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7th International Update on Fabry Disease

29–31 May 2022, Würzburg, Germany

Abstracts

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Conflict of Interest Statement

All of the reviewers are internationally recognized "Key Opinion Leaders" in the diagnosis and treatment of Fabry disease, and serve as consultants and on numerous advisory boards for various pharmaceutical entities involved in Fabry disease. These entities provided financial sponsorship toward the logistical costs of the 7th International Update on Fabry Disease but have had no input into the agenda, content, or arrangements. None of the Abstract Review Group received any compensation from any of these entities related to participation in the 7th Update on Fabry Disease. The sponsoring organization for this event is Kidneys for Life, which is a registered charitable organization in the UK. All of the reviewers declare that their review of the abstracts was independent and not affected by any real or perceived conflict of interest.

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7th Update on Fabry Disease: Biomarkers, Progression and Treatment Opportunities in 2022

May 29-May 31, 2022: Würzburg Germany
Supporting Organization: Kidneys for Life (Manchester, UK)
Secretariat: Kongressmanagement bei *Aey Congresse* GmbH. Berlin, Germany

Venue: Maritim Hotel and Conference Center, Würzburg Germany

Program Steering Committee:

Derralyn Hughes, Royal Free Hospital, London UK
Mirjam Langeveld, MD, AMC, Amsterdam NL
Albina Nowak, MD, University of Zurich, Zurich CH
Juan Politei, MD PhD, FESEN, Buenos Aires, AR
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Chiesi Farmaceutici S.p.A, Idorsia Pharmaceuticals Ltd,
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4D_Molecular Therapeutics, Sangamo Therapeutics
Protalix BioTherapeutics, AceLink Therapeutics, Freeline, uniQure NV

Sunday May 29th May

Workshop on Cell and Gene Therapy for Fabry Disease (Chairs: Michael West MD (Dalhousie University), and Yoshikatsu Eto, MD PhD (Jikei University)

10:00 – 10:20 Update of AAV Approaches to Fabry Disease; Derralyn Hughes MD PhD (Royal Free Hospital)

10:20 – 10:40 (Virtual Presentation): Update of Lenti-Virus Approaches to Fabry Disease; Kathy Nicholls, MD (Royal Melbourne Hospital)

10:40 –11:10 (Virtual Presentation): Knock-In and Knock-Out Approaches to Sickle Cell Disease; Mark C. Walters, MD (UCSF)

11:10 – 11:30 Group Discussion

11:30 – 12:45 Buffet Lunch

12:45 – 13:00 Welcome and Outline of Program: Christoph Wanner, MD and Peter Nordbeck, MD (University of Würzburg)

13:00 – 13:45 Keynote: Fabry Disease – Past, Present and Future; Derralyn Hughes MD PhD (Royal Free Hospital)

Session 1. New Approaches for Optimizing Treatment of Fabry Disease (Chairs: Dominique Germain, MD PhD, (Hôpital Raymond Poincaré) and João Paulo Oliveira, MD PhD (University of Porto)

13:45 – 14:15 Directed Iterative Evolution of Enzymes for Treating Metabolic Diseases: Gjalt Huisman, PhD (Codexis, Inc)

14:15 – 14:45 Structure/Function Insights from Mutation Analysis of ERT: Scott Garman, PhD (University of Massachusetts)

14:45 – 15:15 PEGylation and Immunogenicity of ERT: David G. Warnock, MD (UAB)

15:15 – 15:45 Cardiomyocytes and Novel Treatment Approaches for Fabry Disease: James Shayman, MD (University of Michigan)

15:45 – 16:15 Group discussion

16:15 – 17:30 COFFEE BREAK [Networking]

17:30 – 19:00 Poster Session with Opening Reception (Conference Center: Maritim Hotel Panorama Foyer)

Monday 30st May

Session 2. Artificial Intelligence, Machine Learning and Fabry Disease (Chairs: John Jefferies, MD (University of Tennessee) and Eric Wallace, MD (University of Alabama at Birmingham)

08:00 – 08:30 Keynote: Artificial Intelligence in the Diagnosis of Fabry Disease: Joseph Zabinski, PhD (OM1, Inc.)

08:30 – 09:00 Artificial Intelligence, MRIs and Left Ventricular Mass Index in Fabry Disease: James C. Moon, MD PhD (Barts Hospital London)

09:00 – 09:20 Group Discussion

Session 3. Biomarkers and Clinical Outcomes for Fabry Disease (Chairs: Kevin Mills, PhD (University College London), and Albina Nowak, MD (University of Zurich)

09:20 – 09:50 Molecular Basis, Diagnostics and Therapeutic Avenues for Fabry Disease: Prof. Dr. Hans Aerts (University of Leiden)

09:50 – 10:10 Organ specific biomarkers for Fabry Disease: Christiane Auray Blais, PhD (University of Sherbrooke)

10:10 – 10:30 Clinical and Biomarker Responses to Fabry Therapy: Daniel Bichet, MD (University of Montreal)

10:30 – 11:10 Group discussions

11:10 – 11:30 COFFEE BREAK [Networking and Poster Viewing]

11:30 –13:00 Difficult Cases: Series of Cases with Audience Response Before and After Presentations; Stephen Waldek, MD (University of Sunderland) and Eric Wallace, MD (University of Alabama at Birmingham)

13:00 – 14:15 LUNCH buffet style – working lunch for poster viewing and networking.

Session 4. Neurology and Fabry Disease (Chairs: Alessandro Burlina, MD (San Bassiano Hospital) and Juan Politei, MD (FESEN)

14:15 – 14:35 Nociceptive behavior and central neuropeptidergic dysregulations in Fabry disease: Lessons from a Fabry mice model; Francesco Formaggio, PhD Max Delbrick Center for Molecular Medicine Berlin

14:35 – 14:55 White matter lesions, depression, cognitive decline, and dementia in Fabry disease; Alessandro Burlina, MD (San Bassiano Hospital)

14:55 – 15:15 Understanding Fabry associated pain—a translational approach --- Nurcan Üçeyler, MD (University of Würzburg)

15:15 – 15:30 Group discussion

15:30 – 16:00 COFFEE BREAK

Sesson 5: Gastro-Intestinal Issues and Fabry Disease (Chairs: Renzo Mignani, MD (Ospedale Infermi, Rimini) and Juan Politei, MD (FESEN, Buenos Aires)

16:00 – 16:20 The Microbiome in Fabry disease; Alberto Ortiz, MD (Fundacion Jimenez Diaz)

16:20 – 16:40 Oral alpha-Galactosidase Therapy; Malte Lenders, PhD (University of Münster)

16:40 – 17:00 Group discussion

Session 6: Substrate Reduction Therapy Workshop (Chairs: James Shayman, MD PhD (University of Michigan) and Christoph Wanner, MD (University of Würzburg)

17:00 – 17:30 Substrate Reduction Therapies and Fabry Disease; James Shayman, MD PhD (University of Michigan)

17:30 – 17:45 Clinical Trial Designs for Substrate Reduction Therapy; Christoph Wanner, MD (University of Würzburg)

17:45 – 17:55 Pharmaco-dynamic Responses to Lucerastat in the Phase 3 MODIFY Trial; Peter Nordbeck, MD (University of Würzburg)

17:55 – 18:05 Pre-Clinical Results with a Novel Glucosylceramide Synthase Inhibitor (AL01211); Michael Babcock, PhD (ACELink Therapeutics)

18:05 – 18:15 A Study to Evaluate the Effect of Venglustat on Left Ventricular Mass Index in Adult Fabry Patients; James C. Moon, MD PhD (Bart's Heart Center, London)

18:15 – 18:25 Rational Design of α-1,4-Galactosyltransferase Inhibitors as Substrate Reduction Therapy for the Treatment of Fabry Disease; Nicky de Koster, PhD (Leiden University, Leiden)

18:25 – 18:45 Group discussion

19:00 – 20:30 Reception and Poster Viewing

Tuesday May 31st

Session 7 Inflammation, Autophagy and ROS in Fabry Disease (Chairs: Paula Rozenfeld, MD (CONICET-UNLP) and Sandro Feriozzi, MD (Belcolle Hospital)

08:00 – 08:30 Mitochondrial Structure, Function, and Turnover in Renal Epithelial Cells in Fabry Disease; Anke Schumann, MD PhD (University of Freiburg)

08:30 – 09:00 Novel Insights into Fabry Podocytopathy; Tobias Huber, MD (University of Hamburg-Eppendorf)

09:00 – 09:15 Group Discussion

Session 8 Anti-drug antibodies (Chairs: David Warnock, MD (University of Alabama at Birmingham) and **Mirjam Langeveld, MD (AMC)**

09:00 – 09:25 Impact of neutralizing antibodies on efficacy of ERT; Malte Lenders, PhD (University of Münster)

09:25 – 9:50 Clinical Implications and Treatment Modification for Fabry Patients with Positive ADA Status: Mirjam Langeveld, MD (AMC)

09:50 – 10:15 Group Discussion

10:15 – 11:00 COFFEE BREAK

Session 9 Cardio-Renal Session (Chairs: James Moon, MD (Barts Health NHS Trust) and Christoph Wanner, MD (University of Würz**burg)**

11:00 – 11:30 Renal Outcomes with ERT Treatment in Fabry Disease; Bojan Vujkovac, MD (General Hospital Slovenj Gradec)

11:30 – 12:00 Cardiac Outcomes with ERT Treatment in Fabry Disease; Peter Nordbeck, MD (University of Würz**burg)**

12:00 – 12:30 Cardio-Renal Outcomes with Chaperone Therapy in Fabry Disease; Dominique Germain, MD PhD, (Hôpital Raymond Poincaré))

12:30 – 13:00 Group discussion

13:00 – 14:00 LUNCH buffet style – working lunch for poster viewing and networking.

Session 10 Poster Session (Chairs: Andrew Talbot, MD (Royal Melbourne Hospital) and Richard Steeds MD (Birmingham NHS Trust) with Poster Judging Committee

14:00 – 15:00 4 x 10 minute presentations to poster prize winners preceded by prize presentations

15:00 – 15:15 Closing Remarks

ABSTRACTS

B-1: A Novel *in vivo* Tool Mimicking Fabry Disease in *Drosophila melanogaster*

Authors: Sybille Koehler [1], Lotte Stauch [2], Xiaowen Tang [3], Martin Helmstädter [2,3], Tobias B. Huber [1,2,3]

Institutions: [1] III. Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2] Renal Division, University Hospital Freiburg, D-79106 Freiburg, Germany; [3] Faculty of Biology, University of Freiburg, Germany

Background: A limitation in the identification and characterization of novel pathways is the lack of suitable *in vivo* tools to mimic Fabry nephropathy and cardiomyopathy. The model organism *Drosophila* could prove as a novel and advantageous tool for Fabry research. *Drosophila* has an open circulatory system, with only one body fluid, the haemolymph. The heart tube consists out of a single layer of cardiomyocytes and performs pulsatile contractions, which result in haemolymph flow. Due to its' similarity to the mammalian heart, especially high conservation of genes and proteins, the *Drosophila* heart can be used to investigate pathways and mechanisms influencing heart function also in mammals. The *Drosophila* kidney consists of the Malpighian tubules and the nephrocytes. The latter occur in two different populations, the garland nephrocytes, located around the oesophagus and the pericardial cells, which are localized along the heart tube. Nephrocytes filter the haemolymph and present with a highly similar morphology to mammalian podocytes.

Methods: We performed morphological and functional read-outs in isolated nephrocytes from knockout and controls flies.

Results: Here, we identified the *Drosophila* orthologue of α-GAL, CG7997, and assessed depletion of the enzyme in the fly organism. We were able to reproduce the kidney phenotype observed in Fabry patients, as loss of CG7997 in nephrocytes, the podocyte homologs in flies, caused a severe filtration defect. In addition, we could identify zebra bodies in nephrocytes and observed increased autophagy, lysosome size and disrupted endosome formation upon CG7997 depletion. Preliminary RNAseq data of nephrocytes also revealed a decrease of mitochondria associated genes, but an increase of mitochondria number and ROS.

Conclusion: Our data presented here describes a novel in vivo model to mimic human Fabry disease. We could show that Drosophila nephrocytes express a α-GAL orthologue, CG7997, and its depletion caused a nephrocyte phenotype. Future studies will now focus on other tissues and phenotypes such as the heart, brain, and gut. Moreover, Drosophila is the ideal tool to reintroduce different human mutations in the fly organisms and perform mechanistic and therapeutic assessment in vivo.

Support: None.

B-2: GLA-Mutant Zebrafish Mirror Key Pathological Aspects of Human Fabry Disease Independently of Gb3 Accumulation.

Authors: Hassan Elsaid[1], Mariell Rivedal[1] Einar Svarstad[1], Camilla Tøndel[1,2], Sabine Leh[1,3], Janka Babickova[1,4], Hans-Peter Marti[1,5], Jessica Furriol[1,5]

Institutions: [1]Renal Research Group, Department of Clinical Medicine, University of Bergen, Bergen, Norway, [2]Department of Pediatrics, Haukeland University Hospital, Bergen, Norway, [3]Department of Pathology, Haukeland University Hospital, Bergen, Norway, [4]Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia [5]Department of Medicine, Haukeland University Hospital, Bergen, Norway.

Background: In Fabry disease (FD), complex disease mechanisms interact.[1] It has recently been shown that mitochondria morphology and metabolism is distorted in renal epithelial cell.[2] Although Gb3-independent injury is well acknowledged,[3,4] it remains difficult to make the distinction between Gb3-dependent and Gb3-independent injury in patients with Fabry disease, and the clinical focus remains on the former.[5]

Methods: We investigated the Gb3-independent damage mechanisms by creating α-Galactosidase A mutant zebrafish.[6] We performed proteomics analysis on renal tissue using LC-MS/MS. Additionally, we used electron microscopy to evaluate the mitochondrial morphology parameters in the renal tubules.

Results: Our model imitated distinct features of FD nephropathy; renal damage was indicated by higher plasma creatinine, proteinuria, and podocyte foot process effacement. Our proteomics analysis results revealed 639 dysregulated proteins in mutant compared to wildtype (527 downregulated, 112 upregulated). Down-regulated proteins in the mutant included mitochondria, lysosome, and metabolism-related proteins. Mitochondrial morphology in the renal tubules is shown to be distorted comparing wildtype to mutant zebrafish.

Conclusion: Our results not only provide the first model of FD in Zebrafish, but also show that a Gb3 independent model mirrors distinct features of human FD nephropathy associated with structural and functional mitochondrial alterations, accompanied by cytoskeleton disturbances. Our work supports the role of significant Gb3-independent effect on FD nephropathy.

Support: This work was supported by the Western Norwegian Health Region (Helse Vest) (number HV912233).

References: **[1]**Eikrem, Ø. R. Skrunes, C. Tøndel, S. Leh, G. Houge, E. Svarstad, H.-P. Marti. Cell Tissue Res. 369, 53–62 (2017); **[2]**Schumann, A. K. Schaller, V. Belche, M. Cybulla, S.C. Grünert, N. Moers, J.O. Sass, A. Kaech, L. Hannibal, U. Spiekerkoetter. J. Inherit. Metab. Dis. 44, 1039–1050 (2021); **[3]**Shen, J.-S. X.-L. Meng, D.F. Moore, J.M. Quirk, J.A. Shayman, R. Schiffmann, C.R. Kaneski. Mol. Genet. Metab. 95, 163–168 (2008); **[4]**Braun, F. et al. Cell. Physiol. Biochem. 52, 1139–1150 (2019); **[5]**Svarstad, E. H.P. Marti. Clin. J. Am. Soc. Nephrol. 7, 569-576 (2020); **[6]**Elsaid, H. O. A. et al. Mol. Genet. Metab. Reports 31, 100851 (2022)

B-3: Lyso-Gb3 Affects Energy Metabolism and Elicits a Proteotoxic Effect on a Neuronal Cell Model.

Authors: Wendy E. Heywood[1], Valeria Nikolaenko[1], Justyna Spiewak[1], Ivan Doykov[1], Zak Ibrahim[1], Kevin Mills[1].Inborn Errors of Metabolism Section, Genetic & Genomic Medicine Unit, UCL Institute of Child Health, London, UK.

Background: Lyso-Gb3 is produced by accumulation of Globotriaosylceramide (Gb3) in Fabry disease (FD) patients. Lyso-Gb3 is not a naturally occurring lipid and may be disease causing due to its closer association with disease severity and progression than Gb3.[1] Endogenously administered Lyso-Gb3 has been described to affect healthy sensory neuronal cells which could explain the neuronal pain observed in FD.[2] However, little is understood on the effect of lyso-Gb3 on other neuronal systems and patients are living longer due to treatment. Many of these therapies do not cross the blood brain barrier. Therefore, to investigate the cellular response to exogenous lyso-Gb3 we have profiled the proteome of exposed neuronal SY5HY cells.

Methods: Cells were exposed to low (20 ng/ml) and high (200 ng/ml) levels of lyso-Gb3 over 24 -72 hours and compared with a DMSO control. Cells we lysed, trypsin digested and subjected to label free proteomics analysis. Furthermore, to identify possible protein interactors, we bound lyso-Gb3 to inert supports, incubated with cell lysates and bound proteins were identified using proteomics. Confirmation of the proteomic studies was performed by enrichment of ubiquitinated proteins using anti-ubiquitin immunocapture with the lysate of cells incubated with lyso-Gb3. Finally, proteomic results were confirmed by a multiplex targeted proteomic assay investigating proteins of the protein folding pathway.

Results: Low and high doses of lyso-Gb3 resulted in a 5% and 12% change of the cell proteome respectively. Pathway analysis indicated altered Unfolded Protein Response (UPR), protein ubiquitination, protein translation from mRNA and sirtuin signaling. To investigate UPR/ERAD system and protein ubiquitination, using immunocapture we identified 296 ubiquitinated proteins that are affected after lyso-Gb3 exposure. Many of these proteins were involved in mRNA metabolic processing, protein targeting to the ER and from the mitochondrial ATP synthase complex. Lyso-Gb3 binding studies demonstrated that 157 proteins specifically bound to immobilized lyso-Gb3. These included proteins involved in the chaperone complex and energy metabolism thereby confirming our proteomic analyses. Only four proteins that bound to lyso-Gb3 were also ubiquitinated upon exposure and were mitochondrial and ER membrane proteins in origin. Quantitation of proteins of the ER folding pathway show at low dose an increase of an ER protein transporter and at high dose increase of UPR chaperones HSP90 and PDIA1. High dose lyso-Gb3 groups demonstrated a significant decrease of LAMP1.

Conclusion: Our results indicate the potential disease mechanism of lyso-Gb3 could involve disruption of mitochondrial and ER membranes. This causes misfolding of newly synthesized proteins which elicit a proteotoxic response whereby the ubiquitin system modulates protein translation as well as degradation.[3,4] This could explain the observed ER stress and Zebra inclusion bodies that have been previously described in FD.[5]

Support: Great Ormond Street Biomedical Research Centre

References: [1]Nowak A, et al. Mol Genet Metab. 2018 Feb;123(2):148-153**.** [2]Choi L, Vernon J, Kopach O, et al. *Neurosci Lett*. 2015;594:163-168. [3]Guerra-Moreno A, Isasa M, Bhanu MK, et al. *J Biol Chem*. 2015;290(50):29695-29706. [4]Dougherty SE, Maduka AO, Inada T, Silva GM. *Int J Mol Sci*. 2020;21(3):1151. [5]Hofmann L, Hose D, Grießhammer A, et al. *Elife*. 2018;7:e39300.

B-4: Modeling Fabry disease in Kidney and Heart Organoids from Patient-Derived Human Induced Pluripotent Stem Cells.

Authors: Anna Reinelt[1,2,3], Sandra D. Laufer[1,2,3], Milagros N. Wong[1], Fabian Haas[1], Nastassia Liaukouskaya[1], Katharina von Cossel[4], Luise Förster[4], Benjamin Lohmöller[4], Linda Blomberg[5], Christine E. Kurschat[5], Nicola Wanner[1], Nicole Muschol[4], Victor G. Puelles[1], Arne Hansen[2,3], Tobias B. Huber[1] & Fabian Braun[1]

Institutions: [1]III. Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]University Medical Center Hamburg-Eppendorf, Department of Experimental Pharmacology and Toxicology, Hamburg, Germany; [3]German Center for Cardiovascular Research (DZHK), Partner site Hamburg/Lübeck/Kiel, Germany; [4]Department of Pediatrics, University Medical Center Hamburg, Eppendorf, Hamburg, Germany; [5]Department II of Internal Medicine, Center for Molecular Medicine Cologne and Center for Rare Diseases, University of Cologne, Cologne, Germany.

Background: Currently available animal models fail to adequately resemble the complexity of Fabry disease, particularly concerning the heart and kidney involvement. The aim of this project is, hence, to employ the recent advances in human induced pluripotent stem cells (hiPSC) and organoid differentiation to establish informative human in vitro models for Fabry disease.

Methods: We collected primary urinary cells (PUCs) of 15 Fabry patients with mutations from different categories (classical, classical and migalastat amenable, late onset, unclear significance)[1]. 8 PUC samples were reprogrammed into hiPSC with subsequent creation of respective isogenic control lines by CRISPR Cas9 gene editing. These cell lines were differentiated into kidney and heart organoids as well as cardiomyocytes using published protocols[2–4].

Results: All patient-derived hiPSC lines exhibit decreased α-galactosicase A (aGal A) enzyme activity. Kidney organoids showed tubular structures under bright-field microscopy. Immunofluorescence staining confirmed the presence of marker proteins for different nephron segments, including glomerular structures. Heart organoids showed contractions and contained different cardiac cell types including cardiomyocytes with sarcomeres and Z-Disc formation as demonstrated by transmission electron microscopy.

Conclusion: This project highlights the potential of novel complex human *in vitro* disease models for the study of Fabry disease. Ongoing analyses assess the accumulation of globotriaosylceramide, as well as structural and single cell analyses at high resolution. The functional relevance of GLA mutations for the cardiac phenotype is currently investigated in engineered heart tissue of hiPSC derived cardiomyocytes.

Support: The study was supported by the 3R (Replace, Reduce, Refine) Start-up Funding Program, awarded by the Medical Faculty Hamburg 2018 to AH and FB as well as Amicus Therapeutics through an investigator-initiated proposal awarded to FB and TBH

References: [1]Zhou, T. *et al.* Generation of human induced pluripotent stem cells from urine samples. *Nature protocols* 7, 2080–2089 (2012). [2]Kumar, S. V. *et al.* Kidney micro-organoids in suspension culture as a scalable source of human pluripotent stem cell-derived kidney cells. *Development* 146, (2019). [3]Breckwoldt, K. *et al.* Differentiation of cardiomyocytes and generation of human engineered heart tissue. *Nature protocols* 12, 1177–1197 (2017). [4]Lewis-Israeli, Y. R. *et al.* Self-assembling human heart organoids for the modeling of cardiac development and congenital heart disease. *Nat Commun* 12, 5142 (2021).

B-5: Identification of microRNAs with Possible Involvement in the Development and Progression of Fabry Nephropathy

Authors: Tina Levstek[1], Andreja Cokan Vujkovac[2], Bojan Vujkovac[2], Katarina Trebušak Podkrajšek[1,3]

Institutions: [1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Slovenia, [2]Centre for Fabry Disease, General Hospital Slovenj Gradec, Slovenia, [3]Clinical Institute for Special Laboratory Diagnostics, University Children's Hospital, University Medical Centre Ljubljana, Slovenia

Background: Fabry nephropathy contributes significantly to morbidity and mortality in patients with Fabry disease (FD) (1). However, the pathophysiological processes of Fabry nephropathy have not been fully elucidated. Moreover, currently available biomarkers lack sensitivity and do not predict disease progression (2). Therefore, our study DEFINER aims to identify various early biomarkers of kidney dysfunction. microRNAs (miRNAs) are short noncoding RNAs that regulate gene expression at the posttranscriptional level (3). They have been shown to play an important role in kidney development, maintenance of kidney function, and progression of renal dysfunction (4). Therefore, we aimed to identify candidate miRNAs associated with the development and progression of Fabry nephropathy.

Methods: Total RNA was extracted from urinary extracellular vesicles, which were isolated by a previously optimized method based on size exclusion chromatography. A total of 10 male patients with genetically confirmed FD and 10 age-matched control subjects were included in the discovery cohort and profiled for 87 miRNAs using the miRCURY LNA miRNA Urine Exosomes Focus PCR Panel (Qiagen, Germany). Patients were divided into two groups according to the progression of nephropathy, which was defined by estimated glomerular filtration rate > 3 mL/min/year.

Results: miR-222-3p, -221-3p, -21-5p, -365a-3p, and let-7i-5p differed by more than 1.5-fold and were statistically significant between patients with stable renal function and control subjects. Between patients with progressive nephropathy and control subjects these miRNAs were miR-10b-5p, -22-3p, -30a-5p, -30c-5p, -30d-5p, -15a-5p, -204-5p, -21-5p, and let-7f-5p.

Conclusion: Among the differentially expressed miRNAs, we selected seven candidate miRNAs possibly important for the progression and/or development of Fabry nephropathy based on their fold change and p value. Furthermore, only one miRNA from each family was selected. We plan to independently validate them on chronological samples collected at patients' regular follow-ups over the past 10 years. The validation cohort will include a larger group of patients and their age- and sex-matched control subjects. Chronological correlation of miRNA levels in patients with FD who have different rates of nephropathy progression could identify factors important for the development or progression of kidney injury and thus improve understanding of the pathophysiology of Fabry nephropathy.

Support: The study was funded by the Slovenian Research Agency (research core P1-0170) and donation from Shire. T.L. was granted a scholarship from the University Foundation of ing. Lenarčič Milan.

References: [1]Waldek S et al. Genet Med 2009; 11, 790–6. [2]Levstek T et al. Genes 2020, 11, 1019. [3]Bartel DP et al. Cell 2004, 116, 281–97. [4]Chandrasekaran K et al. Kidney Int. 2012, 81, 617–27.

B-6: Understanding Mechanisms Linking Gb3 Deposits to Endothelial and Neuronal Pathology in Fabry Disease.

Authors: K. Klug[1], M. Spitzel[1], T. Klein[1], N Üçeyler[1,2]

Institutions: [1]Department of Neurology, [2]FAZiT, both University Hospital of Würzburg, GER

Background: The X-linked lysosomal storage disorder Fabry disease (FD) is caused by mutations in the alpha-galactosidase A (GLA) gene. Impaired GLA activity causes intracellular accumulation of the sphingolipid globotriaosylceramide (Gb3). In FD, pain is one of the first symptoms occurring already in childhood; however, the pathophysiological link between Gb3 deposits and pain is not yet understood. Studies revealed a pro-inflammatory phenotype in FD-patients,[1] and hypoperfusion at the level of the dorsal root ganglia (DRG).[2] These studies indicate that inflammation and hypoperfusion may trigger downstream pathways leading to FD pain. We hypothesize that FD-linked endothelial cell (EC) dysfunction and Gb3-associated inflammation promote hypoperfusion at DRG level. This may enhance hypoxia-related gene expression leading to hyperexcitability of DRG neurons and consecutively to pain.

Methods: We generated induced pluripotent stem cells (iPSC) from skin fibroblasts of two patients with a painful (P1) and painless (P2) FD phenotype and compared these data with cells obtained from a healthy control. iPSCs were also differentiated into endothelial cells (EC). Both cell types were cultivated under normoxic, and hypoxic (2% oxygen) conditions for 48 hr to determine effects on gene expression, GLA activity, and vascularization.

Results: qPCR array analysis of normoxic EC of P1 and the control showed 22 differently expressed target genes clustering in pathways of apoptotic stress, endothelial dysfunction, and inflammation. While hypoxia did not further alter the cellular phenotype in FD iPSC, control iPSC displayed a marked reduction in GLA activity and increase in Gb3 accumulations within 48 h of cultivation. Under normoxic conditions, tube formation analysis revealed severely reduced formation of vessel-like structures only in FD EC, which did not recover under enzyme substitution. Since hypoxia induces angiogenesis *in vivo* [3], we repeated the experiment under hypoxic conditions; however, tube formation in FD EC remained impaired. Inhibition of the TGFβ-pathway with the small molecule SB-431542 improved tube formation in FD EC under normoxic and hypoxic conditions as was shown previously.[4, 5]

Conclusion: Our results imply EC dysfunction in FD patients compared to control cells. Hampered tube formation *in vitro* may underlie impaired vascularization and perfusion at DRG level. Reduced oxygen availability for non-EC may be one key factor for the activation of downstream pathways leading to FD pain. SB-431542 as an inhibitor of the TGFβ pathway recovers tube formation in FD EC indicating that FD may lead to increased expression of fibronectin, thrombospondin, and plasminogen,[5,6] which act as negative regulators of EC migration and angiogenesis. In combination with a potential inflammation-induced increase in pain sensitivity, reduced angiogenesis may cause a vicious feedback loop comprising inflammation and hypoxia.

Support: Funding by the German Research Foundation, CRC1158

References: [1]Üçeyler, N. et al. 2019 doi:10.1016/j.ymgme.2019.05.009. [2]Godel, T. et al. 2017 doi:10.1212/WNL.0000000000004396. [3]Liao, D. et al. 2007 doi:10.1007/s10555-007-9066-y. [4]Liu, Z. et al. 2009 doi:10.1242/jcs.048942. [5]Do H. et al. 2020 doi:10.1016/j.ebiom.2020.102633. [6]Goumans, M. et al. 2009 doi:10.1038/cr.2008.326.

B-7: Investigation of a Potential Link Between Hypoxia and Pain in Endothelial Cells of Patients with Fabry Disease

Authors: Clara Hans, Katharina Klug, Nurcan Üçeyler

Institutions: Department of Neurology, University Hospital of Würzburg, Germany FAZiT, University Hospital of Würzburg, Germany

Background: Fabry disease (FD) is a hereditary X-linked storage disorder, which results in a multiorgan disorder and pain. The pathogenic mechanism of acral burning pain, which is one of the FD-associated disease phenotypes,[1] is not yet fully understood. There is evidence for malperfusion in dorsal root ganglia (DRG) of FD patients, which may be related to neurotoxic effects.[2,3] Hence, FD-associated small fiber neuropathy may be directly linked to cellular Gb3 accumulations as was shown in a FD mouse model[4] and may be interlinked with DRG malperfusion. We hypothesize that Gb3 accumulation leads to EC dysfunction, which may cause hypoperfusion and hypoxia in human DRG with consecutive induction of a local inflammation contributing to FD-associated pain.

Methods: We have differentiated induced pluripotent stem cell (iPSC)-derived EC from patients with distinct FD nonsense mutations (with and without FD-associated pain labeled “pain variant” versus “no-pain variant”) and compared these with iPSC-EC from a healthy control. Cells were cultivated under hypoxic conditions (2% oxygen) for 24 h, 48 h, and 72 h. To examine the protein expression of hypoxia- and inflammation-related marker genes such as hypoxia inducible factor (HIF)-1 alpha, HIF2, vascular endothelial growth factor A (VEGFA), and toll-like receptor 4 (TLR4), we applied immunofluorescent labelling and compared fluorescence intensity in patient and control biomaterial using CellProfiler.[5]

Results: Qualitative fluorescence intensity of TLR4 was higher, while that of VEGFA was lower in FD-EC in comparison to the healthy control. The antibody labelling for HIF1 alpha revealed a 10% higher fluorescence intensity in EC of the “pain variant” patient compared to the healthy control. After 48 hr cultivation under hypoxic conditions, the antibody signal of HIF1alpha increased in the nucleus (9% “pain variant”; 5% “no-pain variant”). In the healthy control, the fluorescence intensity of HIF1 alpha was enhanced about 11% in the nucleus after 24 hr, while there was hardly any difference in the FD cells. The fluorescence intensity using antibody labeling against HIF2 did not change under hypoxic conditions.

Conclusion: Our data show a difference between the FD variants and the healthy control group under hypoxic and normoxic conditions, which fits to our hypothesis of a higher hypoxic response in FD. The translocation of HIF into the nucleus under hypoxic conditions is reflected by the higher HIF1 signal in the nucleus of the FD cells after 48 hr. This may be also supported by the higher HIF1 intensity in the cytosol, as HIF1 alpha is stabilized in the cytosol before translocating in the nucleus under hypoxia. HIF2, which is more specific for EC[6] and is mostly stabilized under prolonged hypoxia,[7] does not show changes under hypoxic conditions, which may be due to the short incubation time. Our hypothesis of inflammation triggered by the hypoxic environment in the DRG of FD patients might correspond to the higher TLR4 signal of the different cell lines.

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References: [1]Üçeyler et al. 2014 [2]Godel et al. 2017 [3]Gadoth et al. 1983 [4]Hofmann et al. 2018. [5]Carpenter et al. 2006. [6]Wiesener et al. 2002. [7]Zhao et al. 2015

B-8: Does Lyso-Gb3 Play a Causal Role in Gastrointestinal Symptomology in Fabry Disease?

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Background: Gastrointestinal (GI) symptoms are among the earliest and most difficult to interpret symptoms reported by Fabry disease (FD) patients. Frequent episodes of diarrhea (up to 12 or more times a day) severely impact patients' quality of life.[1] Lyso-Gb3 has emerged as a clinical marker of FD and has been shown to modulate neuronal activity in dorsal root ganglia neurons. However, to date, the effects of lyso-Gb3 on GI physiology have not been examined.[2-3] Therefore, we sought to examine the effects of lyso-Gb3 on the intestinal epithelium and the enteric nervous system which together play important roles in regulating intestinal ion transport and fluid and electrolyte homeostasis.

Methods: We carried out Ussing chamber experiments in colonic mucosa-submucosa preparations from adult C57BL/6 male mice and measured changes in the short circuit current (I_{sc}) and transepithelial resistance (TER) after serosal administration of lyso-Gb3 at increasing concentrations (30nM-10uM) to mimic the blood-derived nature of lyso-Gb3. We also examined the effects of lyso-Gb3 on secretagogue-induced response to carbachol (CCh) and forskolin which stimulate calcium- and cAMP-mediated responses respectively. Tetrodotoxin (TTX; 300nM) was added to assess any neuronal component of lyso-Gb3-induced changes. We also examined the effect of lyso-Gb3 on sensory nerve-mediated responses using capsaicin (3uM), a TRPV1 receptor agonist.

Results: At lower concentrations, lyso-Gb3 did not affect baseline I_{sc} and TER significantly. However, I_{sc} significantly increased after 30 min of incubation at a concentration of 10uM (N = 5-7, ΔI_{sc}(DMSO) = -0.2±0.3 µA.0,12cm^{-2}, ΔI_{sc}(lyso-Gb3) = 1.5±1.3 µA.0,12cm^{-2}, $p < 0.05$). TTX did not influence the response to lyso-Gb3 on baseline I_{sc}. Pre-incubation with lyso-Gb3 significantly increased the response to forskolin, even at lower concentrations ($p < 0.05$) and had no significant effect on CCh-evoked responses. Capsaicin responses were similarly not significantly influenced by lyso-Gb3.

Conclusion: Lyso-Gb3 is not only a valuable marker for diagnosis and follow-up of FD but significantly influences colonic ion transport process which may play a crucial role in the dysregulation of intestinal function in FD in patients.

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B-9: *in vitro* Characterization of iPSC and Sensory Neurons with the *GLA* variant c.376A>G (p.S126G) of Unclear Pathogenicity in Fabry Disease.

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Background: Fabry disease (FD) is an X-linked lysosomal storage disorder caused by mutations in the *GLA* gene coding for the glycosphingolipid-depleting enzyme α-galactosidase A (α-GAL A). The resulting enzyme malfunction is reflected by intracellular globotriaosylceramide (Gb3) deposits and a large symptom spectrum spanning heart failure, renal insufficiency and pain related to small fiber pathology. Symptom severity in FD is directly associated with the type and position of the respective *GLA* mutation.[1] Still, for some variants the effect on α-GAL A activity and therefore pathogenicity is yet inadequately characterized. The *GLA* variant c.376A>G (p.S126G) lacks a distinct classification,[2,3] complicating treatment of affected patients. To investigate the impact of the mutation on patient-derived single cells, especially on sensory neurons potentially associated with FD-specific pain, we used an *in vitro* model based on induced pluripotent stem cells (iPSC).

Methods: Human dermal fibroblasts (HDF) were obtained by skin punch biopsy from a patient carrying the variant p.S126G, reprogrammed to iPSC[4] and subsequently differentiated to sensory neurons. The cell line with the variant p.S126G was compared to a healthy control line and cell lines associated with classic pathogenic FD mutations. To visualize intracellular Gb3 deposits characteristic for FD, we used Shiga toxin subunit B (STxB) coupled with a fluorophore on all three cell types. To determine the effect on enzyme stability, we performed a fluorometric α-GAL A activity assay in iPSC. For the electrophysiological investigation of pain-related neuron function we analyzed action potential parameters and sodium channel kinetics via patch-clamp recordings in iPSC-derived sensory neurons.

Results: In contrast to the classic FD variant p.Q357X, HDF, iPSC and sensory neurons associated with the variant p.S126G were free of Gb3 accumulations measurable by STxB binding. Further, compared to healthy controls α-GAL A activity in p.S126G-iPSC was reduced by 12% while virtually absent in p.Q357X-iPSC. No differences were found between p.S126G associated sensory neurons and healthy controls for the action potential threshold, amplitude, duration as well as sodium current activation and inactivation kinetics applying patch-clamp analysis.

Conclusion: Compared to classic FD causing mutations, a pathogenic role of the variant c.376A>G in FD is not supported by our results. Despite a minor reduction in enzyme activity, Gb3 accumulations, the cellular hallmark of FD, as well as changes in sensory neuron function were not detectable. Patients with FD-like symptoms carrying the variant should therefore be examined for alternative medical causes to provide adequate and early treatment.

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B-10: Is It Fabry Disease or an Apathogenic Polymorphism? Molecular Analysis Comparing Two lines of Patient-Derived Dermal Fibroblasts.

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Background: Fabry disease (FD) is an X-linked multiorgan disorder caused by mutations in the alpha-galactosidase A (GLA) gene. Due to enzyme deficiency, globotriaosylceramide (Gb3) accumulates in various cell types.[1] Symptoms and disease burden vary greatly depending on the type of mutation. D313Y and A143T are two frequent examples of genetic variants with uncertain pathogenicity, as there are arguments pointing towards and against the diagnosis of FD.[1-4] Since FD-specific treatment is lifelong using expensive drugs, thorough characterization of poorly understood variants is crucial. The D313Y variant does not cause severe cardiac, kidney or neurological impairments other than seen in carriers of 'classical' forms of FD, which suggests a pseudo-deficiency of GLA.[5] Patients carrying the A143T variant have reduced residual GLA activities and/or increased Gb3 levels but show less severe FD-like symptoms and no FD-specific renal and cardiac involvement. Hence, it seems to be a neutral variant.[2] To test this hypothesis, we investigated cellular signs of FD in patient-derived dermal fibroblasts (hdF). Our data may have great impact on clinical decision making in these cases, where patients and physicians often remain in doubts about proper diagnosis and treatment.

Methods: hdF from four men and women with either a D313Y or A143T variant and from seven affected patients carrying pathogenic mutations, as well as from five healthy controls were cultivated and analyzed for enzyme activity and intracellular Gb3 load. We used a colorimetric GLA activity assay and visualized Gb3 with fluorescence-labelled shigatoxin subunit B and subsequently performed a quantitative analysis of photomicrographs identifying Gb3 accumulation using CellProfiler.

Results: HdF GLA activity was slightly reduced but Gb3 load did not differ when comparing biomaterial of men and women carrying the D313Y or A143T variant with controls, while GLA activity was markedly reduced and Gb3 load increased in hdF of patients with classic mutations.

Conclusion: Our results suggest that D313Y and A143T do not induce a FD-specific cellular phenotype.

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C-1: Characterization of Early Symptom Progression in Young Pediatric Patients with Classic Pathogenic Variants in the GLA gene: Data from A Prospective, Multicenter Pilot Study Of Fabry Disease Clinical and Biochemical Findings in Young Pediatric Patients (the MOPPet Study)

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Background: Classic Fabry disease is a pan-ethnic, progressive, X-linked lysosomal disorder that commonly presents in childhood and is caused by deficient activity of the lysosomal enzyme alpha-galactosidase A.[1] Symptoms of Fabry disease in the pediatric population are well described for patients over five years of age; however, data are limited for infancy and early childhood .[1]

Methods: In this longitudinal observational study of children under five years of age, prospective phenotypic and urinary biomarker (Gb3 and lyso-Gb3) data were collected annually in young children with pathogenic variants in the GLA gene to learn more about the impact of Fabry disease in this population (Ethics Committee approval IRB00072967). Additionally, the subjects' parents completed annual surveys concerning their child's pain, gastrointestinal issues, and overall symptoms.

Results: The study population includes 15 (12 M and 3 F) subjects with classic GLA pathogenic variants. Longitudinal symptom data were available in 11/15 subjects with classic pathogenic GLA variants. The most common first symptoms reported were gastrointestinal symptoms (10/11), heat intolerance (8/11), and hypohidrosis (5/11). Mapping onset and progression of gastrointestinal, sweating, and heat intolerance reveals a consistent pattern of frequency and severity occurring in the first year of life beginning at an average of 18.5 months of age (onset range 11–45 months). From time of onset, gastrointestinal symptoms progress from chronic bloating to diarrhea to abdominal pain. Sweating and heat intolerance from flushing/average sweating to hypohidrosis to anhidrosis.

Conclusions: This study highlights the pattern of progression of the earliest Fabry related symptoms in children under age five with classic pathogenic GLA variants.

Support: Support for this project is provided through an investigator-initiated grant from Sanofi-Genzyme.

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C-2: Neonatal Screening for Fabry Disease in Brazil: Pilot Study Update

Authors: Giugliani R[1,2,3], Poswar FO[1,2], Sousa I[4], Amorim T[4], Badaró J[4], Pereira D[5], Silva C[5], Brusius-Facchin AC[1], Netto ABO[1], Trometer J[6], Souza A[7,] Ranieri E[8], Polo G[9], Hong X[10], Gelb M[10], Kubaski F[1,11]

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Background: A pilot newborn screening program for six lysosomal storage diseases (LSDs) in Brazil was performed. Here we report the updated results of this program, focusing on the Fabry Disease (FD).

Methods: Dried blood spots (DBS) samples of 20,066 unselected newborns from the state of Bahia were analyzed by the Neo-LSD™ kit (Perkin-Elmer) by tandem mass spectrometry (MS/MS) with a Xevo TQ-S micro (Waters). The activity of α-galactosidase (α-Gal) was measured, alongside other lysosomal enzymes. The samples with low α-Gal activity were submitted to the evaluation of lyso-Gb3, with ultra-performance liquid chromatography (UPLC-MS/MS), followed by molecular analysis of the *GLA* gene. All these tests were performed in the same DBS sample.

Results: Samples from 20,066 newborns have been analyzed. Three samples (all males) showed low α-Gal activity (0.37, 0.38, 1.09 nmoL/h/mL [cutoff < 1.50 nmoL/h/mL]). In one of these newborns, we detected high levels of Lyso-Gb3 (5.7 nmoL/L [cutoff> 0.5-3.5 nmoL/L]) and identified the pathogenic variant (p.Arg356Trp), well-known to be associated with classic Fabry disease[1]. Another newborn with low α-Gal activity showed increased levels of Lyso-Gb3 (5.5 nmoL/L [cutoff> 0.5-3.5 nmoL/L]), but molecular analysis of the *GLA* gene by NGS followed by MLPA could not identify any pathogenic variant. The third newborn showed normal Lyso-Gb3 levels (0.74 nmoL/L [cutoff> 0.5-3.5 nmoL/L]) and a variant of unknown significance (VUS) was found in the *GLA* gene (p.Ile359Thr). This variant was previously described in a female with histological features of FD on kidney biopsy but without the hallmark signs of diffuse angiokeratomas or cornea verticillata[2].

Conclusion: Despite confirmatory studies are still ongoing, we have identified 3 potential cases of FD (1/6,689). This study indicates the usefulness of a three-tier approach with enzyme assay, biomarker measurement, and genetic studies to allow a comprehensive evaluation of newborns using a single DBS sample, providing relevant information before the family is approached.

Support: This study was supported by INAGEMP, CNPq, CAPES, FAPERGS, FIPE, and FundMed

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C-3: Assessing Bone Mineral Density in Fabry Disease

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Objective: To assess the prevalence of low bone mineral density in a cohort of Fabry patients.

Background: Fabry disease is a rare multi-systemic lysosomal storage disease that affects the heart and kidneys most significantly. Other common symptoms may include peripheral neuropathy, hearing loss, and anhidrosis. An underappreciated manifestation of Fabry disease is reduced bone mineral density. A limited number of studies have shown a high prevalence of low bone mineral density in Fabry disease. Bone mineral density is affected by multiple factors including but not limited to renal dysfunction, BMI, and secondary hyperparathyroidism. Treatment of osteopenia and osteoporosis in Fabry patients must take into consideration possible renal dysfunction.

Methods: We studied DEXA scans obtained as part of routine care from our cohort of 24 individuals followed at the UC Irvine Medical Center. 32 DEXA scan results were collected from 11 males and 13 females with a mean age of 51.4 years (± 15.4 years). T-scores were analyzed from the spine, femoral neck, and hip. Quality-of-life measurements using the SF-36 health survey were collected, with reports of physical function, social function, physical role, emotional role, mental health, energy, pain, and general health perception.

Results: Of 15 spine, femoral or hip T-score measurements reported, 47% had osteopenia, defined as a T-score between -1.0 and -2.5 and 33% had osteoporosis defined as T score < -2.5. Of 11 individuals with a femoral neck T-score reported, 63.6% of participants had abnormal results that categorized them as osteopenia or osteoporosis. Of 12 individuals with a hip T-score reported, 66.7% had abnormal results that categorized them as osteopenia or osteoporosis. There was a positive correlation between lowest T-score and BMI (r=0.564), and lowest T-score and calcium levels (r=0.572). We did not find correlations between T-scores and renal markers, vitamin D, and SF-36 quality-of-life measurements.

Conclusion: The effects of Fabry disease on bone mineral density is not yet fully understood. Possible mechanisms to explain osteoporosis/osteopenia in Fabry patients include the effects of renal failure leading to secondary hyperparathyroidism, altered ability to metabolize vitamin D, decreased plasma calcium and lipid deposition in bone. These findings highlight the prevalence of low bone mineral density in Fabry disease and the need for additional research on the etiology, as well as prevention and treatment strategies.

Support: Sanofi Genzyme

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C-4: Gene Variants of Unknown Significance in Fabry Disease: Clinical Characteristics of *c.376A>G (p.Ser126Gly)*

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Background: Anderson–Fabry disease (FD) is an X- linked lysosomal storage disorder with varying organ involvement and symptoms. Over the last years, increasing awareness in FD and easier access to genetic testing led to findings of more patients with genetic variants of unknown significance. It is crucial to evaluate pathogenicity of each genetic variant precisely to determine whether there is or might be not a need for FD - specific therapy in affected patients. In literature the specific *GLA* gene variant *c.376A>G (p.Ser126Gly)* is controversial, and comprehensive clinical data for patients with this variant is rare.

Methods: We examined the clinical impact of the specific GLA gene variant *p.Ser126Gly* in five individuals from different families (one heterozygous and one homozygous female, three males), who visited our center between 2009 and 2021. Comprehensive neurological, nephrological and cardiac examinations were performed in all cases. One patient received a follow-up examination after 12 years.

Results: Index events leading to suspicion of FD were mainly unspecific neurological symptoms. However, FD-specific biomarkers were in normal range (alpha-galactosidase A enzyme activity mean: 0.44 nmol/min/mg protein (norm 0.4-1.0), Lyso-Gb3 mean: 0.62 ng/ml (norm: <0.9 ng/ml). Imaging examinations (i.e., brain MRI, heart MRI), and tissue specific diagnoses, including kidney and skin biopsies, did not reveal evidence for FD-specific symptoms or organ involvement but showed normal results in all cases. This includes findings from 12-year follow-up in one patient with renal biopsy.

Conclusion: Our findings suggest that *p.Ser126Gly* represents a benign *GLA* gene variant, which does not cause clinically significant FD. Precise and detailed evaluation in individuals diagnosed with genetic variations of unknown significance should be performed to distinguish common symptoms broadly prevalent in the general population from those secondary to FD.

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C-5: Clinical Significance of the A143T Mutation: A Review of Two Families.

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Background: Fabry disease (FD) is a highly heterogeneous condition and whilst some patients experience symptoms during childhood others remain asymptomatic after middle age. Furthermore, some patients exhibit full disease symptoms ('classical' FD) whereas others show affection of a single system, usually the heart or the kidney ('non-classical' or 'later onset' FD). Prediction of the course of the disease based on genotype is challenging as some missense GLA mutations with high preserved enzyme activity can result in a spectrum of phenotypes, from classical FD to no symptoms at all.[1] This study explores the clinical significance of the Ala143Thr mutation and compares clinical findings to that of two single recurrent mutations at extremes of the phenotypic spectrum i.e., R227X a non-sense mutation that results in a classical phenotype and the N215S missense variant, linked to a cardiac later onset phenotype.

Methods: The pedigree of two families harboring the A143T mutation were reviewed, and patients' notes under the care of the Royal Free Lysosomal Storage Disorders Unit were retrospectively assessed. These findings were analyzed and compared with other mutations i.e., R22TX and N215S within the male patient cohort at the Royal Free Hospital. Baseline assessments of organ function included left ventricular mass index (LVMI), glomerular filtration rate (GFR), and the presence of white matter lesions (WML). Samples of plasma globotriaosylsphingosine (lyso-Gb3) were available and disease severity scores were calculated utilizing the Mainz severity score index (MSSI) and the age-adjusted severity score (AASS).

Results: This analysis showed that A143T patients showed an intermediate level of residual enzyme activity and plasma lyso-Gb3 when compared to the other mutations. It was also noted that these patients develop cardiac and renal disease equivalent to that of the N215S and R227X variants.

Conclusion: Overall, our indicates the A143T mutation results in a distinctive phenotype that manifests with cardiac and renal disease equivalent to that of the N215S and R227X variants.

Support: None

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C-6: LysoGb3 Normalization: An Achievable Therapeutic Goal in Fabry Disease?

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Background: The optimal timing for initiating enzyme replacement therapy (ERT) in classic Fabry disease (FD) patients is still under discussion. International guidelines suggested waiting for symptoms,[1] or starting after the age of 8 years old,[2] or currently when plasma globotriaosylsphingosine (Lyso-Gb3) level is higher than 20 nm/L.[3] The magnitude of the decrease in plasma lyso-Gb3 levels depends on the timing of starting treatment[4] and dose of ERT.[5] Arends et al.[4] reported in 2017 that the start of ERT before the age of 25 years old resulted in a greater reduction of plasma Lyso-Gb3 compared to those patients who started treatment later in life, but no patient reached normal level in that publication. The early treated group had a age range at treatment onset of 9.5-24.6 years. In 2019 Kritzer et al.[6] reported that an early initiation of agalsidase beta in classic FD normalizes biomarkers in clinically asymptomatic pediatric patients, based in two cases.

We report 2 girls with classic FD who started agalsidase beta 1 mg/kg/EOW before 15 years old because of typical clinical symptoms, high level of plasma Lyso-Gb3, proteinuria and kidney involvement on biopsy.

Age at ERT (y)	Symptoms and signs	Fogo score [7] on renal biopsy	Mutation	Lyso-Gb3 baseline (nmol/L)	Lyso-Gb3 after ERT (nmol/L)/interval baseline-last measure
9	Acrop, AK, Ab pain + diarrhea, cornea vert	Score 2	L415P	69.9	0.7/3 years
14	Acrop, AK, Ab pain + diarrhea, cornea vert, mild proteinuria	Score 3	L415P	9.0	0.8/1 year

Lyso-Gb3 in plasma: reference value < 1.2 nmol/L in females and 1 nmol/L in males. Ab: abdominal, Acrop: acroparesthesias, AK: angiokeratomas, Vert: verticillata.

Results: Clinical follow up of both girls showed resolution of neuropathic pain and gastrointestinal manifestations (abdominal pain and diarrhea), no increase in proteinuria and normalization of plasma Lyso-Gb3 levels, with alpha-galatosidase beta at 1 mg/kg every two weeks.

Conclusion: In older patients with irreversible disease, no clear relation can be expected between clearances of plasma Lyso-Gb3, but for young patients, a decline and normalization in plasma Lyso-Gb3 level may be a predictor of organ function preservation.

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C-7: Plasma Globotriaosylsphingosine (lysoGb3) Correlates Strongly with Disease Severity in Untreated Fabry Disease Patients: A Tool to Aid Clinical Decision Making.

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Background: Fabry disease (FD) manifestations can differ greatly, even between patients of the same sex and with the same mutation type. A biomarker with a strong predictive value for the development of disease manifestations is needed to determine the need for FD specific treatment and appropriate frequency of follow-up, since clinical manifestations of the disorder may take decades to develop.

Methods: We investigated the stability of lysoGb3[1] levels over time and defined correlations between plasma lysoGb3 concentrations and renal function, cardiac imaging and cerebral manifestations in 237 untreated Fabry patients (multiple measurements per patient). Associations were assessed using linear mixed effect models corrected for age and, where appropriate, sex of the patient.

Results: Plasma lysoGb3 is stable over time in untreated patients ($p= 0.8$). Plasma lysoGb3 correlates with several markers of disease severity, including estimates glomerular filtration rate (eGFR, $p = 0.02$), albuminuria (measured as the urinary albumin to creatinine ratio, uACR, $p < 0.001$), left ventricular mass indexed to body surface area (BSA) both on echocardiogram ($p = 0.03$) and on MRI ($p <0.001$), relative wall thickness (RWT, $p = 0.04$), markers for cardiac diastolic dysfunction; septal e' ($p <0.001$), E/e' ($p = 0.008$) and left atrial volume index (LAVI, $p = 0.001$). The association between lysoGb3 and the Fazekas score[2] did not reach statistical significance ($p=0.06$).

Conclusion: We conclude that measuring plasma lysoGb3 in untreated patients provides insight into the expected natural disease course of an individual patient. In addition, it can aid clinical decision making and pave the way towards developing customized treatment strategies for individual patients, more specifically: if, and when to start Fabry specific treatment in individual patients and the frequency of follow-up in asymptomatic patients.

Support: This study was funded by SPHINX, the Amsterdam Lysosome Center. No support or grants from commercial parties were accepted in relation to the submitted work.

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C-8: Echocardiographic Features and Changes in Fabry Disease: Results from a Long-Term Longitudinal Cohort Study

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Background: Cardiac involvement in Fabry disease (FD) can manifest as the development of left ventricular (LV) hypertrophy, left atrial dilatation, LV systolic and diastolic dysfunction. These manifestations can be assessed by echocardiography, and many studies show cross-sectional data on echocardiographic features of FD. However, little is known about the age of onset of abnormalities and the progression over time in male and female FD patients. This knowledge is needed to investigate whether early changes in echocardiographic parameters can serve to identify patients developing cardiac manifestation of FD and whether they can be used to track disease progression. To address these questions, we analyzed the echocardiographic features and alterations in a large FD patient cohort, comparing male and female patients and comparing FD patients to matched healthy control subjects.

Methods: A total of 92 patients with classical FD (37% men, 63% women) were included in the study. For each patient the first and last available echocardiogram were analyzed (mean time between the two studies: 12 years, range 5-15). FD data were compared to data from 147 echocardiograms of healthy individuals (50% men, 50% women) matched for age (± 44 years) and sex. Generalized linear mixed effect models were used to evaluate the effect of FD, age and sex on six echocardiographic parameters (Interventricular septum thickness during diastole (IVSd), relative wall thickness (RWT), left ventricular mass index (LVMi), left atrium volume index (LAVI) and the ratio of early diastolic mitral inflow velocity (E)/ early diastolic septal tissue mitral annulus velocity (E/e')). Regression coefficients (β) were reported to describe the echocardiographic parameters progression for each 10 years increase in FD patients and healthy controls.

Results: Changes over time in IVSd, RWT, LVMi, LAVI and E/e' differed significantly between FD patients and healthy controls ($p<0.05$ for all comparisons). Between male and female FD patients, the increase in LVMi and E/e' over time was greater in men compared to women (β-LVMi= 13.5 g/m2; 95% CI 7.8-19.1 and β-E/e'= 1.1; 95% CI 0.2-2.0). IVSd, RWT, LAVI were higher in male compared to female FD patients throughout follow up, but the rate of change did not differ between the sexes.

Conclusion: From early adulthood, echocardiographic parameters in FD reflecting LV and atrial morphology and LV diastolic function change significantly over time. The rate of change can be used to assess disease development and progression in both male and female patients with classical FD.

Support: This study was funded by SPHINX, the Amsterdam Lysosome Center. No support or grants from commercial parties were accepted in relation to the submitted work.

C-9: ECG Changes During Adult Life in Fabry Disease: Results from a Large Longitudinal Cohort Study.

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Background: Cardiac involvement in Fabry disease (FD) can manifest as electrocardiogram (ECG) changes, both early on and in advanced disease stages. Though several cross sectional and short term studies have been performed [1], the course of the ECG alterations, the differences from the trajectory of healthy individuals and how this differs between men and women with FD is not yet fully understood. This information is needed to detect early cardiac involvement, guiding treatment initiation, and to track the effect of new FD therapies.

Methods: A total of 1,995 ECGs from 133 patients with classical FD, spanning an average of 20 years of follow-up (longitudinal data) were compared to ECGs from 3,893 healthy participants from the HELIUS (Healthy Life in an Urban Setting) cohort (cross-sectional data) [2] .

Generalized linear mixed-effect models (GLM) were used to evaluate the effect of FD, age, and sex on seven ECG parameters (P-wave duration, PR-interval, QRS duration, QTc, Cornell index (Cind), Spatial QRS-T axis and Frontal QRS axis). The regression slope for each parameter was compared between FD patients and healthy controls and between men and women with FD.

Results: In FD patients, the evolution over time of QRS duration, QTc, Cind, Spatial QRS-T angle and frontal QRS axis differed significantly from those of healthy subjects ($p<0.05$). The changes over time of P-wave duration, PR-interval and QTc differed significantly between men and women with FD ($p<0.05$).

Conclusion: This study shows that in FD, several ECG parameters reveal progressive alterations during adult life, representing cardiac disease progression. It indicates that early in the disease, these parameters do not necessarily differ from healthy individuals in absolute value, but the rate of change does set the FD patients apart from the healthy individuals. Later-on these parameters differ between the two groups in both absolute value and rate of change. Monitoring these ECG changes will provide valuable information for timing of treatment initiation and evaluation of the effectiveness of existing and new FD therapies.

Support: This study was funded by SPHINX, the Amsterdam Lysosome Center. No support or grants from commercial parties were accepted in relation to the submitted work.

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C-10: Inflammatory and Cardiac Remodeling Markers for Diagnosis and Staging of Cardiomyopathy in Fabry Disease.

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Background: Cardiovascular complications contribute substantially to morbidity and are the leading cause of premature death in Fabry Disease.[1] The progressive accumulation of globotriaosylceramide (Gb3) and its toxic metabolite lyso-Gb3 trigger a pathologic cascade, continually damaging multiple tissues and organs. In the cardiac tissue, the secretion of inflammatory and transforming growth factors is increases with lyso-Gb3 accumulation.[2] Infiltration of lymphocytes and macrophages into the endomyocardial tissue provides further evidence about the role of inflammation in cardiac damage [3]. NF-κB/TNF-α activation plays a subsequent role in the inflammatory response to cardiac dysfunction.[4] Cardiac hypertrophy corresponds to the expansion of coronary angiogenesis and vascularization with the secretion of growth factors, VEGF, IGF-1, and TGFβ.

Methods: In a cohort of 43 patients with FD and 20 healthy controls, cytokines and growth factors associated with tissue remodeling were studied. Patients were categorized into four different groups based on LVMI and LVPWd measurements: without cardiac complications (1), mild (2), moderate (3), and severe (4) hypertrophic c*ardiomyopathy* (HCM). Inflammatory and growth factors were measured in plasma using commercially available ELISA kits.

Results: Serum levels of TNF*α*, MCP-1, INF-γ, VEGF, IGF-1, TGFβ, and FGF2 were analyzed using enzyme-linked immunosorbent assays (ELISA). Among the biomarkers, MCP-1, IFN-γ, VEGF, TNF*α*, and TGFβ were elevated in FD patients. Moreover, VEGF, TGFβ, INF-γ correlated with severe HCM. VEGF and TGFβ are also elevated in FD patients with a kidney transplant history and pre-diabetes or diabetic complications.

Conclusion: Elevation of pro-inflammatory cytokines TNFα and IFN-γ provides evidence of activation of inflammatory pathways associated with the cardiac hypertrophic remodeling in patients with FD. Tissue fibrosis factors TGFβ and VEGF also correlate with cardiac involvement in FD. This study reveals the potential role of immune activation and cardiac remodeling in the development of FD cardiomyopathy. Selected cytokines and growth factors could be utilized as biomarkers for clinical staging and management of patients with FD and cardiac involvement.

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C-11: Prevalence of Aortic Root Dilatation in Fabry Disease.

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Background: Fabry Disease (FD) patients may develop aortic root dilatation (ARD), which was described to occur in 30-56% of males. Recently, a much lower prevalence of ARD has been reported (16.1%), which was attributed to methodological differences or to possible effects of the advancements in treatment strategies[1]. Here, we assessed the prevalence of aortic root dilatation in a cohort of patients with Fabry Disease from a single center.

Methods: We included patients with FD that performed echocardiograms between 2011 and 2021. Patients younger than 18 years and from families with a *GLA* variant associated to nonclassical phenotype were excluded. Aortic root was measured at the sinus of Valsalva using leading edge to leading edge. Body surface area was measured using the Haycock formula. Standardized Z scores were calculated using the Deveraux formula for body surface area[2]. ARD was defined as a Z score higher than 2.0. For comparison with the study of Barbey et al., 2010[3], we also assessed the prevalence of ARD by using the criteria of >36 mm for females and >40 mm for males. Statistical analysis was performed in the PASW Statistics version 18.0 using the chi-squared test. A p value less than 0.05 was considered significant.

Results: A total of 53 patients (24 males and 29 females) were included. The mean age of the patients was 36.5 years for the males and 45.4 years for the females, and the mean aortic root diameter was 35.3 mm and 31.3 mm for males and females, respectively. The criteria for ARD were met for 6 patients (2 females and 5 males), corresponding to a prevalence of 11.3% (16.4 % in males and 6.9% in females). There was no difference in the classification of the patients by adopting the aortic root Z scores or the aortic root diameters cutoffs. The prevalence of ARD was higher in patients on enzyme replacement therapy (ERT) (6/29 cases vs 0/24 cases in naïve patients; p = 0.027). No statistically significant difference on the prevalence of ARD was found between males and females.

Conclusion: This study corroborates the results from other authors that showed a lower prevalence of ARD in patients with Fabry Disease than previously reported[1]. As the awareness of FD increases, it is possible that patients with a lower disease burden are being included in the more recent studies. It is still unclear if ERT or other advancements in treatment strategies may have resulted in a lower prevalence of ARD. In this study, treated patients had a higher aortic diameter, which may reflect the existence of other clinical criteria for ERT prescription. As ARD is associated to rare yet life-threatening outcomes, careful follow-up with standardized echocardiographic measurements of the aorta remains warranted.

Support: None

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C-12: Family Screening in Fabry Disease

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Institutions: University Hospitals Birmingham NHS Foundation Trust / University of Birmingham

Background: Fabry disease (FD) is a lysosomal storage disorder characterized by a deficiency in the enzyme α-galactosidase A resulting in sphingolipid deposition which causes progressive cardiovascular manifestations. FD is a rare disease affecting 1:40,000-117,000 people worldwide Due to the X-linked inheritance of the condition, men become symptomatic earlier and with more severe phenotype, while female carriers have more variable phenotypic expression. Family screening is critical to identify cases early before significant organ involvement, when therapy is more likely to have a beneficial impact. Although it is a requirement of any commissioned service caring for adult patients with FD in England that pedigree analysis should be carried out in each patient with confirmed disease, there is variable access to genetic counselling and family screening must take place through the proband.

Methods: We conducted a retrospective analysis of 40 adults with FD under an Inherited Metabolic Disease service and screened clinical records and consulted patients in clinic. Our aims were to: 1) Identify if patients had been offered family screen at any point during their care under the service; 2) Establish the degree of pedigree mapping that had taken place, and 3) Identify any mortality associated with family screening not taking place

Results: The mean age of the cohort was 51yrs (range 25-83 SD 12) and 22/40 (55%) were female. Of the 40 patients, 27 had a close family member who died of sudden cardiac death. The mean age of death was 48 years of age (range 35-65 SD 8). Most of these deaths were not recent and happened before the development of family screening and genetic counselling. 19 (47.5%) of patients had been offered family screening either at diagnosis or thereafter. 23 (57.5%) patients reported undiagnosed family members (range 0-5, mean 1.35 per person) of which 12 (52.1%) were due to family members being estranged and 6 (23%) due to refusing testing.

Conclusion: A significant proportion of patients are not offered family screening during their care under the service and there are no data available to indicate routine audit to track numbers identified or refusing contact. Despite this, most episodes of sudden cardiac death happened in relatives 2-3 generations prior with there being no undiagnosed cases of SCD in first degree relatives of patients screened.

Support: This project received no financial support

References: [1]Mehta A, Beck M, Eyskens F, Feliciani C, Kantola I, Ramaswami U, et al. Fabry disease: a review of current management strategies. QJM. 2010;103(9):641-59.

C-13: Kidney Biopsy Findings in Fabry Females with Normal Serum Creatinine Levels.

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Institutions: University of Alabama in Birmingham

Background: Progressive kidney deterioration is a symptom of Fabry disease in males and females. Despite this, many females go undiagnosed or are told they are "carriers". The purpose of our study was to review biopsy findings of female Fabry female adult patients with creatinine values in the normal range and minimal proteinuria.

Methods: Kidney biopsies and clinical parameters were reviewed from female Fabry patients presenting to UAB between years 2000 and 2020 with creatinine values in the normal range and minimal proteinuria (<500mg/day).

Results: All participants had classic mutations (11 nonsense, 2 missense). Despite, creatinine values in the normal range, eGFR values calculated by the CKD-EPI equation ranged from 130 to 79 ml/min/1.73m^2. Serology studies, plasma Lyso-G3 was undetectable marker for the majority, only two patients had values 2.3 and another for 5.1 ng/ml (reference range: < 5.0 ng/ml). GL-3 was below 3 µg/ml for all patients (reference range: < 7.0 µg/ml). The Average Fogo score for the biopsies was 2.4 (reference range of podocyte vacuolization: 0-3).[1] 38% of patients had greater than 50% foot-process effacement despite normal creatinine and minimal proteinuria.

Conclusion: Many females despite having a creatinine in the normal range at time of biopsy and minimal proteinuria had significant histologic evidence of disease. The evidence of histologic damage would not have been suspected given the normal serologic baseline evaluation. Histologic assessment should be performed, when possible, in females with normal baseline serology level to assess tissue involvement and assist with management decisions. Longitudinal follow up of patients is needed to determine if histologic findings on presentation predict long-term outcomes in female patients with Fabry disease with normal serologic findings.

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C-14: Baseline Demographics and Clinical Characteristics of Patients Enrolled in the followME Fabry Pathfinders Registry

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Background: The followME Fabry Pathfinders registry (EUPAS20599) is an observational, patient-focused registry evaluating real-world safety, effectiveness, and patient-reported outcomes for current Fabry disease treatments. Here, we present the baseline patient demographics and clinical characteristics of the registry cohort as of February 2022.

Methods: Patients aged ≥16 years with a confirmed diagnosis of Fabry disease and estimated glomerular filtration rate (eGFR) >30 mL/min/1.73m^2 were enrolled into one of three groups: migalastat-amenable *GLA* variants receiving migalastat; any *GLA* variant receiving enzyme replacement therapy (ERT); migalastat-amenable *GLA* variants not receiving Fabry-disease-specific therapy (untreated, natural history cohort).

Results: As of February 2022, 427 patients were enrolled: migalastat group (n=219 [52.3%]; median [range] age at enrolment, 56.0 [16–77] years; female, 39.7%); ERT group (n=86 [20.1%]; 46.0 [20–73] years; female, 54.7%); untreated group (n=122 [28.6%]; 43.5 [16–82] years; female, 77.9%). Of patients with data available, proteinuria was present in 19.7%, 29.4%, and 5.2% of patients in the migalastat, ERT, and untreated groups, respectively. At enrolment, mean (SD) duration of prior Fabry-disease-specific therapy was 10.4 (8.0) months and 10.4 (8.7) months in the migalastat and ERT arms, respectively. Prior cardiac events (before enrolment) were reported in 11.0%, 4.7%, and 4.9% in the migalastat, ERT, and untreated groups, respectively; prior renal events were reported in 2.7%, 1.2%, and 0%, respectively; prior cerebrovascular events were reported in 3.7%, 8.1%, and 4.1%, respectively.

Conclusion: The followME registry is the largest real-world cohort of migalastat-treated patients; baseline data include a broad range of Fabry disease phenotypes and provide the basis for understanding real-world treatment patterns.

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C-15: Cleared Podocytes and Normal Kidney Function in Classical Fabry Males 15 Years After Start of Enzyme Replacement Therapy at Young Age

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Background: Fabry nephropathy may progress to kidney failure despite enzyme replacement therapy (ERT) when the treatment is initiated at a relatively late stage of the disease. This study evaluates long term effects of agalsidase in serial kidney biopsies and functional measurements in men with classical Fabry disease that commenced treatment with agalsidase alpha or beta at a young age.

Methods: Seven male Fabry patients with an age of 18 years (7-30 years) at start of ERT were monitored over a time of 15 years (12-17 years) with kidney biopsies, measurement of glomerular filtration rate (mGFR) and urinary albumin creatinine ratio (uACR). Values are given in median (range). All patients were treated with both high dose (1 mg/kg/eow) and lower dose (0.2-0.5 mg/kg/eow) of agalsidase.

Results: After 15 years of ERT the podocyte composite score had decreased from 7.0 (6.9-7.0) to 0.56 (0-4.29). The reduction of podocyte-Gb3 was higher on agalsidase 1 mg/kg/eow; change composite score -4.38 (-7.9 to -1.8) and on agalsidase 0.4-0.5 mg/kg/eow; change composite score -4.2 (-0.5 to -5.1) compared to treatment with agalsidase 0.2 mg/kg/eow; change composite score +1.0 (-1.2 to +2.0). mGFR at baseline was 106 (86-113) ml/min/1.73 m^2 and after 15 years 109 (73-134) ml/min/1.73 m^2. uACR was 6.4 (0.8-13.6) mg/mmol at baseline and after 15 years 5.5 (0.6-17.7) mg/mmol.

Conclusion: Initiation of ERT at young age may clear the long living kidney cells of Gb3 and protect the kidneys from significant functional loss over a long-time period. The reduction of Gb3 in podocytes is greater on higher dose compared to low dose of agalsidase.

Support: Western Norway Health Trust

C-16: Sex Differences in Fabry Disease-Related Pain

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Background: Fabry disease is a rare, X-linked inherited lysosomal storage disorder caused by deficient α-galactosidase A enzymatic activity. Loss of functional enzymatic activity results in the lysosomal accumulation of fatty molecules, primarily globotriaosylceramide (Gb3 or Gl-3). Patients typically present with a myriad of symptoms affecting the kidney, heart, and nervous system - including neuropathic pain, hypo- or anhidrosis, angiokeratoma, and microalbuminuria. Despite the prevalence of neuropathic pain in these patients, however, the underlying pathophysiology of Fabry-related pain remains poorly understood, with some studies pointing to glycolipid accumulation in dorsal root ganglia, while others point to potential pathological effects of lysoGb3 on peripheral sensory neurons.

Methods: To determine characteristics of pain in Fabry patients, we followed a cohort of 20 females (mean age 48.13 +/- 19.47 years) and 15 males (mean age 45.33 +/- 17.58 years) and collected SF-36 and BPI surveys for each patient over a period of 2-20 years during bi-annual clinical surveillance visits. Scores from both surveys reflect patients' self-reported pain.

Results: Our results indicate that, among patients who reported having pain, the majority (13/19) experience pain in either their hands or feet, with some differences in spatial distribution of pain among sexes. Interestingly, though males and females report similar pain scores, fewer female patients report using analgesics (4/20) relative to males (8/15), with comparable levels of pain relief as male patients. However, we observed a trend towards increased pain in female patients - while the overall baseline level of pain reported is similar, female patients endorse more frequent severe pain. In addition, though enzyme replacement therapy (ERT) has been shown to significantly improve pathological abnormalities found in Fabry patients, there is limited evidence of pain mitigation. Indeed, in our cohort, we found no significant effect of ERT on patient-reported pain scores. Moreover, we found no correlation between lysoGb3 values and pain scores.

Conclusion: Female patients are often mischaracterized in literature as having an attenuated form of Fabry disease, and thus less-severe Fabry-related pain. However, we find that the pain phenotype is more complicated and deserves more careful attention in the clinic. Similarly, the lack of efficacy of ERT towards relieving pain signifies the need to examine alternative mechanisms that may potentially drive Fabry-related pain.

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C-17: Identified Risk Factors for Stroke in a Large Fabry Disease Cohort

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Background: Stroke is one of the most dreadful manifestations of Fabry disease. However, the risk factors and pathophysiology of this complication is not well known. Some studies suggest large-vessel strokes are caused by cardiac emboli, whereas small vessel disease is most likely to occur due to substrate accumulation within endothelial cells[1].

Methods: We conducted a retrospective longitudinal cohort study in a UK National Reference Centre performing a survival analysis with Cox regression, both univariate and multivariate adjusted by age, p.N215S genotype, gender, and treatment status to find possible risk factors associated with stroke.

Results: There were 380 patients in the cohort: 232 (61.05%) were female, and the mean age at baseline for males and females was 42.1(16.7) and 39.8(16.5) years. The p.N215S genotype was present in 59 (40.1%) males and 64 (27.5%) females. Regarding treatment at baseline, a total of 10 (6.2%) males and 107 (45.7%) females had no Fabry specific treatment. Forty-two patients had had a stroke at baseline (11.0%), and by the end of the study the number rose to 82 (21.6%). Of those, 16.3% were transient ischemic attacks, 55% lacunar, 15% anterior circulation stroke, 12.5% posterior circulation stroke and 1.3% venous thrombosis. Interestingly, 47.8% of strokes were clinically silent and were picked up with brain MRI. The survival analysis revealed a median time to stroke/TIA in males and females of 43 (3.2) and 56 (3.8) years, respectively. Identified risk factors associated with stroke were the presence of white matter lesions (HR 2.2 CI 1.1-4.4), a concomitant autoimmune disease (3.5 CI 1.6-7.7) and a patent foramen ovale (PFO) (5.2 CI 2.5-10.9). The presence of p.N215S genotype (HR 0.30 CI 0.17-0.61) and glomerular filtration rate >90ml/h/m^2 (HR 0.49 CI 0.26-0.92) were associated with less risk of stroke. In the multivariate model, only white matter lesions lost their association, probably because of their relationship with the glomerular filtration rate (χ^2 53.9 $p<0.0001$).

Conclusion: This study centered on a single center big cohort of Fabry disease patients identified several risk factors independently associated with stroke after stratification and age adjustment. These were the presence of a concomitant autoimmune disease and patent foramen ovale, associated with increased risk of stroke, and the presence of glomerular filtration rate greater than 90 ml/h/m2 and genotype p.N215S, which were associated with decreased risk of stroke. Interestingly, gender was not associated with stroke and many strokes were clinically silent.

Support: None

References: Lin J, Wang D, Lan L, Fan Y. Multiple Factors Involved in the Pathogenesis of White Matter Lesions. Biomed Res Int. 2017;2017:9372050.

C-18: MR-Gangliography in Fabry disease

Authors: Magnus Schindehütte, Simon Weiner, Lea Hölzli, Christopher Nauroth-Kreß, Thomas Kampf, Nurcan Üçeyler, Claudia Sommer, Mirko Pham

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Background: Neuropathic pain due to small fiber neuropathy is a distressing symptom within the heterogeneous Fabry disease patient population. The triggering factors as well as the pathogenic mechanisms of the characteristic pain are still not conclusively clarified. In clinical routine, psychophysic examinations such as quantitative sensory testing (QST) and skin punch biopsy are used to detect and characterize small fiber neuropathy. The cell bodies of all small caliber nerve fibers, as well as all other sensory neurons are located in the paired dorsal root ganglia (DRG) next to the spinal cord. In contrast to the central nervous system (CNS), the DRGs have a loose blood-DRG barrier, which allows cytotoxic or larger molecules to reach neurons and lead to inflammatory processes. We hypothesized that DRG from Fabry disease patients would differ morphologically from controls.

Methods: High-resolution nerve imaging of the DRG (MR-gangliography) allows for high-resolution *in vivo* examination of patients. Using optimized MRI sequences (3D-T2w-TSE-SPAIR-SPACE, isotropic voxels of 1.1mm³, TE=301ms), both the DRG volumes and the mean T2w intensity of the DRG can be determined and analyzed by manual 3D segmentation. T2w intensity represents a reliable surrogate marker for edematous or potentially inflammatory processes in MRI imaging. This study was performed in 93 patients with Fabry disease recruited via the Fabry Center for interdisciplinary Therapy (FAZiT) and 55 healthy volunteers. All patients also underwent a clinical examination, QST, electrophysiological testing, two skin biopsies, a pain survey, and genetic testing to determining the exact mutation.

Results: We found an increase in DRG volume of patients with Fabry disease compared to healthy controls ($p<0.05$). When correlating these data with patients` genotypes, we found increased T2w intensity between the clinically milder and more severely affected Fabry patients ($p<0.05$). DRG morphometry data further correlated with Fabry-associated pain ($p<0.05$). Also, alterations in T2w sequences as potential surrogate of inflammation correlated with genotype ($p< 0.05$).

Conclusion: Our results show that noninvasive *in vivo* examination of DRG by MR-gangliography in Fabry patients can detect alterations potentially reflecting local inflammation at DRG level as a possible correlate for the characteristic pain in these patients. We could show that the T2w signal intensity of the DRG correlates with the severity of the disease. MR-gangliography may contribute to improve the understanding of Fabry disease pathophysiology and find its way into clinical diagnostics in the future as a potential non-invasive imaging biomarker for longitudinal examination.

Support: SFB 1158 (DFG), KFO-5001 (DFG)

References: Godel et al. Human dorsal root ganglion in vivo morphometry and perfusion in Fabry painful neuropathy. Neurology;89:1274-1282

C-19: The Interplay of Inflammatory Mechanisms and Ion Channel Gene Expression in the Alpha-Galactosidase A-Deficient Mouse Model of Fabry Disease

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Background: Hallmarks of Fabry disease (FD) are cellular globotriaosylceramide (Gb3) accumulations and triggerable pain. Still, the mechanisms linking Gb3 accumulation and FD pain are elusive. Based on previous studies, we hypothesize that inflammatory mechanisms in response to Gb3 accumulations contribute to alterations of ion channel expression leading to pain-like behavior. To investigate this hypothesis, we worked with the alpha-galactosidase A knockout (GLA KO) mouse model focusing on dorsal root ganglion (DRG) pathology.

Methods: We investigated young (<6 months) and old (>12 months) GLA KO mice and wild type (WT) littermates. DRG were dissected for immunohistochemistry (IHC) and quantitative real-time polymerase chain reaction (qRT PCR) analysis. For Gb3 visualization, we used Shiga toxin, subunit B (STxB) coupled with a fluorophore. Further, we quantified macrophages and T-cells per DRG area using respective antibodies for IHC. For determination of inflammation-associated target and pain-associated ion channel gene expression levels, we used qRT PCR. Pain-associated behaviour was investigated using the von Frey test, Hargreaves test, and cold sensitivity test applying dry ice.

Results: We found higher Gb3 load in DRG of young ($p<0.01$) and old ($p<0.001$) GLA KO mice compared to their age-matched WT littermates. The number of cells positive for Cluster of Differentiation (CD)3 (T-cells) and CD11b, F4/80, and CD80 (macrophages) revealed no differences in DRG of old GLA KO compared to old WT mice (all $p>0.05$). The anti-inflammatory macrophage-specific marker CD206 was lower expressed in DRG of old GLA KO compared to old WT mice ($p<0.01$). The inflammation-associated targets Interleukin (IL)1b, IL10, Leucine-rich alpha-2-glycoprotein 1 (LRG1), and Glial fibrillary acidic protein (GFAP) were lower expressed in DRG of old GLA KO compared to old WT mice (all $p<0.05$). The pain-associated ion channels calcium-activated potassium channel 3.1 (KCa3.1) and transient receptor potential ankyrin 1 (TRPA1) were lower expressed in DRG of old GLA KO compared to old WT mice ($p<0.01$ and $p<0.05$, respectively). Behavioural testing revealed mechanical hypersensitivity of young ($p<0.01$) and old ($p<0.0001$) GLA KO mice compared to their WT littermates. Young GLA KO mice displayed heat hypersensitivity ($p<0.0001$) compared to young WT mice and old GLA KO mice developed an age-dependent heat hyposensitivity ($p<0.0001$) compared to young GLA KO mice. Young ($p<0.0001$) and old ($p<0.001$) GLA KO mice showed cold hyposensitivity compared to their WT littermates.

Conclusion: GLA KO mice show an imbalance of immune mediators in response to elevated Gb3 levels as indicated by reduced numbers of CD206+ macrophages and reduced inflammation-associated gene expression levels. Dysregulation of inflammation-associated targets may contribute to altered gene expression levels of pain-associated ion channels leading to nocifensive behaviour in the GLA KO mouse model.

Support: Funding by the German Research Foundation (Deutsche Forschungsgemeinschaft [DFG], Sonderforschungsbereich SFB1158). N.Ü. was funded by DFG (UE171/15-1).

References: Üçeyler et al., 2019; Ohshima et al., 1997; Üçeyler et al., 2016; Hofmann et al., 2018; Chaplan et al., 1994; Hargreaves et al., 1988; Brenner et al., 2012

T-1: Long-term Safety and Efficacy of Pegunigalsidase Alfa: A Multicenter Extension Study in Adult Patients with Fabry Disease

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Background: Fabry disease (FD), a rare, X-linked lysosomal storage disorder, is caused by deficiency of the enzyme α-galactosidase A (α-Gal-A), leading to low residual enzymatic activity and accumulation of glycosphingolipids such as globotriaosylsphingosine (lyso-Gb3) and progressive end organ failure. Pegunigalsidase alfa is a novel PEGylated α-Gal-A enzyme replacement therapy (ERT) in development for FD, and previous studies report its enhanced bioavailability and favorable safety and efficacy for up to 12 months. The study objective was to investigate long-term safety, tolerability, and efficacy of pegunigalsidase alfa in adults with FD for up to 72 months.

Methods: Patients with FD (ERT-naïve or not receiving ERT for 6 months before inclusion) who completed two phase 1/2 studies were subsequently enrolled in an open-label extension study (NCT01981720). Patients received 1.0 mg/kg pegunigalsidase alfa via intravenous infusion every other week for up to 72 months.

Results: Of 15 patients enrolled, 10 (6 males; 4 females) completed the study. Median age (range: 17–54 years) was 32. Most (97.5%) treatment-emergent adverse events (TEAEs) were mild or moderate. Three patients experienced 4 serious AEs unrelated to treatment. One patient experienced a single clinical event (non-cardiac-related death) following chronic obstructive pulmonary disorder exacerbation unrelated to the treatment. Immunogenicity results showed that 4 patients were transiently positive for anti-pegunigalsidase alfa IgG, and 1 patient was positive from 48 months until study completion. At 60 months, there was a continuous reduction from baseline in plasma lyso-Gb3 concentration (mean [SE] change from baseline was 68.4 [25.0] ng/mL); renal (mean [SE] estimated glomerular filtration rate slope of −1.6 [0.8] mL/min/1.73m^2/year), and cardiac function remained relatively stable.

Conclusion: This is the first assessment of long-term administration of pegunigalsidase alfa. Results are consistent with favorable safety and efficacy findings from earlier studies and suggest long-term pegunigalsidase alfa treatment may provide continued benefits in patients with FD.

Support: This study was sponsored by Protalix Biotherapeutics. Medical writing support was provided by Oxford PharmaGenesis, Inc., and was funded by Chiesi USA, Inc.

T-2: Safety and Efficacy of Pegunigalsidase Alfa Administered Every 4 Weeks in Patients with Fabry Disease: Results from the Phase 3, Open-label, BRIGHT Study

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Background: Fabry disease is caused by reduced activity of the lysosomal enzyme α-galactosidase A (α-Gal A), leading to an accumulation of sphingolipids that can cause organ failure. Current treatments include enzyme replacement therapies (ERTs) that require infusions every 2 weeks. Pegunigalsidase alfa is a PEGylated α-Gal A enzyme with a much longer half-life than other ERTs for Fabry disease developed to reduce infusion frequency. The study objective was to evaluate the safety and efficacy of 2.0 mg/kg pegunigalsidase alfa administered once every 4 weeks for 1 year to patients with Fabry disease, who previously received commercially available ERTs every 2 weeks.

Methods: BRIGHT (NCT03180840) was a phase 3, open-label, switchover study of adults (aged 18–60 years old) with Fabry disease who previously received agalsidase alfa or agalsidase beta every other week for ≥ 3 years. Patients received 2.0 mg/kg pegunigalsidase alfa every 4 weeks for 1 year. Safety outcomes included treatment-emergent (TEAEs) and treatment-related adverse events (TRAEs); efficacy outcomes included eGFR changes and plasma lyso-Gb3 concentration.

Results: This study enrolled 30 adults (mean age of 40.5 years old; 24 males, 6 females). 27 Patients (90%) reported 183 TEAEs with no serious or severe TEAEs attributed to pegunigalsidase alfa treatment. No patients developed de novo antidrug antibodies, and no new safety concerns were identified. Among 413 total infusions, 22 caused 27 mild to moderate infusion-related reactions (IRRs), with no serious or severe IRRs reported. In the efficacy analysis (n=29), eGFR values (mean [SE] change from baseline of −1.27 [1.39] mL/min/1.73m^2), mean [SE] eGFR slope (−1.6 [0.8] mL/min/1.73m^2/year) and plasma lyso-Gb3 concentrations remained stable throughout the study.

Conclusion: Patients with Fabry disease receiving ERT every other week can be successfully transitioned to pegunigalsidase alfa 2.0 mg/kg every 4 weeks as an effective maintenance-therapy schedule with a positive safety profile in this study.

Support: This study was sponsored by Protalix Biotherapeutics. Medical writing support was provided by Oxford PharmaGenesis, Inc., and was funded by Chiesi USA, Inc.

T-3: Safety and Efficacy of Pegunigalsidase alfa vs Agalsidase beta on Renal Function in Fabry Disease: 24-Month Results from the Phase III Randomized, Double-Blind, BALANCE Study

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Background: Pegunigalsidase alfa is a novel PEGylated α-Gal A enzyme replacement therapy in development to treat Fabry disease (FD)—designed to offer enhanced bioavailability, prolonged half-life, lower immunogenicity, and potential tolerability benefits over current treatments. The objective of this Phase III study was to evaluate the safety and efficacy of pegunigalsidase alfa compared to agalsidase beta in FD patients and deteriorating renal function.

Methods: BALANCE (NCT02795676) is a 24-month, randomized, double-blind, active-controlled study of pegunigalsidase alfa in adults with FD. Enrolled patients were previously treated with agalsidase beta for ≥1 year with an estimated glomerular filtration rate (eGFR) slope at screening of -2.0 mL/min/1.73 m^2/y or lower. Patients were randomized 2:1 to receive 1.0 mg/kg pegunigalsidase alfa or 1.0 mg/kg agalsidase beta, administered every two weeks. The primary efficacy analysis was noninferiority based on the median annualized eGFR slope difference between groups, meeting the prespecified noninferiority margin. Treatment-emergent adverse events (TEAEs) and anti-drug antibodies (ADAs) were endpoints for safety, tolerability, and immunogenicity profile.

Results: 77 patients with FD were treated with pegunigalsidase alfa (n=52) or agalsidase beta (n=25); at baseline, mean (range) age was 44.3 (18-60) y; 47 (61%) were men. Discontinuations: 5 (9.4%) patients receiving pegunigalsidase alfa (1 drug-related, 4 drug unrelated) and 1 (4%) patient receiving agalsidase beta (drug unrelated). At baseline, mean ± SD eGFR was 73.3 ± 19.8 mL/min/1.73 m^2 with a mean± SD eGFR slope of -8.2 ± 5.9 mL/min/1.73 m^2/y. At 24 months, eGFR slope CI overlapped: median (95% CI) of -2.5 mL/min/1.73 m^2/y (-3.8, -1.2) for pegunigalsidase alfa and -2.2 mL/min/1.73 m^2/y (-3.8, -0.51) for agalsidase beta; median difference between the two groups was -0.36 with a lower 95% CI of -2.4, meeting the prespecified noninferiority margin. Infusion-related reactions (IRRs): 11 (21%) patients on pegunigalsidase alfa had 13 IRRs (0.5 events/100 infusions) vs 6 (24%) patients on agalsidase beta had 51 IRRs (3.9 events/100 infusions). TEAEs occurred in 21 (40%) patients on pegunigalsidase alfa (43 events/100 patient-years) vs 11 (44%) patients on agalsidase beta (153 events/100 patient-years). Treatment-emergent ADAs: 6 (11.5%) patients on pegunigalsidase alfa (3 titer booster; 3 induced) vs 4 (16.0%) patients on agalsidase beta (1 titer boosted; 3 induced). The % ADA+ patients with neutralizing antibodies declined in the pegunigalsidase alfa arm from 94% at baseline to 64% at 24 months vs an increase from 88% to 100% in the agalsidase beta arm. There were no deaths.

Conclusions: Pegunigalsidase alfa showed noninferiority to agalsidase beta based on the rate of eGFR decline, a key measure of Fabry disease progression. No new additional safety concerns were identified. Overall, the tolerability and immunogenicity profiles were favorable for pegunigalsidase alfa, with a lower rate of TEAEs and treatment-emergent ADAs than those receiving agalsidase beta.

Support: Chiesi Global Rare Diseases and Protalix.

T-4: Switching from Agalsidase Alfa to Pegunigalsidase Alfa to Treat Patients with Fabry Disease: 1 Year of Treatment Data from BRIDGE, a Phase 3 Open-label Study

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Background: Pegunigalsidase alfa is a novel PEGylated alpha-galactosidase A enzyme in development for the treatment of patients with Fabry disease (FD).

Methods: BRIDGE (PB-102-F30; NCT03018730) is a phase 3 open-label, switch-over study designed to assess the safety and efficacy of pegunigalsidase alfa (1 mg/kg every other week) in adults with FD, previously treated with agalsidase alfa for at least 2 years.

Results: Twenty (13 men, 7 women) of 22 enrolled patients (15 men, 7 women) completed 12 months of study treatment and were included in the efficacy analyses. Baseline characteristics (N=22): age 24–60 years; mean estimated glomerular filtration rates (eGFR), 82.5 mL/min/1.73 m^2 (men: 80.8 mL/min/1.73 m^2; women: 86.1 mL/min/1.73 m^2); mean annualized eGFR slopes, −5.3 mL/min/1.73 m^2/y (men: −5.4 mL/min/1.73 m^2/y; women: −5.0 mL/min/1.73 m^2/y); mean residual enzymatic activity in leucocytes, 12.2% (men: 4.8%; women: 27.9%) of normal laboratory means; and mean plasma lyso-Gb3, 38.30 nM (men: 49.73 nM; women: 13.81 nM). At 12 months, plasma lyso-Gb3 mean concentrations decreased from baseline by 31.5%; and mean annualized eGFR slope improved from −5.90 mL/min/1.73 m^2/y for agalsidase alfa to −1.19 mL/min/1.73 m^2/y for pegunigalsidase alfa (men: −6.36 to −1.73 mL/min/1.73 m^2/y; women: −5.03 to −0.21 mL/min/1.73 m^2/y). In this study, 75% (n/N=3/4) of progressing patients and 66.7% (n/N=6/9) of fast-progressing (eGFR slope <–5 mL/min/1.73 m^2/y) patients achieved the proposed therapeutic goals after switching to pegunigalsidase alfa.

Seven of 20 patients (35.0%) in the efficacy population were positive for IgG anti-pegunigalsidase alfa anti-drug antibodies at least at 1 timepoint. Overall, 127 treatment-emergent adverse events (TEAEs) occurred in 21 of 22 patients (95.5%). Most TEAEs (86.4%) were mild or moderate, consistent with the safety profile of the previous phase1/2 study (NCT01678898). Four severe TEAEs (3.1%) occurred in 4 patients (18.2%). Two patients (9.1%) discontinued therapy due to severe TEAEs.

Conclusion: The efficacy results suggest a potential benefit of pegunigalsidase alfa on renal function for patients with FD previously treated with agalsidase alfa. No major safety concerns were reported.

Support: This study was sponsored by Protalix Biotherapeutics. Medical writing support was provided by Oxford PharmaGenesis, Inc., and was funded by Chiesi USA, Inc.

T-5: Tolerability and Infusion Duration of Pegunigalsidase Alfa in Pts with Fabry Disease: Data From 5 Completed Clinical Trials

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Background: Pegunigalsidase alfa (PA) is a novel PEGylated α-Gal A enzyme replacement therapy (ERT) in development for Fabry disease (FD) that was designed to offer enhanced bioavailability, prolonged half-life, and lower immunogenicity with potential tolerability benefits. We characterize PA tolerability in clinical trials completed by 2021, with a focus on the incidence of infusion-related reactions (IRRs) and change in infusion duration over time.

Methods: Patients (pts) were included from 5 PA clinical trials: 2 Phase 1/2 studies (F01; NCT01678898, F02; NCT01769001), their extension (F03; NCT01981720), and 2 Phase 3 studies (BRIDGE [NCT03018730] and BRIGHT [NCT03180840]). We describe: incidence of IRRs, mean duration of infusion at beginning and end of study, number of pts achieving and time to achieve the minimum duration of infusion allowed per protocol.

Results: In the Phase 1/2 studies, 18 treatment-naïve pts received PA once every 14 days; 5 (28%, n=3 female) experienced 24 IRRs (1 hypersensitivity reaction [bronchospasm] during the first infusion). The extension study (F03, completed n=15) had initial infusion of PA 0.2 mg/kg, 1 mg/kg, and 2 mg/kg with mean infusion durations of 4.0h (n=6, SD 0.1), 5.2h (n=5, SD 1.7), and 6.4h (n=4, SD 0.3), respectively, and were reduced at 12 months to mean durations of 1.5h (n=6, SD 0.1), 3.6h (n=5, SD 1.4), and 3.1h (n=4, SD 0.1), respectively ($p<0.001$). In BRIDGE, PA 1 mg/kg was given once every 14 days with initial mean infusion of 2.9h (n=22, SD 0.9). 5 male pts (22.7%) experienced 9 IRRs (7 mild; 2 severe). 2 discontinued due to type 1 hypersensitivity reactions, and 1 was positive for IgE antidrug antibodies at baseline. All other pts reached minimum protocol-allowed infusion of 1.5h (± 10 min, n=20, SD 0.1) by 12 months ($p<0.001$ vs baseline). In BRIGHT, 30 pts initially received PA 2 mg/kg once a month; 5 (17%, all male) pts experienced 27 IRRs (17 mild; 10 moderate) who all completed the study. Mean infusion duration decreased from 4.8h (n=30, SD 0.59) at baseline to 2.3h (n=29, SD 0.7, $p<0.001$) by month 12. At BRIGHT completion, most pts reached targeted infusion duration of 3h (mean 3.0h, SD 0.2) for pts >100kg (n=5, 100% reached target) and 2h (mean 2.1h, SD 0.1) for pts ≤100kg (n=24, 96% reached target); 1 pt discontinued due to motor vehicle accident and 1 pt did not reach target.

Conclusion: This analysis of trials completed by 2021 supports good tolerability of PA. Mean infusion duration at study end and IRR incidence (17% to 28%) compares favorably to other ERTs (14% to 55%). Additional data from the PA clinical program may further characterize its potential to offer both tolerability and quality-of-life benefits for pts with FD.

Support: Chiesi Global Rare Diseases and Protalix.

T-6: Rational Design of Pharmacological Chaperones for α-Galactosidase A for the Treatment of Fabry Disease.

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Background: Fabry disease is a lysosomal storage disorder characterized by the genetic deficiency of lysosomal α-D-galactosidase (α-gal A). Patients are currently treated with enzyme replacement therapy (ERT) which involves the intravenous administration of recombinant human α-gal A (agalsidase beta, Fabrazyme®). However, the clinical efficacy of ERT is limited and its stability in plasma is limited. 1-Deoxy-galactonojirimycin (Migalastat®) has recently been approved as a pharmacological chaperone (PC) for the treatment of Fabry disease in patients with amenable mutations. A promising alternative, comprises the administration of both ERT and an enzyme stabilizer able to stabilize the ERT in circulation such that larger concentrations reach disease affected tissues, which could be translated in extended injections intervals and lower enzyme dosages, and ultimately reduce side effects, treatment costs, and improve the patient's lifestyle.[1]

Methods: We have designed and characterized selective α-gal A stabilizers using metadynamics simulations, 3-D crystal structure analysis, activity-based protein profile (ABPP) as well as *in vitro* and *in situ* activity and stability cell experiments.

Results: Herein, we present a novel class of reversible α-gal A inhibitors. Crystallographic studies in complex with ERT (Fabrazyme) show that α-D-gal-cyclosulfamidate binds in a competitive manner adopting a $^4C_1$ conformation. Remarkably, α-Gal A activity is increased when cells are treated with recombinant enzyme supplemented with α-Gal-cyclosulfamidate and toxic metabolite levels (Gb3 and LysoGb3) are corrected in classical mutant R301X Fabry fibroblasts.

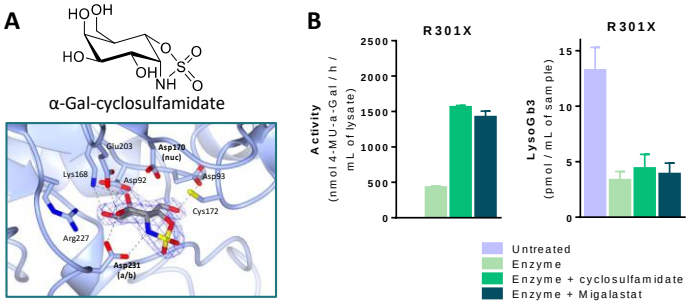

Figure 1. A. Crystal structure of Fabrazyme bound to α-Gal-cyclosulfamidate. B. α-Gal A activity increases when cells are treated with Fabrazyme and α-Gal-cyclosulfamidate, and lysoGb3 levels are corrected.

Conclusion: Reversible α-D-gal-cyclosulfamidate analogues hold promise as pharmacological chaperones for the treatment of Fabry disease.

Support: We thank The Netherlands Organization for Scientific Research (NWO-CW, ChemThem grant), the European Research Council (ERC-2020-SyG-951231) and Sanofi Genzyme for financial support and Fabrazyme supply.

References: [1]M. Artola *et al. Biomolecules* **2021**, *11*, 271. [2]M. Artola *et al. Chemical Science*, **2019**, 10, 9233.

T-7: Long-Term Multi-Systemic Efficacy with Migalastat in ERT-Naive and ERT-Experienced Patients with Amenable *GLA* Variants

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Background: Migalastat has demonstrated multisystemic efficacy and is approved for treating FD in adults with amenable *GLA* variants. We report incidence of predefined cerebrovascular, cardiac, and renal Fabry-associated clinical events (FACEs) as a measure of long-term efficacy of migalastat.

Methods: Phase 3 placebo-controlled FACETS (NCT00925301, ERT-naive), active-controlled ATTRACT (NCT01218659, ERT-experienced), and open-label extension (OLE) studies (N=97; mean age: 46.4 years; males, 38%) data were integrated. FACE incidence (events per 1000 patient-years) was assessed in all patients. Historical comparative data on FACE incidence was obtained from literature review.

Results: Over median (Q1–Q3) follow-up of 5.1 (2.3-6.8) years, 17 (17.5%) patients experienced 22 FACEs with migalastat – an incidence of 48.3/1000. FACE incidence in ERT-naive classic males (<3% α-galactosidase A activity; multiorgan involvement) was 61.5/1000. Separately, incidence of cerebrovascular, cardiac, and renal events was 13.2/1000, 30.7/1000, and 4.4/1000, respectively. Cox proportional hazards identified baseline eGFR as a predictor of FACEs. Patients on migalastat for median (Q1-Q3) 5.9 (4.7-7.0) years (N=78) had stable eGFR (SD) of -1.57 (3.33) ml/min/1.73m^2/year. ATTRACT subjects were randomized to receive migalastat or ERT for 18 months before switching to open-label migalastat. Migalastat (N = 49) was associated with lower FACE incidence versus continued ERT (N = 15) (66.0/1000 vs 326.6/1000, respectively). ATTRACT patients continued migalastat in the OLE (N=49) and experienced sustained low FACE incidence of 47.9/1000 over median (Q1–Q3) 4.9 (3.0–5.7) years, comparing favorably with historical reports of ERT.

Conclusion: Low FACE incidence in migalastat-treated patients with amenable variants supports long-term multisystemic efficacy with migalastat.

Support: Amicus Therapeutics

T-8: Development of AL01211, a novel Glucosylceramide Synthase Inhibitor, to treat Fabry disease.

Authors: Michael Babcock, Jianhong Zheng, Li Li, Marvin Garovoy, Yuqiao Shen

Institutions: AceLink Therapeutics

Background: Fabry disease, a lysosomal storage disorder caused by reduced activity of the enzyme α-galactosidase A (GalA), is characterized by accumulation of globotriaosylceramide (GL3) and lyso-globotriaosylceramide (lyso-GL3) and a wide range of symptoms from pain, gastrointestinal issues, kidney failure, heart disease, and stroke[1]. Enzyme replacement therapy (ERT), the standard of care for Fabry disease, reduces GL3 and improves many symptoms but patients can continue to develop progressive kidney and heart disease[2]. Glucosylceramide synthase inhibitors (GCSi) reduce glycosphingolipid (GSL) production, including GL3 and lyso-GL3, and have the potential to slow Fabry disease progression. Clinical development of other GCSi have shown that GCS can be safely targeted therapeutically[3]. We are developing AL01211, a GCSi with excellent drug-like properties for the treatment of Fabry disease.

Methods: The IC50 of AL01211 against GCS was determined in cell and cell-free assays. PK, pharmacodynamic (PD), and tissue distribution were conducted in mice, rats, and dogs. The pharmacological profile of AL01211 was characterized *in vitro* including off-target selectivity panels designed to predict clinical drug related adverse effects (SAFETYscan, *Eurofins*), and GBA1 and GBA2. PD studies were conducted in murine disease models including Gaucher and Fabry disease mouse models.

Results: AL01211 inhibits GCS activity with an IC50 of approximately 7 nM in both enzyme and cellular GCS activity assays without significant off-target activity to other targets and pathways of safety concern. PK support once daily, oral administration. AL01211 efficiently reduces GSL production in mouse, rat, and dog kidney with minimal effects on brain GSL levels. Importantly, AL01211 reduces GL3 and lyso-GL3 levels in kidney and heart of a Fabry disease mouse model.

Conclusion: AL01211 is a novel, orally available, potent, and selective GCSi that efficiently reduces GSL in the kidneys and heart of animal models but does not significantly enter the brain. The increased potency and low brain penetration make AL01211 a potentially safer and more efficacious molecule for treating Fabry disease patients, especially in young Fabry patient seeking a convenient, lifelong treatment option. A Phase I clinical trial, consisting of daily oral administration of AL01211 (single ascending dose and 14-day multiple ascending dose study) in healthy volunteers is currently underway.

Support: AceLink Therapeutics

References: [1]Desnick RJ, Ioannou YA, Eng CM. α-Galactosidase A deficiency: Fabry disease. In: Beaudet AL, Vogelstein B, Kinzler KW, et al., eds. *The Online Metabolic and Molecular Bases of Inherited Disease.* 2014. [2]Rombach SM, Smid BE, Bouwman MG, Linthorst GE, Dijkgraaf MG, Hollak CE. Long term enzyme replacement therapy for Fabry disease: effectiveness on kidney, heart and brain. *Orphanet J Rare Dis.* 2013 Mar 25;8:4. [3]Coutinho, M., Santos, J., Alves, S. Less Is More: Substrate Reduction Therapy for Lysosomal Storage Disorders. *IJMS* **17**, 1065 (2016).

T-9: Rational Design of α-1,4-Galactosyltransferase Inhibitors as Substrate Reduction Therapy for the Treatment of Fabry Disease.

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Institutions: Medical Biochemistry Department, LIC, Leiden University, 2300 RA Leiden, The Netherlands.

Background: Fabry disease (FD) is a lysosomal storage disorder, caused by a deficiency of the enzyme α-galactosidase A. The disease is characterized by the build-up of the substrate globotriaosylceramide (Gb3) and its deacylated metabolite lyso-Gb3, which become toxic at high concentrations. FD patients are currently treated by enzyme replacement therapy (ERT) and/or the administration of a pharmacological chaperone to restore basic enzymatic function. However, at this moment, these therapies offer only limited clinical efficacy. The success of Eliglustat as substrate reduction therapy (SRT) in Gaucher disease has led to the search of a similar target for FD. A suitable target is the enzyme that generates Gb3, α-1,4-galactosyltransferase (A4GALT). Reducing the activity of this relatively isolated enzyme in the glycosphingolipid pathway may have only limited effects on further down/upstream metabolism. This hypothesis is supported by the observation that some patients deficient for this enzyme show no significant clinical manifestations[1]. SRT targeting A4GALT requires potent inhibitors, that so far have not been described.

Methods: Our rational design of A4GALT inhibitors is based on the two known substrates, namely the glycolipid lactosylceramide (LacCer) and nucleoside uridine 5′-diphospho-α-D-galactose (UDP-Gal). We have generated a compound library based on LacCer with a range of reactive (electrophilic and nucleophilic) moieties with the aim of blocking the glycosylation at 4-OH. In addition, several sulfamidate- and phosphate-linked UDP-Gal analogues with similar properties have also been included. We have also developed a cell-based assay to assess the activity of these potential inhibitors by determining glycosphingolipid levels in fibroblasts.

Results: A family of 6 UDP-Gal and 15 LacCer mimetics have been synthesized and are currently under evaluation. The inhibitory activity against A4GALT of each compound is measured based on the feeding of a ^{13}C-sphingosine isotope to fibroblasts and following short-term fluctuations in its metabolism up to ^{13}C-Gb3. Two adamantyl-substituted LacCer analogues which have been described in literature to lower glycosphingolipids in cells[2], AdaGalCer and AdaGlcCer, have been used for assay optimization and are now applied as positive controls.

Conclusion: Here, we provide the means to study A4GALT inhibition in cells. Effective and non-toxic A4GALT inhibitors, able to reduce Gb3 levels, could represent a novel approach for the treatment of FD. More extensive screenings could yield alternative scaffolds and broaden our scope of active compounds. Furthermore, these studies might help to provide structural and mechanistic insight in A4GALT, and improve future rational drug design.

Support: We thank The Netherlands Organization for Scientific Research (NOW-CW ChemThem grant) and the European Research Council (ERC-2020-SyG-951231 “Carbocentre”).

References: [1]Kok *et al. Biomolecules* **2021**, 11(2), 271. [2]Kamani *et al. J. Biol.Chem.* **2011**, 286(24), 21413-21426

T-10: A study to Evaluate the Effect of Venglustat on Left Ventricular Mass Index in Adult Fabry Patients with Cardiac Manifestations

Authors: James C Moon[1,2], Joseph Kupferman[3], Pascal Minini[3], Harmonie Goyeau[3], Igor Antonshchuk[3], Pronabesh DasMahapatra[3]

Institutions: [1]Cardiology Department, Barts Heart Centre, London, United Kingdom; [2]Institute of Cardiovascular Science, University College London, United Kingdom**;** [3]Sanofi, Cambridge, MA, USA

Background: Fabry disease (FD) is an X-linked lysosomal storage disorder caused by mutations in the *GLA* gene leading to reduced or absent alpha-galactosidase A activity. Progressive cardiac damage results from glycosphingolipid deposition in the heart. Attention is increasingly focusing on Fabry cardiac involvement, a major source of morbidity and the leading cause of premature death for FD patients. Venglustat is an oral investigational glucosylceramide synthase (GCS) inhibitor that reduced globotriaosylceramide (GL-3) and lyso-GL-3 in plasma, and decreased GL-3 accumulation in all tissues (including kidney and heart) in Fabry mouse models. Additionally, venglustat prevented accumulation of lysosomal GL-3 and led to GL-3 clearance in pluripotent stem cell-derived cardiomyocytes generated from skin fibroblasts of classic FD patients. In a completed Phase 2 study and its long-term extension (NCT02228460), venglustat treatment of adult males with newly diagnosed classic FD for up to 3 years reduced skin capillary endothelial GL-3, plasma GL-3, and plasma lyso-GL-3.

Methods: Accordingly, we designed a phase 3, randomized, open-label, parallel-group trial of venglustat versus standard of care over 18 months in subjects with cardiac FD. The primary endpoint is cardiac – specifically change in left ventricular mass index. Key inclusion criteria include males and females, 18-65 years of age, with classic or late-onset FD, with left ventricular hypertrophy. Exclusion criteria will be the presence of advanced cardiac fibrosis, asymmetric hypertrophy, or with advanced renal, cardiovascular and/or cerebrovascular disease. After the 18-month randomized period, there will be open label extension. Additional endpoints will be safety, pharmacokinetic, and secondary efficacy outcomes (renal function, cardiac function, and storage).

Conclusion: The study will address a key emerging “care gap” for Fabry disease and assesses the effect of novel and available Fabry treatments on myocardial structure and function.

Support: This study is sponsored by Sanofi.

T-11: Preliminary Results of the STAAR Study, a Phase I/II study of Isaralgagene Civaparvovec (ST-920) Gene Therapy in Adults with Fabry Disease.

Authors: Cristobal Passalacqua[1], Jaya Ganesh[2], Ozlem Goker-Alpan[3], Robert J. Hopkin[4], John Bernat[5], Patrick Deegan[6], Liching Cao[1], Michael Chen[1], Sravan Jaggumantri[1], Emma Bowden[1], Lisa Shiue[1], Bernard Souberbielle[1], Bettina M. Cockroft[1]

Institutions: [1]Sangamo Therapeutics, Inc., Brisbane, CA, USA, [2]The Icahn School of Medicine at Mount Sinai, New York, NY, USA, [3]Lysosomal and Rare Disorders Research and Treatment Center, Fairfax, VA, USA, [4]University of Cincinnati College of Medicine, Cincinnati, OH, USA**,** [5]University of Iowa, Iowa City, IA, USA**,** [6]Addenbrooke's Hospital, Cambridge, UK,

Background: Fabry disease is caused by pathogenic variants in the GLA gene, resulting in insufficient alpha-galactosidase A (α-Gal A) activity. Adeno-associated virus (AAV)–mediated gene transfer enables delivery of a functional GLA coding sequence to hepatocytes that consequently synthesize α-Gal A. STAAR is a Phase I/II dose-ranging study (NCT04046224) of ST-920, a recombinant AAV2/6 vector containing the human GLA cDNA that encodes for α-Gal A, administered as a single infusion in adults ≥18 years with Fabry disease.

Methods: Key endpoints include safety, circulating α-Gal A activity, and substrate levels. We present preliminary data (cutoff October 21, 2021) from 3 ascending dose cohorts.

Results: Five male classic Fabry disease subjects were dosed (mean age [SD], 35.2 [11.2] years); two Cohort 1 (0.5e13 vg/kg) Cohort 1 Subject 1 (C1S1) on ERT since 2003, baseline a-Gal (at trough) 1.54 (nmol/h/mL), plasma lyso-Gb3 22.1 ng/ml, C1S2 pseudo-naïve off ERT since 2018, baseline a-Gal 0.92 (nmol/h/mL), plasma lyso-Gb3 18.1 ng/ml; two Cohort 2 (1e13 vg/kg) C2S1 pseudo-naïve off ERT for several years, baseline a-Gal below quantification, plasma lyso-Gb3 83.2 ng/ml, C2S2 on ERT since 2019, baseline a-Gal (at trough) 2.44 (nmol/h/mL), plasma lyso-Gb3 11.1 ng/ml; one Cohort 3 (3e13 vg/kg) C3S1 on ERT since 2016, baseline a-Gal (at trough) 0.85 (nmol/h/mL), plasma lyso-Gb3 32.7 ng/ml. Follow-up was up to 12 months. Eleven treatment-related AEs, all mild, were reported: Cohort 1 Subject 1 (C1S1) anemia, thrombocytosis, rash; C2S1 fever (twice); C3S1 fever, frequent bowel movements, abdominal pain, fatigue, headache, and myalgia. No treatment-related serious AEs were reported. No subject experienced liver enzyme elevations requiring steroid treatment. Cohort 1 and 2 subjects produced supraphysiologic α-Gal A activity from 2- to 15-fold of normal at last sampling timepoint, C1S1 Week 52: 86.3 (nmol/h/mL) (at trough), C1S2 Week 52: 14.3 (nmol/h/mL); C2S1 Week 40: 21.2 (nmol/h/mL), C2S2 Week 25: 58.8 (nmol/h/mL) (withdrawal ERT for 1 week); C3S1 Week 2: 6.1 (nmol/h/mL) (at trough). C1S1, C1S2, C2S2 had relatively low baseline plasma lyso-Gb3 levels that remained stable. C2S1 showed reduction from 83.7 ng/mL pre-dose to 48.1 ng/mL (average, weeks 10-36) after dosing; C2S2 withdrew from ERT at week 24. Cardiac progression was evaluated by cardiac MRI (cMRI); at week 52, C1S1 revealed unchanged concentric hypertrophy versus baseline. Baseline mild biventricular dilation for C1S2 was absent at week 52. C2S1, C2S2 presented normal cMRI at baseline and week 24.
Conclusion: A single infusion of ST-920 up to 3e13 vg/kg was generally well tolerated, providing supraphysiologic α-Gal A activity in plasma.

Support: Sangamo Therapeutics, Inc

T-12: An open-label, phase 1/2 trial of gene therapy 4D-310 in adult males with Fabry disease

Authors: Jinsong Shen[a], Jerry Vockley[b], William R. Wilcox[c], Ozlem Goker-Alpan[d], Damara Ortiz[b], Molly Nie[a], David Kirn[a], Robert Fishman[a], Raphael Schiffmann[a]

Institutions: [a]4D Molecular Therapeutics, Inc., Emeryville, California; [b]University of Pittsburgh, Pittsburgh, Pennsylvania; [c]Emory University School of Medicine, Atlanta, Georgia; [d]Lysosomal Disorders Research and Treatment Center, Inc., Fairfax, Virginia

Background: 4D-310 is an AAV gene therapy product candidate designed to treat Fabry disease systemically via both normalization of secreted α-galactosidase A (AGA) levels in the blood and directly expressing AGA within target organs. 4D-310 includes a codon-optimized *GLA* transgene and utilizes the C102 targeted vector that was invented through directed evolution in non-human primates. In non-human primates, intravenous infusion of 4D-310 efficiently transduces cardiomyocytes, kidney cells, and hepatocytes.

Methods: The Phase 1/2 trial (NCT04519749) assesses 4D-310 in adult males with Fabry disease. The primary endpoints are safety and maximum tolerated dose of 4D-310. Secondary endpoints include serum AGA enzymatic activity and lyso-Gb3 levels, and measures of biologic activity in the heart, including cardiac MRI. A single dose of 4D-310 was administered intravenously on day 1. Corticosteroid prophylaxis is administered for 10 weeks post-treatment.

Results: Three patients with classic Fabry disease were enrolled in cohort 1 (dose 1E13 vg/kg; time on study: 20, 34, and 45 weeks; data cutoff: 1/13/2022). Patients 1 and 3 were receiving long-term enzyme replacement therapy (ERT) at study entry; patient 2 discontinued ERT 13 months prior to enrollment. All three patients had positive anti-AGA antibody titers at baseline (range, ~1:1,000 – 1:100,000). No dose-limiting toxicities were reported. Patient 2 developed transient, self-limited atypical hemolytic uremic syndrome, received IV fluids for 4 days and no other interventions, and recovered completely. No other serious adverse events occurred. Patients 1 and 3 (low to medium pre-treatment anti-AGA antibody titers) demonstrated an increase in serum AGA enzyme activity, which remained significantly above the normal range at all timepoints through last follow-up. Following discontinuation of ERT, serum AGA enzyme activity was 139.7 nmol/hr/mL (14-fold mean normal) at week 37 and 98.8 nmol/hr/mL (10-fold mean normal) at week 20 in patients 1 and 3, respectively. Patient 2 (high pre-treatment anti-AGA antibody titer [~1:100,000] and elevated baseline lyso-Gb3) demonstrated a significant increase in serum AGA enzyme activity (0 nmol/hr/mL at baseline, 4.1 nmol/hr/mL [42% mean normal] at week 13). Serum lyso-Gb3 decreased significantly (>50%) in patient 2 within the first four weeks following 4D-310 treatment and subsequently remained stable (101.0 ng/mL at baseline, 45.5 ng/mL at week 13). Preliminary clinical data showed increased native T1 on cardiac MRI (950 ms at baseline, 980 ms at week 26) and improved global longitudinal strain (-16.4% at baseline, -18.7% at week 26) in patient 1.

Conclusion: 4D-310 shows promise as a potential gene therapy for Fabry disease broadly, including ERT-experienced patients with positive anti-AGA antibody titers at baseline.

Support: This study was supported by 4D Molecular Therapeutics

References: ASGCT 2021 Abstract #220; WORLD Symposium 2022 Abstract #318

T-13: Lentivirus-Mediated Gene Therapy in Fabry Disease; Persisting Polyclonal Engraftment and Increased Alpha-Galactosidase A Activity at 3-5 Year Follow-Up.

Authors: Michael L. West[1], Aneal Khan[2], Armand Keating[3], Dwayne L. Barber[4], Ronan Foley[5], William M. McKillop[6], C. Anthony Rupar[7], Christiane Auray-Blais[8], Jeffrey A. Medin[9].

Institutions: [1]Department of Medicine, Dalhousie University, Halifax, NS, Canada. [2]MAGIC Department of Medical Genetics, Metabolics and Pediatrics, Alberta Children's Hospital, Cumming School of Medicine, Research Institute, University of Calgary, Calgary, AB, Canada. [3]University of Toronto, Princess Margaret Cancer Centre, Toronto, ON, Canada. [4]University Health Network, Toronto, ON, Canada. [5]Department of Pathology and Molecular Medicine, McMaster University and Juravinski Hospital and Cancer Centre, Hamilton, ON, Canada. [6]Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI, USA. [7]Departments of Pediatrics and Pathology and Laboratory Medicine, Western University, London, ON, Canada. [8]Division of Medical Genetics, Department of Pediatrics, CIUSSS de l'Estrie-CHUS Hospital Fleurimont, Université de Sherbrooke, Sherbrooke, QC, Canada, [9]Departments of Biochemistry and Pediatrics, Medical College of Wisconsin, Milwaukee, WI, USA.

Background: Current therapies for Fabry disease have limited benefits and problems of antidrug antibodies, iv access and infusion-associated reactions. Gene therapy is a promising treatment that may avoid some of these issues. We report the 3 to 5 year follow up of a phase 1 trial of lentivirus-mediated gene therapy in Fabry disease (NCT02800070)(Khan et al 2021).

Methods: 5 adult males, mean age 38.4 years with Type 1 (classical) phenotype Fabry disease were receiving standard dose enzyme replacement therapy (ERT). All were infused with autologous lentivirus-transduced, CD34+-selected, hematopoietic stem/progenitor cells engineered to express human alpha-galactosidase A (α-gal A). The non-myeloablative preparative regimen consisted of intravenous melphalan 100 mg/m^2. ERT was stopped 60 days prior to the autologous stem cell transplant and resumed at 30 days post in 4 of 5 patients. ERT could be withdrawn by agreement of the treating physician and patient if patients were stable.

Results: All patients showed stable polyclonal engraftment by day 13 which persisted through follow-up, mean 3.6, range 3-5 years. Plasma and WBC a-gal A activity increased above baseline in all and persisted throughout the observation period. Vector copy number slowly declined over time. Gb3 and lysoGb3 levels fell in urine and plasma in all patients, After ERT withdrawal in 3 of 5 (at times -41, +214, +545 days), Gb3 levels increased in both plasma and urine; plasma lysoGb3 increased in 2/3, while urine lysoGb3 stayed low. All patients showed no change in clinical status from pre-gene therapy status and are feeling well with no treatment-related adverse events.

Conclusion: Lentivirus-mediated gene therapy for Fabry disease is a safe and efficacious treatment with disease stabilization over 3 to 5 years of follow up. There is persistent polyclonal engraftment with plasma and leukocyte a-gal activity above baseline in all with fall in Gb3 and lysoGb3 levels. There is discordance between Gb3 and lysoGb3 levels in urine and plasma in the patients who stopped ERT. Patients will be followed for 10 years.

Support: This work was supported by Canadian Institutes of Health Research (CIHR, grant number 119187), The Kidney Foundation of Canada, and the MACC Fund. Financial support for the study was also provided by AVROBIO, Inc.

Reference: Khan A et al. Lentivirus-mediated gene therapy for Fabry disease. Nature Communications 2021;12:1178. doi.org/10.1038/s41467-021-21371-5.

T-14: Safety and Efficacy of FLT190 for the Treatment of Patients with Fabry Disease: Results from the MARVEL-1 Phase 1/2 Clinical Trial

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Institutions: [1]Department of Medicine, Division of Nephrology, Charité, Universitätsmedizin Campus Mitte, Berlin, Germany; [2]Lysosomal Storage Disorders Unit, Royal Free London NHS Foundation Trust, London, United Kingdom; [3]University College London, London, United Kingdom; [4]Freeline Therapeutics, Stevenage, United Kingdom

Background: FLT190 is an investigational adeno-associated virus (AAV) gene therapy for the treatment of Fabry disease (FD). It consists of a rationally designed capsid (AAVS3) containing an expression cassette with a codon-optimized human *GLA* cDNA under the control of a liver-specific promoter. FLT190 is being evaluated in a Phase 1/2 clinical trial in adult male FD patients (MARVEL-1).

Methods: MARVEL-1 is an open-label, multi-center, ascending single-dose, Phase 1/2 clinical trial. The primary endpoint is safety, and secondary efficacy endpoints include production of alpha-galactosidase A (α-Gal A) in plasma and globotriaosylceramide (Gb3)/ globotriaosylsphingosine (LysoGb3) clearance in plasma/urine.

Results: As of December 2021, two patients have received the lowest dose of FLT190 at $7.5x10^{11}$ vg/kg in Cohort 1. FLT190 infusion has been well tolerated. Transaminitis seen in Patient 1 was not seen in Patient 2 following an updated immune management regimen. Increases in troponin-T levels consistent with mild transient myocarditis were reported in both patients; neither patient required treatment and there were no enduring clinical sequelae on cardiac MRI or arrhythmias. Efficacy data from the two patients might suggest a dose-dependent increase in plasma α-Gal A levels. Patient 1 had a subtherapeutic response to FLT190 (α-Gal A: 1.3 nmol/hr/mL) prior to restarting enzyme replacement therapy (ERT) at Week 6, with a trough α-Gal A of 0.8 nmol/hr/mL (approximately three times baseline) at 2 years. Patient 2, who received a 46% higher absolute total dose than Patient 1 and did not show transaminitis, showed an increase in α-Gal A from 0.0 nmol/hr/mL at baseline to a mean of 3.4 nmol/hr/mL (Weeks 6-24 [assay normal range: 4.0-21.9 nmol/hr/mL]) and remains off ERT.

Conclusion: MARVEL-1 demonstrates that FLT190 is well tolerated and has promising efficacy with durable α-Gal A levels sustained for up to 2 years in the lowest dose cohort.

Support: The study is sponsored by Freeline Therapeutics.

Reference: Hughes DA, Canaan-Kühl S, Barton S, Collis R, Sherry N. Safety and efficacy of FLT190 for the treatment of patients with Fabry disease: Results from the MARVEL-1 Phase 1/2 clinical trial. Paper presented at the 18th Annual WORLD Symposium; February 11, 2022; San Diego, CA USA.

T-15: AAV5-GLA Gene Therapy Results in Sustained Long-Term GLA Transgene Expression and Cross-Correction of Target Organs in Fabry Disease Mouse Model.

Authors: Jolanda M. P. Liefhebber[1], Giso Brasser[1], Ilma Revers[1], Lieke Paerels[1], Maria J. Ferraz[2], Roelof Ottenhoff[3], Lukas K. Schwarz[1], Giorgia Squeri[1], Ines L. Pereira[1], Leonie Allart[1], Shrijana Tripathi[1], Nikoleta Efthymiopoulou[1], Greg Dobrynin[1], Paula S. Montenegro-Miranda[1], Carlie J. M. de Vries[3], Johannes M. F. G. Aerts[2], Ying Poi Liu[1]

Institutions: [1]Research, uniQure, Amsterdam, Netherlands, [2]Medical Biochemistry, Leiden institute for Chemistry, Leiden University, Leiden, Netherlands, [3]Medical Biochemistry, Amsterdam UMC, University of Amsterdam, Amsterdam, Netherlands

Background: Fabry disease is an X-linked hereditary metabolic disorder caused by mutations in the gene that encodes alpha-galactosidase A (GLA). Deficiency of GLA leads to accumulation of globotriaosylceramide Gb3 and its deacylated derivative globotriaosylsphingosine (lysoGb3) in cells and plasma, resulting in cell abnormalities and organ dysfunction affecting heart, kidney, and brain. Current enzyme-replacement therapy must be administered frequently, has infusion related side-effects, and has limited therapeutic efficacy because of poor cross-correction of heart and kidney phenotypes.

Methods: GLA-knockout mice were injected with AAV5-GLA. Plasma and tissues were analyzed by qPCR for vector concentration, ELISA for protein concentration, immunohistochemistry for GLA tissue localization, 4MU-activity assay to determine GLA activity and Mass Spectrometry for Gb3 and lyso-Gb3 reduction. Additionally, animals were tested for nociception to assess phenotypic improvement.

Results: Injection of increasing concentrations of AAV5-GLA in GLA-knockout mice showed a dose-dependent increase in GLA-activity and lowering of (lyso)Gb3 in plasma and liver, kidney, heart, and brain. Tissue staining confirmed the presence of GLA-protein and image quantification showed a dose dependent increase of GLA-protein in kidney glomeruli, a key structure for kidney function. Additionally, 10 weeks after AAV5-GLA injection, GLA-knockout mice showed improved nociception approaching that of wild-type animals. Moreover, treatment efficacy and durability were confirmed at 6 months post-IV injections demonstrating sustained long term GLA transgene expression and efficacy of the AAV5-GLA vector in the Fabry disease mouse model.

Conclusion: The long-term reduction of substrate in target organs, the phenotypic improvement in GLA-knockout mice, and the favorable profile of AAV5-based gene therapy in humans strongly suggest that AAV5-GLA is an attractive approach for treating human Fabry disease.