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Abstracts
5th Update on Fabry Nephropathy:

Biomarkers, Progression and Treatment Opportunities

April 25–27, 2017, Mexico City, Mexico

Editor
David G. Warnock, Birmingham, AL
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### Tuesday April 25, 2017: Mexico City, Mexico
Hotel NH Collection, Mexico City Airport

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:00</td>
<td>Onsite Registration</td>
</tr>
<tr>
<td>12:30</td>
<td>Opening Session</td>
</tr>
<tr>
<td>3:00</td>
<td><strong>Ricardo Correa-Rotter</strong> and <strong>Gregorio Obrador:</strong> Welcome</td>
</tr>
<tr>
<td>3:15</td>
<td>Introduction of Keynote Speakers and Stage of the Art Lectures – <strong>Rafael Schiffmann</strong> (USA) and <strong>David Warnock</strong> (USA)</td>
</tr>
<tr>
<td>3:35–4:30</td>
<td><strong>Tobias Huber</strong> (Germany): Fabry Disease, from Drosophila to Rats to Humans</td>
</tr>
<tr>
<td>4:30–5:15</td>
<td><strong>Christoph Wanner</strong> (Germany): Classic and Non-Classic Fabry Disease</td>
</tr>
<tr>
<td>5:15–6:00</td>
<td><strong>Giuseppe Remuzzi</strong> (Italy): Progression of Proteinuric CKD: Lessons for Fabry Nephropathy</td>
</tr>
<tr>
<td>6:00–8:30</td>
<td>Poster Sessions and Opening Reception</td>
</tr>
</tbody>
</table>

### Wednesday April 26, 2017: Mexico City, Mexico
Hotel NH Collection, Mexico City Airport

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:30</td>
<td>Phenotypic Variation in Fabry Disease; <strong>Chairs:</strong> <strong>Stephen Waldek</strong> (UK) and <strong>David Warnock</strong> (USA)</td>
</tr>
<tr>
<td>8:30–9:05</td>
<td>Phenotypic Variation and Mutation Analysis: <strong>João Paulo Oliveira</strong> (Portugal)</td>
</tr>
<tr>
<td>9:05–9:40</td>
<td>Phenotypic Variation in Alport’s Syndrome: <strong>Roser Torra-Balcells</strong> (Spain)</td>
</tr>
<tr>
<td>9:40–10:15</td>
<td>Phenotypic Variation with the IVS4+919G Variant: <strong>Dau-Ming Niu</strong> (Taiwan)</td>
</tr>
<tr>
<td>10:15–10:45</td>
<td>Group Discussion</td>
</tr>
<tr>
<td>10:45–11:15</td>
<td>Coffee Break</td>
</tr>
<tr>
<td>11:15–12:30</td>
<td>Moderated Discussion on Histologic Assessment of Fabry Nephropathy; <strong>Chairs:</strong> <strong>Luiz A. Moura</strong> (Brazil) and <strong>Agnes Fogo</strong> (USA)</td>
</tr>
<tr>
<td>11:15–11:30</td>
<td>Biopsies from Children with Fabry Disease; Lessons Learned and Unanswered Questions: <strong>Camilla Tøndel</strong> (Norway)</td>
</tr>
<tr>
<td>11:30–12:15</td>
<td>Discussion Participants: <strong>Laura Barisoni</strong> (USA), <strong>Randy Hennegar</strong> (USA), <strong>Robert Hopkin</strong> (USA)</td>
</tr>
<tr>
<td>12:15–12:30</td>
<td>Summary and Conclusions; <strong>Agnes Fogo</strong> (USA) and <strong>Luiz A. Moura</strong> (Brazil)</td>
</tr>
<tr>
<td>12:30–14:00</td>
<td>Working Lunch and Poster Viewing</td>
</tr>
<tr>
<td>14:00</td>
<td>Molecular Mechanisms in Fabry Disease; <strong>Chairs:</strong> <strong>Alberto Ortiz</strong> (Spain) and <strong>James Shayman</strong> (USA)</td>
</tr>
<tr>
<td>14:00–14:45</td>
<td>Structural Basis for Inhibition of Alpha-Galactosidase A: <strong>Scott Garman</strong></td>
</tr>
<tr>
<td>14:45–15:15</td>
<td>Lyso-Gb3 and Analogues in Cardiac Variant Mutations: <strong>Christiane Auray-Blais</strong></td>
</tr>
<tr>
<td>15:15–15:45</td>
<td>Vascular and Cardiac Hypertrophy in Fabry Disease: <strong>Peter McCullough</strong></td>
</tr>
<tr>
<td>15:45–16:15</td>
<td>Coffee Break</td>
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<td>Event</td>
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<tr>
<td>16:15–16:45</td>
<td>Cellular Injury and Responses; Chairs: <strong>Camilla Tøndel</strong> (Norway) and <strong>Suichi Ito</strong> (Japan)</td>
</tr>
<tr>
<td>16:45–17:15</td>
<td>Molecular Mechanisms of Podocyte Injury: <strong>M.D. Sanchez Niño</strong> (Spain)</td>
</tr>
<tr>
<td>17:15–17:45</td>
<td>Podocyte Biomarkers and Response to ERT: <strong>Kevin Mills</strong> (UK)</td>
</tr>
<tr>
<td>17:45–18:45</td>
<td>Oral Presentations from Poster Sessions; Chairs: <strong>Hernán Trimarchi</strong> (Argentina) and <strong>Kathy Nicholls</strong> (Australia). The top 5 posters will be presented as 8 min presentations with 3 minutes for questions. Poster Selection Committee: <strong>Ricardo Correa-Rotter</strong> (Mexico); <strong>Giovanni Duro</strong> (Italy); <strong>Luis E. Figuera</strong> (Mexico); <strong>Richard J. Glassock</strong> (US); <strong>Ana Maria Martins</strong> (Brazil); <strong>Gregorio Obrador</strong> (Mexico); <strong>Juan Politei</strong> (Argentina); <strong>Andrew Talbot</strong> (Australia); <strong>Bojan Vujkovac</strong> (Slovenia)</td>
</tr>
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<td><strong>Wednesday Evening:</strong> Fiesta (Onsite)**</td>
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<tr>
<td><strong>Thursday April 27, 2017: Mexico City, Mexico</strong></td>
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<td></td>
<td><strong>Hotel NH Collection, Mexico City Airport</strong></td>
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<tr>
<td>8:00</td>
<td>Immune Responses to ERT; Chairs: <strong>Robert Hopkin</strong> (USA) and <strong>Christoph Wanner</strong> (Germany)</td>
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<tr>
<td>8:00–8:25</td>
<td>Clinical Significance of Antibodies: <strong>Malte Lenders</strong> (Germany)</td>
</tr>
<tr>
<td>8:25–8:50</td>
<td>Secondary Membranous Nephropathy and ERT: <strong>Pierre Ronco</strong> (France)</td>
</tr>
<tr>
<td>8:50–9:15</td>
<td>Predicting Immunogenicity from Genotype, Lessons from Pompe Disease: <strong>Deeksha Bali</strong> (USA)</td>
</tr>
<tr>
<td>9:15–9:40</td>
<td>Immune Modulation with Bortezomib: <strong>Derralyn Hughes</strong> (UK)</td>
</tr>
<tr>
<td>9:40–10:00</td>
<td>Discussion</td>
</tr>
<tr>
<td>10:00–10:20</td>
<td>Coffee Break</td>
</tr>
<tr>
<td>10:20–10:45</td>
<td>New Therapeutic Approaches; Chairs: <strong>Yoshi Eto</strong> (Japan) and <strong>Michael West</strong> (Canada)</td>
</tr>
<tr>
<td>10:45–11:10</td>
<td>Extending the Range of &quot;Amenable Mutations&quot;: <strong>Elfrida Benjamin</strong> (Amicus)</td>
</tr>
<tr>
<td>11:10–11:35</td>
<td>Immunogenicity and Pharmacokinetics of PegUNIGALsidas: <strong>Einat Almon</strong> (Protalix)</td>
</tr>
<tr>
<td>11:35–12:00</td>
<td>Gene Therapy Approaches: <strong>Jeffery Medin</strong> (USA)</td>
</tr>
</tbody>
</table>
| 12:00–12:45  | Debate: Lyso-GB3(s) – Fit for What Purpose(s); Moderators: **Christiana Auray-Blais** (Canada) and **Christoph Wanner** (Germany)  
Con: **Raphael Schiffmann** (USA)  
Pro: **Derralyn Hughes** (UK) |
| 12:45–13:00  | Announcements: Poster Prize Winners; **Hernán Trimarchi** (Argentina) and **Kathy Nicholls** (Australia) |
| 13:00        | Departures                                                                                           |
MicroRNAs as Biomarkers in Fabry Disease: Nephropathy: The Missing Pathophysiological Link?

Patricio Aguiar, Marina C. Costa, Olga Azevedo, Manuela Fiuza, Jacira Marino, José Luis Ducla Soares, Derralynn Hughes, Francisco J. Enguita

1Centro Hospitalar Lisboa Norte, Lisbon, Portugal; 2Instituto de Medicina Molecular – Lisbon Medicine Faculty, Lisbon, Portugal; 3Hospital Senhora da Oliveira, Guimarães, Portugal; 4Royal Free London NHS Foundation Trust and University College London, London, United Kingdom

Background: Renal involvement in Fabry disease (FD) is a major determinant of overall disease prognosis and tubule-interstitial fibrosis, glomerulosclerosis are major determinants of enzyme replacement therapy response. Thus, accurate biomarkers for early prediction of activation of fibrotic and other pathophysiological pathways are crucial. MicroRNAs (miRNAs) are short noncoding RNAs and key players in the regulation of gene expression at the post-transcriptional level and may regulate several pathways involved in FD nephropathy. In a previous study evaluating the entire miRNAnome in FD we found that 31 miRNAs were significantly differentially expressed between FD patients and controls, remarkably all down-regulated. Here, we present the preliminary results of these miRNAs evaluation in a larger cohort of FD patients.

Methods: In this multicentre, prospective, cross-sectional and diagnostic test study, we determined the 31 differentially expressed miRNAs in a cohort of 50 FD patients and 19 healthy controls. MiRNAs were quantified by reverse quantitative PCR using specific LNA primers (Exiqon). Expression levels were calculated by normalization against an internal spike-in synthetic control. MiRNAs were compared with the reference standard (albuminuria) as predictors of estimated glomerular filtration rate (eGFR). In a cohort of 50 FD patients (42% males, mean age 52.3±14.6 years, 80% in ERT), 14 out of the 31 miRNAs showed a significant direct correlation with eGFR, which was stronger than the inverse correlation between albuminuria and eGFR (rho = −0.392, p = 0.007) for 5 of them (hsa-let-7b-5p; hsa-miR-126-3p; hsa-miR-126-5p; hsa-miR-150-5p; hsa-miR-30c-5p). Moreover, comparing the results across chronic kidney disease (CKD) stages, all these 14 miRNAs were significantly different comparing stage 1, 2 and ≥3, which was not true for albuminuria (p = 0.091). We also found a significant decrease in all 14 miRNAs in patients with FD and CKD stage 1, compared with the controls. In a multivariate regression model (including albuminuria and the 14 miRNAs, weighted for age) to predict eGFR, only the hsa-miR-23a-3p retained statistical significance (p = 0.005).

Conclusion: MiRNAs may play a key role in the regulation of several pathophysiological pathways in FD nephropathy and may be attractive candidates as biomarkers for the identification of patients at increased risk of a progressive nephropathy (probably outperforming albuminuria). Moreover, confirming that miRNAs down-regulation is associated with more advanced CKD stages further supports that impaired exocytosis of the miRNAs (due to disruption of the Golgi/endosomal, lysosomal trafficking in an environment of lysosomal dysfunction) may be an alternative pathophysiological pathway in FD.

Support: an investigator-initiated research grant from Shire Pharmaceuticals.

References


Extending the Range of Amenable Mutations


1Amicus Therapeutics, Cranbury, NJ, USA; 2Hôpital du Sacré-Coeur, Montréal, Québec, Canada; 3University of Versailles, Univ. Paris-Saclay, Montigny, France; 4Medical Genetics Service, HCPA/UFRGS Porto Allegre, Brazil; 5Royal Free Campus, Univ College London, London, London, UK; 6Baylor Research Institute, Dallas, TX, USA; 7Emory Univ, Atlanta, GA, USA

Background: Fabry disease is an X-linked lysosomal storage disorder caused by GLA mutations, resulting in deficient lysosomal α-galactosidase A (α-Gal A) activity and accumulation of globotriaosylceramide (GL-3) and plasma globotriaosylsphingosine (lyso-Gb3). Migalastat, a pharmacological chaperone, can reversibly bind to the active site of specific mutant forms of α-Gal A that are physically unstable, prone to aberrant folding, and/or have deficient lysosomal trafficking. Binding of migalastat stabilizes such mutant forms, thereby increasing the ability of newly synthesized enzyme to pass the endoplasmic reticulum quality control system, traffic to lysosomes, and reduce substrate. Missense, small in-
frame insertions/deletions, and multiple-site missense mutations may result in mutant forms amenable to the mechanism of action of migalastat. However, mutations that impair the synthesis of α-Gal A, severely affect protein structure (e.g., frameshift, truncation, large insertion/deletion), and/or catalytic activity are not expected to lead to amenable mutant forms. Splice site mutations have complex molecular consequences (e.g., cryptic splice-site usage, intron retention, exon skipping, and/or leaky wild-type splicing) resulting in severe effects on protein structure and/or catalytic activity that may or may not include some residual wild-type protein expression.

Methods: The Good Laboratory Practice (GLP)-validated in vitro assay (GLP HEK/Migalastat Amenability Assay), is used to identify α-Gal A mutant forms amenable to migalastat. Fabry disease-associated missense, small in-frame insertion/deletion, and multiple-site missense mutations are each expressed in HEK-293 cells, and increases in α-Gal A activity in response to migalastat are measured. Mutants that do not qualify for testing in the GLP HEK assay, including large deletions, insertions, truncations, frameshift, and splice site mutations, are categorized as non-amenable.

Results: The predictive value of the assay was assessed based on pharmacodynamic responses to migalastat in phase 2 and 3 clinical studies. Comparison of mutant α-Gal A activity fold over baseline with an absolute increase of ≥3.0% wild-type α-Gal A activity in the presence of 10 μmol/l migalastat are categorized as amenable. Mutations that do not qualify for testing in the GLP HEK assay, including large deletions, insertions, truncations, frameshift, and splice site mutations, are categorized as non-amenable.

Conclusion: The GLP HEK assay is used to identify migalastat-amenable mutations. Male and female Fabry disease patients with amenable mutations are candidates for treatment with migalastat.

References


3 Evaluation of the Distribution of Gb3 Isoforms and Analogues in Fabry Mouse Tissues Using a Tandem Mass Spectrometry Approach

Michel Boutin1, Philippe Provencal2, Shaalee Dworski1, Bryan Au3, Jeffrey A. Medin2,3,4, Christiane Auray-Blais1

1Department of Pediatrics, Division of Medical Genetics, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, Québec, Canada; 2Institute of Medical Science, University of Toronto and 3University Health Network, Toronto, Ontario Canada; 4Medical College of Wisconsin, Milwaukie, Wisconsin, USA

Background: Fabry disease leads to the storage of globotriaosylceramide (Gb3) isoforms and analogues, and lyso-Gb3 and analogues in different tissues and biological fluids. Recent urine [1] and plasma [2] metabolomic studies performed in our laboratory from untreated Fabry males revealed a panel of 22 different Gb3 isoforms/analogues as Fabry disease biomarkers. Gb3 isoforms showed different fatty acid chains, whereas Gb3 analogues were characterized by chemical modifications on the sphingosine chain, including the methylation of the amide linkage. The purpose of this study was to evaluate the relative abundance of Gb3 isoforms/analogues in plasma and various organs, such as in the kidney, brain, liver, heart, lung, small intestine and spleen of a Fabry mouse model [3].

![Fig. 1. Relative abundance of total Gb3 (22 isoforms/analogues) in organs from NOD/SCID Fabry mice (n = 18) (for Abstract no 3).](http://galafoldamenabilitytable.com)
Methods: Tissue and plasma specimens were analyzed from non-obese diabetic (NOD) with severe combined immune deficiency (SCID) Fabry mice (9 males, 9 females) and control mice (3 males, 2 females) (3). Tissue samples were homogenized using a bead mill (Omni Bead Ruptor). Tissue homogenates and plasma samples were extracted with tert-butyl methyl ether (MTBE). Gb3 isoforms/analogues were separated by ultra-performance liquid chromatography (Acquity I-Class, Waters Corp.) and analyzed by tandem mass spectrometry using the multiple reaction monitoring mode (Xevo TQ-S, Waters) in positive electrospray [4].

Results: A total of 22 Gb3 isoforms/analogues were analyzed from the organs of NOD/SCID Fabry (n = 18) and NOD/SCID control (n = 5) mice. As expected, Gb3 levels were significantly higher in Fabry mouse compared to controls. Surprisingly, the total Gb3 levels measured were significantly higher in the spleen and small intestine tissues compared to kidney and heart tissues, the latter organs being involved in premature death or morbidity for Fabry patients (Figure 1).

Conclusion: A mass spectrometry methodology was developed and validated for the analysis of 22 Gb3 isoforms/analogues in Fabry and control mouse tissues. A marked variation in the relative abundance of Gb3 isoforms/analogues was detected in some mouse organs. If the same applies to humans, the high levels of Gb3 stored in some organs, such as the spleen, might explain the persistence of clinical manifestations for Fabry patients, even following ERT treatment.

Financial Support: Canadian Institutes of Health Research (CIHR).

References
reversible. The ADA+ patient in Cohort 2 had a low ADA titer and had stable PK parameters throughout the study.

In summary, pegunigalsidase alfa has an extended circulatory half-life and higher AUC, plus there is a low immune response toward pegunigalsidase alfa. The reduced immunogenicity of pegunigalsidase alfa is associated with improved PK profiles that may reflect long-term induction of tolerance in previously seroconverted patients and has the potential for clinical benefit in treating FD patients.

The study was supported by Protalix Ltd.

5 A Pilot Study of Circulating miRNAs as Potential Biomarkers of Fabry Disease

Giuseppe Cammarata1, Simone Scalia1, Paolo Colomba1, Carmela Zizzo1, Antonio Pisani1, Eleonora Riccio1, Simona Taverna1, Riccardo Alessandro1, Antonello Giordano1, Giovanni Duro1

1Institute of Biomedicine and Molecular Immunology (IBIM), National Research Council, Palermo, Italy; 2Department of Public Health, Section of Nephrology, Federico II University of Naples, Naples, Italy; 3Department of Biopathology and Medical Biotechnology, University of Palermo, Palermo, Italy; 4Department of Neurology, Guzzardi Hospital, Vittoria (RG), Italy

Background: Fabry disease (FD) is an X-linked lysosomal storage disease caused by a deficiency of the lysosomal hydrolase α-galactosidase A (α-GalA). In patients with Fabry disease, the progressive intra-lysosomal accumulation of the enzyme substrate globotriaosylceramide (Gb3) in multiple cell types leads to a multisystem disorder, culminating in stroke, progressive renal and cardiac dysfunction and premature death. Clinical manifestations of FD are heterogeneous, and could be misdiagnosed with other pathologies. Although the enzyme replacement therapy (ERT) offers a specific treatment for patients affected by FD, the monitoring of treatment is hindered by a lack of surrogate markers of response. The occurrence of circulating microRNAs (miRNAs) in blood components (including serum and plasma) was observed in several diseases. Considering the significance of miRNA in some pathological process involved in lysosomal storage disease, circulating miRNAs in blood may be unique biomarkers for early and minimally invasive diagnosis of FD. The objective of this pilot study was to discover a panel of circulating miRNAs as potential novel FD biomarkers.

Methods: using high-throughput nCounter based miRNAs expression profiling (NanoString Technologies) followed by Real-Time quantitative Polymerase Cycle Reaction (RT-qPCR) validation, we compared the levels of circulating miRNAs in plasma samples from patients with classical form of FD and controls.

Results: Using the significance level of p = 0.05 and at least 1.5-fold expression change as selection criteria, we found that 15 miRNAs were differentially expressed between 10 FD subjects and 10 normal controls (NC). This data were confirmed in a new cohort of 10 Fd and 10 NC. Using receiver operational curve (ROC) analysis, we showed that these up-regulated miRNAs can discriminate patients with FD from healthy controls with reasonable sensitivity and specificity. To further test the sensitivity and specificity of these circulating miRNAs in FD diagnosis we compared the levels of these 15 miRNAs in plasma sample of FD, with 20 subject with left ventricular hypertrophy (LVH), and/or with chronic kidney disease (CKD). We noticed that most of miRNAs overlap with those of symptomatic controls but 2 microRNAs mir-199 a-5p and mir126 a-3p are specific for FD. Analyzing the 15 miRNAs in 10 subject constantly under therapy we found that the levels of 15 miRNAs decrease as consequence of therapy. A systematic literature and database search revealed that these microRNAs are involved in processes affecting macrovascular and microvascular endothelium.

Conclusion: In this study, we identified a common microRNA signature in FD patients regardless of gender and age. The aberrantly expressed plasmatic miRNAs are probably linked to microvascular or macrovascular damage involved in the typical FD vasculopathy and could be attractive as diagnostic markers as well as candidates for the monitoring of the pharmacological treatment.

Acknowledgements: this study was funded by Genzyme, a Sanofi company.

6 A Multiplex Targeted Proteomic Assay Identifies Altered Sortilin and GM2 Activator Prior to Kidney Damage in Females With Fabry Disease

Ivan Doykov1, Justyna Spiewak1, Jenny Hallqvist1, Valeria Nikolaenko1, Albina Nowak2, Kevin Mills1, Wendy Heywood4

1Centre for Translational Omics, UCL Great Ormond Street Institute of Child Health, 2Internal Medicine Unit, University of Zurich, Switzerland

Background: The timing of placing Fabry patients on ERT can be a difficult choice. Patients who appear asymptomatic for renal or cardiac disease may not necessarily need ERT early in life. However, patients require frequent monitoring to detect the first signs of disease before being placed on ERT. Female Fabry patients are typically more difficult to diagnose and identify when treatment is needed. In this work we describe the identification of a potentially more sensitive marker of pre-symptomatic renal damage. We have developed a targeted multiplex assay to monitor a panel of urine proteins and have applied this to Fabry patients divided into normal (stage 1) to stage 5 eGFR, in an attempt to identify proteins that may change prior to reduced eGFR that could be used as an early indicator of kidney damage.

Methods: Heavy labelled peptide standards were added to 1 ml of urine which was filtered, trypsin digested and C18 cleaned. Peptides were injected onto a Xevo TQ-S mass spectrometer and analysed using dynamic multiple reaction monitoring over a 10 min gradient. Peptide levels were standardized to creatinine.

Results: Healthy Controls with an age range of 22–61 yrs were included. Patients were grouped according to eGFR stages 1–5. Patients not on ERT who were group 1 were sub grouped into pa-
Biomarkers, Progression and Treatment Opportunities

Nephron 2017;136:163–182
DOI: 10.1159/000475511

Methods: The study was carried out in a cohort of 192 women with clinical suspicion or positive familial history of FD. AGAL enzyme activity in DBS was measured by fluorometric method with deproteination. The routine test used in our lab as control enzyme to assess the quality of all the DBS samples, Beta-Galactosidase (BGAL) activity, was also measured. BGAL/AGAL ratio was calculated in all women. The study was completed with GLA genetic test (sequencing + MLPA) in all women.

Results: By genetic test, 61 of 192 women were detected as HF. Only 19 of these 61 HF showed low AGAL activity (31%), but 55 of 61 (90%) showed an increased BGAL/AGAL ratio. BGAL/AGAL ratio was normal in 127 of 192 women. (ROC curve: AUC = 0.959, BGAL/AGAL cutoff: 8.25, Positive Predictive Value: 85% and Negative Predictive Value: 95%).

Conclusion: BGAL/AGAL ratio in DBS reduced significantly false negatives, improving the detection of HF in 189%. This allowed detect 90% of HF for FD versus 31% detected by AGAL activity in DBS. This strategy does not mean any extra cost because of the use of BGAL as control enzyme.

Support: None.

References


7

A Simple Tool to Enhance the Detection of Heterozygous Females for Fabry Disease through Enzymatic Activities in Dried Blood Spots

Joaquin Frabasil, Consuelo Durand, Silvia Sokn, Daniela Gaggioli, Patricia Carozza, Andrea Beatriz Schenone
Laboratorio de Neuroquímica “Dr. N.A. Chamoles” – FESEN, Buenos Aires, Argentina

Background: Fabry Disease (FD) is an X-linked lysosomal disease caused by mutations in the GLA gene that encodes the Alpha-Galactosidase A enzyme (AGAL). In vitro assays, Heterozygous Females (HF) for FD show a spread range of AGAL activity from very low to normal values regardless of the type of sample analyzed as Dried Blood Spots (DBS), plasma, leukocytes or fibroblasts. As consequence, the Gold Standard method for diagnosis in women is GLA gene sequencing with deletion/duplication search, an expensive and time consuming method. HF may be asymptomatic or likely to suffer manifestations, even similar to men with FD. Detection is therefore essential for an early diagnosis and treatment, and for proper genetic counseling. The aim of this study was to improve the detection of HF for FD by measuring enzymatic activities in DBS.

Methods: The study was carried out in a cohort of 192 women with clinical suspicion or positive familial history of FD. AGAL enzyme activity in DBS was measured by fluorometric method with deproteination. The routine test used in our lab as control enzyme to assess the quality of all the DBS samples, Beta-Galactosidase (BGAL) activity, was also measured. BGAL/AGAL ratio was calculated in all women. The study was completed with GLA genetic test (sequencing + MLPA) in all women.

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Support: None.

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7

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Laboratorio de Neuroquímica “Dr. N.A. Chamoles” – FESEN, Buenos Aires, Argentina

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Support: None.

References

Lucerastat, an Inosimog for Substrate Reduction Therapy for Glycolipid Storage Disorders: Safety, Tolerability, and Pharmacokinetics in Healthy Subjects

N. Güérard¹, O. Morand², J. Dingemanse¹

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Background: Lucerastat, an inhibitor of glucosylceramide synthase, has the potential to reduce the balance between synthesis and degradation of glycosphingolipids with glycolipid storage disorders such as Gaucher disease and Fabry disease. The safety, tolerability, and pharmacokinetics of oral lucerastat were evaluated in two separate randomized, double-blind, placebo-controlled, single- and multiple-ascending dose studies (SAD and MAD, respectively) in healthy male subjects.

Methods: In the SAD study, 31 subjects received placebo or a single oral dose of 100, 300, 500, or 1000 mg lucerastat. Eight additional subjects received two doses of 1000 mg lucerastat or placebo separated by 12 h. In the MAD study, 37 subjects received placebo or 200, 500, or 1000 mg b.i.d. lucerastat for 7 consecutive days. Six subjects in the 500 mg cohort received lucerastat in both absence and presence of food.

Results: In the SAD study, 15 adverse events (AEs) were reported in 10 subjects. Eighteen AEs were reported in 15 subjects in the MAD study, in which the 500 mg dose cohort was repeated because of elevated alanine aminotransferase (ALT) values in 4 subjects, not observed in other dose cohorts. No severe or serious AE was observed. No clinically relevant abnormalities regarding vital signs and 12-lead electrocardiograms were observed. Lucerastat Cmax values were comparable between studies, with geometric mean Cmax 10.5 (95% CI: 7.5, 14.7) and 11.1 (8.7, 14.2) µg/mL in the SAD and MAD study, respectively, after 1000 mg lucerastat b.i.d. tmax (0.5–4 h) and t1/2 (3.6–8.1 h) were also within the same range across dose groups in both studies. Using the Gough power model, dose proportionality was confirmed in the SAD study for Cmax and AUCCmax, and for AUCC0-12 in the MAD study. Fed-to-fasted geometric mean ratio for AUCC0-12 was 0.93 (90% CI: 0.80, 1.07) and tmax was the same with or without food, indicating no food effect.

Conclusions: Incidence of drug-related AEs did not increase with dose. No severe or serious AEs were reported for any subject. Overall, lucerastat was well tolerated. These results warrant further investigation of substrate reduction therapy with lucerastat in patients with glycolipid storage disorders.

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Lucerastat, an Inosimog for Substrate Reduction Therapy for Fabry Disease

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Background: Lucerastat, an inhibitor of glucosylceramide synthase (GCS), has the potential to provide substrate reduction therapy (SRT) in glycosphingolipid storage disorders such as Fabry disease (FD). The safety, tolerability, pharmacodynamics, and pharmacokinetics (PK) of oral lucerastat were evaluated in a Phase 1b study in adult FD subjects receiving enzyme replacement therapy (ERT).

Methods: In this single-center, open-label, randomized study, 10 subjects received 1000 mg lucerastat b.i.d. for 12 weeks on top of ERT (lucerastat group). Four subjects received ERT only (control group). The safety and tolerability of lucerastat, and effects on cardiac function, renal function, and biomarkers were evaluated every 4 weeks.

Results: Nine subjects reported 18 adverse events (AEs); 17 in the lucerastat group and 1 in the control group. A serious AE of atrial fibrillation was observed in the lucerastat group and part of medical history and, therefore, considered not related to lucerastat. No clinically relevant abnormalities in vital signs, safety laboratory, and 12-lead ECG were observed. Cardiac (left ventricular ejection fraction and myocardial mass index) and renal function (estimated glomerular filtration rate, urine albumin-to-creatinine ratio) remained stable. Plasma glucose, lactate, and globotriaosylceramide (Gb3) were significantly decreased in the lucerastat group. At end-of-study, the mean (SD) change from baseline was -49.0 (16.5)% for GlcCer, -32.7 (13.0)% for LacCer, and -55.0 (10.4)% for Gb3 (p < 0.0001 for all). Urinary Gb3 was reduced by 52.5 (21.2)%%. No statistically significant changes were observed for biomarkers in plasma and urine of the control group. PK parameters were comparable to those in healthy subjects.

Conclusions: 1000 mg b.i.d. lucerastat was well tolerated in FD subjects over 12 weeks. A marked decrease in plasma GlcCer, LacCer, Gb3, and urinary Gb3 was observed, indicating inhibition of GCS by lucerastat and suggesting clinical potential for SRT in FD.

Support: This study was funded by Actelion Pharmaceuticals Ltd, Allschwil, Switzerland. N. Guérard, O. Morand, R. Welford,
Lucerastat, an Iminosugar for Substrate Reduction Therapy: Pharmacokinetics, Tolerability, and Safety in Subjects with Mild, Moderate, and Severe Renal Function Impairment

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Background: Lucerastat, an inhibitor of glucosylceramide synthase, has the potential for substrate reduction therapy in glycosphingolipid storage disorders such as Fabry disease. In pharmacokinetic (PK) studies in rats, dogs, and healthy subjects the main route of elimination was renal.

Methods: The PK, tolerability, and safety of lucerastat were evaluated in subjects with mild (group A), moderate (group B), and severe (group C) renal impairment. Group D included healthy subjects. Thirty-two subjects (8 per group) were included in this single-center, open-label study. Subjects had an estimated glomerular filtration rate (eGFR), as per the Modification of Diet in Renal Disease formula, between 60 and 89 mL/min/1.73 m² in group A, 30 to 59 mL/min/1.73 m² in group B, and <30 mL/min/1.73 m² in group C. Group D subjects had a screening eGFR ≥90 mL/min/1.73 m², and were matched for age, body weight, and sex to group C subjects.

Results: Subjects received a single oral dose of 1000 mg lucerastat in groups A and B and 500 mg in groups C and D. Plasma lucerastat concentrations were higher in group B and C compared to group D. The elimination phase (t1/2) was slower in group B (9.6 h) and C (16.1 h) compared to group D (7.0 h). Increased exposure (AUC0–∞) to lucerastat was observed in subjects from groups B and C with ratio of geometric means (90% CI) of 1.60 (1.29, 1.98) for group B vs D and 3.17 (2.76, 3.65) for group C vs D. There were no clinically relevant abnormalities in vital signs, 12-lead ECGs, and clinical laboratory values. Four non-serious adverse events were reported by 4 subjects (1 in group A, 3 in group D).

Conclusion: Lucerastat was well tolerated in all dose groups. Dose adjustment is warranted in subjects with moderate and severe renal impairment.

Support: This study was funded by Actelion Pharmaceuticals Ltd, Allschwil, Switzerland. N. Guérard and J. Dingemanse are employees of Actelion Pharmaceuticals Ltd. C. Zwingelstein is a former employee of Actelion Pharmaceuticals Ltd.
Characterization of a Novel Transgenic Mouse that Over-Expresses Human Wild-Type Alpha-Galactosidase A

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Background: Alpha-Galactosidase A (α-Gal A) cleaves α-galactosyl moieties from glycosphingolipids and its many mutations cause deficient enzymatic activity in Fabry disease. While α-Gal A enzyme replacement therapy (ERT) is the current gold standard of treatment for Fabry disease there is an omnipresent need for novel therapeutic strategies, in particular those that are able to increase α-Gal A activity to organs/tissues (e.g. CNS) in which accessibility of ERT is precluded. A previous publication described generation of transgenic mice that over-expressed human wild-type α-Gal A but this line was no longer available. Thus the goals of this study were to generate a viable mouse line that over-expresses human wild-type α-Gal A and to confirm its relative increase in plasma, kidney and brain compared to wild-type littermates.

Methods: Transgenic mice were established with a ~13 kilobase human genomic clone that contained 246 bp of the 5’ untranslated region, the entire coding sequence, and approximately 3 kb of the 3’ untranslated region and which was inserted into pGEM-3Z vector. This DNA-containing vector was microinjected into FVB/NJ eggs for production of transgenic founder mice by the Mouse Genetics Shared Resources Facilities at Mount Sinai School of Medicine. Founder mice were screened for the correct genotype. Offspring from one of these founders (with a relative CNV of 10.6 and 1.6 fold increase in plasma α-Gal A enzyme activity) were used for subsequent breeding and experimental endpoints. Alpha-Gal A was assessed in 4-week and 8-week old weanling mice via western blot, activity assay and/or immunohistochemistry (IHC).

Results: Analysis of α-Gal A enzymatic activity (fluorescent 4MU cleavage product) in brain homogenates of 4-week-old transgenic mice indicated a 2-fold increase in ventral midbrain and a 1.8-fold increase in hippocampus compared to WT littermate controls (n = 3–7 mice/group). Western blot analysis revealed a 3-fold increase in α-Gal A 46 kDa “active” species (2) normalized to GAPDH loading control in hippocampal homogenates of transgenic vs. WT mice. IHC analysis of tissues from 8-week old transgenic male weanlings indicated a remarkable increase in α-Gal A immunoreactivity in their kidneys and throughout their brains compared to those of their wild-type littermates. We are currently investigating α-Gal A levels and activity in brain/kidney homogenates from these 8-week-old weanlings.

Conclusion: We have generated a viable line of mice that transgenically over-expresses human wild-type α-Gal A that can be used for subsequent pre-clinical investigations of Fabry disease and other diseases in which increasing α-Gal A activity could be a viable therapeutic strategy.

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sensation deficit in Fabry mice, which was assessed by hot-plate test at 55°C, was not corrected by either treatment in this experimental setting. We further tested the effect of ERT on preventing the peripheral neuropathy in earlier disease stage. Recombinant α-galactosidase A (agalactosidase-alfa, -beta and PRX-102) at doses of 0.2 or 1.0 mg/kg was administered to 2 months old Fabry mice every 2 weeks for a total 6 injections. Compared to untreated Fabry mouse controls, ERT led to slower progression of the thermosensation abnormality, and the effect was likely dose-dependent. In conclusion, this study suggested that 1) SRT may be more effective than ERT in correcting pre-existing cardiac and renal pathologies; 2) ERT may slow the onset and progression of small-fiber neuropathy in a dose-dependent manner; 3) Once the neurological deterioration occurs the damage may be irreversible and thus early treatment is probably critical for Fabry disease neuropathy.

Reference


14. Efficacy and Safety of Migalastat, an Oral Pharmacologic Chaperone for Fabry Disease: Results from Two Randomized Phase 3 Studies, FACETS and ATTRACT

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Background: Fabry disease is an X-linked disorder of lysosomal α-galactosidase A (α-Gal A) deficiency, leading to substrate accumulation and multi-organ disease. Migalastat, an oral pharmacological chaperone, stabilizes amenable forms of α-Gal A, increasing trafficking to lysosomes.

Methods: Two randomized phase 3 studies of migalastat HCl 150 mg every other day were conducted. FACETS (NCT00985301) is a randomized, placebo-controlled, 24-month study that included a 6-month double-blind placebo-controlled phase in 67 enzyme replacement therapy (ERT)–naive patients; 50 of these patients had amenable mutations. ATTRACT (NCT01218659) is an active-controlled, 18-month trial in 60 ERT–experienced patients with a 12-month open-label extension (OLE). In ATTRACT, 57/60 patients received treatment and 53/57 had amenable mutations. Efficacy analyses focused on patients with amenable mutations.

Results: In FACETS, the mean annualized rate of change over 24 months in eGFR (± standard error of the mean with migalastat was −0.3 ± 0.7 mL/min/1.73 m², mean change from baseline in left ventricular mass index (LVMi) was −7.7 g/m² (95% confidence interval [CI] −15.4, −0.009; P < 0.05), and improvements were observed in diarrhea and indigestion (P < 0.05). In ATTRACT, migalastat and ERT had comparable effects on renal function during the 18-month controlled period. Compared to natural history, the rate of GFR decline in FACETS was slower than that of untreated patients when matched for gender and baseline proteinuria. LVMi was significantly decreased with migalastat, with an average change from baseline over 18 months of −6.6 g/m² (95% CI −11.0, −2.2); there was no significant change with ERT. In FACETS, 6 months of treatment with migalastat resulted in significant reductions in substrate from baseline to month 6 vs placebo and from month 6 to month 12 in placebo-arm patients switching to migalastat. Over 18 months in ATTRACT, patients experienced renal (migalastat vs ERT, 24% vs 33%) or cardiac (6% vs 17%) events, while only one cerebrovascular event was reported in the ERT group.

Conclusion: During the OLE, eGFR remained stable and LVMi reductions were maintained. Migalastat was well tolerated and effective across patient subgroups in both studies.

Support: Support was provided by Amicus Therapeutics, Inc.

15. A Multiplex Glycosphingolipid Assay Identifies Changes in Hydroxylated Isoforms of Glycosphingolipids in Adult Fabry Patients in Comparison to Gender and Early Kidney Damage

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Background: The glycosphingolipid (GSL) Gb3 is elevated in Fabry disease due to defects in alpha galactosidase. The down and upstream effects of reduced alpha galactosidase activity on other glycosphingolipids (GB1, GB2 and GB4) in the GSL degradation pathway have not been investigated previously. We have developed a 10 minute multiplex LC-MS/MS GSL assay to quantitate all of these GSL’s and their isoforms. This test has the ability to not only diagnose Fabry disease but also Sandhoff and Gaucher disease. We have applied this assay to Fabry patient plasma and urine samples to correlate plasma Gb3 and lyso-Gb3 with urine Gb3 and

Biomarkers, Progression and Treatment Opportunities

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the other GSL’s. Our aim being to investigate any correlation of these 4 glycosphingolipids in Fabry disease and whether they can be informative for the detection of female Fabry patients or response to kidney damage as monitored by eGFR.

Methods: Plasma GSLs (Gb1–Gb4) were prepared from 50 μl of Fabry patients’ plasma. Extraction solution containing internal standards for Gb3 and Gb2 (lactosylceramide) was added, and then samples were shaken, sonicated to disintegrate the cells and then shaken again. After that PBS was added, samples were cortexed and centrifuged. Two phases appeared divided by an interphase. The bottom layer was collected, dried under N2 and reconstituted in chloroform. Extracted samples were then cleaned on C18 SPE cartridges. After elution sample were dried and reconstituted in methanol.

Plasma lyso-Gb3 was prepared from 100 μl of Fabry patients’ plasma. Extraction solution containing di-methyl psychosine internal standard was added, and then samples were shaken, sonicated, shaken again and centrifuged. Supernatant was taken out and dried in rotational evaporator. Prior to analyses samples were reconstituted in methanol.

Fig. 1. Heat map correlation matrix figure of all plasma Gb3 isoforms indicates poor correlation of C16:OH isoforms of Gb3 with unmodified Gb3 isoforms (for Abstract no 15).
Urinary GSLs (Gb1–Gb4) were prepared from 50 μl of Fabry patients’ urine. Extraction solutions containing internal standards for Gb3 and Gb2 was added, and then samples were shaken, centrifuged, supernatant was taken out and dried under N₂. After that samples were reconstituted in methanol prior to analysis on a Xevo TQ-S mass spectrometer and analyzed using multiple-reaction-monitoring (MRM). Data was acquired for lyso-Gb3 analogues and Gb1 (glucosylceramide), Gb2, Gb3, and Gb4 (globotetraosylceramide) isoforms to create a profile of Fabry GSLs.

Results: Sixty eight Fabry adult patient samples were analysed and were grouped according to ERT status, sex and eGFR stage. Multiple correlation analysis of all isoforms for Gb3 and lyso-Gb3 in plasma and urine showed good correlation with each other in both plasma and urine apart from hydroxylated forms C:16 Gb3. Statistical analysis revealed significant changes of urinary C24:1 Gb4 (p < 0.008) and hydroxylated isoforms of plasma Gb1, C18 and C26:Gb3 between female Fabry patients either on or not on ERT. Changes in urinary Gb1 and most Gb2 isoforms were observed between patients at eGFR stage 1 vs stage 2. Hydroxylated isoforms of Gb3, and Gb1 and Gb2 (P < 0.05) -were altered between patients who had unaffected eGFR but showed signs of proteinuria.

Conclusion: This work describes a rapid assay for quantitating Gb1-Gb4 and has been used to analyse patients with Fabry disease. Results indicate that concentrations of urinary hydroxylated isoforms of Gb3, Gb2 and Gb1, are independent of the unmodified levels of Gb3. These results may also indicate kidney damage as well if not better than conventional plasma lyso-gb3. The origin of these hydroxylated species of GSL is unknown but may be due to other biological processes such as oxidative stress.

Support: This project was funded by the UCL Biological Mass Spectrometry Centre and the GOSH BRC.

Reference
1 Guérard: Orphanet J Rare Dis 2017.

Clinical Science
Abstracts are alphabetical by first author

Kidney Injury in a Family with Fabry Disease Due To Mutation c.352C>T (p.Arg118Cys)
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Background: Fabry Disease (FD) is a lysosomal storage disorder caused by mutations in the GLA gene coding for α-galactosidase A (α-GalA). These mutations lead to the accumulation of α-GalA substrates, including globotriaosylceramide (Gb3). As a consequence of lipid storage, Fabry patients can suffer from neuropathic pain, impaired kidney function and cardiomyopathy. Existing treatments for FD either require bi-weekly intravenous infusions of replacement enzyme, or are effective in a limited number of patients with specific “amenable” mutations. Substrate reduction therapy with lucerastat, an orally-available small molecule inhibitor of glucosylceramide synthase (GCS) is an alternative mechanism to reduce Gb3 accumulation, that would be suitable for all FD patients.

Methods: Fabry patient-derived fibroblasts with the genotypes R301G (residual α-GalA activity; 20%) R220X (<3%) and W162X (<1%) were obtained from the Coriell Institute and cultured for 9 days in the presence of 9 concentrations in duplicate of either lucerastat, migalastat or agalsidase alfa. Lysosomes were stained using LysoTracker® Red DND-99 and area was quantified. Sphingolipids were extracted with methanol and quantified with LC-MS/MS.

Fabry mice (Gla<sup>−/−</sup> and Gla<sup>−/+</sup>, n = 5 or 6 for each gender) were treated from 5 weeks of age with lucerastat (1200 mg/kg/day food admix) or normal food for 20 weeks. Mice were sacrificed and sphingolipids were quantified in various organs.

Results: In Fabry patient-derived fibroblasts, lucerastat dose-dependently inhibited GCS, reducing glucosylceramide and increasing sphinomyelin, while ceramide remained unchanged. The downstream consequence of GCS inhibition was reduction of Gb3 and lysosome staining, including in cells from patients with no residual α-GalA activity.

In Fabry mice, lucerastat treatment reduced lipid storage in two major organs affected by FD: mean Gb3 in the kidneys (−33%, p < 0.001) and α-galactose-terminated glycosphingolipids in the dural root ganglia (−48%, p < 0.05). In the liver of the Fabry mice, mean glucosylceramide (GlcCer (24:0)) was reduced (−59%, p < 0.001) in addition to Gb3 (24:1) (−37%, p < 0.05), demonstrating substrate reduction through GCS inhibition.

Conclusion: Lucerastat, a GCS inhibitor, reduces Gb3 in the absence of residual α-GalA activity both in vitro and in vivo. Lucerastat has potential to provide an oral substrate reduction therapy for all Fabry patients independent of genotype. A 12-week exploratory clinical study with lucerastat in Fabry patients has been completed, and a pivotal clinical efficacy study in Fabry patients is being designed.

Support: None.

Reference
1 Guérard: Orphanet J Rare Dis 2017.
>200 mutations with different clinical profiles. We describe a family with kidney injury associated to a not nephritogenic c.352C>T (p.Arg118Cys) mutation with undetectable LysoGL-3 and normal GLA.

**Methods and Results:** The index case of this family with FD was maternal grandfather, who had c.352C>T (p.Arg118Cys) mutation and died of chronic renal failure. He had 5 daughters who are carriers and several children inherited this mutation but only 2 of them have been studied: 1. A 7-year-old male with neuropathic pain in hands and feet, abdominal pain with diarrhea, glomerular filtration rate (GFR) of 89(mL/min)1.73 m 2, LysoGL-3: BQL and GLA: 1.61 μmol/L/h and 2. An asymptomatic and mild malnourished 9-year-old male with tricuspid insufficiency and right bundle branch block, GFR of 94(mL/min)1.73 m2, LysoGL-3: BQL and GLA 3.01 μmol/L/h. Both patients had renal biopsy with storage of GL-3 in the podocytes and received enzymatic replacement therapy with β-agalsidase (1 mg/k/2 weeks).

**Conclusion:** Ferreira described 12 adults with FD. 5 of them with c.352C>T (p Arg118Cys) mutation, normal level of LysoGL-3 and normal renal biopsy. This mutation has been as not nephritogenic. The nephritogenic mutations cause absence of GLA function and high levels of LysoGL-3. Our patients had a not nephritogenic mutation but they have GL-3 inclusions in their podocyte associated to normal GLA, undetectable GL-3 and normal GFR. Instead, these children don’t have criteria to start treatment, they had histological renal changes that need treatment to avoid progression to renal failure. This is the first report of renal injury associated to this mutation.

**Support:** None.

**References**


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**Clinical Follow Up of Female Fabry Disease Patients Receiving Enzyme Replacement Therapy for up to 2 Years**


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**Background:** Fabry disease (FD) is an X-linked lysosomal storage disorder resulting from a deficiency of the hydrolytic enzyme α-galactosidase A (α-Gal-A). Heterozygous female patients may develop clinical manifestations requiring enzyme replacement therapy (ERT).

**Methods:** A review of clinical and laboratorial data of females FD patients under ERT (agalsidase-beta; 1 mg/kg; eow). Only patients whose serum creatinine and albuminuria were measured before ERT were included. Cardiac and cerebral involvement was evaluated by echocardiography and/or magnetic resonance imaging.

**Results:** Seventeen female patients (age: 29.1 ± 16.2 years) were included. The median age at diagnosis was 21 (13–65) yrs. The most frequent clinical manifestations were as follows: left ventricular hypertrophy (59%), septal hyperthrophy (29%), cardiac fibrosis (35%), white matter lesion (43%), acroparestesia (59%) and nephropathy (59%). 6/17 and 2/17 patients were at CKD stage 2 and 3a at baseline, respectively. ERT was initiated after 5 yrs (median) of the diagnosis. All patients received anti proteinuric medication. Renal function remained stable for most patients at the two different time points of follow up, including the patient with the lowest eGFR (49.5 ml/min) and the highest proteinuria (2500 mg/24 h) at baseline. Kidney biopsy performed before ERT in a patient without overt proteinuria and normal eGFR unveiled effacement of foot processes of the podocytes. No cardiac or cerebrovascular event was observed during the follow up.

**Conclusions:** Heterozygous Female FD patients may present disease burden and should not be neglected. ERT combined with renin-angiotensin system blockade seems to be a safe and efficacy an effective strategy to prevent progression of Fabry nephropathy.

**References**

2. Wang R: Heterozygous Fabry women are not just carriers, but have a significant burden of disease and impaired quality of life. Genet Med 2007;9:34–45.
Relationships Between Cardiac Myocyte Structural Parameters, Left Ventricular Mass Index, Plasma Lyso-GL3 and Age in Patients with Cardiac Variant Fabry Disease

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Background: Cardiovascular complications are the most common cause of mortality in patients with Fabry disease. Fabry cardiomyopathy is characterized by left ventricular hypertrophy with increased left ventricular mass index (LVMI) on imaging and globotriaosylceramide (GL3) accumulation in cardiac myocytes (CM). In order to better understand relationships between CM structural changes and clinical characteristics of the disease we developed novel unbiased stereological methods. The findings were correlated with clinical and imaging parameters.

Methods: Cardiac biopsies from 10 treatment-naïve male Fabry patients (age 62 [44–68], median [range]) with later-onset IVS4 + 919G>A mutation were studied by electron microscopy stereology. Clinical parameters included plasma AGAL-A activity, globotriaosylphosphingosine (lyso-GL3), LVMI, septal thickness and ejection fraction (EF) measured by MRI. Structural parameters included total [V(Inc/CM)] and fractional [Vv(Inc/CM)] GL3 content in CM, average CM volume [V(CM)], volume of CM nuclei [V(CMN)] and volume density of vessels per myocardium.

Results: All patients had GL3 inclusions in CM, but not in endothelial cells, consistent with the later-onset Fabry phenotype. LVMI was directly related to plasma lyso-GL3 (r = 0.76, p = 0.01). Among structural parameters, LVMI showed direct relationship with V(CMN) (r = 0.66, p = 0.04) and a trend of direct association with V(Inc/CM) (r = 0.58, p = 0.08). Septal thickness directly correlated with V(Inc/CM) (r = 0.69, p = 0.03). On multiple regression analysis, among the clinical and structural variables studied, V(CMN) was the only statistically significant independent predictor of LVMI and septal thickness. V(CMN) was also the only structural parameter correlating with lyso-GL3 (r = 0.69, p = 0.03). On the other hand, V(Inc/CM) was the only structural variable inversely related EF (r = -0.68, p = 0.03). V(CM) (r = 0.73, p = 0.02), V(Inc/CM) (r = 0.74, p = 0.02) and the volume of CM which is not GL3 (r = 0.69, p = 0.03) and V(CMN) (r = 0.71, p = 0.02) were all directly associated with age. AGAL-A activity was inversely related to Vv(Inc/CM) and directly with volume density of vessels in myocardium.

Conclusion: We developed novel methods for estimation of various structural parameters relevant to Fabry cardiomyopathy. Correlations observed between these structural parameters and clinically important variables, such as age, lyso-GL3, LVMI, septal thickness and EF suggest that these biopsy parameters can be used to better understand pathophysiology of Fabry cardiomyopathy. Inverse relationship between EF and V(Inc/CM) is suggestive of a role for GL3 accumulation in heart failure in Fabry disease. Our findings that V(CMN), perhaps a reflection of cardiac myocyte injury or activation, was the only independent predictor of LVMI and septal thickness and correlated with lyso-GL3, suggest that this parameter may be a novel structural biomarker of Fabry cardiomyopathy.

Support: None.

Long Term Immune Responses in Fabry Disease Patients who were Switched from Agalsidase Alfa to Agalsidase Beta

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Background: In Fabry disease (FD) patients, the mutations in GLA gene lead to lack of or faulty α-galactosidase A (α-gal A) enzyme causing accumulation of globotriaosylceramide (Gb3) and related glycosphingolipids in lysosomes of multiple cell types. Due to the fact that some glycolipids serve as antigens, various immune abnormalities have been associated with several lysosomal storage disorders. Treatment options for FD include enzyme replacement therapy with two different recombinant α-gal A enzymes, where agalsidase beta has been approved by FDA for use in the USA while both agalsidase beta and agalsidase alfa are being prescribed in the European nations. Several FD patients in the US were switched to agalsidase alfa for a certain period of time due to supply shortage but were reverted back upon availability of the enzyme. In the present IRB approved clinical study (NCT01745185) we evaluated the effect of their treatment status on peripheral immune cell abnormalities in FD patients.

Methods: Flow cytometry based immunophenotyping was performed using direct immunofluorescence on peripheral blood samples drawn at various time points. Lyso-Gb3 and analogues were measured from plasma and urine using tandem mass spectrometry. Anti-agalsidase antibody and neutralizing antibody titrations were performed from plasma samples.

Results: FD patients showed skewed T helper to cytotoxic T cell ratio, elevated expression of T cell activation markers-CCR4, CCR6 and CXCR3 as well as significant increase in CD16/CD56+ NKT cells. No significant alterations were seen in B cells, NK cells and dendritic cells. Further, the study elucidated the effect of switching from agalsidase alfa to agalsidase beta on immunophenotype as well as other clinical markers like urine and plasma lysoGb3 and their analogues, compared to untreated FD patients who were later administered agalsidase beta. The extent of T cell and NKT cell abnormalities did not differ based on the type of ERT administered. After ERT treatment, there was a decrease in plasma lysoGb3 (mean value of 40 to 29 nmol/L) as well as urine lysoGb3 (mean value of 27 to 21 pmol/mmol of creatinine).

Conclusion: FD patients present with alterations in T cells and NKT cells with no notable effect on B cells indicating persistent immune abnormalities mostly in cellular rather than on humoral adaptive immune system. No significant alterations were seen in innate immune systems components, NK cells and dendritic cells.

Support: None.
In addition, the extent of T cell and NKT cell abnormalities did not differ based on treatment status or type of ERT administered on the FD subjects. The immune abnormalities were not affected by gender and age as well indicating that these persistent abnormalities were inherent to FD subjects irrespective of the extent of substrate accumulation. LysoGb3 levels, though reduced after ERT, still remained above normal reference ranges.

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1 Key Signs and Symptoms Associated with GLA Mutation Detection in Relatives of an Individual with a Known GLA Mutation

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Background: Fabry Disease is an X-linked lysosomal storage disorder resulting from mutations in the α-galactosidase gene (GLA) encoding the α-galactosidase A enzyme. Individuals affected by Fabry disease report signs and symptoms including neuropathic extremity pain, hypohidrosis, fatigue, decreased exercise tolerance, angiokeratomas, cornea verticillata, gastrointestinal issues, proteinuria, cardiac problems, and hearing loss. However, these symptoms are non-specific and occur in non-Fabry related conditions. To gain a better understanding of the Fabry disease symptom profile that predicts a positive finding of a familial mutation in GLA, we investigated the associations between self-reported symptoms and Fabry disease in genetic relatives of an identified Fabry disease proband (N = 565). (Wang et al 2011).

Methods: Genetically related family members of individuals with a known GLA mutation submitted a saliva or blood sample to the laboratory for genetic testing. They also completed questionnaires about demographics, disease family history, and their self-reported Fabry symptoms. Each sample was analyzed for the family specific mutation based on a provided molecular genetic test report. We examined associations between self-reported symptoms and detection of the family GLA mutation.

Results: Testing positive for the familial GLA mutation was associated with self-reported corneal changes/whorls (OR: 6.30, 95% CI: 1.79, 22.1). Males with pain in their hands/feet and a first degree relative with Fabry were at a six fold increased odds of testing positive (OR: 6.37, 95% CI: 2.55, 15.91). Additionally, males experiencing decreased sweating (OR: 5.05, 95% CI: 1.78, 14.32) or angiokeratomas (OR: 6.4, 95% CI 2.3, 17.82) were at an increased odds for testing positive.

Conclusion: These findings suggest that patients presenting with corneal changes, pain in hands/feet, decreased sweating or angiokeratomas may be prioritized in testing relatives of Fabry disease patients and counseled accordingly.

Support: This work was funded by the American Association of Kidney Patients and an educational grant from Genzyme-Sanofi.

Reference

significant correlations were seen for the non-N215S group. When correlating the same parameters to life time exposure lyso-Gb3 levels, we found even stronger associations for the N215S group.

When looking into the impact of ageing on the above parameters, we found strong correlations for LVMI, GFR and MSSI in the N215S group and in the female non-N215S.

**Conclusion:** In conclusion, life time exposure to lyso-Gb3 seems to have a significant impact on N215S overall severity in both genders.

**Support:** None.

**References**


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**Carpal Tunnel Syndrome in Fabry Disease**

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**Background/Methods:** Carpal tunnel syndrome (CTS) is a common peripheral mononeuropathy affecting up to 4% of the general population. Incidence in patients with Fabry disease is unclear but may affect up to 25% of patients. Diagnosis of CTS can be delayed as paresthesia in hands is common among Fabry patients due to underlying small fiber neuropathy. Here, we describe a case of Fabry patient with severe CTS.

**Results:** 37 years old right handed male with Fabry disease and small fiber neuropathy was referred to neuromuscular clinic for evaluation of hand pain. He has not received enzyme replacement therapy in the past. He was enrolled in Fabry disease treatment study with GZ/SAR402671 oral treatment 9 months prior to the evaluation. 1 month in to the treatment, he developed numbness and tingling in the first 3 fingers of the right hand along with difficulties grasping small objects. He did not endorse similar symptom in his left hand. He also denied numbness, tingling or burning in his feet. In the past, he went through periods of time when he had burning pain in his fingers and toes, mostly in his childhood. This has not been a problem for him at the time he was getting involved in this treatment study. He has worked as a truck driver for years and carpal tunnel syndrome was suspected. His physical examination showed decreased light touch and pinprick in the right 2nd digit compared to the 5th digit. Two-point discrimination was also increased to 6 mm. Bilateral abductor pollicis brevis muscle strength was diminished to 4/5 and 4+/5. Examination was otherwise normal. Electromyography of the bilateral upper extremities showed bilateral median neuropathy at the wrist (carpal tunnel syndrome), severe on the right and moderately severe on the left. Carpal tunnel release surgery was recommended.

**Conclusion:** Carpal tunnel syndrome can be overlooked in Fabry disease patients as pain, tingling and numbness in the fingers are common manifestation of Fabry disease owing to small fiber neuropathy. Detailed history, physical examination and electrodiagnostic testing is required for timely diagnosis and appropriate management.

**Support:** No funding.

**Reference**

Pathic pain occurred in childhood in four patients, as has been
reported in other series. Although the timing of initiation of en-
zeyme therapy has been debated, presence of neuropathic pain
alone and because it reduces the quality of life, justified the initia-
tion of enzyme therapy. By backgrounds and due to the segrega-
tional pattern of FD, the mother was probably heterozygous.

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25
A Review of Bone Disease in Fabry’s Patients
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Background: Fabry’s disease is an X-linked, inborn error of
metabolism caused by the deficiency of the enzyme alpha-galacto-
sidase A, which leads to the accumulation of globotriaosylce-
ramide (Gb3) in multiple organs. Here, we briefly review the avail-
able information about bone involvement in Fabry’s disease.

Methods: We performed a review of literature about bone dis-
ease in Fabry’s disease through PubMed.

Results: Three major studies have investigated bone disease in
Fabry’s patients using DEXA scan (two cross-sectional and one
retrospective). A total of 116 patients have been studied in these
studies. The major consistent finding in all of these studies is that
bone mineral density (BMD) in male patients with Fabry’s disease
is comparable to postmenopausal females. Also, two of these studies
pointed out carbamazepine as an important contributor of
bone disease in Fabry’s disease. (Germain, Benistan, Boutouyrie, &
Mutschler, 2005; Mersebach et al., 2007; Talbot, Ghali, & Nicholls,
2014).

Discussion: These studies leave a few important questions un-
answered: 1). No tissue studies have been done in Fabry’s bone
disease, and we are not aware of any possible changes in bone
structure; 2). The nutritional status has not been consistently in-
vestigated and reported in these studies; 3). Lack of physical activ-
ity as a result of heat intolerance and hypohydrosis has been re-
ported as one of the possible etiologies for the bone disease, but has
never been investigated; 4). Some of the well-known factors in
bone disease, including FGF-23 have not been investigated; and 5).
The enzyme replacement therapy (ERT), and its possible effect on
BMD has not been reported in the third study, which could give us
a better idea of an effective treatment for bone disease.

Conclusion: Considering that the introduction of ERT has sig-
nificantly increased the life expectancy in Fabry’s patients, it is im-
perative to investigate the basic etiology of bone disease in these
patients. Therefore treatment strategies can be developed in order
to decrease the incidence of complications, specifically pathologi-
ical fractures.

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