Fabry Disease Biomarker: Lyso-GL-3 (Lyso-Gb3)

Disease Overview

Fabry disease is a progressive, genetic disorder caused by a deficiency or absence of lysosomal α-galactosidase A activity due to variants in GLA., located on the X chromosome.[1,2] Lack of sufficient α-galactosidase A activity leads to progressive accumulation of glycosphingolipids globotriaosylceramide (denoted GL3 or Gb3) and globotriaosylsphingosine (Lyso-GL-3 or Lyso-Gb3) within lysosomes in a variety of cell types, including microvascular endothelium, podocytes, arterial smooth muscle cells, and cardiomyocytes.[1,2]

Patients with Fabry disease are typically classified as classic or late-onset (non-classic):

- Classic males primarily present in childhood/adolescence with neuropathic pain, angiokeratomas, corneal opacities, hypohidrosis, and GI disturbances that progress to kidney failure, cardiomyopathy, cardiovascular disease, arrhythmias, and stroke/TIA.[1,2]
- Late-onset patients present with variable age of onset and manifestations, and may not have multiple organ involvement.[1]
- Female Fabry patients have a wide spectrum of disease manifestations from asymptomatic to severe phenotype similar to classic.[1]

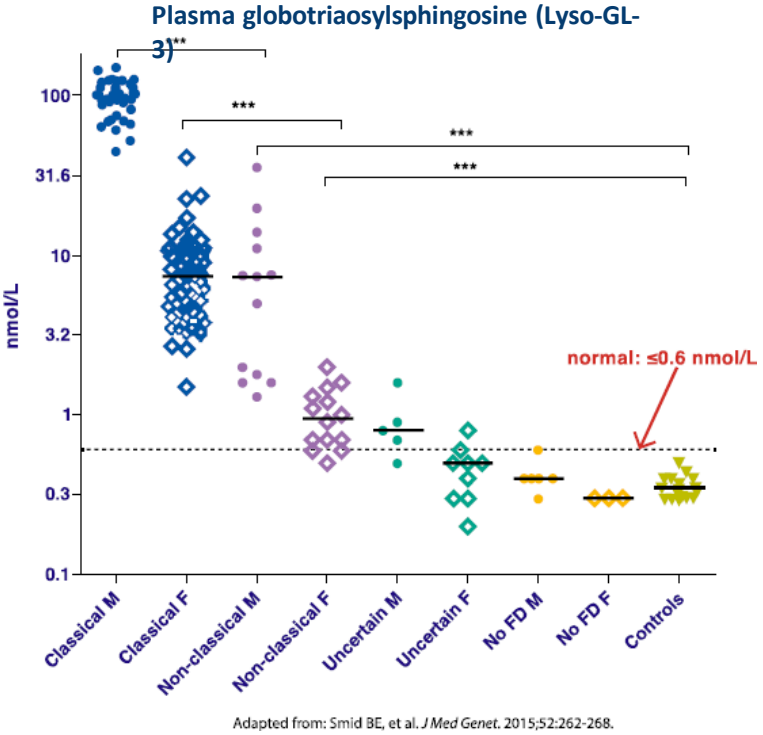
Phenotypic heterogeneity and overlap with more common conditions can make predicting genotype-phenotype correlations challenging.

Biomarker: Globotriaosylsphingosine (Lyso-GL-3, Lyso-Gb3)

Distinguishing phenotypes:

First reported in 2008 by Aerts et al, globotriaosylsphingosine or Lyso-GL-3 (also referred to as Lyso-Gb3) is a pathogenic, vasoactive metabolite, which may be a useful biomarker for diagnosing Fabry, monitoring disease progression, and differentiating between clinical phenotypes.[3,4]

Plasma Lyso-GL-3 was measured in a retrospective Dutch adult Fabry disease (FD) cohort comprising individuals with classic and non-classic FD phenotype. One hundred fifty-four subjects were classified into four groups: classic FD, non-classic FD, uncertain, and no FD.[4]



Adapted from: Smid BE, et al. *J Med Genet*. 2015;52:262-268.

Males (M)=dots, Females (F)=diamonds, horizontal line per group=median, Uncertain FD: Biopsies unavailable to confirm diagnosis, No FD: Negative biopsy or D313Y variant, ***P<0.01

- Plasma Lyso-GL-3 values differ between FD subjects (both male and female subjects with the classic and non-classic phenotype) and controls (P<0.01 for all separate groups versus controls). There was no overlap in Lyso-GL-3 value between men and women with a classic phenotype or between men with a classic and a non-classic phenotype.[4]
- All men and women with a classic phenotype and men with a nonclassic phenotype had higher plasma Lyso-GL-3 values than controls. Lyso-GL-3 values in non-classic female subjects showed some overlap with control values: three out of fourteen women with a non-classic phenotype had normal Lyso-GL-3 values although they were close to the upper limit of the normal range.[4]
- Concentrations of >45 nmol/L are strongly indicative of classic FD phenotype in men with FD.[4]

  Classic FD was defined as α-GalA enzyme activity in leukocytes <5% of the mean reference value (men) and GLA variant and either one or both of the following criteria: ≥1 of the described characteristic features of FD (neuropathic pain, corneal verticillata, clustered angiokeratoma) or family member with definite diagnosis of classic FD. Eighty percent of the women in the classic group had ≥1 characteristic sign or symptom, while 20% was grouped as such because of a family member with a classic FD phenotype.[4]

Did You Know? Case reports have demonstrated that Lyso-GL-3 can be elevated in asymptomatic children.[5,6] In a study of 237 naïve FD patients, plasma Lyso-GL-3 levels remain unchanged from childhood over decades.[16] Further research is warranted.

MAT-US-2023886 v5.0 - P Expiration Date: 03/27/2027

Lyso-GL-3 is a Bioactive Molecule

Cardiac:
- Stimulates proliferation of vascular smooth muscle cells and cardiomyocytes *in vitro*.[7]
- May be instrumental to development of intra-media thickness and left ventricular hypertrophy[3]
- Correlated with left ventricular mass in females[3]

Renal: *(All studies performed in vitro)*
- Induces mechanisms of renal interstitial fibrosis[8]
- Increases RIPK3 expression resulting in increases in albuminuria, podocyte loss, and foot process effacement[9]
- Lyso-GL-3 increases TGF-β1 and CD74 mRNA levels in cultured podocytes in a time and dose-dependent manner. TGF-β1 and CD74 are critical mediators of glomerulosclerosis and interstitial fibrosis in diabetic nephropathy.[10]
- Promotes Notch1-mediated inflammatory and fibrogenic responses in podocytes that may contribute to Fabry nephropathy.[11]

Other:
- Modulates gut microbiota *in vitro*[12]
- Dorsal root ganglion cells showed enhanced voltage-dependent calcium currents *in vitro*[13]
- Increased sensitivity to pain in mice[13]

Correlation of Lyso-GL-3 with Disease Progression and Clinical Events

- Arends and colleagues (2017) retrospectively assessed event-free survival in 499 treatment-naïve adult patients (mean age 43 years old; 41% males, 57% with classic phenotype). Plasma Lyso-GL-3 concentrations were available for 351 patients. Taking all patients together, higher Lyso-GL-3 concentration at baseline was associated with higher FD-related event rate in the past (P<0.001).[14]

- Nowak and colleagues (2022) analyzed plasma Lyso-GL-3 as an independent factor associated with adverse clinical outcomes* in 26 males and 40 females. In the Kaplan-Meier analysis, the median of plasma Lyso-GL-3 levels and the cumulative Lyso-GL-3 pre-treatment exposure separated patients with clinical events from patients without clinical events better than age, sex or phenotype.[15] *See Kaplan-Meier figure to the right:*

  * Outcomes were a composite endpoint of kidney replacement therapy, atrial fibrillation, pacemaker and/or implantable cardioverter defibrillator, cerebrovascular events or death

- Van der Veen and colleagues (2023) reviewed changes in plasma Lyso-GL-3 over time in 237 treatment-naïve patients with confirmed FD. Regardless of sex, higher plasma Lyso-GL-3 levels were significantly associated with steeper eGFR decline, faster increase in left ventricular mass on echocardiography, diastolic dysfunction as measured by echocardiography parameters and increased white matter lesions over time on cerebral MRI.[16]

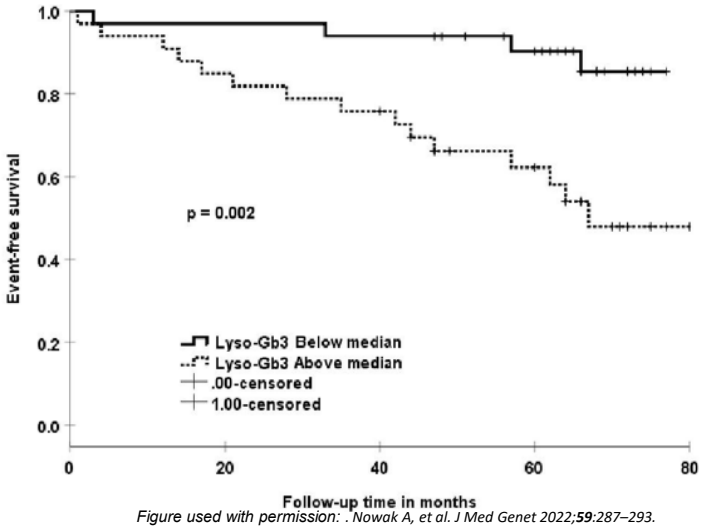


*Figure used with permission: . Nowak A, et al. J Med Genet 2022;**59**:287–293.*

Lyso-GL-3 Testing Options

Sanofi does not review or control the content of non-Sanofi websites. These listings do not constitute an endorsement by Sanofi of information provided by any other organizations. The following is a selection of laboratories whose Fabry testing programs include Lyso-GL-3. This is not an exhaustive list of labs or an endorsement of any one lab. Other testing options may be found at www.concertgenetics.com or www.ncbi.nlm.gov/gtr. Content is current at time of printing and tests may not be available in all states; please call the laboratory to confirm test availability, sample shipping information, and all other logistics.

Lab	Test Name and Code	Sample Requirements	Kits	Avg TAT	Mobile Blood Draw	Billing	Contact
Centogene	Lyso-Gb3	WB: 1mL EDTA (lavender) tube; DBS card: 10 circles	Blood, DBS	15 d	No	Inst, Self-Pay, Ins	P: 617-580-2102 W: www.centogene.com
Duke University	Lyso - GB3 (LAB9035)	WB: 4mL EDTA (lavender) tube; Plasma: 1mL	No	Up to 28 d	No	Inst	P: 919-613-8400 W: https://testcatalog.duke.edu
The Lantern Project (Revvity Omics)	Lyso-GL3 (for diagnostic purposes, must be bundled with enzyme and/or GLA sequencing)^	DBS: 2 spots WB: 5ml EDTA (lavender) tube	Blood, DBS	3 d	Yes	No Charge*	P: 866-354-2910 E: genomics@revvity.com W: www.LanternProjectDx.com
Mayo Clinic Laboratories	Globotriaosylspingosine (LGB3S, LGBWB, or LGBBS)	WB: 1mL EDTA (lavender) tube; DBS: 2 spots; Serum: 1mL red top tube	DBS (in some cases),	8-15 d	Yes	Inst (acct required) Ins (some cases)	P: 800-533-1710 E: mcl@mayo.edu W: www.mayocliniclabs.com
Revvity Omics	Globotriaosylsphingosine (Lyso-Gb3), 80029	DBS: 2 spots	DBS	3 d	No	Inst, Self-Pay	P: 866-354-2910 W: www.revvity.com
Sanofi Rare Disease Specialty Testing Program (Labcorp)	Lyso-GL3	Plasma: 1mL sodium heparin (green) tube	Blood+	14 d	No	No Charge*~ (account required)	P: 888-681-1701 E: RareDiseaseProgram@labcorp.com

d=days, DBS=dried blood spots, Ins=Insurance, Inst=Institutional, TAT = turnaround time from receipt in lab, WB=whole blood, w=weeks

*Testing is performed at no charge; local charges may apply for sample collection, processing, or shipping. ^Lyso-GL-3 as part of the Lantern Project is for diagnostic assistance. It is not for ongoing monitoring. +Individual testing supplies can be ordered. ~Phlebotomy is covered if performed at a Labcorp Patient Service Center (PSC)

1. Ortiz A, et al. *Mol Genet Metab* 2018;123:416–427. 2. Schiffman R, et al. *Kidney Int.* 2017;91: 284-293. 3. Aerts JM, et al. *Proc Natl Acad Sci* U S A. 2008;105(8):2812-2817. 4. Smid BE, et al. *J Med Genet* 2015;52:262–268. 5. Kritzer A, et al. Mol Genet Metab Rep. 2019;21:100530. 6. Spada M, et al. *Italian J Pediatr* 2017;43:1. 7. Barbey F, et al. *Arterioscler Thromb Vasc Biol*. 2006;26(4):839-844. 8. Jeon YJ, et al. *PLoS One*. 2015;10(8):e0136442. 9. Kim SY, et al. *Cells.* 2021;10(2):245. 10. Sanchez-Niño et al. *NDT*. 2011. 11. Sanchez-Niño et al. *Hum Mol Genet*. 2015. 12. Aguilera-Correa JJ, et al., Sci Rep. 2019 Aug 19;9(1):12010; 13. Choi et al. *Neurosci Lett*. 2015. May 6; 594:168-8. 14. Arends M, et al. *J Am Soc Nephrol.* 2017;28: 1631–1641. 15. Nowak A, et al. J Med Genet 2022;**59**:287–293. 16. van der Veen SJ, et al. Clin J Am Soc Nephrol. 2023:18(10):1272-1282.

MAT-US-2023886 v 5.0 - P
Expiration Date: 03/27/2027