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KS-462-282

Krabbe disease biomarker of unknown identity

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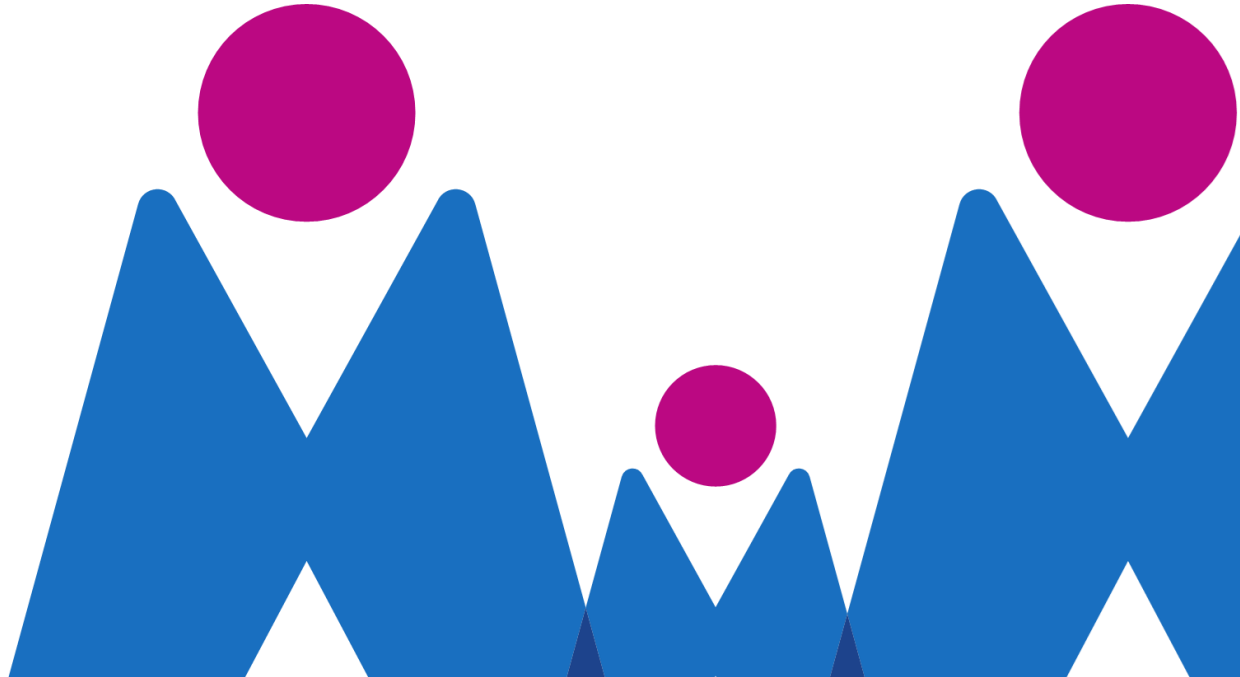

Krabbe Disease

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- Initially described by Dr Knud H Krabbe as a familial “diffuse **brain** sclerosis”

- A rare, autosomal recessive (prevalence 1-9 / 100000) LSD
- Deficient Galactocerebrosidase (GALC) activity

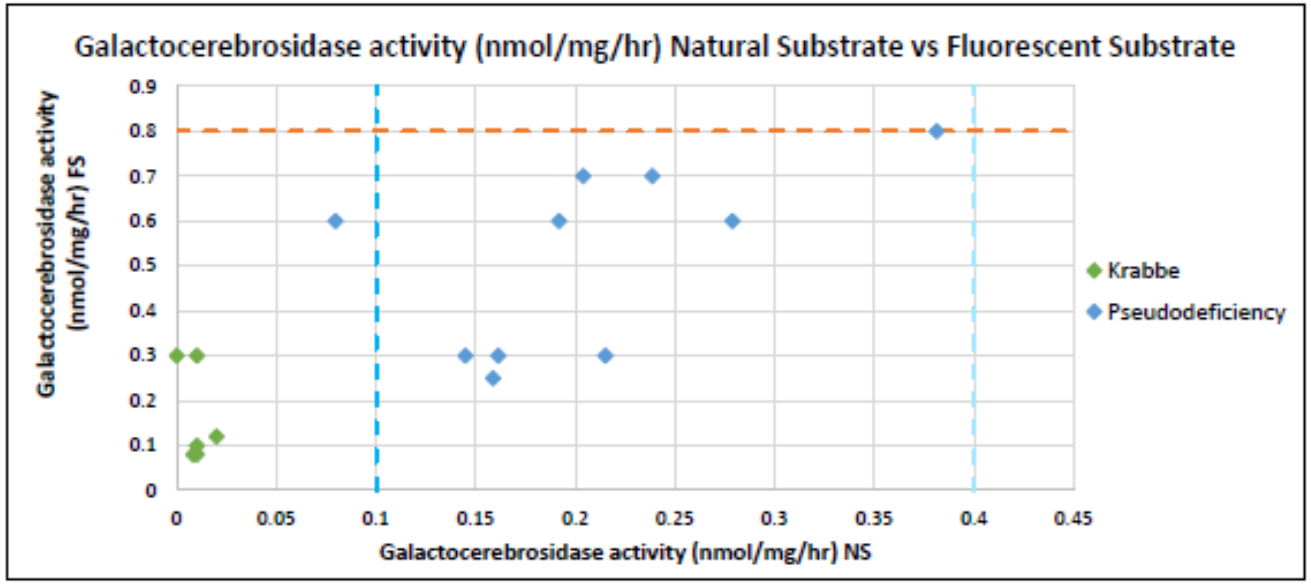
- Variable phenotypic severity (neurological features and rate of progression/decline) from
  - Early-infantile (onset <6m)
  - Late-infantile (onset 6m – 3y)
  - Juvenile (onset 3-16y)
  - Adult onset (16y +)

- White matter typically affected
  - Gigantic polynuclear glial cells – globoid cells
  - Globoid cell leukodystrophy = Krabbe disease

- No curative treatment
  - Bone marrow transplant modifies phenotype

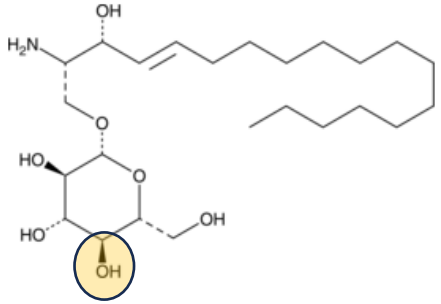
Diagnosis by Enzymology

- Psuedodeficiency alleles relatively common
- Enzymology for diagnosis of Krabbe disease
  - Fluorogenic substrate
    - Readily available
    - Simpler analysis, higher throughput (broad LSD enzyme screen)
    - Pseudodeficiency a problem
  - Natural substrate
    - Difficult to source
    - Analytically more challenging
    - Not suited to higher throughput
    - Substrate availability?
    - More reliable differentiation of pseudodeficiency from affected
- Can plasma biomarkers be used to support this pathway?

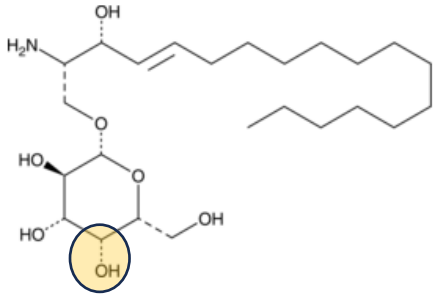


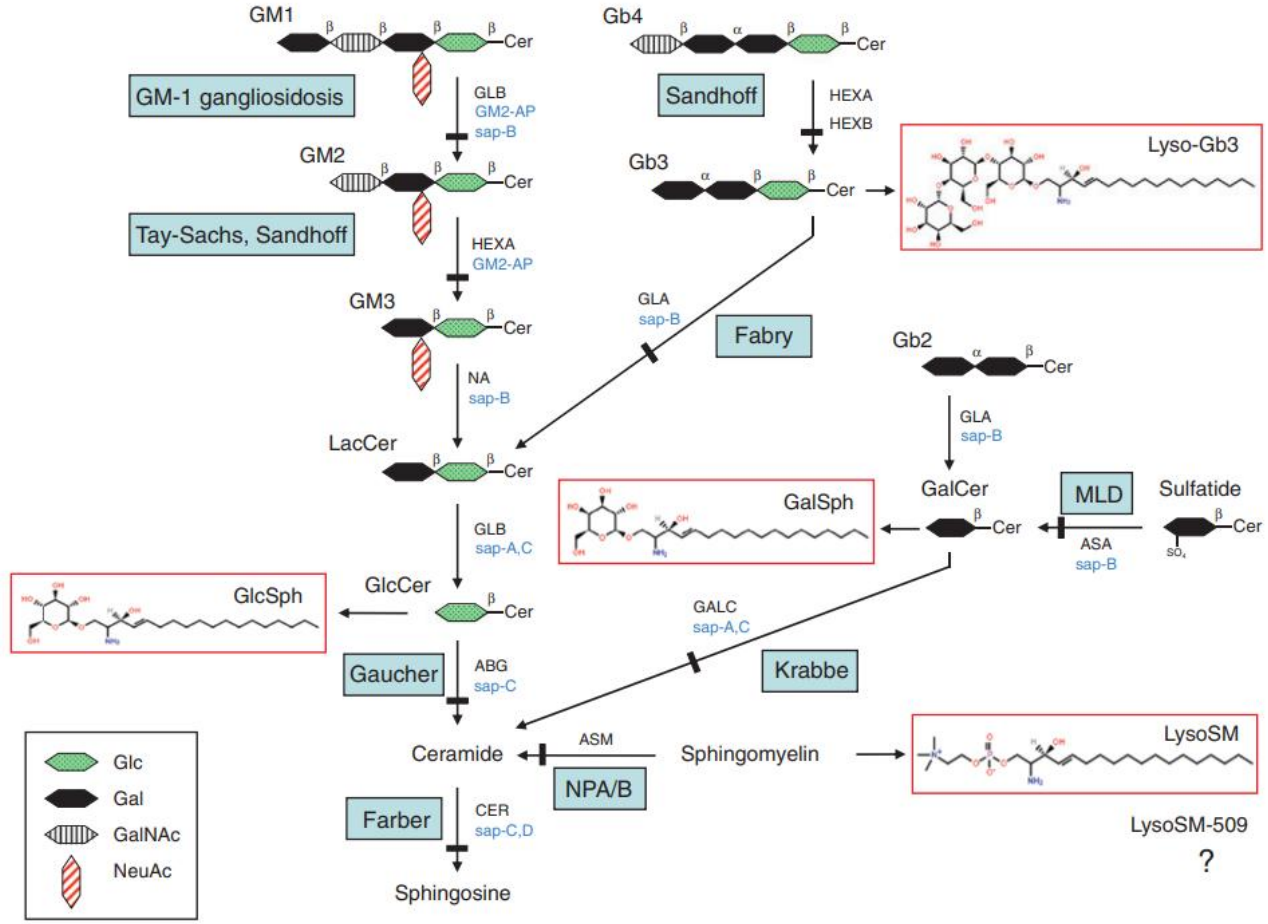
Psychosine – Glucosyl- and Galactosyl- stereoisomers (MS indistinguishable)

- Glucosyl-sphingosine (Lyso-GB1, glucosyl-psychosine) – Gaucher disease



- Galactosyl-sphingosine (galactosyl-psychosine) – Krabbe disease





Polo, Giulia, Burlina, Alessandro P., Kolamunnage, Thilini B., Zampieri, Michele, Dionisi-Vici, Carlo, Strisciuglio, Pietro, Zaninotto, Martina, Plebani, Mario and Burlina, Alberto B.. "Diagnosis of sphingolipidoses: a new simultaneous measurement of lysosphingolipids by LC-MS/MS" *Clinical Chemistry and Laboratory Medicine (CCLM)*, vol. 55, no. 3, 2017, pp. 403-414.

Krabbe Biomarkers - Gal-Sph / Glc-Sph

Total hexosyl-sphingosine

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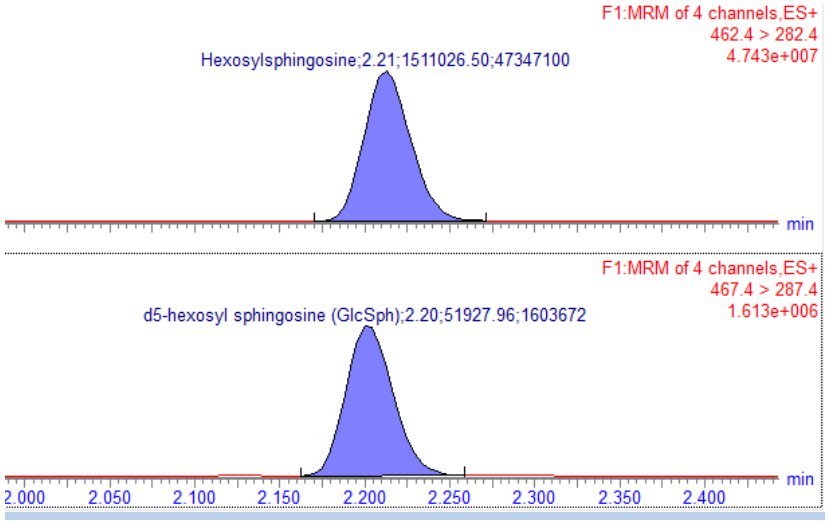
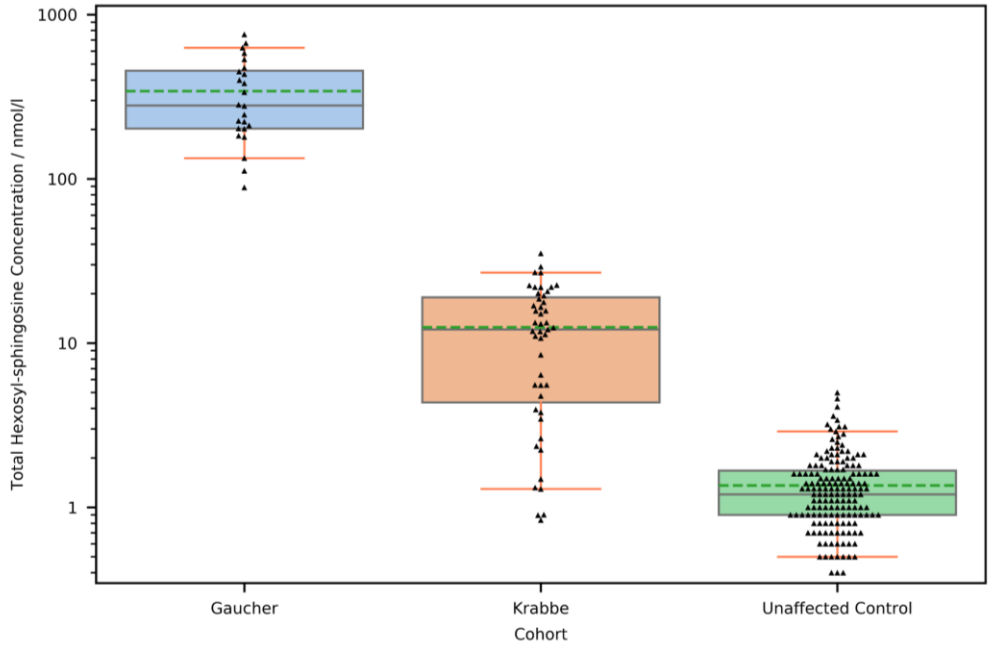
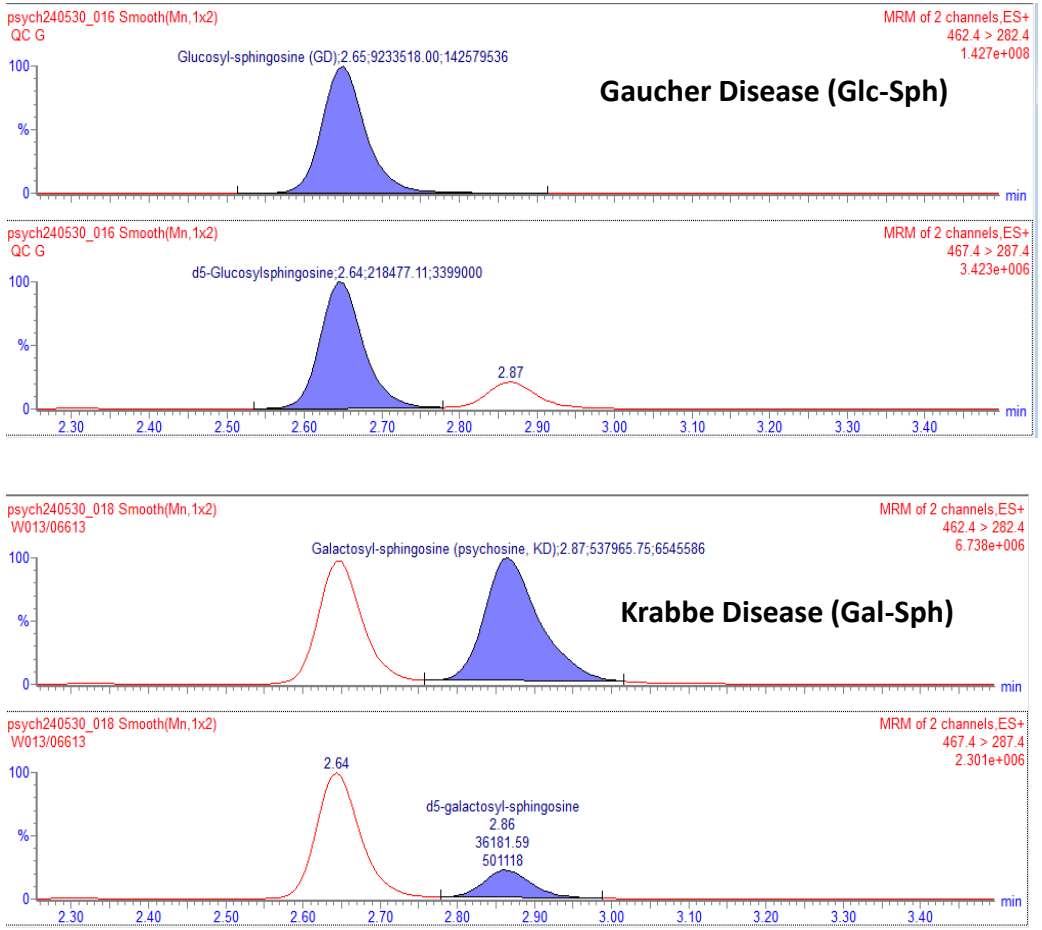
- UPLC-MS/MS
  - Reversed-phase UPLC, fairly simple retention mechanism, technically undemanding
  - Glc-sph (GD) and Gal-sph (KD) cannot be resolved
  - Glc-sph (GD) and Gal-sph (KD) mass spectrally identical





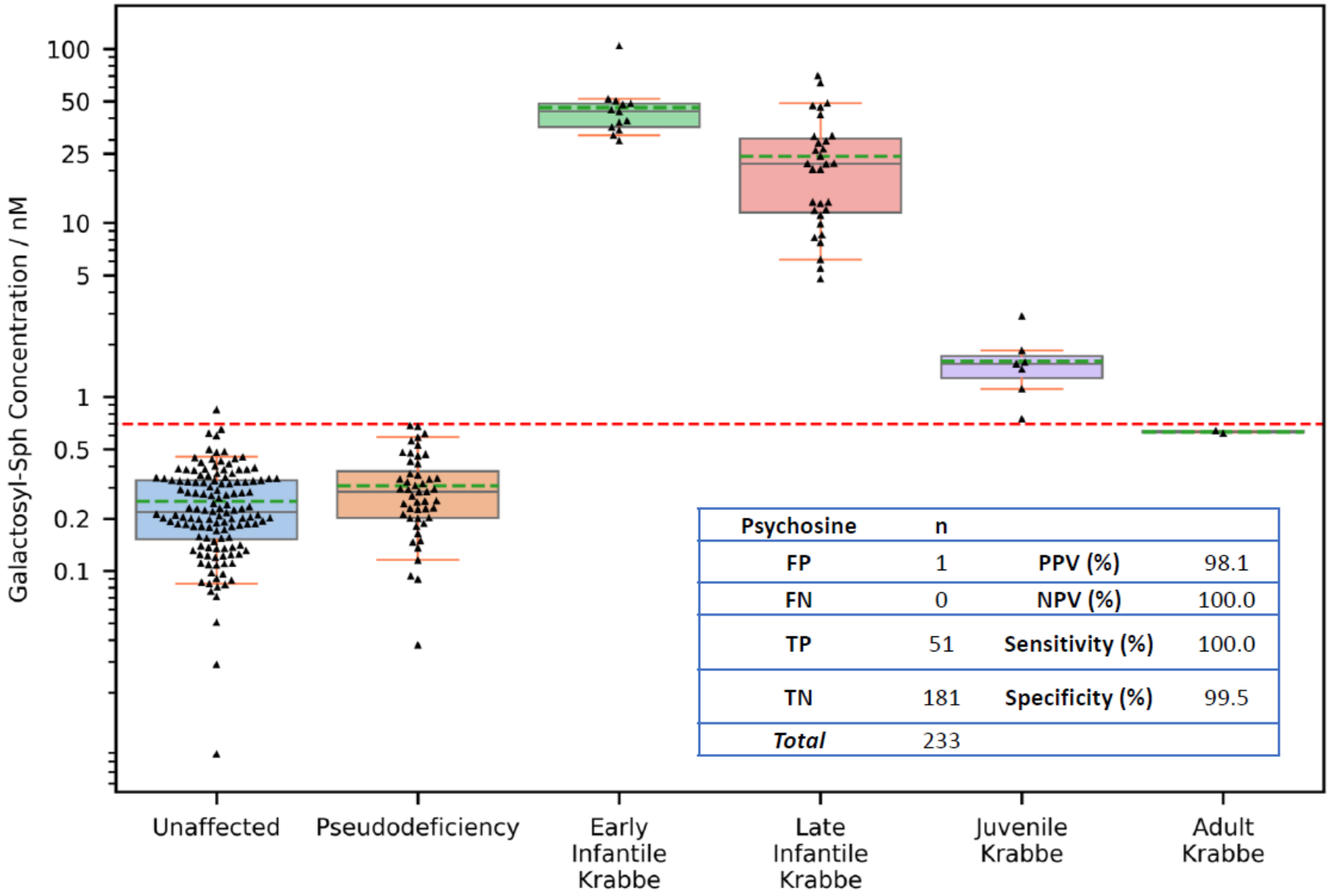
Resolving the isomers – Polar retention mechanisms e.g. HILIC

Psychosine – galactosyl-sphingosine



- HILIC retention mechanism - analytically much more challenging
  - Sample preparation considerations
    - Injection solvent / starting MP conditions
    - Sensitive detection required (physiological concentrations of psychosine 100-500 pmol/l
  - Balance of several chromatographic mechanisms required
    - Polar-polar interactions
    - Peak shape – tailing of basic species can be controlled by buffering
    - Resolution of Gal/Glc isomers by secondary ion-exchange interactions
    - Buffer concentration affects:
      - Detection sensitivity
      - Peak shape / tailing
      - Resolution of the critical pair

Plasma psychosine biomarker performance



Psychosine	n		
FP	1	PPV (%)	98.1
FN	0	NPV (%)	100.0
TP	51	Sensitivity (%)	100.0
TN	181	Specificity (%)	99.5
Total	233		

Something else…

Resolved from hexosyl-sphingosine (Glc + Gal-Sph) in reversed-phase

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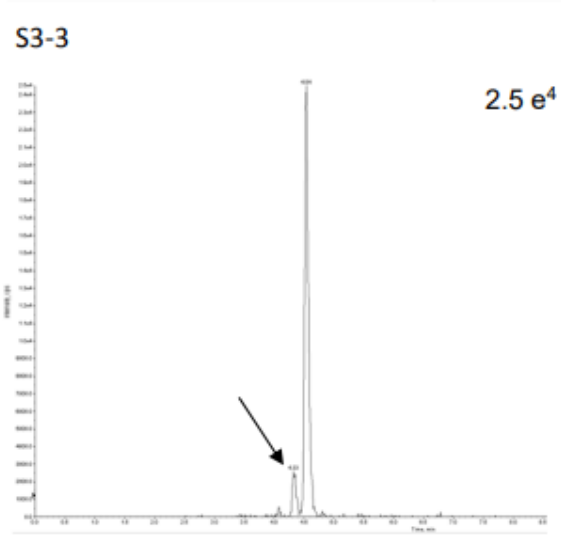
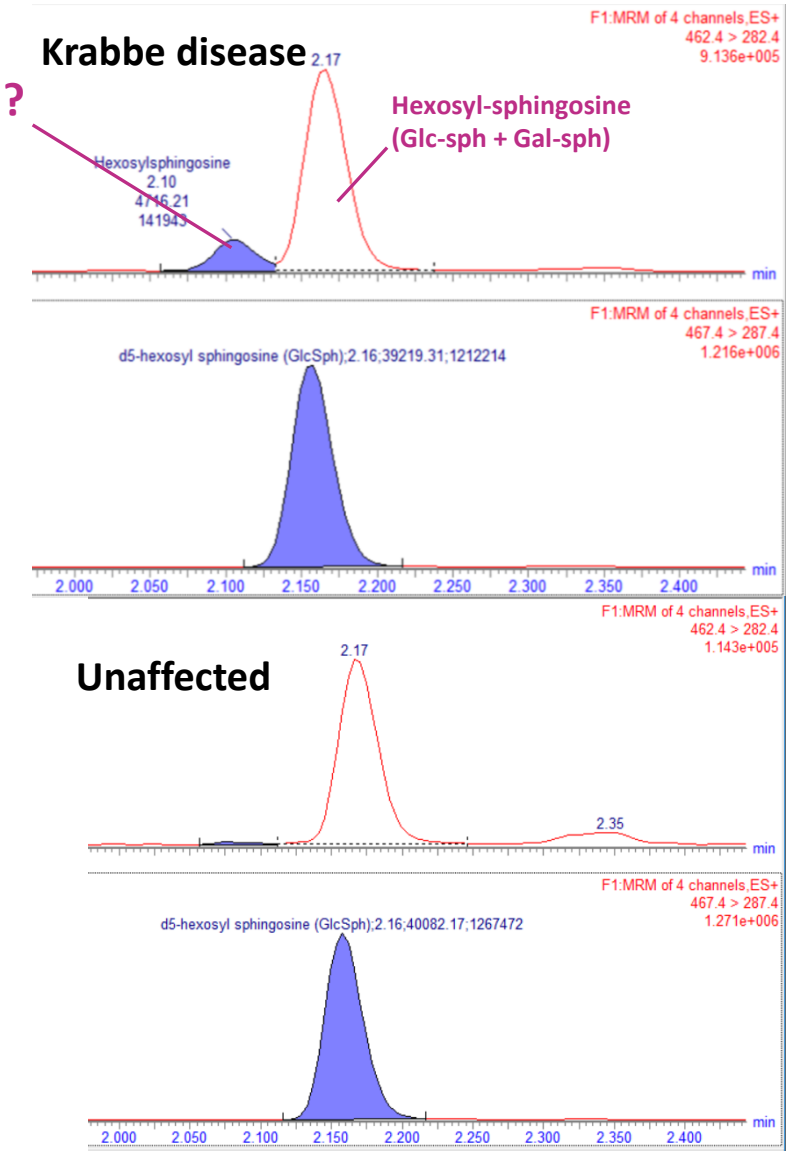
- Lysosphingolipid analysis – reversed-phase UPLC-MS/MS
- Galactosyl and glucosyl-sphingosine unresolved = hexosyl-sphingosine
- Hexosyl-sphingosine 462.4 > 282.4

- Anecdotally reported previously:

"An additional peak was systematically observed … with slightly shorter RT than LysoHexCer (see S3 Fig). This peak was absent in GD, in controls, and in patients affected with other lysosomal or peroxisomal disorder"

Pettazzoni M, Froissart R, Pagan C, Vanier MT, Ruet S, Latour P, Guffon N, Fouilhoux A, Germain DP, Levade T, Vianey-Saban C, Piraud M, Cheillan D. LC-MS/MS multiplex analysis of lysosphingolipids in plasma and amniotic fluid: A novel tool for the screening of sphingolipidoses and Niemann-Pick type C disease. PLoS One. 2017 Jul 27;12(7):e0181700. doi: 10.1371/journal.pone.0181700. PMID: 28749998; PMCID: PMC5531455.





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Examples

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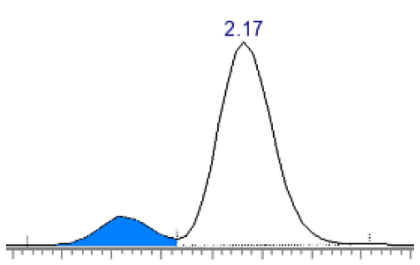
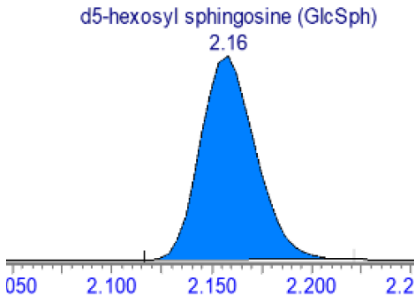
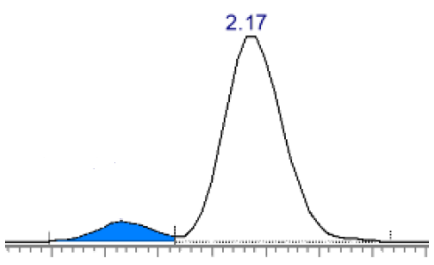
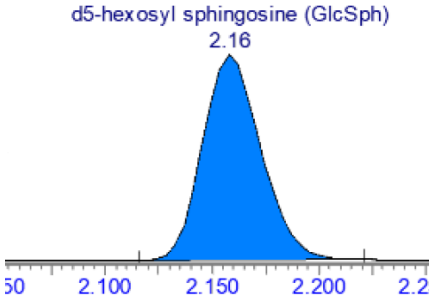


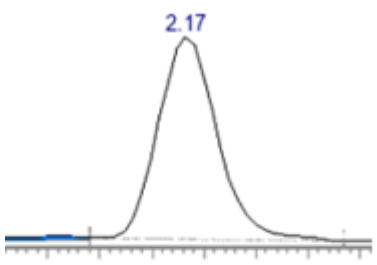


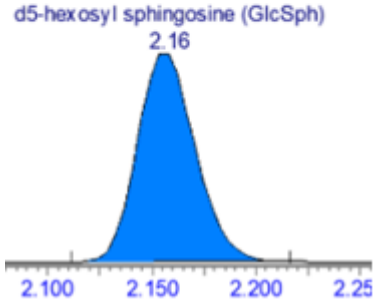
Early infantile KD



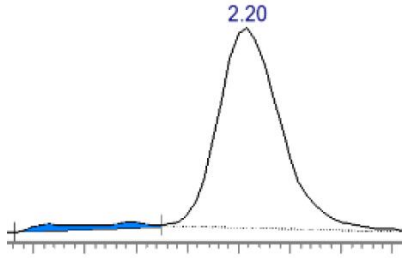


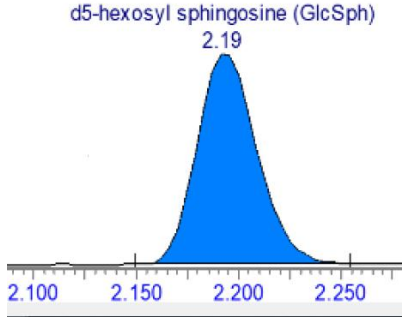
Juvenile KD





GALC pseudodeficiency





Unaffected

* Adult onset KD - equivocal

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Semi-quantitation

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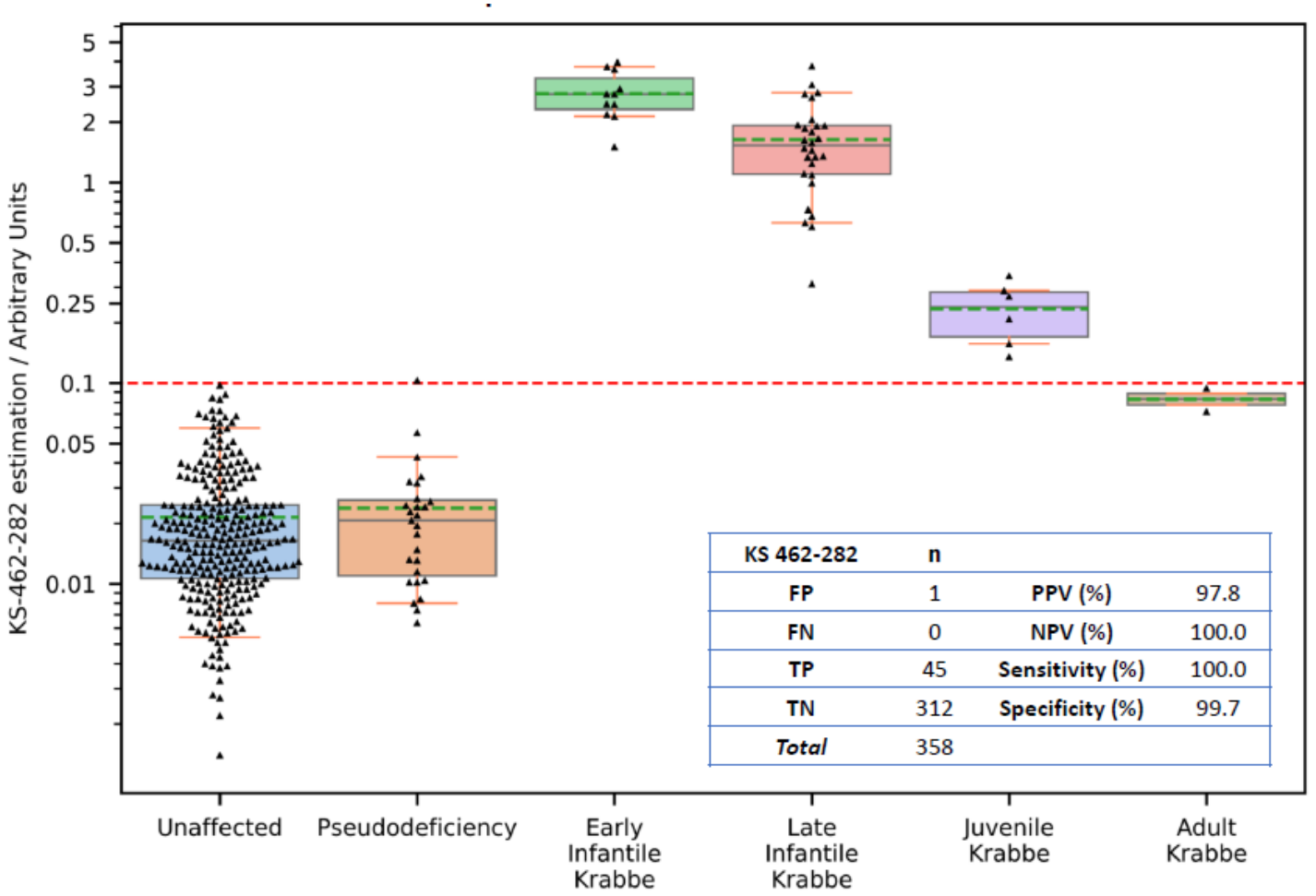
- Semi-quantitation?
  - Calibrate using glucosyl-sphingosine as surrogate reference material and d5-glucosyl-sphingosine as IS
- Potential?
  - Concentration of this species increases with severity of phenotype, similar to galactosyl-sphingosine
  - Very simple analysis compared with gal/glc isomer separation (which is quite a bit harder)
- Limitations
  - We don't know what it is!
    - No authentic standard or internal standard
      - Cannot tune / optimise detector response
      - ? Reliable quantitation



KS-462-282 Vs Psychosine

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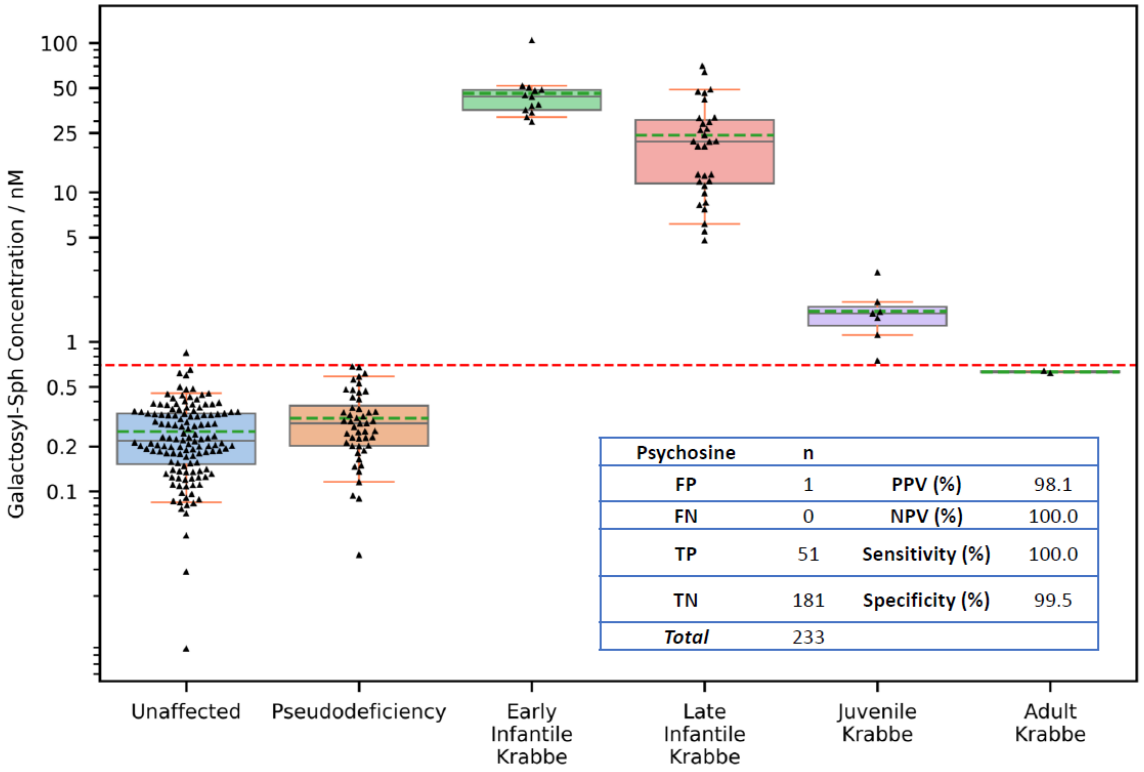

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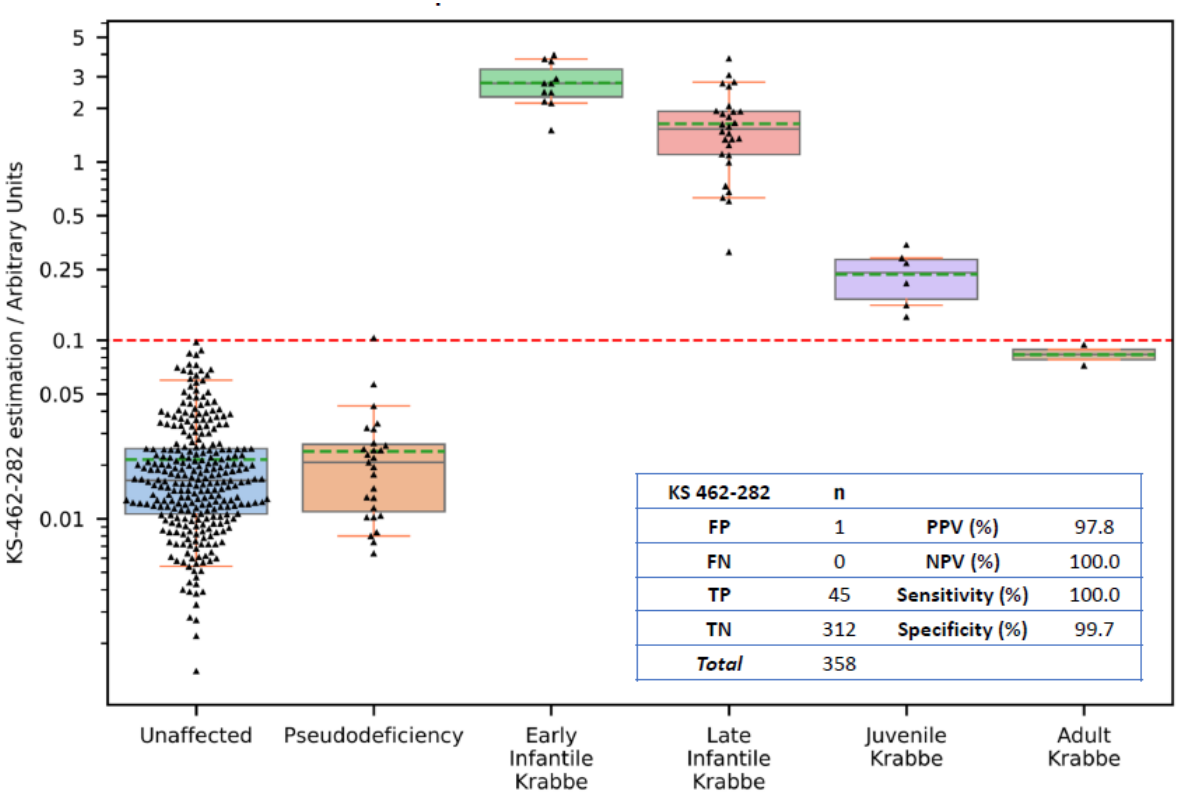

KS 462-282	n		
FP	1	PPV (%)	97.8
FN	0	NPV (%)	100.0
TP	45	Sensitivity (%)	100.0
TN	312	Specificity (%)	99.7
Total	358		

Summary

- Krabbe disease enzymology in leucocytes
  - Often give false positives by measurement with fluorogenic substrate due to pseudodeficiency
  - Repeat sample often required for analysis with natural substrate

- Plasma biomarkers as tier 2 of a 3-tier strategy (lysosomal enzyme screen)
  - Eliminate unnecessary patient rebleeds (non-adults)
  - Reduce delay to diagnosis and exclusion of KD

- Plasma psychosine
  - An excellent biomarker for KD
  - Analytically difficult

- KS-462-282
  - A very promising alternative KD biomarker
  - Analytically simple
  - Huge potential to change diagnostic strategy - ? Present / extractable from DBS samples
  - Need for structural elucidation and synthesis of reference material

Acknowledgements

- Kate Neal - Willink lab, did most of this work.
- Willink laboratory – contributions
  - Christine Egerton
  - Oliver Parkes
  - Heather Church
  - Teresa Wu
- GEM appeal – Instrument via fundraising and charitable donations