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Abstracts
4th Update on Fabry Nephropathy:

Biomarkers, Progression and Treatment Opportunities

June 1–2, 2015, Manchester, UK

Editor
David G. Warnock, Birmingham, Ala.
### Monday June 1, 2015: Manchester, UK
Radisson Blu Edwardian Manchester Hotel, Manchester, UK

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<td>Poster Awards and Opening of Poster Session – (5 presentations of selected poster abstracts (8 min each)) – Selection Committee: Stephen Waldek (Manchester), Laura Barisoni (Miami), Ricardo Correa-Rotter (Mexico City), Dominique Germain (Paris), Paul Hwu (Taipei), Gere Sunder-Plassmann (Vienna)</td>
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Tuesday June 2, 2015: Manchester UK

Radisson Blu Edwardian Manchester Hotel, Manchester UK

Chairs: Christiane Auray-Blais (Sherbrooke) and Kevin Mills (London): Lyso-GB3 and Urinary Peptidomics in Fabry Disease

08:00 Christiane Auray-Blais (Sherbrooke): Urinary and Plasma Lyso GB3 and Analogues

08:30 Kevin Mills (London): New Biomarkers: Effects of ERT in Fabry Disease

09:00 Carla Hollak (Amsterdam): Plasma Lyso_GB3 and Fabry Phenotypes

09:30–10:00 Tea/Coffee Break in Poster Viewing Area

Chairs: Alberto Ortiz (Madrid) and Michael Mauer (Minneapolis): Podocytes and Fabry Nephropathy

10:00 Tobias Huber (Freiburg): Autophagy, Podocyte Injury and Fabry Nephropathy

10:30 Rachel Lennon (Manchester): Podocyte Adhesion to Glomerular Basement Membrane

11:00 Behzad Najafian (Seattle): Structural Studies of Podocytes in Fabry Nephropathy

11:30 Scott Garman (Amherst): Novel Approaches to Podocyte Treatment

12:00 Erik I. Christensen (Aarhus): ERT Uptake Pathways in Different Cells

Chairs: Christoph Wanner (Würzburg) and David Warnock (Birmingham): Rare Disease Perspective

12:30 Peter Harris (Rochester) Allelic Complexity and Treatment Prospects in ADPKD: Parallels with Fabry Disease Harris (Rochester); Polycystic Kidney Disease and Fabry Nephropathy

13:00–14:00 Lunch and Departures
1 Complete Elimination of Renal Glycosphingolipid Deposition by 3 Years of Treatment with Agalsidase Beta in a Boy with Fabry Disease

Shuichi Ito1,2, Masao Ogura2, Koichi Kamei2, Kentaro Matsuoka3

1Department of Pediatrics, Graduate School of Medicine, Yokohama City University, Yokohama, Japan; 2Division of Pediatric Nephrology and Rheumatology, National Center for Child Health and Development, Tokyo, Japan; 3Division of Pathology, National Center for Child Health and Development, Tokyo, Japan

Introduction: Enzyme replacement therapy (ERT) using agalsidase is a unique and effective therapy against Fabry disease. However, early diagnosis is difficult if there is a lack of family history. A delay in diagnosis often results in irreversible organ damage, but initiation of ERT in children or young adults without apparent organ involvement is still controversial. We report here the clinical course and renal pathological findings in a boy with Fabry disease before and 3 years after ERT.

Results: A 10-year-old boy was referred to our hospital because of pain on the soles of his feet since 7 years old. The pain was intermittent, but sometimes endurable, and disturbed his school life and sleep. Despite many consultations with pediatricians and orthopedists, he was undiagnosed. However, we suspected Fabry disease and performed biochemical and genetic analysis. His serum globotriaosylceramide (GL3) level was markedly increased. His alpha-galactosidase A activity was also markedly reduced (2.5 AgalU; normal (male) <17 AgalU) because of a truncated M1T mutation in the GLA gene. We found that he also suffered from hypohidrosis and angiokeratoma on his foot and hips. Although he showed neither a decreased eGFR nor microalbuminemia, his renal biopsy showed typical features of Fabry disease, with GL3 accumulation in podocytes, endothelial cells, mesangial cells, and tubules. Partial foot process effacement of podocytes was characteristic, suggesting podocytopathy. We treated him with 1.0 mg/kg of agalsidase beta, pregabalin, and carbamazepine biweekly. Three years after initiation of ERT, the pain on his soles had disappeared and his hypohidrosis had improved. His daily and school activities were normalized. Three years after ERT, his hypohidrosis had improved. His daily and school activities were normalized.

Conclusion: Appropriate timing of initiation of ERT is still controversial. However, a recent report on a 5-year high dose of ERT (0.4–1.0 mg/kg/2W) showed a marked reduction in renal GL3 accumulation, including podocytes, in children or young adults. Our patients also showed that early indication of high-dose ERT for male children with truncated mutation might improve their prognosis and quality of life. Therefore, awareness of early clinical signs by pediatricians and orthopedists could be indispensable for early diagnosis and treatment.

2 The Utility of Pharmacogenetics in the Identification of Fabry Patients Eligible for Treatment with Migalastat

E.R. Benjamin1, C. Della Valle1, X. Xu1, E. Katz2, K.J. Valenzano1, D.G. Bichet2, D.P. Germain1, R. Giugliani3, D.A. Hughes4, R. Schiffermann4, W.R. Wilcox5, J. Yu1, J. Kirk1, J. Barth6, J. Castelli1

1Amicus Therapeutics, Cranbury, NJ, USA; 2Hôpital du Sacré-Coeur, Montréal, Québec, H4J1C5, Canada; 3Hôpital Raymond Poincaré (AP-HP), Univ of Versailles – St. Quentin en Yvelines (UVSQ), Garches, France; 4Medical Genetics Service, HCPA/UFRGS Porto Allegre, Brazil; 5Royal Free Campus, Univ College London, London, UK; 6Baylor Research Institute, Dallas, TX; 7Department of Human Genetics, Emory Univ, Atlanta, GA, USA

Introduction: Fabry disease (FD) is caused by mutations in the gene (GLA) that encodes α-galactosidase A (α-Gal A). Deficient α-Gal A activity leads to accumulation of globotriaosylceramide (GL-3) and globotriaosylsphingosine (lyso-Gb3). Migalastat is an investigational pharmacological chaperone that was evaluated in Phase 3 studies AT1001-011 and AT1001-012. Migalastat binds, stabilizes, and increases cellular levels of specific mutant forms of α-Gal A in patients, predominantly with missense mutations, most of which express the classic phenotype. A bioanalytically validated human embryonic kidney (HEK)-293 cell-based assay (GLP HEK assay) is used to individually express FD mutations and measure increases in mutant α-Gal A activity in response to 10 μM migalastat. Amino acid mutations are defined as those having a ≥1.20-fold relative increase and ≥3.0% absolute increase in α-Gal A activity. The current objective was to identify pharmacogenetic variants that allow the prediction of patient response to migalastat treatment.

Methods: The relationship between mutant α-Gal A responses in the GLP HEK assay and corresponding clinical response in Phase 2 and clinical studies of migalastat (NCT01218659 ‘011, NCT00283959, NCT00283933, NCT00304512, NCT00925301 ‘011’, NCT01218659 012’) were evaluated.

Results: Comparison of mutant α-Gal A responses in the GLP HEK assay and in WBCs of male Fabry patients treated with migalastat showed a high degree of consistency between the two sets of results (ranges in predictive values across clinical studies: sensitivity
0.882–1.0; specificity 0.75–1.0; positive predictive value 0.875–1.0; negative predictive value 0.75–1.0). In Study 011, subjects with amenable mutations randomized to migalastat demonstrated statistically significant (±SEM) decreases in the number of kidney GL-3 inclusions per capillary (IPC) (−0.25±0.10) at month 6, compared to the placebo group (+0.07±0.15; p=0.008). Subjects randomized to placebo and switched to migalastat also demonstrated statistically significant decreases in IPC (−0.31±0.10; p=0.013). Statistically significant reductions in plasma lyso-Gb3 were observed after 6 months of treatment with migalastat, in both groups (p=0.0033, p<0.0001). However, substrate levels were not reduced in subjects with non-amenable mutations. In Study 012 subjects with amenable mutations switched from ERT, plasma lyso-Gb3 levels remained low and stable in both males and females for at least 18 months. However, plasma lyso-Gb3 increased in two male subjects with non-amenable mutations following switch from ERT to migalastat. The GLP HEK assay responses to migalastat in the amenable mutant forms evaluated in the Phase 2 and 3 clinical studies (n=51) were not significantly different from the responses of all amenable mutant forms (n=224). A majority of all amenable mutations (68%) and those represented in the clinical studies of migalastat (67%) were associated with classic Fabry disease based on literature review.

Conclusions: Categorization of GLA mutations as amenable according to the GLP HEK assay is effective in identifying Fabry patients who would be eligible for treatment with migalastat. Approximately 30–50% of patients with FD are estimated to have amenable mutations; the majority of amenable mutations are associated with the classic phenotype of the disease.

3
Investigation of Biomarkers in Immune Response Against Human Recombinant Alpha-GAL-A
Tabitha Taber1, Christiane Auray-Blaiz2, Michel Boutin2, Pamela Lavio1, Michelle Hoard3, Shawn Lipinski4, Ozlem Goker-Alpan1
1Lysosomal and Rare Disorders Research and Treatment Center, Fairfax, VA, USA; 2Université de Sherbrooke, Division of Medical Genetics, Department of Pediatrics, QC, Canada

Introduction: Fabry disease (FD) is characterized by the abnormal deposition of glycosphingolipids (Gb3) in organs and tissues due to the deficiency of lysosomal alpha-galactosidase A (αGAL-A). It has recently been suggested that levels of deacylated globotriaosylsphingosine (lyso-Gb3) and its related analogues may correlate with FD biomarkers. Levels of deacylated globotriaosylceramide (Gb3) in organs and tissues due to the deficiency of lysosomal alpha-galactosidase A (αGAL-A). It has recently been suggested that levels of deacylated globotriaosylsphingosine (lyso-Gb3) and its related analogues may correlate with FD biomarkers. Levels of deacylated globotriaosylceramide (Gb3) in organs and tissues due to the deficiency of lysosomal alpha-galactosidase A (αGAL-A). It has recently been suggested that levels of deacylated globotriaosylsphingosine (lyso-Gb3) and its related analogues may correlate with FD biomarkers. Levels of deacylated globotriaosylceramide (Gb3) in organs and tissues due to the deficiency of lysosomal alpha-galactosidase A (αGAL-A). It has recently been suggested that levels of deacylated globotriaosylsphingosine (lyso-Gb3) and its related analogues may correlate with FD biomarkers. Levels of deacylated globotriaosylceramide (Gb3) in organs and tissues due to the deficiency of lysosomal alpha-galactosidase A (αGAL-A). It has recently been suggested that levels of deacylated globotriaosylsphingosine (lyso-Gb3) and its related analogues may correlate with FD biomarkers. Levels of deacylated globotriaosylceramide (Gb3) in organs and tissues due to the deficiency of lysosomal alpha-galactosidase A (αGAL-A). It has recently been suggested that levels of deacylated globotriaosylsphingosine (lyso-Gb3) and its related analogues may correlate with FD biomarkers. Levels of deacylated globotriaosylceramide (Gb3) in organs and tissues due to the deficiency of lysosomal alpha-galactosidase A (αGAL-A). It has recently been suggested that levels of deacylated globotriaosylsphingosine (lyso-Gb3) and its related analogues may correlate with FD biomarkers. Levels of deacylated globotriaosylceramide (Gb3) in organs and tissues due to the deficiency of lysosomal alpha-galactosidase A (αGAL-A). It has recently been suggested that levels of deacylated globotriaosylsphingosine (lyso-Gb3) and its related analogues may correlate with FD biomarkers. Levels of deacylated globotriaosylceramide (Gb3) in organs and tissues due to the deficiency of lysosomal alpha-galactosidase A (αGAL-A). It has recently been suggested that levels of deacylated globotriaosylsphingosine (lyso-Gb3) and its related analogues may correlate with FD biomarkers. Levels of deacylated globotriaosylceramide (Gb3) in organs and tissues due to the deficiency of lysosomal alpha-galactosidase A (αGAL-A). It has recently been suggested that levels of deacylated globotriaosylsphingosine (lyso-Gb3) and its related analogues may correlate with FD biomarkers. Levels of deacylated globotriaosylceramide (Gb3) in organs and tissues due to the deficiency of lysosomal alpha-galactosidase A (αGAL-A). It has recently been suggested that levels of deacylated globotriaosylsphingosine (lyso-Gb3) and its related analogues may correlate with FD biomarkers. Levels of deacylated globotriaosylceramide (Gb3) in organs and tissues due to the deficiency of lysosomal alpha-galactosidase A (αGAL-A). It has recently been suggested that levels of deacylated globotriaosylsphingosine (lyso-Gb3) and its related analogues may correlate with FD biomarkers. Levels of deacylated globotriaosylceramide (Gb3) in organs and tissues due to the deficiency of lysosomal alpha-galactosidase A (αGAL-A). It has recently been suggested that levels of deacylated globotriaosylsphingosine (lyso-Gb3) and its related analogues may correlate with FD biomarkers. Levels of deacylated globotriaosylceramide (Gb3) in organs and tissues due to the deficiency of lysosomal alpha-galactosidase A (αGAL-A). It has recently been suggested that levels of deacylated globotriaosylsphingosine (lyso-Gb3) and its related analogues may correlate with FD biomarkers. Levels of deacylated globotriaosylceramide (Gb3) in organs and tissues due to the deficiency of lysosomal alpha-galactosidase A (αGAL-A). It has recently been suggested that levels of deacylated globotriaosylsphingosine (lyso-Gb3) and its related analogues may correlate with FD biomarkers. Levels of deacylated globotriaosylceramide (Gb3) in organs and tissues due to the deficiency of lysosomal alpha-galactosidase A (αGAL-A). It has recently been suggested that levels of deacylated globotriaosylsphingosine (lyso-Gb3) and its related analogues may correlate with FD biomarkers.

Methods: In an IRB approved study, immune response against human recombinant alpha-GAL-A (h-roGALA) was studied prospectively among 30 subjects (13M and 17F) (age range: 8–58 y). Gb3 and lyso-Gb3 and its analogues were followed in plasma with consecutive measurements for at least one year from the onset of hypersensitivity reaction (HSR) as described by Boutin and Auray-Blaiz, Anal. Chem. 2014. Clinically, HSRs were graded according to the NCI criteria, and neutralizing antibodies were assessed by the in vitro assay.

Results: 8 male and 3 female patients with FD developed HSR against h-roGALA. Immediate HSR was observed in male subjects, where as only delayed HSR occurred in females. Clinical NCI criteria grading of immediate HSRs were 3 in 6, and 2 in 2 subjects. There was evidence of complement activation in two subjects and one subject had elevated trypase levels. None of the subjects had IgE type antidrug antibodies; where as the majority (6/8) had IgG type ADA, all with in vitro neutralizing antibodies. While plasma Gb3 was not elevated (mean 5 μg/ml, median 5.45 μg/ml, normal <7 μg/ml), our results show that the mean plasma lyso-Gb3 was 60.9 nM and the median was 48.8 nM (<2.4 nM), with increased levels of all analogues at −28, −2, +16, +18, +34, and +50.

Conclusions: The importance of sphingolipids, not only as components of plasma membranes, but also as key players in different cellular events, with growing evidence of their role on the development, activation and regulation of the immune system, are now emerging. The elevated plasma levels of lyso-Gb3 and all related analogues among subjects with HSR merits further exploration whether elevated FD biomarkers contribute further to the immune response or increased lyso-Gb3 levels reflect decreased efficacy of h-roGALA secondary to the immune response.

Acknowledgements: This study has received an investigational grant support from Shire, and is listed under NCT01745185.

4
Reduction of Plasma Globotriaosylsphingosine Levels After Switching from Agalsidase Alfa to Agalsidase Beta as Enzyme Replacement Therapy for Fabry Disease
Ozlem Goker-Alpan1, Michael J. Gambello2, Gustavo H.B. Maegawa3, Khan J. Nedd4, Daniel J. Gruskin5, Larry Blankstein5, Neal J. Weinreb6
1LSD Unit, CFCT, Fairfax, VA, 2Emory University, Atlanta, GA, 3JHU, Baltimore, MD, 4Infusion Associates, Grand Rapids, MI, 5Genzyme, Cambridge, MA, 6University Research Foundation, Coral Springs, F, USA

Introduction: Agalsidase alfa and agalsidase beta, recombinant enzyme preparations for treatment of Fabry disease (FD), have different approved dosing schedules: 0.2 mg/kg and 1.0 mg/kg every other week (EOW), respectively.

Methods: This open-label, multicenter, exploratory phase 4 study evaluated plasma globotriaosylsphingosine (lyso-GL-3), and plasma and urine globotriaosylceramide (GL-3) levels at baseline and 2, 4, and 6 months after the switch from agalsidase alfa (0.2 mg/kg EOW for ≥12 months) to agalsidase beta (1.0 mg/kg EOW) in 15 male patients with FD. Immunoglobulin (Ig)G antidrug antibody titers were assessed, and safety monitored throughout the study.

Results: Plasma lyso-GL-3 concentrations decreased significantly within 2 months after switch, and reductions continued through Month 6 (mean absolute changes: −12.8, −16.1, and −16.7 ng/ml at 2, 4, and 6 months, respectively; all P<0.001). The mean percentage reduction from baseline was 39.5% (P<0.001) at Month 6. For plasma GL-3, the mean absolute change from base-
line (~0.9 μg/ml) and percentage reduction (17.9%) at Month 6 were both significant (P < 0.05). Urine GL-3 measurements showed intra-patient variability and changes from baseline were not significant. There were no differences in IgG antidrug antibody titers between the two enzymes. The switch from agalsidase alfa to agalsidase beta was well tolerated.

**Conclusions:** Plasma lyso-GL-3 and GL-3 levels reduced after switching from agalsidase alfa to agalsidase beta, indicating that agalsidase beta has a greater pharmacodynamic effect on these markers at the recommended dose. These data further support the use of agalsidase beta 1.0 mg/kg EOW as enzyme replacement therapy in FD.

**Acknowledgements:** The study was sponsored by Genzyme, a Sanofi company, and is registered at www.ClinicalTrials.gov under the identifier NCT01650779.

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5

**Rapid Immunochromatographic Detection of Serum Anti-α-Galactosidase a Antibodies in Fabry Patients After Enzyme Replacement Therapy**

Tadayasu Togawa¹, Futoshi Shibasaki², Takahiro Tsukimura¹, and Hitoshi Sakuraba³

Departments of ¹Functional Bioanalysis and ³Clinical Genetics, Meiji Pharmaceutical University, ²Department of Molecular Medical Research, Tokyo Metropolitan Institute of Medical Science, Japan

**Objectives:** Fabry patients have been successfully treated with recombinant α-Galactosidase A (GLA). However, recurrent injections of GLAs often cause the production of antibodies against these recombinant proteins among Fabry male patients, leading to allergic reactions and/or reduction of the ERT efficacy.

**Methods:** Agalsidase alfa (Aga-A) or Agalsidase beta (Aga-B) was immobilized on the IC membrane (fig. 1, Test line; T), and anti-goat IgG antibody was immobilized on the control line (fig. 1, Control line: C). In the first step, serum was diluted tenfold with sample buffer and dropped on the IC chip (fig. 1 (1)). In the second step, the reservoir unit (Reservoir) containing the conjugation buffer (alkaline phosphatase (AP) labeled-goat anti-human IgG) was snapped open to allow the antibody/antigen reaction development. The dry substrate of AP was fixed on the membrane and mixed with conjugation buffer after opening the Reservoir (fig. 1 (2)). In the final step, anti-GLA antibodies captured by the immobilized Aga-A or Aga-B on the membrane were detected by AP-conjugated goat anti-human IgG and visualized with the enzymatic reaction of AP to a substrate (fig. 1 (3)). The level of color strength (score) was evaluated by the visual determination according to a control color paper.

**Results:** The evaluation of serum samples from 29 Fabry patients, who had received enzyme replacement therapy (ERT) with agalsidase alfa (Aga-A) and/or agalsidase beta (Aga-B), was performed using this assay. Our results clearly revealed that patients exhibited the same level of antibodies against both Aga-A and Aga-B, regardless of the species of recombinant GLA proteins used for ERT.

**Conclusions:** We developed an easy and rapid IC-based assay method for detecting anti-GLA antibodies in serum. It will be useful for a quick evaluation or first screening of serum antibodies against Aga-A or Aga-B.
WSI Quantitative (BLISS) and Semiquantitative (FSS) Assessment for Cortical PTC Gb3 Inclusions in Fabry Disease Patients Following IV Administration of Plant Derived Alpha-GAL-A Enzyme (PRX-102)


1U.Miami, USA; 2MGH/Harvard Medical School, USA; 3UNC, USA; 4Instituto Privado De Hematologia Investigacion Clinica, Paraguay; 5Emory University, USA; 6Baylor University MC, USA; 7O&O Alpan LLC, USA; 8Johns Hopkins, USA; 9University of Iowa Hospital, USA; 10Protalix Biotherapeutics, Israel

Introduction: Previous studies on Fabry disease patients receiving enzyme replacement therapy (Fabrazyme) showed reduction of Gb3 inclusions in renal peritubular capillaries (PTC) endothelium by using a semiquantitative approach (Fabrazyme Scoring System – FSS). A subsequent study with male and female Fabry patients evaluating the efficacy of a pharmacological chaperone (AT1001) demonstrated that the application on annotated whole slide images (WSI) of a quantitative scoring methodology (Barisoni Lipid Inclusion Scoring System – BLISS) increases sensitivity, accuracy and reproducibility. The aim of this study is to evaluate the efficacy of a novel therapeutic option for Fabry disease, a plant derived α-galactosidase A enzyme (PRX-102), by applying both BLISS and FSS on annotated WSI.

Methods: 5 symptomatic Fabry patients (2/3, F/M), either native or from ERT in the last 6 months, with negative anti PRX-102 antibodies, and with eGFR ≥60 ml/min/1.73 m² received IV-PRX-102 (0.2 mg/kg every 2 weeks). Plastic embedded 1 μ thick sections stained with toluidine blue from kidney biopsies obtained prior the first IV treatment and at 6 months of treatment, were scanned into WSI (Aperio) at 100×. 300 PTC/biopsy were first annotated by one renal pathologist and then scored by 2 different renal pathologists, using BLISS and FSS in a random and blinded manner. Individual PTCs with discrepant score were rescored for adjudication by the annotator.

Results: Substantial reduction of Gb3 inclusions was noted in PTC using both BLISS and FSS (figure 1).

Conclusions: The application of BLISS and FSS on annotated WSI to assess PRX-102 IV infusion efficacy shows significant reduction of PRC Gb3 renal PTC inclusions after 6 months of therapy.

Acknowledgements:*Pathologists who equally contributed to the study; Victoria Madden; Microscopy Services Laboratory, Pathology and Laboratory Medicine CB# 7525 Brinkhouse-Bullitt Bldg., University of North Carolina at Chapel Hill Josh Staples, Image Analysis Specialist, Leica Biosystems, 1360 Park Center Dr, Vista, CA.

Introduction: Fabry disease (FD) is an X-linked lysosomal storage disease caused by the deficiency of α-galactosidase A, resulting in the accumulation of glycosphingolipids in body fluids and different organs. Globotriaosylceramide (Gb3), globotriaosylsphingosine (Lyso-Gb3) and their analogues have been identified and quantified as biomarkers for the disease severity and treatment efficacy. The current study aimed to develop HPLC-MS methods in order to identify and quantify FD biomarkers.

Methods: Human plasma and urine samples were collected from healthy controls, Fabry patients and patients of other 5 disorders including (renal, adrenal, vascular, liver autoimmune and inflammatory bowel disorders). The samples were processed using

![Fig. 1.](color version available online)

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solid phase extraction before being analysed for Lyso-Gb3 and its analogues levels using HPLC-ESI-micrOTOFMS.

Results: Lipids extraction from urine and plasma samples showed a recovery of 90% & 70% respectively. Reverse phase-HPLC methods were optimised with an isocratic elution of (0.1% formic acid/40% acetonitrile) and flow rate of 3 μL/min. A multiple reaction monitoring mode MS method was optimised for metabolites analysis showing limit of detection and quantification of 10 & 20 fmol Lyso-Gb3 respectively. Lyso-Gb3 and its analogues levels and their correlations with vital factors were studied by details.

Conclusion: We have developed an HPLC-ESI-MS approach for analysis of Lyso-Gb3 and its analogues. Pilot data shows low levels of these biomarkers are quantified in urine and plasma.


Fig. 1. Extracted ion chromatogram of (a) Lyso-Gb3 standard (made in-house), (b) Lyso-Gb3 in a plasma sample from a classical Fabry male, (c) Lyso-Gb3 in a urine sample from a classical Fabry male (for Abstract 7).

8 Fabry Disease Diagnosed in Living Donor Kidney Transplant Biopsy

R. Skrunes¹,2, K.K. Larsen²,4, S. Leh²,4, E.H. Strøm³, T.G. Jenessen⁶, C. Tøndel¹,2, E. Svarstad¹,2

¹Haukeland University Hospital, Department of Medicine, Bergen, Norway, ²University of Bergen, Department of Clinical Medicine, Bergen, Norway, ³Haukeland University Hospital, Department of Pathology, Bergen, Norway, ⁴Haukeland University Hospital, Department of Pathology, Bergen, Norway, ⁵Oslo University Hospital, Department of Pathology, Oslo, Norway, ⁶Metabolic and Renal Research Group, UiT The Arctic University of Norway, Tromsø, Norway

Introduction: A 33 year old woman donated a kidney to her 52 year old brother, a hemodialysis patient with presumed IgA nephropathy, not biopsied. Comorbid conditions included a diagnosis of multiple sclerosis, atrial fibrillation, left ventricular hypertrophy and a transitory ischemic attack. The transplanted kidney was biopsied shortly after transplantation due to proteinuria. Fabry disease was diagnosed based on GL3 deposits in the podocytes, and the recipient was started on Agalsidase Beta 1.0 mg/kg/eow one year after transplantation.
after the kidney transplantation. The donor was found to have cornea verticillata and mild acroparesthesias, but no signs of kidney or heart disease. In retrospect, symptoms of Fabry disease were present in several family members. We present the long term outcome of the kidney donor (sister) and the kidney transplant recipient (brother) with a classical Fabry mutation c.800T>G[p.M267R].

**Results:** At diagnosis enzyme levels in the donor and recipient were 9.5 and 2.1 μkat/kg protein respectively (ref: 40;29–61). The donor developed minimal albuminuria 2 years after donation. Enzyme replacement therapy (ERT) and angiotensin II receptor blockade were started 12 years after donation due to increasing albuminuria. Kidney function is stable in the donor. Two months after transplantation measured glomerular filtration rate (Cr-EDTA) was 46 ml/min/1.73 m² in the recipient. 12 years after transplantation, and after 11 years of ERT (Agalsidase Beta 1.0 mg/kg/eow), measured glomerular filtration rate (lohexol) was 49 ml/min/1.73 m². Before ERT albumin/creatinine ratio (ACR) was 66.4 mg/mmol, compared to ACR 1.1 mg/mmol after 11 years of ERT. All podocytes were completely cleared of all GL3 deposits after 11 years of ERT. Mesangial and endothelial cells were cleared of GL3 deposits after 5 years of ERT. The recipient is immunosuppressed with Prednisolone, Tacrolimus and Mycophenolate mofetil. Severe cardiomyopathy progressed, necessitating an implantable cardioverter-defibrillator.

**Conclusions:** Classical Fabry disease was diagnosed in siblings after living kidney donation. With increasing albuminuria the sister (donor) started ERT 12 years after donation, kidney function is well preserved. The donor kidney has done exceptionally well in the recipient after initiation of ERT. Kidney function (CKD3) has remained stable. During long-term high-dose ERT all glomerular involvement was so prominent that mean podocyte score was almost 3. Arterial/artiolar hyalinosis and interstitial inflammation were absent. Tubulointerstitial fibrosis was involved only in 5%. Electron microscopy showed not only characteristic myelin-like podocyte inclusions but also foot process effacement. These results in electron microscopy were consistent with the findings in the recent case series in young normoalbuminuric classical type of FD (Nephron 129: 16–21, 2015).

**Conclusions:** Even if urinalysis and renal function is normal, renal morphological changes, especially in podocytes, are prominent also in late-onset variant type of FD.

### 9 Morphological Details of Renal Lesions in Late-Onset Normoalbuminuric Fabry Disease

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**Introduction:** We previously published a case report of 36-year-old normoalbuminuric Fabry disease (FD) patient discovered with urinary mulberry cells (Nakamichi et al., CEN Case reports 2: 49–52, 2013), in which we showed typical abnormalities in podocytes with light and electron microscopy. The main object of our current study is to evaluate further renal morphological details in this late-onset normoalbuminuric FD patient.

**Methods:** Firstly, we assessed our case on periodic acid-Schiff stain slide with International Study Group of Fabry Nephropathy Score Sheet, the novel scoring system for renal pathology in FD (Nephrol Dial Transplant 25: 2168–2177, 2010). Secondly, we focused on the podocyte abnormalities in electron microscopy. Additional scoring on the epoxy-embedded toluidine blue-stained semi-thin section slides was not done.

**Results:** Total 33 glomeruli were observed. Podocyte vacuolization was so prominent that mean podocyte score was almost 3. Arterial/artiolar hyalinosis and interstitial inflammation were absent. Tubulointerstitial fibrosis was involved only in 5%. Electron microscopy showed not only characteristic myelin-like podocyte inclusions but also foot process effacement. These results in electron microscopy were consistent with the findings in the recent case series in young normoalbuminuric classical type of FD (Nephron 129: 16–21, 2015).

**Conclusions:** Even if urinalysis and renal function is normal, renal morphological changes, especially in podocytes, are prominent also in late-onset variant type of FD.

### 10 Post-Mortem Pathology of Two Males with Fabry Disease After 13 Years on Enzyme Replacement Therapy

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**Introduction:** We describe the post-mortem findings of 2 males with Fabry disease, an X-linked lysosomal storage disease.

**Patient Characteristics:** See table 1.

**Results:**

**Renal Histology:**

- Case 1 (figure 1a and b) – Global glomerular sclerosis, podocyte vacuolation and interstitial fibrosis similar pre- and post-treatment.
- Case 2 – Pre-ERT renal biopsy showed 90% globally sclerosed glomeruli, extensive tubular atrophy and vacuolated cytoplasm.

<table>
<thead>
<tr>
<th>Table 1. (for Abstract 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
</tr>
<tr>
<td>Age – mutation</td>
</tr>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>Renal involvement</td>
</tr>
<tr>
<td>Cardiac involvement</td>
</tr>
<tr>
<td>Cerebral lesion(s)</td>
</tr>
<tr>
<td>Cause of death</td>
</tr>
<tr>
<td>Biopsies</td>
</tr>
</tbody>
</table>
Renal transplant biopsy after 8 years on ERT showed moderate to severe arteriosclerosis.

Cardiac Histology: Post-ERT – Both cases showed hypertrophied myocytes, intracellular vacuolation and patchy interstitial fibrosis.

Conclusion: Renal function did not deteriorate and renal parenchyma remained viable in the patient commenced on ERT prior to reaching end-stage renal disease. ERT did not prevent cerebrovascular disease or cardiac events in either case.

### Table 1. (for Abstract 11)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>ERT (years)</th>
<th>mGFR ml/ min/1.73 m²</th>
<th>UACR mg/mmol</th>
<th>Podocyte score</th>
<th>Fibrosis</th>
<th>Vasculopathy</th>
<th>Cardiomyopathy</th>
<th>Cum. dose ERT at last biopsy</th>
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<td>=</td>
<td>=</td>
<td>=</td>
<td>104.4 mg/kg</td>
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</table>

*Age is at start of ERT. 0: normal; =: unchanged level; ⇓: improvement; ⇑: deterioration.

### 11 Long-Term Enzyme Replacement Therapy (ERT) Benefits the Glomeruli More Than the Vasculature in Younger Fabry Nephropathy

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1Haukeland University Hospital, Department of Medicine, Bergen, Norway, 2University of Bergen, Department of Clinical Medicine, Bergen, Norway, 3Haukeland University Hospital, Department of Pediatrics, Bergen, Norway, 4Haukeland University Hospital, Department of Pathology, Bergen, Norway

Introduction: ERT has been available for more than 10 years and seems to prevent or attenuate progressive kidney disease in younger patients. ERT induced reduction of globotriaocylceramide (Gb3) deposits has been shown in mesangial and endothelial cells and in podocytes. Beneficial dose-dependent structural effects have been accompanied by similar effects on microalbuminuria in children. We have examined glomerular and vascular changes in kidney biopsies at baseline and after an average of 8.5 (range 5–13)
years of ERT in 11 consecutive classical males with microalbuminuria and normal GFR at baseline.

Results: Patient 1 had progressive fall in GFR and increasing albuminuria due to de novo glomerulonephritis. The other patients had stable GFR (except patient 10), and variable microalbuminuria. None of the patients had overt proteinuria. Progressive vasculopathy was seen in the majority of patients who were above the age of 16 at baseline.

Conclusion: ERT confer beneficial long-term effects on glomerular structure with generally preserved kidney function in children and young adults. Increasing vascular damage despite ERT was seen in the majority of patients above 16 years of age at initiation of ERT.

12 Differential Kidney Effects of High and Low Enzyme Dose in Male Siblings Treated for 13 Years
R. Skrunes1,2, E. Svarstad1,2, K.K. Larsen2,4, S. Leh2,4, C. Tøndel2,3
1Haukeland University Hospital, Department of Medicine, Bergen, Norway, 2University of Bergen, Department of Clinical Medicine, Bergen, Norway, 3Haukeland University Hospital, Department of Pediatrics, Bergen, Norway, 4Haukeland University Hospital, Department of Pathology, Bergen, Norway

Introduction: Two brothers with classical Fabry disease started enzyme replacement therapy (ERT) in 2001, the younger brother was 13 years old (M13), the older brother 15 (M15). At start of ERT both brothers had severe acroparesthesias, angiokeratomas and cornea verticillata. Kidney biopsies were performed 2 years after starting ERT due to development of albuminuria in both brothers. The brothers were started on Agalsidase Alpha (AA) 0.2 mg/kg/eow, and received double dose after 2 y of ERT. After 6 years of ERT, M13 was switched to Agalsidase Beta (AB) 1 mg/kg/eow due cardiac complications, necessitating a pacemaker.

Results: Both brothers had significant GL3 deposits in their baseline kidney biopsies, with full podocyte GL3 scores. Over time M13 has nearly cleared the podocytes of sphingolipids and albuminuria normalized. M15 still has a full score and increasing albuminuria. Total clearance of mesangial and glomerular endothelial deposits was seen in both patients on AA.

Measured GFR-level (mGFR, iohexol clearance) is preserved in M13, while M15 has lost 10 ml/min/1.73 m² from first to final biopsy. The cumulative dose of ERT was almost twice as high in M13 compared to M15.

Conclusion: Dose of ERT seems to matter when clearing of sphingolipids from podocytes are evaluated in long-term kidney biopsies. The higher dose of ERT normalized kidney histology and albuminuria. Whereas the lower dose failed to clear the podocytes, and was associated with early decline of mGFR and progressive albuminuria, it may still be sufficient to attenuate the rate of progressive kidney disease.

13 Does Lisinopril Without Enzyme Replacement Therapy (ERT) Prevent the Progression of Fabry Nephropathy?
Vinay Narasimha Krishna1, Huma Fatima2, Dana V. Rizk1, Monica Tucci3, Leslie Jackson1, Eric L. Wallace1, David G. Warnock1
1University of Alabama at Birmingham, 1Nephrology, 2Pathology and Children’s Hospital, 3Pediatrics, Birmingham AL, USA

Introduction: The timing of initiating ERT in oligosymptomatic children with Fabry disease is debated. Conservative measures such as renin-angiotensin blockade can control proteinuria but may not stop the progression of nephropathy. We describe a 17-

<table>
<thead>
<tr>
<th></th>
<th>M13</th>
<th>M15</th>
</tr>
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<tbody>
<tr>
<td>Baseline, 2 years of ERT</td>
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</tr>
<tr>
<td>mGFR ml/min/1.73m²</td>
<td>107</td>
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<td>Albumin/Creatinine ratio mg/mmol</td>
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<td>7 years of ERT</td>
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<tr>
<td>mGFR ml/min/1.73m²</td>
<td>115</td>
<td>111</td>
</tr>
<tr>
<td>Albumin/Creatinine ratio mg/mmol at 7 years of ERT</td>
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<td>19.5</td>
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<tr>
<td>Composite podocyte score (0–7)</td>
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<td>7.0</td>
</tr>
<tr>
<td>13 years of ERT</td>
<td></td>
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<tr>
<td>mGFR at 13 ml/min/1.73m²</td>
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<td>7.0</td>
</tr>
<tr>
<td>Cum. dose of Agalsidase</td>
<td>233.0 mg/kg</td>
<td>121.7 mg/kg</td>
</tr>
</tbody>
</table>
89

year old female with Fabry disease (R277X mutation) who showed segmental foot process effacement despite 5 years of lisinopril therapy with control of her proteinuria before ERT was started.

Methods: This was a retrospective case review.

Results: The patient had mild acroparesthesias, as well as angio-keratomas over the palmar surface of her fingers. Baseline electrocardiogram, cardiac 2D echo and brain magnetic resonance imaging were normal. The patient was receiving lisinopril (5 mg/day) with consistent urine protein to creatinine ratio <0.2 g/g. Her estimated GFR was stable at 139 ml/min/1.73 m². The renal biopsy showed numerous lamellated lipid inclusions in visceral epithelial cells, tubular epithelial cells and peritubular capillary endothelial cells. Furthermore, podocyte foot processes showed focal effacement. Based on these findings ERT with Fabrazyme was initiated.

Conclusions: Control of proteinuria with lisinopril without ERT did not prevent progression of Fabry nephropathy and podocyte foot process effacement in this patient. Proteinuria control is an important adjunctive therapy to slow progression of any form of proteinuric chronic kidney disease but does not replace the need for treating the underlying cause of the disease.

Introduction: Factors relating to clinical variability in Anderson-Fabry disease (AFD) heterozygotes are not fully known. Understanding molecular pathways related to onset, progression and severity of disease in individuals would be valuable in genetic counselling of patients, optimising treatment to limit progression of renal, cardiac and cerebrovascular disease in individuals, and providing adjunctive therapy in patients. Non-coding GLA gene polymorphisms are identified in AFD patients, but their role in the disease is unclear.

Aim: To establish whether the –10 C›T GLA gene polymorphism plays a role in clinical variability of AFD females.

Methods: Seven different AFD families were assessed to determine global clinical severity scores in females with –10 C›T GLA gene polymorphism. Clinical data was analysed in relation to biochemical, molecular and an expression assay analysis in human embryonic kidney epithelial cells (HEK 293T) to determine the effect of –10T GLA allele on expression of wild-type and mutant (c.644 A›G, p.N215S) enzyme.

Results: Seven families analysed contained various pathogenic mutations all of which affected the enzyme activities with severe to moderate reduction in plasma and leukocyte alpha-galactosidase A (α-Gal A). Eight females were identified with pathogenic GLA mutant allele and a separate GLA –10T allele.

Two groups of AFD females with –10 T allele were identified: group one females (n = 4) were severely affected by disease whilst group two females (n = 4) were affected relatively mildly by the disease. Analysis of clinical data in group one females showed there were severe presentations of cardiovascular and neurological symptoms at an early age in three out of four females in the group. Analysis by mutation type confirmed that individuals with the same mutation and a –10T allele displayed considerable clinical variability. We hypothesized that the –10T allele may act as a potential modifier of the disease in some of AFD females.

Our hypothesis was examined by analysing effect of –10T allele in an in vitro enzyme over-expression assay in HEK 293T cells using a newly developed plasmid vector. We established the –10T allele caused a strong reduction in α-Gal A activity compared to the wild-type enzyme. The variant N215S enzyme expression indicated this was associated with high residual enzyme activity. The function of a mutant enzyme was severely reduced when –10T allele was associated with a pathogenic c.644 A›G allele.

Conclusions: Our family studies indicate clinical variability in females is partly related to the GLA promoter –10T allele. Evidence derived from in vitro expression confirmed the –10T allele modified α-Gal A activity in vitro and suggests that the extent of attenuated cellular enzyme expression in individuals may relate to severe disease.
Fabry Disease: Early and Severe Presentation in Women Carriers


Division of Nephrology, Department of Internal Medicine, Hospital das Clínicas, Universidade Federal de Goiás, Goiânia, Brazil

Introduction: Fabry Disease (FD) is a rare lysosomal X-linked disease that leads to alphagalactosidase A (alpha-Gal A) deficiency and accumulation of globotriaosylsphingosine (Gb-3). It causes cellular dysfunction and several clinical abnormalities.

Retrospective Case Series: from a male index patient with FD in hemodialysis was possible to diagnose, after genotyping, 8 female carriers. The genetic investigation shows the mutation p. G35V in homozygosis in exon 1. The oldest one, with 71 y, died of mesenteric ischemia two months after diagnosis. The average age at diagnosis was 31 y. Only one of the patients, the daughter of the index case is oligosymptomatic. The other 6 patients had characteristic symptoms such as headache, hypoacusia and acroparesthesia. They were screened for kidney dysfunction such as proteinuria and serum creatinine. They all had normal serum creatinine, but 4 of them already have proteinuria >150 mg/24 hours. They underwent percutaneous kidney biopsy with optic, electronic microscopic and immunofluorescence evaluation. They identified deposits of Gb-3 in the podocytes in all samples and signs of tubular atrophy and interstitial fibrosis in one patient with gross proteinuria. They were all screened for cardiac and neurologic involvement. Three sisters of the index case have cardiologic involvement. It was also noted that this new mutation has a more aggressive neurologic involvement, reaching central and peripheral nervous system. Five of these patients already started enzymatic replacement therapy.

Discussion: Recent evidences points that women in heterozygosis are potential patients and not only carrier of the new mutation. These patients can present the disease as severe as men, although the progression is slower. In this case series, the average age of diagnosis was 31 y; about one decade earlier literature reports though the progression is slower. In this case series, the average age at diagnosis was 31 y. Only one of the patients, the daughter of the index case is oligosymptomatic. The other 6 patients had characteristic symptoms such as headache, hypoacusia and acroparesthesia. They were screened for kidney dysfunction such as proteinuria and serum creatinine. They all had normal serum creatinine, but 4 of them already have proteinuria >150 mg/24 hours. They underwent percutaneous kidney biopsy with optic, electronic microscopic and immunofluorescence evaluation. They identified deposits of Gb-3 in the podocytes in all samples and signs of tubular atrophy and interstitial fibrosis in one patient with gross proteinuria. They were all screened for cardiac and neurologic involvement. Three sisters of the index case have cardiologic involvement. It was also noted that this new mutation has a more aggressive neurologic involvement, reaching central and peripheral nervous system. Five of these patients already started enzymatic replacement therapy.

Conclusion: As FD presents an important decrease in quality of life and important organs dysfunctions, the early detection is crucial. It is a big challenge making early detection of FD and simple exams such as ophthalmologic exam and ECG are large diagnostic weapons in this case. The main point is to find out the correct time to start treatment, before irreparable complications.

Enzyme Replacement Therapy Dose Adjustments and Biomarker Trends in a Male with Classic Fabry Disease

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1Duke University Hospital Biochemical Genetics Lab and 2Division of Medical Genetics, Dept of Pediatrics, Duke School of Medicine, Durham, NC, USA

Overview: Urinary and plasma biomarkers of disease burden were monitored in patients with Fabry disease on enzyme replacement therapy (ERT) with agalsidase beta (Fabrazyme®). We report disease burden biomarker trends before and after a dose adjustment in a male patient with progression of renal disease.

Methods: Patients with a confirmed diagnosis of Fabry disease were consented to the study. Patients were enrolled onto the study while on a reduced dose, or prior to the start of ERT, and were monitored for up to 4 years. Urinary and plasma biomarkers of disease burden including globotriaosylceramide (Gb3) and deacylated-Gb3 (lyso-Gb3) were quantified using UPLC-MS/MS. Plasma Gb3, antibody titers and clinical information were obtained from review of medical records.

Results: A male subject with classic Fabry disease (c.1018_1021delGTGGins24) reportedly had an onset of symptoms at approximately 7 years of age and was treated with agalsidase beta from the age of 31 years. At the time of enrollment, the patient had received ERT at the standard dose of 1 mg/kg/biweekly for seven years, but had a reduction of the dose to 0.5 mg/kg/biweekly because of a drug shortage. While on the reduced dose over approximately two years, an increase in urinary microalbumin was observed from 91 to 177 mg/g creatinine. Despite a return to the standard dose of 1 mg/kg/biweekly, microalbumin continued to increase over the next twelve months. The dosing frequency was increased to 1 mg/kg/weekly; microalbumin peaked at 916 mg/g creatinine shortly after this dose adjustment, and subsequently decreased after one year on the increased dosing frequency. Urinary Gb3 and lyso-Gb3, and plasma lyso-Gb3 were markedly elevated, and plasma Gb3 was mildly elevated at enrollment. Urinary Gb3 (687±246 μg/mmol creatinine) and plasma lyso-Gb3 (66±8 nmol/L) remained relatively unchanged over four years despite the alterations in dose, whereas urinary lyso-Gb3 showed an upward trend over the first three years after enrollment. Plasma Gb3 trends were also stable over the first three years (8.1±0.7 μg/ml), but then showed a rapid increase to 14 μg/ml coinciding with the peak in microalbuminuria. Subsequently, plasma Gb3 normalized after 1 year of increased dosing frequency. During the study period the patient had chronic pain, and frequent and severe gastrointestinal symptoms, in addition to the microalbuminuria. Antibody titers against the infused enzyme were chronically mildly elevated (6400 to 12800).

Conclusions: Persistent marked elevations of biomarkers of disease burden in a patient on ERT for more than seven years was associated with an increase in microalbuminuria. After one year of an increased frequency of dose, the microalbuminuria was reduced and plasma Gb3 normalized. No change was observed in plasma lyso-Gb3 or urinary Gb3.
Three Significant Milestones and a Review of the A143T Mutation Within One Family with Anderson–Fabry Disease

P. Rohman, Uma Ramaswami, A. Mehta, D.A. Hughes
Royal Free Hospital, Lysosomal Storage Disorders Unit, Pond St, London, NW3 2QG

Introduction: The X-linked nature of Anderson-Fabry disease (AFD) has enabled the pedigree of the original patient described by Dr William Anderson in 1898 to be traced well over a century after the condition was first published. Described almost simultaneously by Anderson and Fabry it is now known that mutations in the GLA gene result in decreased production of the lysosomal enzyme α-galactosidase A. One centre has identified 3 significant milestones in the timeline of AFD within one family who are known to have the A143T mutation. These milestones form the basis of discussion as to the clinical significance of this gene mutation.

Aim: To highlight the clinical significance of the A143T mutation within one family and compare these findings to current available information on the A143T mutation.

Methods: A literature review was done all information around the A143T gene in patients with AFD. The pedigree of the first described case by Anderson was reviewed and patients’ notes under the care of the Royal Free Lysosomal Storage Disorders Unit were retrospectively reviewed. 3 affected males and 2 obligate carrier females made up the patient cohort under this centres care.

Case Discussion: The A143T mutation is a previously reported missense mutation first described in 1997 after being incidentally found in a 1 month old infant screened for α-Gal A deficiency with no known family history of AFD. Since then the A143T mutation has been described multiple times – with extremely varied clinical expression resulting in differing views of the significance of this mutation. The pedigree of the patient described by Anderson in 1898 have subsequently shown to have the A143T mutation. In this family three significant milestones within the history of this disease have occurred. The first of these milestones being the original case described by Anderson in 1898. The second milestone of this report occurred four generations on, with a descendant of the original patient being one of the first men started Enzyme Replacement Therapy in 1999. Diagnosed at birth in 1973 on skin biopsy, enzyme results and confirmed to have the A143T mutation, this patient displayed a number of symptoms from an early age. He was randomised to active drug and remained on treatment for 15 years until 2014 when he died expectantly of cardiac complications. Despite significant cardiac and renal involvement these both remained stable but progressive for many years on treatment. The third milestone of this report occurred 5 generations after the first description of the disease. A descendant of the original patient became the youngest child in the United Kingdom to start ERT at the age of three. He was diagnosed soon after birth on routine testing and carries the A143T mutation. This child presented with abdominal symptoms in infancy and multiple pain crises-usually associated with infection. Before three years of age he had clear evidence of acroparasthesia. At this stage he also had typical facial features and started on ERT in 2003 due to his severe phenotype. He remains on treatment 12 years later.

Many different authors have described this mutation and its clinical significance. When first described in 1997 the gene was recognised to be pathological but the clinical significance remains unknown. Reports on the significance of the mutation vary; with suggestions including the mutation expressivity is extremely variable and no data suggests the mutation is pathogenic. Other suggestions link specific organ involvement to this mutation suggesting it predisposes to cardiac, renal, neurological involvement.

Conclusion: Within this one family huge variation exists in the phenotypic expression of this mutation, both in homozygous and heterozygous affected members. Observations based on this family demonstrate consistently through multiple generations this mutation is linked to significant skin, neurological, cardiac and abdominal involvement in both homozygous and heterozygous individuals.