Signatures of Altered Gene Expression in Dorsal Root Ganglia of a Fabry Disease Mouse Model

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Fabry disease is an X-linked lysosomal storage disorder with involvement of the nervous system. Accumulation of glycosphingolipids within peripheral nerves and/or dorsal root ganglia results in pain due to small-fiber neuropathy, which affects the majority of patients already in early childhood. The α-galactosidase A deficient mouse proved to be an adequate model for Fabry disease, as it shares many symptoms including altered temperature sensitivity and pain perception. To characterize the signatures of gene expression that might underlie Fabry disease-associated sensory deficits and pain, we performed one-color based hybridization microarray expression profiling of DRG explants from adult α-galactosidase A deficient mice and age-matched wildtype controls. Protein-protein interaction (PPI) and pathway analyses were performed for differentially regulated mRNAs. We found 812 differentially expressed genes between adult α-galactosidase A deficient mice and age-matched wildtype controls, 506 of them being upregulated, and 306 being downregulated. Among the enriched pathways and processes, the disease-specific pathways “lysosome” and “ceramide metabolic process” were identified, enhancing reliability of the current analysis. Novel pathways that we identified include “G-protein coupled receptor signaling” and “retrograde transport” for the upregulated genes. From the analysis of downregulated genes, immune-related pathways, autoimmune, and infection pathways emerged. The current analysis is the first to present a differential gene expression profile of DRGs from α-galactosidase A deficient mice, thereby providing knowledge on possible mechanisms underlying neuropathic pain related symptoms in Fabry patients. Therefore, the presented data provide new insights into the development of the pain phenotype and might lead to new treatment strategies.

Keywords: Fabry disease, alpha Galactosidase A, lysosomal storage disorder, neuropathy, neurodegeneration, neuropathic pain

INTRODUCTION

Fabry disease (FD) is an X-linked lysosomal storage disorder with estimated incidence rates of 1:37,000 for the classical Fabry phenotype and 1:3,100 for a late-onset disease variant (Spada et al., 2006; Mechtler et al., 2012). It can be caused by more than 500 different mutations of the lysosomal α-galactosidase A (α-Gal A) gene (Gal et al., 2006; Saito et al., 2011). Those mutations lead to deficient activity, reduction or depletion of α-Gal A, followed by impaired degradation of glycosphingolipids and subsequent accumulation of globotriaosylceramide (Gb3) in a variety
of tissues, including vascular endothelial cells and neurons (Desnick et al., 2001; Bangari et al., 2015). In general, males are more affected by the α-Gal A mutations, but also heterozygote females have a significant risk for major organ involvement due to random X-inactivation causing variable expression of α-Gal A and decreased quality of life (Wilcox et al., 2008). One of the earliest symptoms of FD is pain due to small-fiber neuropathy, which affects the majority of patients already in early childhood. It can manifest as episodic crises with pain attacks originating in the extremities that can last for several days or even weeks, or chronic pain characterized by burning and tingling paraesthesia (Germain, 2010; Ginsberg, 2013). The origin of this pain phenotype presumably lies in accumulation of glycolipids within peripheral nerves and/or dorsal root ganglion (DRG) somata that might lead to degeneration of small sensory fibers (Kocen and Thomas, 1970; Ohnishi and Dyck, 1974; Bangari et al., 2015; Godel et al., 2017).

Investigating FD specific pathogenesis in Fabry patients is difficult and limited to molecular analyses of tissue biopsies and clinical neurophysiology techniques. It has been found that both motor and sensory conduction velocities are decreased, whereas vibratory, cold, and heat thresholds are elevated in Fabry patients (Sheth and Swick, 1980; Dutsch et al., 2002; Uceyler et al., 2013; Namer et al., 2017). In addition, the proportion of mechano-responsive C-fibers is reduced in patients compared to healthy controls (Namer et al., 2017). To investigate the molecular and physiological mechanisms underlying the pathology of FD, α-galactosidase A deficient mice [α-Gal A(−/−)] were generated which share many symptoms with Fabry patients, including altered temperature sensitivity and pain perception (Ohshima et al., 1997; Lakoma et al., 2014; Uceyler et al., 2016; Namer et al., 2017).

Although FD constitutes a monogenic disease with loss of function mutations of the α-Gal A gene causing the disease, other genes and/or gene products might be indirectly regulated during disease progression and could play important roles in the manifestation of disease-specific pathologies and symptoms, like the development of small-fiber neuropathy. In the current study we therefore performed mRNA microarray expression profiling of DRG samples from α-Gal A(−/0) mice aged > 20 weeks when the disease is fully developed to investigate the mRNA signatures associated with FD peripheral nerve neuropathy.

**METHODS**

**Animals**

Male α-galactosidase A(−/0) (α-Gal A(−/0); background C57BL/6; provided by Dr. A. Kulkarni, National Institute of Health, NIDCR, Bethesda, USA) (Ohshima et al., 1997) and wildtype C57BL/6J mice aged 20-24 weeks were inbred and housed under specific pathogen-free (SPF) conditions. For microarray expression profiling mice from the separate inbred colonies were used, whereas for RT-qPCR validation, α-Gal A(−/0) mice backcrossed with wildtype C57BL/6J mice and wildtype C57BL/6J mice were used to control for inbred colony effects. Animals were maintained at constant room temperature of 24°C on a 12 h light/dark cycle with lights on from 07:00 to 19:00 and had *ad libitum* access to autoclaved pelleted food and water.
water. All animals were treated in accordance with the Ethics Guidelines of Animal Care (Medical University of Innsbruck), as well as the European Communities Council Directive of 22 September 2010 on the protection of animals used for scientific purposes (2010/63/EU), and approved by the Austrian National Animal Experiment Ethics Committee of the Austrian Bundesministerium für Wissenschaft und Forschung (permit number BMWF-66.011/0054-WF/V/3b/2015).

### Tissue Collection

For microarray expression profiling eight adult mice (aged between 20 and 24 weeks) per group, whereas for RT-qPCR validation six adult mice (aged between 20 and 24 weeks) per group, were deeply anesthetized with isoflurane and euthanized by decapitation. Spinal cords were removed, lumbar DRGs L3-L5 (containing the cell bodies of primary afferents that project into the hind paw) harvested and flash-frozen in liquid nitrogen. Samples were kept at −80°C until further processing. For microarray expression profiling, DRGs from two mice were pooled for the final tissue sample.

### Microarray Expression Profiling

Genome-wide expression profiling was carried out by IMGM Laboratories (Munich, Germany) using Agilent SurePrint G3 Mouse GE 8×60K Microarrays in combination with a one-color based hybridization protocol. Microarray signals were detected using the Agilent DNA Microarray Scanner.

Total RNA including small RNAs was isolated using the miRNeasy Mini Kit (Qiagen) according to the manufacturer's instructions and eluted in 40 µl RNase-free water. RNA concentration and purity was determined on a NanoDrop ND-1000 spectral photometer (Peqlab). Samples were analyzed using the RNA 6000 Nano LabChip Kit (Agilent Technologies) on a 2100 Bioanalyzer (Agilent Technologies). For mRNA analysis, total RNA samples were spiked with *in vitro* synthesized polyadenylated transcripts (One-Color RNA Spike-In Mix, Agilent Technologies), reverse transcribed into cDNA and then converted into Cyanine-3 labeled complementary RNA (Low Input Quick-Amp Labeling Kit One-Color, Agilent Technologies) according to the manufacturer's instructions. cRNA concentration, RNA absorbance ratio, and Cyanine-3 dye concentration were recorded using a NanoDrop ND-1000 UV-VIS spectral photometer, and quality of labeled cRNA was analyzed using the RNA 6000 Nano LabChip Kit (Agilent Technologies) on a 2100 Bioanalyzer (Agilent Technologies). Following cRNA clean-up and quantification, Cyanine-3-labeled cRNA samples were fragmented and prepared for one-color-based hybridization (Gene Expression Hybridization Kit, Agilent Technologies) and hybridized at 65°C for 17 h on Agilent SurePrint G3 Mouse GE 8×60K Microarrays. After hybridization, microarrays were washed with increasing stringency using Triton X-102 supplemented Gene Expression Wash Buffers (Agilent Technologies) followed by drying with acetonitrile (Sigma). Fluorescence signals were detected on an Agilent DNA Microarray Scanner and extracted using feature extraction software (Agilent Technologies). The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (Edgar et al., 2002) and are accessible through GEO Series accession number GSE104625 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE104625).

### Bioinformatics Analyses

GeneSpring GX 13.0 analysis software (Agilent Technologies) was used to normalize and analyze the microarray raw data. Data were normalized using non-parametric quantile normalization. Groups were compared using Welch's approximate *t*-test (unpaired unequal variances) and *p*-values corrected for multiple testing using the algorithm of Benjamini and Hochberg (Benjamini and Hochberg, 1995), controlling for false discovery...
TABLE 1 | Raw expression values, fold changes and statistical analysis for significantly upregulated genes.

| NCBI RefSeq ID | Gene symbol | Gene name                              | Expression wildtype | Fold change | p-value | FDR         |
|----------------|-------------|----------------------------------------|---------------------|-------------|---------|-------------|
| NM_001277925   | Ecel1       | Endothelin converting enzyme-like 1    | 3,723               | 12.3        | 0.0021  | 0.1166      |
| NM_001099632   | Rnf39       | Ring finger protein 39                 | 1,048               | 7.2         | <0.0001 | 0.0038      |
| NM_007870      | Dnase113    | Deoxynucleobase 1-like 3              | 286                 | 7.1         | <0.0001 | 0.0028      |
| BC096660       | Tmemb181b-ps| Transmembrane protein 181B, pseudogene| 290                 | 5.1         | 0.0054  | 0.1676      |
| NM_173405      | Amz1        | Archaeysin family metallopeptidase 1   | 1,865               | 3.7         | 0.0001  | 0.0327      |
| NM_013887      | Opn4        | Opin 4 (melanopalin)                  | 149                 | 3.7         | <0.0001 | 0.0117      |
| XR_105403      | A930033H14Rik| RIKEN cDNA A930033H14 gene             | 215                 | 3.6         | <0.0001 | 0.0089      |
| DQ459435       | Gm4924      | Predicted gene 4924                   | 2,969               | 3.4         | 0.0003  | 0.0548      |
| NM_013811      | Dnah8       | Dynein, axonemal, heavy chain 8       | 552                 | 3.3         | <0.0001 | 0.0044      |
| NM_053110      | Gpnmb       | Glycoprotein (transmembrane) nmb      | 446                 | 3.0         | 0.0081  | 0.1909      |
| NM_026528      | 2700060602Rik| RIKEN cDNA 2700060602 gene             | 242                 | 3.0         | 0.0015  | 0.0992      |
| NM_133762      | Ncapg2      | Non-SMC condensin II complex, subunit G2| 641                | 2.8         | <0.0001 | 0.0181      |
| NM_007489      | At3         | Activating transcription factor 3     | 1,776               | 2.7         | 0.0003  | 0.0524      |
| NM_001177470   | Gm7325      | Predicted gene 7325                   | 845                 | 2.6         | 0.0001  | 0.0324      |
| NM_008579      | Meig1       | Melosis expressed gene 1              | 712                 | 2.5         | <0.0001 | 0.0168      |
| NM_019465      | Crtam       | Cytotoxic and regulatory T cell molecule| 102                | 2.4         | 0.0009  | 0.0784      |
| NM_008579      | Meig1       | Melosis expressed gene 1              | 287                 | 2.4         | <0.0001 | 0.0059      |
| NM_025876      | Cdk5rap1    | CDK5 regulatory subunit associated protein 1| 487              | 2.4         | 0.0010  | 0.0839      |
| NM_023434      | Toyx        | TOX high mobility group box family member 4| 480                | 2.4         | 0.0004  | 0.0609      |
| NM_013473      | Anxa8       | Annexin A8                            | 142                 | 2.3         | 0.0076  | 0.1879      |
| NM_008682      | Neddf       | Neural precursor cell expressed, developmentally down-regulated gene 1| 192         | 2.3         | 0.0001  | 0.0321      |
| NM_001037928   | Gm11992     | Predicted gene 11992                  | 172                 | 2.3         | 0.0025  | 0.1233      |
| NM_026251      | Pat2        | Protein associated with topoisomerase II homolog 2 (yeast)| 226            | 2.2         | 0.0002  | 0.0392      |
| NM_031202      | Tyrp1       | Tyrosinase-related protein 1          | 2,358               | 2.2         | 0.0008  | 0.0755      |
| NM_011933      | Decr2       | 2-4-dienyl-Coenzyme A reductase 2, peroxisomal| 3,844          | 2.2         | 0.0020  | 0.1141      |
| NM_010871      | Nai6p       | NLR family, apoptosis inhibitory protein 6| 145                | 2.2         | 0.0017  | 0.1073      |
| NM_177576      | Sun3        | Sad1 and UNC84 domain containing 3    | 109                 | 2.2         | 0.0067  | 0.1815      |
| NM_026798      | Wdr65       | WD repeat domain 65                   | 1,875               | 2.1         | 0.0001  | 0.0290      |
| NM_001033293   | Uap111      | UDP-N-acetylglucosamine pyrophosphorylase 1-like 1| 3,662          | 2.1         | 0.0001  | 0.0291      |
| NM_026358      | Mgarp       | Mitochondria localized glutamic acid rich protein| 421             | 2.1         | 0.0001  | 0.0279      |
| NM_009659      | Alox12b     | Arachidonate 12-lipoxygenase, 12R type| 135                 | 2.1         | 0.0011  | 0.0869      |
| NM_001166630   | Dynl1c      | Dynin light chain Tctex-type 1C       | 16,719              | 2.1         | 0.0081  | 0.1909      |
| NM_007413      | Adora2b     | Adenosine A2B receptor                | 1,478               | 2.1         | 0.0008  | 0.0771      |
| AK031397       | Hps1        | Hermansky-Pudlak syndrome 1 homolog (human)| 156             | 2.0         | 0.0010  | 0.0850      |
| NM_020574      | Kcnj3       | Potassium voltage-gated channel, Isk-related subfamily, gene 3| 159             | 2.0         | 0.0001  | 0.0332      |
| NM_001145953   | Lgals3      | Lectin, galactose binding, soluble 3  | 19,457              | 2.0         | 0.0010  | 0.0850      |
| NM_183187      | Fam107a     | Family with sequence similarity 107, member A| 277              | 2.0         | 0.0067  | 0.1813      |
| NM_013710      | Fgd2        | FYVE, RhoGEF and PH domain containing 2| 314               | 2.0         | 0.0053  | 0.1650      |
| NM_026283      | Samd8       | Sterile alpha motifs domain containing 8| 362               | 2.0         | <0.0001 | 0.0065      |
| NM_001199948   | Dynl1f      | Dynin light chain Tctex-type 1F       | 12,240              | 2.0         | 0.0029  | 0.1333      |
| XR_002334      | Lnr31       | Leucine rich repeat containing 31     | 689                 | 2.0         | 0.0008  | 0.0755      |

Differential expression between the two groups was determined by calculating fold changes of the averaged normalized expression values. Significantly regulated mRNAs were identified by applying filters on fold changes (absolute fold change ≥ 1.2 or ≥2) and p-values (p ≤ 0.01). Chip array data were further processed by R statistics statistical software package (R Development Core Team, 2008) and Volcano plots prepared using R statistics ggplot function. Only genes with uncompromised hybridization values in all individual samples were used for the current analysis.
### Protein-Protein Interaction Analysis

Protein-protein interactions (PPIs) were investigated for the significantly regulated mRNAs using the STRING Database v. 10.5 (http://www.string-db.org) (Szklarczyk et al., 2017), which includes direct and indirect protein associations collected from different databases. Interaction networks were prepared using medium confidence scores (0.40) and clustered using MCL clustering algorithm (inflation parameter: 3). Disconnected nodes were hidden from the network.

### Functional Enrichment and Pathway Analysis

Functional enrichment and pathway analyses were also performed using the STRING Database v. 10.5 (http://www.string-db.org). Classification systems tested were Gene Ontology and KEGG functional annotation spaces, employing Fisher's exact test followed by a correction for multiple testing (FDR).

### RT-qPCR Validation of Regulated Genes

Reverse transcription quantitative polymerase chain reaction (RT-qPCR) validation of regulated genes was performed using TaqMan Gene Expression Assays (Thermo Fisher Scientific) in an Applied Biosystems 7500 Fast-Real-Time PCR System (Thermo Fisher Scientific).

Table 2 summarizes the results of RT-qPCR validation for downregulated genes.

#### Table 2: Raw expression values, fold changes, and statistical analysis for significantly downregulated genes.

| NCBI RefSeq ID | Gene symbol | Gene name | Expression α-Gal A<sup>+/−</sup> | Expression wildtype | Fold change | p-value | FDR  |
|----------------|-------------|-----------|----------------------------------|---------------------|-------------|--------|------|
| NR_003506      | Gm3893      | Predicted gene 3893 | 74 | 1,582  | −22.4 | <0.0001 | 0.0006 |
| NR_003123      | 4933409K07Rik | RIKEN cDNA 4933409K07 gene | 134  | 1,332 | −10.5 | <0.0001 | 0.0002 |
| NM_001085530   | Gm13298     | Predicted gene 13298 | 214  | 2,104  | −10.4 | <0.0001 | 0.0028 |
| NM_001085530   | Gm13298     | Predicted gene 13298 | 217  | 1,989  | −9.8  | <0.0001 | 0.0002 |
| NM_003506      | Gm3893      | Predicted gene 3893 | 92   | 628    | −7.1  | <0.0001 | 0.0044 |
| AK046830       | Prune2      | Prune homolog 2 (Drosophila) | 256  | 1,279  | −5.3  | 0.0012 | 0.0905 |
| NM_001085530   | Gm13298     | Predicted gene 13298 | 143  | 883    | −5.0  | <0.0001 | 0.0038 |
| NM_008228      | Hdac1       | Histone deacetylase 1 | 577  | 2,465  | −4.6  | <0.0001 | 0.0065 |
| AK009987       | 2310058N22Rik | RIKEN cDNA 2310058N22 gene | 342  | 1,408  | −4.4  | <0.0001 | 0.0117 |
| AK147155       | S100pbp     | S100P binding protein 100 | 2274 | 5,394  | −22.4 | <0.0001 | 0.0006 |
| NM_134041      | 4930427A07Rik | RIKEN cDNA 4930427A07 gene | 74   | 303    | −4.2  | <0.0001 | 0.0102 |
| NM_145932      | S100pbp     | Solute carrier family 51, alpha subunit | 130  | 513    | −4.1  | 0.0001 | 0.0376 |
| NM_181529      | Syt15       | Synaptotagmin XV | 56   | 201    | −3.7  | 0.0001 | 0.0254 |
| NM_001193667   | Gm1987      | Predicted gene 1987 | 981  | 3,294  | −3.6  | <0.0001 | 0.0120 |
| NM_027865      | Tmem25      | Transmembrane protein 25 | 5609 | 18,317 | −3.6  | <0.0001 | 0.0005 |
| NM_010923      | Nrat        | Neuronatin | 185  | 620    | −3.5  | <0.0001 | 0.0005 |
| NM_010923      | Nrat        | Neuronatin | 233  | 773    | −3.5  | <0.0001 | 0.0001 |
| NM_015521      | 1700030C10Rik | RIKEN cDNA 1700030C10 gene | 150  | 466    | −3.2  | 0.0015 | 0.1002 |
| NM_172803      | Dock4       | Cytoskeletal protein 4 | 1,369 | 3,479  | −2.8  | 0.0003 | 0.0541 |
| XM_003945355   | LOC101056136 | Disks large homolog 5-like | 51   | 135    | −2.7  | 0.0001 | 0.0326 |
| NM_008228      | Hdac1       | Histone deacetylase 1 | 159  | 407    | −2.7  | 0.0004 | 0.0591 |
| NM_145563      | Zfp932      | Zinc finger protein 932 | 1,101 | 2,666  | −2.6  | <0.0001 | 0.0076 |
| XM_001135567   | 119000707Rik | RIKEN cDNA 119000707 gene | 571  | 1,387  | −2.6  | 0.0008 | 0.0763 |
| NM_022995      | Pmepa1      | Prostate transmembrane protein, androgen induced 1 | 2,274 | 5,394  | −2.6  | 0.0001 | 0.0378 |
| AK139097       | S100Pbp     | S100P binding protein | 100  | 239    | −2.5  | <0.0001 | 0.0117 |
| NM_175475      | Cyp26b1     | Cytochrome P450, family 26, subfamily b, polypeptide 1 | 583  | 1,264  | −2.3  | 0.0008 | 0.0756 |
| NM_178420      | Nr1x        | NLR family member X1 | 383  | 793    | −2.2  | 0.0015 | 0.0991 |
| NM_018857      | Meln        | Mesothelin | 81   | 172    | −2.2  | 0.0054 | 0.1674 |
| NM_011109      | Usp18       | Ubiquitin specific peptidase 18 | 226  | 451    | −2.1  | 0.0045 | 0.1552 |
| NM_029011      | Pyroxd2     | Pyridine nucleotide-disulfide oxidoreductase domain 2 | 57   | 115    | −2.1  | 0.0066 | 0.1804 |
| NM_198026      | Iqcc        | IQ motif containing C | 556  | 1,068  | −2.0  | <0.0001 | 0.0130 |
TABLE 3 | Enrichment-analysis for upregulated mRNAs in α-Gal A(−/−) vs. wildtype mice using gene ontology and KEGG pathway annotations.

| Pathway ID | Pathway description | Count in network | False discovery rate |
|------------|---------------------|------------------|----------------------|
| 04142      | Lysosome            | 12               | 0.0011               |
| 05204      | Chemical carcinogenesis | 9              | 0.0073               |
| 00980      | Metabolism of xenobiotics by cytochrome P450 | 7 | 0.0184 |
| 00480      | Glutathione metabolism | 6 | 0.0317 |
| 00511      | Other glycan degradation | 4 | 0.0231 |
| **KEGG PATHWAYS** |                     |                  |                      |
| 04142      | Lysosome            | 12               | 0.0011               |
| 05204      | Chemical carcinogenesis | 9              | 0.0073               |
| 00980      | Metabolism of xenobiotics by cytochrome P450 | 7 | 0.0184 |
| 00480      | Glutathione metabolism | 6 | 0.0317 |
| 00511      | Other glycan degradation | 4 | 0.0231 |
| **BIOLOGICAL PROCESSES (GO)** |                     |                  |                      |
| GO:0008150 | Biological_process | 254              | 0.0328               |
| GO:0009987 | Cellular_process    | 242              | 0.0139               |
| GO:0044763 | Single-organism cellular process | 204 | 0.0139 |
| GO:0008152 | Metabolic process   | 184              | 0.0328               |
| GO:1901564 | Organonitrogen compound metabolic process | 46 | 0.0139 |
| GO:0033993 | Response to lipid   | 30               | 0.0462               |
| GO:0006872 | Ceramide metabolic process | 8 | 0.0328 |
| **CELLULAR COMPONENT (GO)** |                     |                  |                      |
| GO:0005575 | Cellular_component  | 299              | 0.0004               |
| GO:0005623 | Cell                | 273              | 0.0004               |
| GO:0044484 | Cell part           | 273              | 0.0004               |
| GO:0005622 | Intracellular       | 256              | 0.0001               |
| GO:0043226 | Organelle           | 251              | <0.0001              |
| GO:0044424 | Intracellular part  | 251              | 0.0001               |
| GO:0043227 | Membrane-bounded organelle | 239 | <0.0001 |
| GO:0005737 | Cytoplasm           | 228              | <0.0001              |
| GO:0043229 | Intracellular organelle | 222 | 0.0010 |
| GO:0043231 | Intracellular membrane-bounded organelle | 208 | 0.0009 |
| GO:0016020 | Membrane            | 172              | 0.0053               |
| GO:0044444 | Cytoplasmic part    | 164              | <0.0001              |
| GO:0044422 | Organelle part      | 151              | 0.0309               |
| GO:0044425 | Membrane part       | 134              | 0.0088               |
| GO:0031224 | Intrinsic component of membrane | 116 | 0.0149 |
| GO:0016021 | Integral component of membrane | 112 | 0.0211 |
| GO:0005576 | Extracellular region | 98               | 0.0222               |
| GO:0031982 | Vesicle             | 97               | 0.0001               |
| GO:0005888 | Plasma membrane     | 96               | 0.0095               |
| GO:0071944 | Cell periphery      | 96               | 0.0186               |
| GO:0031988 | Membrane-bounded vesicle | 94 | 0.0001 |
| GO:0044421 | Extracellular region part | 91 | 0.0044 |
| GO:0070062 | Extracellular exosome | 77 | 0.0004 |
| GO:0031090 | Organelle membrane  | 69               | 0.0260               |
| GO:0044459 | Plasma membrane part | 57 | 0.0041 |
| GO:0005829 | Cytosol             | 49               | 0.0335               |
| GO:0098805 | Whole membrane      | 49               | 0.0378               |
| GO:0005739 | Mitochondrion       | 48               | 0.0222               |
| GO:0031226 | Intrinsic component of plasma membrane | 33 | 0.0170 |
| GO:0005887 | Integral component of plasma membrane | 30 | 0.0434 |

TABLE 3 | Continued

| Pathway ID | Pathway description | Count in network | False discovery rate |
|------------|---------------------|------------------|----------------------|
| GO:0005773 | Vacuole             | 25               | 0.0006               |
| GO:0048471 | Perinuclear region of cytoplasm | 25 | 0.0041 |
| GO:0005764 | Lysosome            | 23               | 0.0004               |
| GO:0042470 | Melanosome          | 8                | 0.0309               |
| GO:0030904 | Retromer complex    | 4                | 0.0200               |
| GO:0097422 | Tubular endosome    | 3                | 0.0044               |
| GO:1906222 | CHOP-ATF3 complex   | 2                | 0.0186               |

Whole genome was used as statistical background.

RESULTS

mRNA Expression Profile of Fabry Mouse Dorsal Root Ganglia

Using microarray expression profiling we found that in total 812 genes from the overall 21,736 detected mRNAs were significantly different between DRG samples from wildtype and α-Gal A(−/−) mice (criteria p ≤ 0.01, absolute fold change ≥ 1.2) (Figure 1). Of those, 506 genes were significantly upregulated and 306 genes were significantly downregulated as compared to wildtype controls. More stringent filtering (criteria p ≤ 0.01, absolute fold change ≥ 2) of those significantly regulated genes revealed an assessable number of 78 genes in total (Figure 2). Using these criteria 41 genes were significantly upregulated, of which 29 showed FDR corrected p ≤ 0.1 (Table 1). Furthermore, 31 genes remained significantly downregulated, of which 27 showed FDR corrected p ≤ 0.1 (Table 2). PPI analysis (STRING Database) neither revealed clusters of interacting proteins nor enriched pathways, due to the small number of input genes. Thus, for in
FIGURE 3 | STRING database protein-protein interaction (PPI) network of significantly upregulated genes. Cut-off values, \( p \leq 0.01 \), fold change \( \geq 1.2 \).

Enriched Pathways and Protein-Protein Interactions for Upregulated mRNAs

Enrichment analysis of the 506 significantly upregulated genes revealed that a number of KEGG pathways and Gene Ontology processes were enriched, including the KEGG pathway “Lysosome” (KEGG:04142) and the biological process “Ceramide metabolic process” (GO:0006672), both known to constitute major hallmarks of FD pathogenesis (Table 3).

Protein-protein interaction (PPI) analysis of significantly upregulated mRNAs revealed a significant PPI enrichment (\( p < 0.0001; \) Figure 3). The number of actually observed edges (\( n = 328 \)) exceeded the expected number of edges (\( n = 231 \)) by 42%. Furthermore, three clusters of at least five interconnected proteins became apparent. Enrichment analysis of those clusters showed that those genes were involved in different pathways (Table 4), the red cluster was related to “G-protein coupled receptor signaling” (e.g., GO:0007186), the pink cluster was involved in “retrograde transport” (GO:0042147) and the orange cluster was related to “glutathione transferase activity” (GO:0004364).

Enriched Pathways and Protein-Protein Interactions for Downregulated mRNAs

Enrichment analysis for the 306 significantly downregulated genes revealed a variety of regulated pathways, including immune related pathways (e.g., Complement and coagulation cascades, Antigen processing and presentation, Immune system process, Immune responses, etc.), autoimmune diseases (e.g., Systemic lupus erythematosus, Diabetes mellitus Type 1, Autoimmune thyroid disease, Asthma, etc.) and different infection pathways (e.g., Herpes simplex, Staphylococcus aureus, Leishmaniasis, etc.). In addition, “Neuroactive ligand-receptor interaction” (KEGG:04080) and “Vesicle” (GO:0031982) were enriched in the downregulated mRNAs (Table 5).

Also, the PPI analysis of significantly downregulated mRNAs revealed a significant PPI enrichment (\( p < 0.0001; \) Figure 4). Actually observed edges (\( n = 250 \)) exceeded the expected number of edges (\( n = 134 \)) by 87%. Also for the downregulated mRNAs clusters of interconnected proteins emerged. Enrichment analysis showed three clusters (i.e., green, purple, and cyan) related to the immune system (e.g., Immune system process—GO:0002376, Immune response—GO:0006955). The blue cluster was associated with gene regulation (e.g., Chromatin modification—GO:0016568) and the rose cluster was related to “G-protein coupled receptor activity” (GO:0004930) (Table 6).

Ion Channel Regulation

As the sensory deficits of Fabry patients are generally accepted to be caused by changes in the excitability of sensory neurons, we...
TABLE 5 | Enrichment-analysis for downregulated mRNAs in α-Gal A<sup>−/−</sup> vs. wildtype mice using gene ontology and KEGG pathway annotations.

| Pathway ID | Pathway description | Count in network | False discovery rate |
|------------|---------------------|------------------|---------------------|
| **KEGG PATHWAYS** | | | |
| 05168 | Herpes simplex infection | 12 | 0.0003 |
| 04080 | Neuroactive ligand-receptor interaction | 10 | 0.0220 |
| 05164 | Influenza A | 9 | 0.0050 |
| 05322 | Systemic lupus erythematosus | 8 | 0.0007 |
| 05150 | Staphylococcus aureus infection | 7 | 0.0003 |
| 05164 | Antigen processing and presentation | 7 | 0.0247 |
| 04145 | Cell adhesion molecules (CAMs) | 7 | 0.0319 |
| 05332 | Graft-versus-host disease | 6 | 0.0010 |
| 05330 | Allograft rejection | 5 | 0.0058 |
| 04940 | Type I diabetes mellitus | 5 | 0.0100 |
| 05322 | Autoimmune thyroid disease | 5 | 0.0159 |
| 05140 | Leishmaniasis | 5 | 0.0193 |
| 05164 | Antigen processing and presentation | 5 | 0.0220 |
| 04145 | Phagosome | 5 | 0.0247 |
| 05332 | Asthma | 4 | 0.0050 |
| 04672 | Intestinal immune network for IgA production | 4 | 0.0220 |
| **BIOLOGICAL PROCESSES (GO)** | | | |
| GO:0050896 | Response to stimulus | 93 | <0.0001 |
| GO:0044707 | Single-multicellular organism process | 75 | 0.0471 |
| GO:0044852 | Negative regulation of cellular process | 61 | 0.0268 |
| GO:0006950 | Response to stress | 54 | 0.0005 |
| GO:0051239 | Regulation of multicellular organismal process | 43 | 0.0301 |
| GO:0010033 | Response to organic substance | 38 | 0.0351 |
| GO:0002376 | Immune system process | 36 | <0.0001 |
| GO:0006952 | Defense response | 31 | 0.0000 |
| GO:0051240 | Positive regulation of multicellular organismal process | 30 | 0.0222 |
| GO:0006955 | Immune response | 24 | 0.0007 |
| GO:0007155 | Membrane | 22 | 0.0465 |
| GO:0016064 | Immunoglobulin mediated immune response | 16 | 0.0080 |
| GO:0006959 | Humoral immune response | 7 | 0.0280 |
| GO:0002455 | Humoral immune response mediated by circulating immunoglobulin | 6 | 0.0015 |
| GO:0048002 | Antigen processing and presentation of peptide antigen | 5 | 0.0280 |
| GO:0019886 | Antigen processing and presentation of exogenous peptide antigen via MHC class II | 4 | 0.0080 |
| GO:0070268 | Cytokine activity | 2 | 0.0471 |

**CELLULAR COMPONENT (GO)**

| Pathway ID | Pathway description | Count in network | False discovery rate |
|------------|---------------------|------------------|---------------------|
| GO:0005775 | Cellular_component | 183 | 0.0002 |
| GO:0044486 | Cell part | 160 | 0.0052 |
| GO:0005823 | Cell | 160 | 0.0057 |
| GO:0044287 | Membrane | 112 | 0.0004 |
| GO:004425 | Membrane part | 91 | 0.0003 |
| GO:0031224 | Intrinsinc component of membrane | 82 | 0.0002 |
| GO:0046021 | Integral component of membrane | 80 | 0.0002 |
| GO:005886 | Plasma membrane | 68 | 0.0003 |
| GO:0071444 | Cell periphery | 68 | 0.0005 |
| GO:0005676 | Extracellular region | 62 | 0.0252 |
| GO:004421 | Extracellular region part | 58 | 0.0054 |
| GO:0031982 | Vesicle | 57 | 0.0041 |
| GO:0031988 | Membrane-assisted vesicle | 52 | 0.0252 |
| GO:0070062 | Extracellular exosome | 49 | 0.0016 |
| GO:004459 | Plasma membrane part | 40 | 0.0011 |
| GO:005615 | Extracellular space | 25 | 0.0252 |
| GO:005887 | Integral component of plasma membrane | 24 | 0.0026 |
| GO:008797 | Plasma membrane protein complex | 16 | 0.0011 |
| GO:0045121 | Membrane raft | 10 | 0.0252 |
| GO:0072562 | Blood microvesicle | 6 | 0.0252 |
| GO:0042611 | MHC protein complex | 5 | 0.0002 |
| GO:0042613 | MHC class II protein complex | 4 | 0.0001 |
| GO:0035998 | ESC/E(Z) complex | 3 | 0.0252 |

Whole genome was used as statistical background.

Specifically searched our dataset for genes related to ion channels, ion channel function, and trafficking. Besides downregulation of voltage-gated sodium and calcium channels (i.e., Scn7a and Cacna1h), we found that several potassium channels and potassium channel associated proteins were differentially expressed (Table 7). Voltage-gated (i.e., Kcnb2) and calcium activated potassium channel subunits (i.e., Kcnn1 and Kctn1), as well as potassium channel tetramerization and interacting proteins (i.e., Kcnip2, Pctd16, and Kctd11) were downregulated in DRGs from FD mice. In contrast, the potassium channel ancillary beta subunit Kcne3 was upregulated. Last but not least, the mechanosensitive ion channel Piezo2 was significantly downregulated. Against all expectation, we found none of
the pain-associated transient receptor potential (TRP) channels regulated.

**RT-qPCR Validation of Regulated Genes**

To validate the differentially expressed genes from the microarray expression profiling, we performed RT-qPCR analysis of the top 10 up- and downregulated genes in a separate set of samples from α-Gal A(−/−) mice backcrossed with C57BL/6J mice and C57BL/6J wildtype mice. We found that 9/10 of the upregulated genes (i.e., Rnf39, Opn4, Ecel1, Dnah8, Amz1, Dnase1l3, Meig1, Atf3, and Ncapg2) showed significant upregulation, whereas only one gene (i.e., Samd8) was not regulated (Figure 5A). For the downregulated genes, 6/10 genes (i.e., Slc51a, Zfp932, Gm1987, Syt15, Nnat, and Hdad1) were significantly downregulated, and four genes (i.e., Tmem25, S100pbp, Pmepa1, and Dock4) did not show regulation (Figure 5B). Thus, differential expression of 75% of the genes selected for RT-qPCR validation could be verified.

**DISCUSSION**

Neuropathic pain and small-nerve fiber neuropathy are among the first symptoms of Fabry disease and affect the majority of patients already in early childhood. Therefore, the involvement of sensory neurons, whose cell somata are located in DRGs, is evident. However, our study is the first to present a differential gene expression profile of DRGs from α-Gal A(−/−) mice, a recognized mouse model for FD (Ohshima et al., 1997; Lakoma et al., 2014; Uceyler et al., 2016), and wildtype controls. We performed microarray expression profiling and found that 812 genes were significantly deregulated, 506 of them being upregulated and 306 being downregulated. Enrichment analysis revealed that the two pathways “lysosome” and “ceramide metabolic process” were significantly enriched. As FD is part of the broad family of lysosomal storage disorders that all show defects in ceramide metabolism (Platt et al., 2012), our results demonstrate the involvement of these two pathways also in DRG neurons and therefore enhance the reliability of the current analysis.

When taking a closer look at the significantly downregulated genes the “immune system” emerged as another disease specific entity. Lysosomal storage disorders in general are associated with deficits in processing of protein antigens and antibody production (Daly et al., 2000), and Hawkins-Salsbury et al. (2011) specifically report an immune deficit in Fabry patients. In the present study, enrichment analysis of downregulated genes revealed mainly immune system related pathways and processes, for example different autoimmune diseases, infection pathways and processes like “immune responses” or “antigen processing and presentation” (Table 5). In this regard, it might be noted that the downregulated purple cluster which includes serine-protease inhibitors (Serpins) might also be involved in nervous system related symptoms. Serpins are known to play a role in coagulation, and loss of serpins might induce a variety of bleeding disorders (Kaiserman et al., 2006). It has recently been
shown that angiokeratoma, one of the first dermatologic disease presentations in Fabry patients, if present in gastrointestinal mucosa can lead to life-threatening bleeding episodes during coagulation therapy (Oh et al., 2016; Kang et al., 2017). Interestingly, 30% of Fabry patients show cerebral microbleeds (Kono et al., 2016), which together with the downregulated Thrombospondin 1 (Thsd7a) and Thromboxane a2 receptor (Tbxa2r) can be related to a general deficit in blood coagulation pathways. Further analysis of the regulation and impairment of those genes might open up new treatment strategies for cerebral vasculopathy, including cerebral hemorrhage, stroke, or other cerebral lesions associated with FD (Schifffmann and Moore, 2006). In a mouse model of neuropathic pain, it has been shown that mice that underwent surgery for chronic constriction injury showed activation of the immune system in higher brain structures (Koks et al., 2008). Based on these results it would be interesting to see if this immune activation is also present in brains of FD mice and/or patients.

Enrichment analysis of the upregulated clusters revealed significant enrichment of the “G-protein coupled receptor signaling” and “retrograde transport” pathways. Upregulation of genes in these clusters could be related to hypersensitivity and changes in excitability of DRG nerve fibers as a possible underlying cause of the frequent pain attacks experienced by Fabry patients (Schifffmann and Moore, 2006; Uceyler et al., 2014). Although the genes in the reported clusters are not directly related to changes in excitability, a number of ion channels were significantly deregulated and could be responsible for the hyperexcitability. Besides downregulation of voltage-gated sodium and calcium channels, different potassium channels and associated proteins showed regulation. In contrast, only Kcn3—a potassium channel ancillary beta subunit known to increase excitability (Abbott et al., 2001)—was upregulated. To date, knowledge on changes in ion channel expression and function in FD are sparse and controversial. Lakoma et al. (2014) reported increased immunoreactivity for a voltage-gated sodium channel Na1.8 (Scn10a) in skin samples of FD mouse sensory neurons including the free nerve endings. Recently, decreased conductance of sodium currents in dissociated DRG neurons from FD mice was demonstrated (Namer et al., 2017). This latter publication also reported activation of voltage-gated potassium channels at more depolarized potentials, supporting a general reduction in FD neuron excitability (Namer et al., 2017). With regard to calcium channels it has been shown that Lyso-Gb3 enhances voltage-gated calcium currents in DRGs of FD mice (Choi L. et al., 2015), whereas Namer et al. (2017) report decreased voltage-gated calcium currents in α-Gal A(−/−) nociceptors. We also found a downregulation of the mechanosensitive ion channel Piezo2 mRNA, which may possibly be correlated to the decreased number of mechanosensitive fibers found in both human patients and FD mice (Namer et al., 2017). With regards to temperature sensitive ion channels it has been shown that expression of Trpv1 was increased, whereas expression of Trpm8 was decreased in skin biopsies of FD mice (Lakoma et al., 2014, 2016), which may be related to the changed thermal thresholds reported in both Fabry patients and mice (Sheth and Swick, 1980; Dutsch et al., 2002; Uceyler et al., 2013; Namer et al., 2017). The present unbiased screen for differentially expressed ion channels did not confirm deregulation of Trpv1 or Trpm8 though.

Previous gene expression studies were not performed in neuronal tissues but could still be affected by the same regulating pathways. In α-Gal A(−/−) fibroblasts and endothelial cells Kc_Ca.3.1 (Kccn4) was downregulated (Choi et al., 2014; Choi J. Y. et al., 2015) and the conductance of calcium-activated potassium channels was reduced (Olivan-Viguera et al., 2017). Additional gene expression studies have been performed in hepatic, renal and human blood cells (Park et al., 2009; Cigna et al., 2013; Shin et al., 2015). Thrombospondin 2 and 4 have been found to be upregulated in FD kidney cells (Park et al., 2009), whereas our results show a downregulation of both Thrombospondin 1 (Thsd7a) and Thromboxane a2 receptor (Tbxa2r) in FD DRGs. Both observations point towards impaired blood coagulation pathways in FD. In the same screen Neuropeptide Y (NPY) was found to be upregulated (Park et al., 2009). In the current dataset, a different neuropeptide, Neuropeptide B, was significantly upregulated, which has been shown to be functionally connected with NPY at least in fish.
(Yang et al., 2014). Furthermore, different types of S100 calcium binding proteins, i.e., S100a4/a8/a9 are upregulated in liver and kidney (Park et al., 2009), whereas S100bp (a S100P binding protein) was decreased in FD DRGs in the present screen.

The deregulated genes that emerged from our analysis largely overlap with genes from previous reports on other painful disorders, although the direction of regulation does not always match. Uregulation of the transcription factor Atf3 is in line with previous reports that showed induction of Atf3 in DRGs in different models of nerve injury (Tsujino et al., 2000; Shortland et al., 2006; Matsuura et al., 2013), as well as upon exposure to noxious stimuli (Braz and Basbaum, 2010). Also, the adenosine receptor Adora2b which was upregulated in FD mice promotes chronic pain through neuro-immune interactions (Hu et al., 2016). The Tyrp1 gene has been associated with thermal nociception, and loss of function mutations generate deficits in thermal nociception (Fortin et al., 2010). Furthermore, Cdk5-mediated phosphorylation modulates Trpv1 function (Jendryke et al., 2016). Upregulation of these two latter genes in FD may therefore be associated with burning and tingling paraeesthesias reported in Fabry patients (Germain, 2010; Ginsberg, 2013). Neuronatin (Nnat), which is significantly downregulated in the current screen, was upregulated in DRGs after sciatric nerve injury and associated with mechanical hypersensitivity (Chen et al., 2010). Several genes in the clusters that emerged from the current analysis, are associated with G-protein signaling and are controversially discussed (Pan et al., 2008). The somatostatin receptor Strr2 in the red cluster is downregulated after sciatric nerve ligation (Shi et al., 2014), but elevated in response to intestinal inflammation (Van Op den Bosch et al., 2009). The endothelin receptor Ednrb attenuates cancer-induced pain (Viet et al., 2011), and the angiotensin receptor Agrp1b has been proposed as a biomarker for pain (Grace et al., 2012). All clusters involve genes that have been associated with exacerbated pain phenotypes in clinical or preclinical studies. Single nucleotide polymorphisms in the serotonin receptor gene Htr2a in the rose gene cluster are associated with pain-phenotypes as a genetic predisposition to musculoskeletal pain (Nicholl et al., 2011). The hypocretin receptor Hcrtr1 is associated with migraine (Rainier et al., 2011), and Kalirin (Klrrn), a Rho guanine nucleotide exchange factor, is required for persistent nociceptive activity dependent synaptic long-term potentiation (Lu et al., 2015). The pink cluster contains genes that are mainly associated with retrograde transport. Vps26a is increased following spinal nerve ligation in the spinal dorsal horn and is required for recycling of mGluR5 and plasticity at excitatory synapses (Lin et al., 2015). Vps35, another regulated gene product from our screen, forms a complex with Vps26a (Kim et al., 2010) and is also highly associated with members from the sorting nexin family (e.g., Snx6 and Snx8 from our screen). Individuals with polymorphisms in Gluthatione-S-transferase genes found in the orange gene cluster are more likely to develop neuropathy during

### TABLE 7 | Raw expression values, fold changes, and statistical analysis for significantly regulated ion channels.

| NCBI RefSeq ID | Gene symbol | Gene name | Expression α-Gal A+/−/0 | Expression wildtype | Fold change | p-value | FDR |
|---------------|-------------|-----------|-------------------------|---------------------|------------|---------|-----|
| NM_020574     | Kcone3      | Potassium voltage-gated channel, isk-related subfamily, gene 3 | 160 | 77 | 2.0 | 0.0001 | 0.0332 |
| NM_001190870  | Kcone3      | Potassium voltage-gated channel, isk-related subfamily, gene 3 | 142 | 77 | 1.8 | 0.0001 | 0.0236 |
| NM_001042489  | Hvcn1       | Hydrogen voltage-gated channel 1 | 215 | 133 | 1.6 | 0.0045 | 0.1545 |
| NM_146037     | Kcnk13      | Potassium channel, subfamily K, member 13 | 1,721 | 1,049 | 1.5 | 0.0000 | 0.0122 |
| NM_001042489  | Hvcn1       | Hydrogen voltage-gated channel 1 | 312 | 217 | 1.4 | 0.0046 | 0.1565 |
| NM_031169     | Kcnnb1      | Potassium large conductance calcium-activated channel, subfamily M, beta member 1 | 1,759 | 2,621 | −1.6 | 0.0000 | 0.0059 |
| NM_011028     | P2x6        | Purinergic receptor P2X, ligand-gated ion channel, 6 | 523 | 682 | −1.4 | 0.0045 | 0.1546 |
| NM_175462     | Kcnt1       | Potassium channel, subfamily T, member 1 | 11,165 | 13,232 | −1.3 | 0.0056 | 0.1701 |
| NM_145703     | Kcnip2      | Kv channel-interacting protein 2 | 1,321 | 1,598 | −1.3 | 0.0027 | 0.1273 |
| NM_026135     | Kctd16      | Potassium channel tetramerisation domain containing 16 | 947 | 1,140 | −1.3 | 0.0029 | 0.1327 |
| NM_001039485  | Piezo2      | Piezo-type mechanosensitive ion channel component 2 | 1,089 | 1,258 | −1.2 | 0.0085 | 0.1966 |
| NM_021415     | Cacna1h     | Calcium channel, voltage-dependent, T type, alpha 1H subunit | 3,547 | 4,085 | −1.3 | 0.0029 | 0.1327 |
| NM_009135     | Scn7a       | Sodium channel, voltage-gated, type VII, alpha | 1,193 | 1,366 | −1.2 | 0.0071 | 0.1837 |
| NM_001098528  | Kcnb2       | Potassium voltage-gated channel, Shab-related subfamily, member 2 | 1,600 | 1,799 | −1.2 | 0.0061 | 0.1744 |
| NM_153143     | Kctd11      | Potassium channel tetramerisation domain containing 11 | 1,445 | 1,624 | −1.2 | 0.0051 | 0.1637 |
oxaliplatin treatment (Kanat et al., 2017). In addition, activation of Aldh2, a gene associated with the glutathione pathway, reduces nociception in acute inflammatory pain (Zambelli et al., 2014). This gene is regulated by Aldh3a1 which was deregulated in the current analysis (Chen et al., 2015). The green cluster contained the gene Tnfsf10, a member of the Tumor necrosis factor superfamily. Tnfsf10 is increased by excitotoxic spinal cord injury (Plunkett et al., 2001), downregulated in inflamed tissue (Yang et al., 2007), and associated with migraine susceptibility (Jia et al., 2015). Parp10, a poly(ADP-ribose) polymerase upregulates pro-inflammatory pathways, and its inhibition attenuates neuropathy and neuroinflammation (Komirishetty et al., 2016a,b). Interferon regulatory factor Irf5 is increased in spinal microglia after peripheral nerve injury and drives P2X4R+ reactive microglia thereby gating neuropathic pain (Masuda et al., 2014). In Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE), a disease exhibiting joint pain symptoms, mutations have been found in proteasome subunit genes Psmb8 and Psmb9 (Arimochi et al., 2016). Genes from the Oas dsRNA sensor family, in particular Oas1a and Oas1b, are induced by lipopolysaccharides, which induce inflammatory pain (Lee et al., 2013). The E3 ubiquitin ligase Nedd4 is decreased in DRGs of SNI mice (Laedermann et al., 2013), and ribosomal protein Rps25, as well as other ribosomal proteins are downregulated in a model for HIV-associated neuropathic pain (Maratou et al., 2009). In line with the downregulation of Hdac1 in the blue cluster, HDAC inhibitors attenuate the development of hypersensitivity (Denk et al., 2013), restore C-fiber sensitivity (Matsushita et al., 2013), and induce behavioral anti-nociception (Tao et al., 2016). In addition, nerve injury increases the activity of Hdac1 and Ezh2 (Laumet et al., 2015). Pain responses depend on genes from the major histocompatibility complex (MHC; Guo et al., 2015), and the MHC-2 haplotype is involved in the incidence of postherpetic pain (Sato-Takeda et al., 2006). Further, MHC-2 molecules synergize with Toll-like receptor Tlr4 in inducing an innate immune response (Frei et al., 2010), and the lymphocyte antigen Ly86 is required in DRG neurons for functional Toll-like receptor
Tlr4 signaling (Grace et al., 2014). Serpin3n is upregulated in mouse DRGs following nerve injury and attenuates neuropathic pain. Mice lacking Serpin3n develop more severe neuropathic pain symptoms than wildtypes (Vicuna et al., 2015). Another member of the Serpin family—Serping—has been implicated in hereditary angioedema, as mutations in this gene are associated with abdominal pain symptoms (Andrejevic et al., 2015). Finally, the Complement component genes C1r, C1s and C3 are upregulated after spinal nerve ligation (Levin et al., 2008). When comparing the emerging FD pain related genes with the global pain systems network for heat nociception (Neely et al., 2012) only epidermal growth factor receptor pathway substrate 8 (Eps8), alpha-N-acetylgalactosaminidase (Naga) and the proteasome subunit gene Psmb8 were contained. Together, this comprehensive literature search demonstrates considerable overlap of the current FD expression profile with genes implicated in nociception and pain disorders, suggesting relevant common pathogenesis components of FD pain and other pain disorders.

Despite constituting the first presentation of differentially expressed genes in DRG explants of α-Gal A(−/−) mice, some limitations of the current analysis need to be considered. For instance, concerns have been raised that the α-Gal A(−/−) mouse model might only resemble the later-onset phenotype of FD (Bangari et al., 2015). In kidney, Gb3 concentrations only reach 25% of that found in patients, and FD mouse life expectancy is normal (Taguchi et al., 2013). Therefore, the G3Stg/GLA knockout mouse has been generated and evaluated as a new FD mouse model, in which α-Gal A(−/−) mice were crossedbred with transgenic mice expressing the human Gb3 synthase. This resulted in symptomatic animals with increased Gb3 accumulation and progressive renal impairment (Taguchi et al., 2013). Another FD mouse model is the NOD/SCID immune deficiency mouse model with tissue specific Gb3 accumulation, but without clinical manifestation (Pacienza et al., 2012). Few data are available for these genetic models yet, but it would be important to know to what extent the three FD mouse models share the same differential gene expression. In addition, it would be of interest to explore the deregulation of gene expression in heterozygote females, which in humans and mice exhibit a considerably weaker phenotype than males (Ucelýer et al., 2013, 2016). Screening of homozygote females could be helpful to better understand the mechanisms and degree of X-chromosomal inactivation in female Fabry patients (Wilcox et al., 2008). Finally, it should be noted that gene targeting experiments are prone to a general phenomenon of background dependence that might confound the interpretation of results (Schalkwyk et al., 2007). In this study we controlled for this effect by using α-Gal A(−/−/−) mice that had been backcrossed to an inbred C57BL/6j colony for the RT-qPCR validation of regulated genes.

Our in-depth bioinformatics analysis revealed a new set of genes and pathways that might be involved in the FD-associated small-nerve fiber neuropathy. These data give rise to subsequent functional studies on the importance of these deregulated genes for the pathogenesis of FD small fiber disease and neuropathic pain, and are expected to lead to the identification of novel treatment strategies, especially for neuropathic pain related symptoms in Fabry patients.

**AUTHOR CONTRIBUTIONS**

KK, MK, and ML: designed the study; KK, TK, and ML: performed the data collection, analyzed, and interpreted the data; KK: wrote the manuscript. TK, MK, and ML: critically reviewed the contents of the paper and suggested substantial improvements; All authors have approved the final version of the manuscript.

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