

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 pre-clinical 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 available³ 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 cells⁵ 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 brain¹⁰ 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 A δ , and unmyelinated C-fibers. Fiber hypofunction with a preference for A δ 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 A δ 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 A δ 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 reports¹⁸ 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 newborns 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 transfected 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
	IMEF1 cell line transfected with a plasmid which encodes α -Gal A.	Increase in α -Gal A activity up to 4-fold vs non-treated IMFEI cells.	Ruiz De Garibay et al. ²⁹
	FD Human BM CD341-enriched cells transfected with α -Gal A retrovirus	Increased α -Gal A activity, secretion and correction of lipid accumulation.	Takenaka et al. ³⁰
	FD mouse BM cells transfected with α -Gal A and human IL-2Ra chain (retroviral vector).	Multilineage corrected hematopoietic cells in transplanted animals.	Qin et al. ³¹
	FD Skin fibroblasts infected with human α -Gal A cDNA retroviral vector.	Secreted enzyme observed.	Medin et al. ³²
Fibroblasts cell lines	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
	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
	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
	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
	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
Podocyte Cell Culture	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
	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
Tobacco cells	Fabry podocytes treated with α -Gal A.	High Gb3 clearance, but deregulated signaling pathways unchanged.	Braun et al. ⁵⁰
Renal epithelial 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
	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
Induced pluripotent stem cells (iPSC)	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
	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
Neuronal	FD patients' peripheral blood cells differentiated into vascular endothelial-like cells expressing CD31, VE-cadherin, and von WF.	Excess Gb3 suppression 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
Neuronal	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
	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
<i>Caenorhabditis elegans</i>	GANA-1 is a single <i>C. elegans</i> 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
G3Srg/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
Nav _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; Uçeyler 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 Glako 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 Ca ₂₊ channels)	Primary cultures of DRG neurons from WT mice incubated with LysoGb3 DRG neurons from WT or Glako mice (from > 18 weeks)	Lyso-Gb3 in clinical concentrations increased Ca ₂₊ levels in capsaicin-sensitive small-diameter peptidergic neurons. Conductance of VGCC reduced (both, low and high voltage).	Gb3 or lyso-Gb3 administration induces mechanical allodynia in healthy 7-8 weeks mice (von Frey filament test). Heat and mechanical hyposensitivity from altered excitability (Von Frey filaments and Hargreaves and hot plate test) in 20-24 week old mice.	Choi et al. ¹⁰² Namer et al. ¹⁷
HCN2 (Hcn2)	DRG neurons from WT or Glako mice	Hyperpolarization-activated (I _h) 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 1/2 pathway activated, and prevented by blocking ASIC1a channels (Psalmitoxin-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
KCNIN4 (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 EEAI-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. ^{69,8)}). 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 old group, 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) Gait analysis	Heat hypersensitivity in young vs hyposensitive in old GLAko mice 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. 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 (≥ 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 ≥ 18 months.		
	Physical activity and body weight			
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 hypersensitivity; 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 A δ Fibers. Reduced excitability of cultured DRG neurons,	Namer et al. ¹⁷

(continued)

Table 4. Continued.

Model	Behavioural test	Main findings	Others	References	
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.	Hargreaves test (paw withdrawal)	Hyposensitivity to heat.	reduced conductance of Nav _v VGCC currents, with activation of K _v currents was at more depolarized potentials.	Hofmann et al. ¹³²	
	Hot plate test	Hyposensitivity to heat.			
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.	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. ⁶	
	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.			
	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.p. Capsaicin in old GLAko mice. In turn, GLAko mice are protected from heat and mechanical hypersensitivity;		
	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.	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.		
	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 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.	Substantial serum and tissue accumulation of α -galactosyl glycolipids 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
	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 $\text{Na}_v1.7$ in zebrafish sensory neurons led to decreased small fiber density and increased sensitivity to temperature changes⁶⁷ 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: A δ -fibers with cold, cold-pain, and mechanical pain detection thresholds; C-fibers with heat and heat-pain detection thresholds; and large fiber (A $\alpha\beta$ -) 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 nociceptive behavior in response to an innocuous stimulus

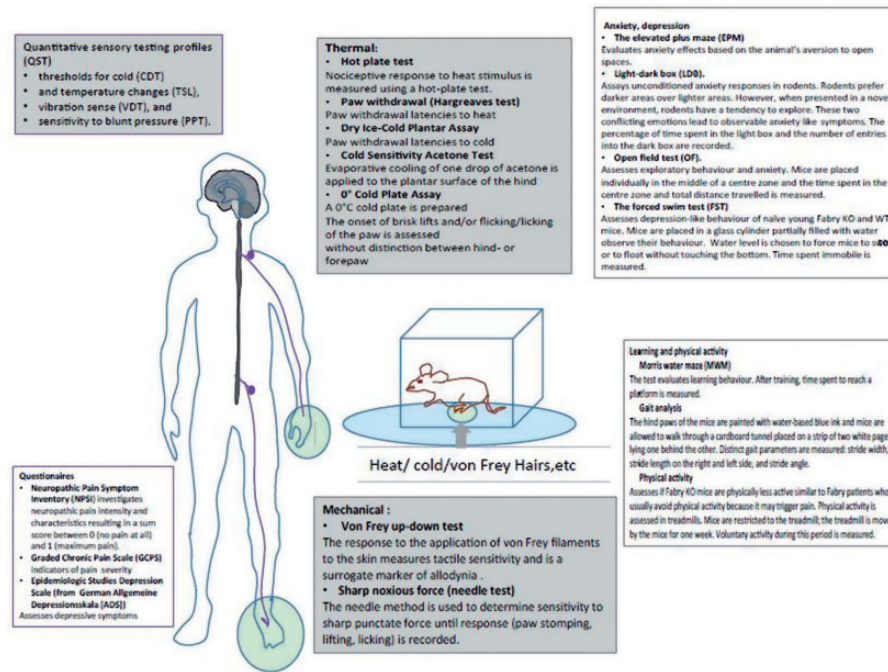


Figure 1. Pain assessment: Comparison of tests performed on human patients and animal models to assess pain. Abbreviations: QST quantitative sensory testing; description of mouse tests according to Refs.^{86–91}

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 $\alpha 2\delta 1$ 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 $Ca_v 2.1$ 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 ($\text{Na}_v1.7$ and $\text{Na}_v1.8$), 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 $\text{Na}_v1.7$, 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 $\text{Na}_v1.8$ channels in nociceptors has been associated with neuropathy, while ablation of $\text{Na}_v1.8$ 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 stimuli¹⁷ Consistent with this finding, Hofmann et al. determined a reduction of $\text{Na}_v1.7$ 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 $\text{Na}_v1.8$ 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 non-selective 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 I_h 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 (I_h) 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 1996¹³⁴ 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 accordingly⁶ (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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