## Supplementary information

### Genetic analysis of 59 cardiomyopathy related genes

### Genetic analyses of the *GLA* gene

Genomic DNA was isolated from peripheral blood samples using standard methods for the detection of germline mutations. Haloplex Target Enrichment System (Agilent Technologies, Santa Clara, CA, USA) was used to capture the all exons of the *GLA* gene for next generation sequencing. Online design tool SureDesign (https://earray.cham.agilent. com/suredesign/) was used for capturing the probe design. Target regions consisted of exons, UTRs and 10bp flanking regions of the GLA gene from the RefSeq database (GRCh37/hg19). Library preparation was performed using HaloPlex Target Enrichment Kit following the manufacturer's instructions. Paired-end sequencing (2300 bp) was performed on MiSeq instrument (Illumina, San Diego, CA, USA) using the MiSeq Reagent Kits v3 (600 cycles). The gene variant found was confirmed with Sanger sequencing (Genome Center of Eastern Finland).

### Targeted next-generation sequencing

Haloplex Target Enrichment System (Agilent Technologies) was used to capture regions of interest for next generation sequencing.

Online design tool SureDesign (https://earray.chem.agilent.com/suredesign/) was used to capture of probe design. Target regions consisted of exons, UTRs and 10bp flanking regions of 59 cardiomyopathy associated genes from the RefSeq and Ensembl databases (GRCh37/hg19) (Supplementary Table S1). Total length of the target regions was 496 kbp and involved 1478 regions. The design yielded 18767 amplicons covering 491 kbp of target regions. Library preparation was performed using HaloPlex Target Enrichment Kit by following the manufacturer's instructions. Paired-end sequencing (2 × 300 bp) was performed on MiSeq instrument (Illumina) using the MiSeq Reagent Kits v3 (600 cycles). After sequencing, the average sequencing depth was 316 fold and 92% of the target regions were sequenced at least 15-fold.

### Data analysis and variant calling

In-house developed analysis pipeline was used for the analysis of raw fastq files generated by the MiSeq-sequencer. Cutadapt (https://code.google.com/p/cutadapt/) software was applied for

Illumina sequencing adapter removal and read trimming. Reads shorter than 20bp were abandoned. Remaining reads were mapped to human reference genome hg19 using BWA-MEM algorithm (http://bio-bwa.sourceforge.net/). Variant calling (SNVs and indels) was performed using four different variant callers: GATK HaplotypeCaller (https://www.broadinstitute.org/gatk/), SAMTools mpileup (http://samtools.sourceforge.net/), Atlas2 (http://sourceforge.net/projects/atlas2/) and Platypus (http://www.well.ox.ac.uk/platypus). All called variants were annotated using SnpEff (http://snpeff.sourceforge.net/), ANNOVAR (http://annovar.openbioinformatics.org/) and different public databases (eg, 1000 Genomes, dbSNP and ClinVar). Variants had to meet the following conditions to be included in the downstream analysis: 1) located within the exonic or splicing regions, 2) have high or moderate effect on gene function, and 3) have unknown or variant allele frequency below 1% in the 1000 genomes variant database. The alignments at variant positions were visually inspected using the Integrative Genomics Viewer (https://www.broadinstitute.org/igv/). Variants that met these conditions and passed visual inspection were annotated using Condel (http://bg.upf.edu/fannsdb/).

**Sanger sequencing**

Cascade mutation screening of all available relatives was performed with Sanger sequencing.

**Molecular structure of mutated GLA protein**

**In silico variant pathogenecity analyses**

In order to determine the pathogenicity of selected rare variants in *GLA*, different programs were used, i.e., Sorting Intolerant From Tolerant (SIFT) (http://sift.bii.a-star.edu.sg/)[1] Polymorphism Phenotyping V2 (PolyPhen-2) (http://genetics.bwh.harvard.edu/pph2/)[2], Mutation taster (http://www.mutationtaster.org/)[3] and Mutation prediction (MutPred) (http://mutpred1.mutdb.org/).[4] The variant pathogenicity was further evaluated on the basis of nucleotide conservation score, i.e., PhyloP >2.7 was selected based on the report of Visser et al. [5] The difference in amino acid physiochemical properties of wild type and mutated residue was assessed by considering Grantham distance. In addition, the frequency of a variant in general and Finnish population was determined using Genome aggregation database (http://gnomad.broadinstitute.org/). These analyses, except for MutPred, were performed using ALAMUT, a licensed program from “interactive Biosoftware”. Additional information on previously

published variants was taken from scientific literature and public databases like Human Gene Mutation Database (HGMD)[6] and ClinVar.[7]

***In silico* structural analysis**

The structural analysis of missense variants was performed using a next generation web service program "HOPE: Have your Protein Explained.[8] This program is based on protein 3D structure centered approach. It takes input query sequence and does BLAST search against sequences in protein databank (PDB) and uniprot database to identify a perfectly matched template, which is selected based on its resolution, experimental method and length of protein covered. For structural analysis, HOPE uses WHATIF calculations on PDB file or Yet Another Scientific Artificial Reality Application (YASARA) homology model, uniprot sequence annotations, Homology-derived Structures of Proteins (HSSP) conservation scores and Direct Attached Storage (DAS) server sequence based predictions. Protein features are then analyzed for each residue in terms of its contacts, physiochemical properties like hydrophobicity and accessibility, torsion angles, domain or motifs, and other variations in the sequence. HOPE combines all available information from these sources, analyze it and generates a user-friendly report.

References:

1. Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. Genome Res2001; 11: 863-874.
2. Adzhubei IA, Schmidt S, Peshkin L et al. A method and server for predicting damaging missense mutations. Nat Methods2010; 7: 248-49.
3. Schwarz JM, Cooper DN, Schuelke M et al. MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods2014; 11: 361-2.
4. Li B, Krishnan VG, Mort M et al. Automated inference of molecular mechanisms of disease from amino acid substitutions. Bioinformatics2009; 25: 2744-50.
5. Vissers L E, de Ligt J, Gilissen C et al. A de novo paradigm for mental retardation. Nat Genet2010; 42: 1109-12.
6. Stenson PD, Mort M, Ball EV et al. The Human Gene Mutation Database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. Hum Genet2017; 136: 665-677.
7. Landrum MJ, Lee JM, Benson M et al. ClinVar: public archive of interpretations of clinically relevant variants. Nucleic Acids Res2015; 4: 44(D1): D862-8.

8. Venselaar H, Te Beek TA, Kuipers RK et al. Protein structure analysis of mutations causing inheritable diseases. An e- Science approach with life scientist friendly interfaces. BMC Bioinformatics2010;11: 548.

**Alpha-galactosidase A (GLA) enzyme activity and lyso-Gb3 levels**

GLA enzyme activity was determined in leukocytes by a fluorometric enzyme assay using an artificial 4-methylumbelliferyl-alpha-D-galactoside as substrate. Active enzyme liberates 4-methylumbelliferyl from the substrate which was measured fluorometrically (excitation at 360 nm, emission at 465 nm) (Islab, Kuopio University Hospital).

For lyso-Gb3 quantification dried Blood Spot (DBS) samples were measured using a fully validated high-sensitive electrospray ionization liquid chromatography tandem mass spectrometry (ESI LC-MS/MS). Hereby, a seven-point DBS calibrator for lyso-Gb3 quantification (covering the analytic range from 0-120ng/mL; lower limit of quantification: 1.5ng/mL), as well as three level quality controls (3, 30 and 100ng/mL) were used (ARCHIMED Life Science GmbH, Vienna, Austria; www.archimedlife.com). The normal range of lyso-Gb3 was defined < 3.5 ng/ml.

**Finnish national Fabry disease protocol**

The Finnish FD- protocol[1] includes careful patient examination by specialist (cardiologist, internist, nephrologist, neurologist or pediatrician), an interview (symptom and depression questionnaire, quality of life measurements, cognitive tests) and several laboratory tests (complete blood count, plasma levels of sodium, potassium, creatinine, urea, cystatin C, alanine aminotransferase, gamma-glutamyl transpeptidase, brain natriuretic peptide (proBNP), troponin T, high-sensitivity C-reactive protein (hsCRP) and thyroid stimulating hormone, fasting plasma glucose, glycohemoglobin (Hba1C) and lipids, albumin-to-creatinine ratio, estimated GFR, creatinine clearance, ECG, plasma and urine levels of globotriaosylsphingosine (lyso-Gb3) and antibodies if on ERT) at least annually. In addition to the above, individually at the discretion of a physician, cardiac stress test, 24-hour ECG monitoring, cardiac MRI including LGE and T1 mapping, brain MRI, spirometry, audiogram and an examination by an ophthalmologist and dermatologist will be performed.

Reference:

1. Kantola I, Penttinen M, Nuutila P et al. Fabryn tauti. Duodecim2012;128:729-39.

**Imaging studies**

**CMR**

**Cine imaging**

Cardiac magnetic imaging was acquired with 1.5 T MR scanner (Magnetom Aera, Siemens, Erlangen, Germany) by using an 18-channel body array coil. Breath-hold cine MR was performed using retrospectively electrocardiographically gated segmented true fast imaging with balanced steady-state free precession (bSSFP) TrueFISP sequence. To assess left ventricular (LV) and right ventricular (RV) volumes and ejection fractions (EF) cine MR images were obtained in two-chamber, three-chamber and four-chamber long-axis planes and in short-axis planes covering both ventricles from basis to apex. The typical imaging parameters were TR/TE 35/1.2, flip angle 55 º, 218x256 matrix, 294 x 294 mm2 field of view (FOV). Slice thickness was 6 mm and inter-slice gap 20 %.

**Myocardial T1 mapping**

Myocardial T1 mapping was performed before contrast agent injection in a mid-ventricular short-axis slice using a shortened Modified Look-Locker Inversion-recovery (ShMOLLI) sequence. The short axis view imaging plane was chosen based on standard Siemens cardiac imaging routines. Slice thickness was set for 8 mm, in-plane resolution 1.4 x 1.4 mm2; FOV 360 x 306 mm2; inversion times between 100 and 2910 ms; and flip angle of 35°.

**LGE images**

Ten minutes after injection of contrast agent (gadoteratemeglumine, Dotarem® 0,2 mmol/kg) LGE images were acquired in three-chamber, four-chamber and short axis planes similarly to cine images, using inversion recovery spoiled gradient echo (IR-SPGR) sequence. The imaging parameters were TR/TE 2.58/2.3 ms, flip angle 40°, 256 x 256 matrix, 240 x340 FOV. Slice thickness was 8 mm and interslice gap 0%. Inversion time was optimized for each measurement to null the signal intensy of normal myocardium (240-360 ms).

**CMR image analysis**

Image analysis was performed by using Sectra IDS7/dx workstation. Images were analyzed by one cardiologist specialized to cardiac MR imaging (MH) and one radiology fellow (LL-R). The analyzers were blinded to genetic and clinical findings of the study subjects. To evaluate left ventricular ejection fraction (LVEF), left ventricular end-diastolic volume (LVEDV), left ventricular mass (LVM) and left ventricular end-systolic volume (LVESV), the endocardium and the epicardium were traced by using semiautomatic quantification program (Syngo.ViaSRV, Siemens Healthineers, Erlangen, Germany), with the papillary muscles and trabeculations excluded. LVESV was analyzed from the cine images with smallest LV cavity. Cardiac hypertrophy was defined as increased LV wall thickness in one or more myocardial segments of more than 12 mm. Body surface area (BSA) was calculated by using Du Bois method.

Motion corrected T1 maps were generated and T1 estimates were computed on a per-pixel basis by performing a non-linear curve fitting using the three parameter signal model. Midventricular segmental analysis was used to calculate the mean T1-relaxation values separately from the anterior, anteroseptal, inferoseptal, inferior, inferolateral and anterolateral segments of myocardium. In addition to segmental analysis we also calculated the mean T1 value of the mid-myocardium. T1 values were compared with published values for normal healthy myocardium. [1]

The amount and location of LGE were analyzed by visual evaluation.

Reference:

1. Piechnik S, Ferreira V, Lewandowski A et al. Normal variation of magnetic resonance T1 relaxation times in the human population at 1.5 T using ShMOLLI. J Cardiovasc Magn Reson2013;15(1):13.

**18F-FDG PET/CT**

Maximum standardized uptake value (SUV) was determined. To calculate the metabolic volumes of abnormal FDG uptake threshold of SUV 2.7 was used.

**Acquisition**

A whole-body 18F-FDG PET/CT scan (Siemens Biograph mCT PET/CT, Siemens/CTI, Knoxville, TN) was performed on the patients. In order to reduce physiological glucose uptake of the myocardium, the patients were on low-carbohydrate diet for 24 hours before the PET-scan and fasted at least 12 hours before the study. Blood glucose levels were measured prior to injection of the tracer. Injected dose of 18F-FDG was 4 MBq/Kg, with a maximum dose of 350 MBq. FDG is

produced from onsite cyclotron and radiochemistry facilities. An uptake period of 60 min followed the injection in recumbent position was allowed before the scan. Both patients underwent a low dose whole body CT scan (120 kV, 45 mAs, CARD Dose 4D) for anatomical reference and attenuation correction. No i.v. contrast material was used for the CT. Subsequently a wholebody PET scan with an acquisition time of 2.5 – 4.5 min per bed position according to body mass index was performed in supine. In addition, one PET scan limited to the heart (one bed position) was performed with an acquisition time of 4 min

**Imaging reconstruction and analysis**

PET images were reconstructed iteratively with and without attenuation correction, for the whole body with 200 × 200 matrix size and for the region limited to the heart LIST MODE 400 x 400 (ultra HD, PSF-correction). Images were also reformatted into coronal, transaxial and sagittal sections for viewing. The PET/CT images were interpreted by an experienced nuclear medicine specialist. Visual analysis was performed for regions of focally increased glucose metabolism. An abnormal focus of FDG uptake was defined as focal activity relatively higher than that of liver. Furthermore, images were evaluated semiquantitatively by maximal standardized uptake values normalized for lean body mass (max SUV/lbm). In addition, metabolic volume was measured using FDG-positive voxels above threshold of 2.7 g/ml.

**Endomyocardial biopsy (EMB)**

Myocardial specimens for histological analysis and immunohistochemistry were obtained from two sons of the index patient. Endomyocardial biopsy samples of the elder son were obtained under fluoroscopic guidance from the basal septum of the right ventricle and of the younger son of the right and the left ventricles with the standard bioptomes. Several representative biopsies were taken. Standard light microscopy finding of the elder son was originally interpreted as non-specific myodegeneration. Because of suspect FD, the pathologist analyzed EMB specimen once again.

Specimens for light microscopy were immersed in 10% phosphate-buffered formalin, dehydrated in ethanol, cleared in xylene and embedded in paraffin. The sections were stained with HE, PAS for carbohydrates, Congo for amyloid, and iron. Congo and iron stains were negative.

For immunohistochemical staining an anti-Gb3 antibody (TCI Chemicals, Portland, OR, USA) was applied on paraffin sections of the younger son in dilution 1:20. Visualization was done using

Dako's Envision Flex system in a Dako Autostainer Link 48 stainer (Dako, Agilent Technologies, Santa Clara, CA, USA). The staining was controlled with a Gb3-positive kidney specimen and a normal cardiac specimen. The electron microscopical specimen of the elder son of the index patient was prepared from a paraffin block by reversing the light microscopical process. Paraffin was dissolved in xylene which was removed and water restored by descending series of ethanols down to distilled water. Thus, the Gb3 stain could not be done. The specimen was thereafter fixed in the same way as the fresh cardiac sample from the younger son, i.e. in a mixture of glutaraldehyde and formalin[1] and routinely processed into blocks of epon resin (Ladd Research, VT, USA). Epon sections of 1 um thickness were first cut and stained with toluidine blue for light microscopy. Ultrathin sections were cut on grids and stained with uranyl acetate and lead citrate. The sections on grids were examined and photographed in a Jeol JEM 2100F electron microscope (Jeol Ldt., Japan).

Reference:

1. McDowell & Trump, Arch Pathol Lab Med1976; 100: 405-14.