguori R, Caprini M. Increased expression of trpv1 in peripheral terminals mediates thermal nociception in Fabry disease mouse model. *Mol Pain* 2016; 12: 174480691666372–174480691666316.
36. Welford RWD, Mühlemann A, Garzotti M, Rickert V, Groenen PMA, Morand O, Üçeyler N, Probst MR. Glucosylceramide synthase inhibition with lucerastat lowers globotriaosylceramide and lysosome staining in cultured fibroblasts from Fabry patients with different mutation types. *Hum Mol Genet* 2018; 27: 3392–3403.
37. Oliván-Viguera A, Lozano-Gerona J, de Frutos LL, Cebolla JJ, Irún P, Abarca-Lachen E, García-Malinis AJ, García-Otín ÁL, Gilaberte Y, Giraldo P, Köhler R. Inhibition of intermediate-conductance calcium-activated K channel (KCa3.1) and fibroblast mitogenesis by α-linolenic acid and alterations of channel expression in the lysosomal storage disorders, Fabry disease, and Niemann pick C. *Front Physiol* 2017; 8: 1–10.
38. Andreotti G, Citro V, De Crescenzo A, Orlando P, Cammisa M, Correra A, Cubellis MV. Therapy of Fabry disease with pharmacological chaperones: from in silico predictions to in vitro tests. *Orphanet J Rare Dis* 2011; 6: 66.
39. Fan JQ, Ishii S, Asano N, Suzuki Y. Accelerated transport and maturation of lysosomal α-galactosidase A in Fabry lymphoblasts by an enzyme inhibitor. *Nat Med* 1999; 5: 112–115.
40. Benjamin ER, Flanagan JJ, Schilling A, Chang HH, Agarwal L, Katz E, Wu X, Pine C, Wustman B, Desnick RJ, Lockhart DJ, Valenzano KJ. The pharmacological chaperone 1-deoxygalactonojirimycin increases α-galactosidase a levels in Fabry patient cell lines. *J Inherit Metab Dis* 2009; 32: 424–440.
41. Kase R, Bierfreund U, Klein A, Kolter T, Utsumi K, Itoh K, Sandhoff K, Sakuraba H. Characterization of two α-galactosidase mutants (Q279E and R301Q) found in an atypical variant of Fabry disease. *Biochim Biophys Acta Mol Basis Dis* 2000; 1501: 227–235.
42. Tajima Y, Kawashima I, Tsukimura T, Sugawara K, Kuroda M, Suzuki T, Togawa T, Chiba Y, Jigami Y, Ohno K, Fukushige T, Kanekura T, Itoh K, Ohashi T, Sakuraba H. Use of a modified α-N-Acetylgalactosaminidase in the development of enzyme replacement therapy for Fabry disease. *Am J Hum Genet* 2009; 85: 569–580.
43. Tian W, Ye Z, Wang S, Schulz MA, Van Coillie J, Sun L, Chen Y-H, Narimatsu Y, Hansen L, Kristensen C, Mandel U, Bennett EP, Jabbarzadeh-Tabrizi S, Schiffmann R, Shen J-S, Vakhrushev SY, Clausen H, Yang Z. The glycosylation design space for recombinant lysosomal replacement enzymes produced in CHO cells. *Nat Commun* 2019; 10: 1785.
44. Song HY, Chiang HC, Tseng WL, et al. Using CRISPR/Cas9-Mediated GLA Gene Knockout as an In Vitro Drug Screening Model for Fabry Disease. *Int J Mol Sci* 2016; 17: 2089.

45. Benjamin ER, Della Valle MC, Wu X, Katz E, Pruthi F, Bond S, Bronfin B, Williams H, Yu J, Bichet DG, Germain DP, Giugliani R, Hughes D, Schiffmann R, Wilcox WR, Desnick RJ, Kirk J, Barth J, Barlow C, Valenzano KJ, Castelli J, Lockhart DJ. The validation of pharmacogenetics for the identification of Fabry patients to be treated with migalastat. *Genet Med* 2017; 19: 430–438.
46. Biancini GB, Morás AM, Reinhardt LS, Busatto FF, de Moura Sperotto ND, Saffi J, Moura DJ, Giugliani R, Vargas CR. Globotriaosylsphingosine induces oxidative DNA damage in cultured kidney cells. *Nephrology (Carlton)* 2017; 22: 490–493.
47. Salinas LCC, Rozenfeld P, Gatto RG, Reisin RC, Uchitel OD, Weissmann C. Upregulation of ASIC1a channels in an in vitro model of Fabry disease. *Neurochem Int* 2020; 140: 104824.
48. Liebau MC, Braun F, Höpker K, Weitbrecht C, Bartels V, Müller R-U, Brodesser S, Saleem MA, Benzing T, Schermer B, Cybulla M, Kurschat CE. Dysregulated autophagy contributes to podocyte damage in Fabry's disease. *PLoS One* 2013; 8: e63506.
49. Pereira EM, Labilloy A, Eshbach ML, Roy A, Subramanya AR, Monte S, Labilloy G, Weisz OA. Characterization and phosphoproteomic analysis of a human immortalized podocyte model of Fabry disease generated using CRISPR/Cas9 technology. *Am J Physiol Renal Physiol* 2016; 311: F1015–F1024.
50. Braun F, Blomberg L, Brodesser S, Liebau MC, Schermer B, Benzing T, Kurschat CE. Enzyme replacement therapy clears GB3 deposits from a podocyte cell culture model of Fabry disease but fails to restore altered cellular signaling. *Cell Physiol Biochem* 2019; 52: 1139–1150.
51. Kizhner T, Azulay Y, Hainrichson M, Tekoah Y, Arvatz G, Shulman A, Ruderfer I, Aviezer D, Shaaltiel Y. Characterization of a chemically modified plant cell culture expressed human α-Galactosidase-A enzyme for treatment of Fabry disease. *Mol Genet Metab* 2015; 114: 259–267.
52. Labilloy A, Youker RT, Bruns JR, Kukic I, Kiselyov K, Halfter W, Finegold D, do Monte SJH, Weisz OA. Altered dynamics of a lipid raft associated protein in a kidney model of Fabry disease. *Mol Genet Metab* 2014; 111: 184–192.
53. Slaats GG, Braun F, Hoehne M, Frech LE, Blomberg L, Benzing T, Schermer B, Rinschen MM, Kurschat CE. Urine-derived cells: a promising diagnostic tool in Fabry disease patients. *Sci Rep* 2018; 8: 1–11.
54. Lenders M, Stappers F, Niemietz C, Schmitz B, Boutin M, Ballmaier PJ, Zibert A, Schmidt H, Brand S-M, Auray-Blais C, Brand E. Mutation-specific Fabry disease patient-derived cell model to evaluate the amenability to chaperone therapy. *J Med Genet* 2019; 56: 548–556.
55. Itier J-M, Ret G, Viale S, Sweet L, Bangari D, Caron A, Le-Gall F, Bénichou B, Leonard J, Deleuze J-F, Orsini C. Effective clearance of GL-3 in a human iPSC-derived cardiomyocyte model of Fabry disease. *J Inherit Metab Dis* 2014; 37: 1013–1022.
56. Birket MJ, Raibaud S, Lettieri M, Adamson AD, Letang V, Cervello P, Redon N, Ret G, Viale S, Wang B, Biton B, Guillemot J-C, Mikol V, Leonard JP, Hanley NA, Orsini C, Itier J-M. A human stem cell model of Fabry disease implicates LIMP-2 accumulation in cardiomyocyte pathology. *Stem Cell Reports* 2019; 13: 380–393.
57. Tseng W-L, Chou S-J, Chiang H-C, Wang M-L, Chien C-S, Chen K-H, Leu H-B, Wang C-Y, Chang Y-L, Liu Y-Y, Jong Y-J, Lin S-Z, Chiou S-H, Lin S-J, Yu W-C. Imbalanced production of reactive oxygen species and mitochondrial antioxidant SOD2 in Fabry disease-specific human induced pluripotent stem cell-differentiated vascular endothelial cells. *Cell Transpl* 2017; 26: 513–527.
58. Chou S-J, Yu W-C, Chang Y-L, Chen W-Y, Chang W-C, Chien Y, Yen J-C, Liu Y-Y, Chen S-J, Wang C-Y, Chen Y-H, Niu D-M, Lin S-J, Chen J-W, Chiou S-H, Leu H-B. Energy utilization of induced pluripotent stem cell-derived cardiomyocyte in Fabry disease. *Int J Cardiol* 2017; 232: 255–263.
59. Song HY, Chien CS, Yarmishyn AA, Chou SJ, Yang YP, Wang ML, Wang CY, Leu HB, Yu WC, Chang YL, Chiou SH. Generation of GLA-Knockout human embryonic stem cell lines to model autophagic dysfunction and exosome secretion in Fabry disease-associated hypertrophic cardiomyopathy. *Cells* 2019; 8: 327.
60. Do H-S, Park S-W, Im I, Seo D, Yoo H-W, Go H, Kim YH, Koh GY, Lee B-H, Han Y-M. Enhanced thrombospondin-1 causes dysfunction of vascular endothelial cells derived from Fabry disease-induced pluripotent stem cells. *EBioMedicine* 2020; 52: 102633.
61. Kaneski CR, Brady RO, Hanover JA, Schueler UH. Development of a model system for neuronal dysfunction in Fabry disease. *Mol Genet Metab* 2016; 119: 144–150.
62. Castellanos LCS, Rozenfeld P, Gatto RG, Reisin RC, Uchitel OD, Weissmann C. Upregulation of ASIC1a channels in an in vitro model of Fabry disease. *Neurochem Int* 2020; 140: 104824.
63. Abe A, Gregory S, Lee L, Killen PD, Brady RO, Kulkarni A, Shayman JA. Reduction of globotriaosylceramide in Fabry disease mice by substrate deprivation. *J Clin Invest* 2000; 105: 1563–1571.
64. Shui B, Hernandez Matias L, Guo Y, Peng Y. The rise of CRISPR/cas for genome editing in stem cells. Synnergren J, editor. *Stem Cells Int* 2016; 2016: 8140168.
65. Calvo M, Davies AJ, Hébert HL, Weir GA, Chesler EJ, Finnerup NB, Levitt RC, Smith BH, Neely GG, Costigan M, Bennett DL. The genetics of neuropathic pain from model organisms to clinical application. *Neuron* 2019; 104: 637–653.
66. Jeong Y, Holden JE. Commonly used preclinical models of pain. *West J Nurs Res* 2008; 30: 350–364.
67. Eijkenboom I, Sopacua M, Otten ABC, Gerrits MM, Hoeijmakers JGJ, Waxman SG, Lombardi R, Lauria G, Merkies ISJ, Smeets HJM, Faber CG, Vanoevelen JM, PROPANE Study Group. Expression of pathogenic SCN9A mutations in the zebrafish: A model to study small-fiber neuropathy. Exp Neurol 2019; 311: 257–264.

68. Hujová J, Sikora J, Dobrovolný R, Poupetová H, Ledvinová J, Kostrouchová M, Hrebícek M. Characterization of gana-1, a Caenorhabditis elegans gene encoding a single ortholog of vertebrate α-galactosidase and α-N-acetylgalactosaminidase. *BMC Cell Biol* 2005; 6: 5–13.
69. Ohshima T, Murray GJ, Swaim WD, Longenecker G, Quirk JM, Cardarelli CO, Sugimoto Y, Pastan I, Gottesman MM, Brady RO, Kulkarni AB. alpha-Galactosidase a deficient mice: a model of Fabry disease. *Proc Natl Acad Sci U S A* 1997; 94: 2540–2544.
70. Pacienza N, Yoshimitsu M, Mizue N, Au BCY, Wang JCM, Fan X, Takenaka T, Medin JA. Lentivector transduction improves outcomes over transplantation of human HSCs alone in NOD/SCID/Fabry mice. *Mol Ther* 2012; 20: 1454–1461.
71. Taguchi A, Maruyama H, Nameta M, Yamamoto T, Matsuda J, Kulkarni ABB, Yoshioka H, Ishii S. A symptomatic Fabry disease mouse model generated by inducing globotriaosylceramide synthesis. *Biochem J* 2013; 456: 373–383.
72. Miller JJ, Aoki K, Moehring F, Murphy CA, Hara CLO, Tiemeyer M, Stucky CL, Dahms NM. Neuropathic pain in a Fabry disease rat model. *JCI Insight* 2018; 3: 1–19.
73. Miller JJ, Aoki K, Mascari CA, et al. α-galactosidase a-deficient rats accumulate glycosphingolipids and develop cardiorenal phenotypes of Fabry disease. *FASEB J* 2019; 33: 418–429.
74. Miller JJ, Aoki K, Reid CA, Tiemeyer M, Dahms NM, Kassem IS. Rats deficient in α-galactosidase A develop ocular manifestations of Fabry disease. *Sci Rep* 2019; 9: 1–9.
75. Zhu X, Yin L, Theisen M, Zhuo J, Siddiqui S, Levy B, Presnyak V, Frassetto A, Milton J, Salerno T, Benenato KE, Milano J, Lynn A, Sabnis S, Burke K, Besin G, Lukacs CM, Guey LT, Finn PF, Martini PGV. Systemic mRNA therapy for the treatment of Fabry disease: preclinical studies in Wild-Type mice, Fabry mouse model, and Wild-Type non-human primates. *Am J Hum Genet* 2019; 104: 625–637.
76. Kinghorn KJ, Grönke S, Castillo-Quan JI, Woodling NS, Li L, Sirka E, Gegg M, Mills K, Hardy J, Bjedov I, Partridge L. A drosophila model of neuronopathic gaucher disease demonstrates lysosomal-autophagic defects and altered mTOR signalling and is functionally rescued by rapamycin. *J Neurosci* 2016; 36: 11654–11670.
77. Biegstraaten M, Binder A, Maag R, Hollak CEM, Baron R, Schaik IV. The relation between small nerve fibre function, age, disease severity and pain in Fabry disease. *Eur J Pain* 2011; 15: 822–829.
78. Üçeyler N, He L, Schönfeld D, Kahn A-K, Reiners K, Hilz MJ, Breunig F, Sommer C. Small fibers in Fabry disease: baseline and follow-up data under enzyme replacement therapy. *J Peripher Nerv Syst* 2011; 16: 304–314.
79. Steven H. Horowitz, Neuropathic pain: is the emperor wearing clothes?, In: Howard S. Smith (ed) *Current Therapy in Pain*, W.B. Saunders, 2009. Chapter 3. pp. 9–14. ISBN 9781416048367, https://doi.org/10.1016/B978-1-4160-4836-7.00003-1. (https://www.sciencedirect.com/science/article/pii/B9781416048367000031)
80. Üçeyler N, Kahn A-K, Kramer D, Zeller D, Casanova-Molla J, Wanner C, Weidemann F, Katsarava Z, Sommer C. Impaired small fiber conduction in patients with Fabry disease: a neurophysiological case-control study. *BMC Neurol* 2013; 13: 47.
81. Ren K, Dubner R. Inflammatory models of pain and hyperalgesia. *Ilar J* 1999; 40: 111–118.
82. Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain* 1987; 30: 103–114.
83. López-Cano M, Fernández-Dueñas V, Llebaria A, Ciruela F. Formalin murine model of pain. *Bio-Protocol* 2017; 7: 1–8.
84. Colleoni M, Sacerdote P. Murine models of human neuropathic pain. *Biochim Biophys Acta* 2010; 1802: 924–933.
85. Sewell RDE. Neuropathic pain models and outcome measures: a dual translational challenge. *Ann Transl Med* 2018; 6: S42.
86. Bradman MJG, Ferrini F, Salio C, Merighi A. Practical mechanical threshold estimation in rodents using von frey hairs/Semmes-Weinstein monofilaments: towards a rational method. *J Neurosci Methods* 2015; 255: 92–103.
87. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods* 1994; 53: 55–63.
88. Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* 1988; 32: 77–88.
89. Brenner DS, Golden JP, Gereau RW. A novel behavioral assay for measuring cold sensation in mice. Sakakibara M, editor. *PLoS One* 2012; 7: e39765.
90. Golden JP, Hoshi M, Nassar MA, Enomoto H, Wood JN, Milbrandt J, Gereau RW, Johnson EM, Jain S. RET signaling is required for survival and normal function of nonpeptidergic nociceptors. *J Neurosci* 2010; 30: 3983–3994.
91. Rogers DC, Fisher EMC, Brown SDM, Peters J, Hunter AJ, Martin JE. Behavioral and functional analysis of mouse phenotype: SHIRPA, a proposed protocol for comprehensive phenotype assessment. *Mamm Genome* 1997; 8: 711–713.
92. Vicario N, Turnaturi R, Spitale FM, Torrisi F, Zappalà A, Gulino R, Pasquinucci L, Chiechio S, Parenti C, Parenti R. Intercellular communication and ion channels in neuropathic pain chronicization. *Inflamm Res* 2020; 69: 841–850.
93. Bennett DL, Clark XAJ, Huang J, Waxman SG, Dib-Hajj SD. The role of voltage-gated sodium channels in pain signaling. *Physiol Rev* 2019; 99: 1079–1151.
94. Trimmer JS. Ion channels and pain: important steps towards validating a new therapeutic target for neuropathic pain. *Exp Neurol* 2014; 254: 190–194.
95. Lipscombe D, Lopez-Soto EJ. Epigenetic control of ion channel expression and cell-specific splicing in nociceptors: chronic pain mechanisms and potential therapeutic targets. *Channels* 2021; 15: 156–164.

96. Raouf R, Quick K, Wood JN. Review series pain as a channelopathy. *J Clin Invest* 2010; 120: 3745–3752.
97. Hendrich J, Van Minh AT, Heblich F, Nieto-Rostro M, Watschinger K, Striessnig J, Wratten J, Davies A, Dolphin AC. Pharmacological disruption of calcium channel trafficking by the $\alpha 2\delta$ ligand gabapentin. *Proc Natl Acad Sci U S A* 2008; 105: 3628–3633.
98. Uchitel OD, Inchauspe CG, Urbano FJ, Di Guilmi MN. Ca V2.1 voltage activated calcium channels and synaptic transmission in familial hemiplegic migraine pathogenesis. *J Physiol Paris* 2012; 106: 12–22.
99. Weissmann C, Di Guilmi MN, Urbano FJ, Uchitel OD. Acute effects of pregabalin on the function and cellular distribution of CaV2.1 in HEK293t cells. *Brain Res Bull* 2013; 90: 107–113.
100. Lakomá J, Rimondini R, Donadio V, Liguori R, Caprini M. Pain related channels are differentially expressed in neuronal and non-neuronal cells of glabrous skin of Fabry knockout male mice. *PLoS One* 2014; 9: e108641.
101. Üçeyler N, Biko L, Hose D, Hofmann L, Sommer C. Comprehensive and differential long-term characterization of the alpha-galactosidase a deficient mouse model of Fabry disease focusing on the sensory system and pain development. *Mol Pain* 2016; 12: 174480691664637–174480691664610.
102. Choi L, Vernon J, Kopach O, Minett MS, Mills K, Clayton PT, Meert T, Wood JN. The Fabry disease-associated lipid lyso-Gb3 enhances voltage-gated calcium currents in sensory neurons and causes pain. *Neurosci Lett* 2015; 594: 163–168.
103. Rickert V, Kramer D, Schubert AL, Sommer C, Wischmeyer E, Üçeyler N. Globotriaosylceramide-induced reduction of KCa1.1 channel activity and activation of the Notch1 signaling pathway in skin fibroblasts of male Fabry patients with pain. *Exp Neurol* 2020; 324: 113134.
104. Park S, Kim JA, Joo KY, Choi S, Choi EN, Shin JA, Han KH, Jung SC, Suh SH. Globotriaosylceramide leads to K (Ca)3.1 channel dysfunction: a new insight into endothelial dysfunction in Fabry disease. *Cardiovasc Res* 2011; 89: 290–299.
105. Choi S, Kim JA, Na H-Y, Cho S-E, Park S, Jung S-C, Suh SH. Globotriaosylceramide induces lysosomal degradation of endothelial K Ca3.1 in Fabry disease. *Arterioscler Thromb Vasc Biol* 2014; 34: 81–89.
106. Choi JY, Shin MY, Suh SH, Park S. Lyso-globotriaosylceramide downregulates KCa3.1 channel expression to inhibit collagen synthesis in fibroblasts. *Biochem Biophys Res Commun* 2015; 468: 883–888.
107. Choi JY, Park S. Role of protein kinase A and class II phosphatidylinositol 3-kinase C2β in the downregulation of KCa3.1 channel synthesis and membrane surface expression by lyso-globotriaosylceramide. *Biochem Biophys Res Commun* 2016; 470: 907–912.
108. Namer B, Ørstavik K, Schmidt R, Mair N, Kleggetveit IP, Zeidler M, Martha T, Jorum E, Schmelz M, Kalpachidou T, Kress M, Langeslag M. Changes in ionic conductance signature of nociceptive neurons underlying Fabry disease phenotype. *Front Neurol* 2017; 8: 335.
109. Klein MC, Oaklander AL. Ion channels and neuropathic pain. *Elife* 2018; 7
110. Scholz A. Mechanisms of (local) anaesthetics on voltage-gated sodium and other ion channels. *Br J Anaesth* 2002; 89: 52–61.
111. Shields SD, Deng L, Reese RM, Dourado M, Tao J, Foreman O, Chang JH, Hackos DH. Insensitivity to pain upon adult-onset deletion of nav1.7 or its blockade with selective inhibitors. *J Neurosci* 2018; 38: 10180–10201.
112. Liu XD, Yang JJ, Fang D, Cai J, Wan Y, Xing GG. Functional upregulation of Nav1.8 sodium channels on the membrane of dorsal root ganglia neurons contributes to the development of cancer-induced bone pain. *PLoS One* 2014; 9: e114623.
113. Toyoda H. Ion channels involved in spontaneous pain. *Neurol Neurobiol* 2018; 1: 2–7.
114. Activation of the TRPM8 channel can generate analgesia in chronic neuropathic pain. *Nat Clin Pract Neurol* 2006; 2: 643.
115. Dini L, Del Lungo M, Resta F, Melchiorre M, Spinelli V, Di Cesare Mannelli L, Ghelardini C, Laurino A, Sartiani L, Coppini R, Mannaioni G, Cerbai E, Romanelli MN. Selective blockade of HCN1/HCN2 channels as a potential pharmacological strategy against pain. *Front Pharmacol* 2018; 9: 1252.
116. Geevasinga N, Tchan M, Sillence D, Vucic S. Upregulation of inward rectifying currents and Fabry disease neuropathy. *J Peripher Nerv Syst* 2012; 17: 399–406.
117. Busserolles J, Gasull X, Noël J, Busserolles J, Gasull X, Noël J. Potassium channels and pain. *The Oxford Handbook of the Neurobiology of Pain*. 2019, pp. 1–83. doi: 10.1093/oxfordhb/9780190860509.013.19
118. Park S, Kim JA, Joo KY, Choi S, Choi E-N, Shin J-A, Han K-H, Jung S-C, Suh SH. Globotriaosylceramide leads to KCa3.1 channel dysfunction: a new insight into endothelial dysfunction in Fabry disease. *Cardiovasc Res* 2011; 89: 290–299.
119. Ghlichloo I, Gerriets V. Nonsteroidal Anti-inflammatory Drugs (NSAIDs). In: *StatPearls [Internet]*. Treasure Island (FL): StatPearls Publishing, 2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK547742/
120. Voilley N, De Weille J, Mamet J, Lazdunski M. Nonsteroid anti-inflammatory drugs inhibit both the activity and the inflammation-induced expression of acid-sensing ion channels in nociceptors. *J Neurosci* 2001; 21: 8026–8033.
121. Voilley N. Acid-sensing ion channels (ASICs): new targets for the analgesic effects of non-steroid anti-inflammatory drugs (NSAIDs). *Curr Drug Targets Inflamm Allergy* 2004; 3: 71–79.
122. Cruz CDC, Cruz F. The ERK 1 and 2 pathway in the nervous system: from basic aspects to possible clinical applications in pain and visceral dysfunction. *Curr Neuropharmacol* 2007; 5: 244–252.

123. Pang XY, Liu T, Jiang F, Ji YH. Activation of spinal ERK signaling pathway contributes to pain-related responses induced by scorpion Buthus martensi Karch venom. *Toxicon* 2008; 51: 994–1007.
124. Rodrigues LG, Ferraz MJ, Rodrigues D, Pais-Vieira M, Lima D, Brady RO, Sousa MM, Sá-Miranda MC. Neurophysiological, behavioral and morphological abnormalities in the Fabry knockout mice. *Neurobiol Dis* 2009; 33: 48–56.
125. Bangari DS, Ashe KM, Desnick RJ, Maloney C, Lydon J, Piepenhagen P, Budman E, Leonard JP, Cheng SH, Marshall J, Thurberg BL. α-Galactosidase a knockout mice: progressive organ pathology resembles the type 2 later-onset phenotype of Fabry disease. *Am J Pathol* 2015; 185: 651–665.
126. Jabbarzadeh-Tabrizi S, Boutin M, Day TS, Taroua M, Schiffmann R, Auray-Blais C, Shen J-S. Assessing the role of glycosphingolipids in the phenotype severity of Fabry disease mouse model. *J Lipid Res* 2020; 61: 1410–1423.
127. Burand A, Stucky C. Fabry disease pain: patient and preclinical parallels. *Pain* 2021; 162: 1305–1321.
128. Mogil JS. Qualitative sex differences in pain processing: emerging evidence of a biased literature. *Nat Rev Neurosci* 2020; 21: 353–365.
129. Traub RJ, Ji Y. Sex differences and hormonal modulation of deep tissue pain. *Front Neuroendocrinol* 2013; 34: 350–366.
130. Di Noto PM, Newman L, Wall S, Einstein G. The hermunculus: what is known about the representation of the female body in the brain? *Cereb Cortex* 2013; 23: 1005–1013.
131. Echevarria L, Benistan K, Toussaint A, Dubourg O, Hagege AA, Eladari D, Jabbour F, Beldjord C, De Mazancourt P, Germain DP. X-chromosome inactivation in female patients with Fabry disease. *Clin Genet* 2016; 89: 44–54.
132. Hofmann L, Karl F, Sommer C, Üçeyler N. Affective and cognitive behavior in the alpha-galactosidase a deficient mouse model of Fabry disease. *PLoS One* 2017; 12: e0180601–e0180614.
133. Rigaud M, Gemes G, Barabas M-E, Chernoff DI, Abram SE, Stucky CL, Hogan QH. Species and strain differences in rodent sciatic nerve anatomy: implications for studies of neuropathic pain. *Pain* 2008; 136: 188–201.
134. Gerlai R. Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype? *Trends Neurosci* 1996; 19: 177–181.
135. Wilson SG, Mogil JS. Measuring pain in the (knockout) mouse: big challenges in a small mammal. *Behav Brain Res* 2001; 125: 65–73.
136. Mogil JS, Wilson SG, Bon K, Lee SE, Chung K, Raber P, Pieper JO, Hain HS, Belknap JK, Hubert L, Elmer GI, Chung JM, Devor M. Heritability of nociception I: responses of 11 inbred mouse strains on 12 measures of nociception. *Pain* 1999; 80: 67–82.
137. Kummer KK, Kalpachidou T, Mitrić M, Langeslag M, Kress M. Altered gene expression in prefrontal cortex of a Fabry disease mouse model. *Front Mol Neurosci* 2018; 11: 201.