Development of a Plasma Lyso-Gb1 Clinical Assay and its Application to Gaucher and Krabbe Disease Patient Plasma

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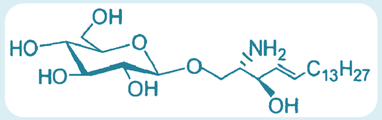
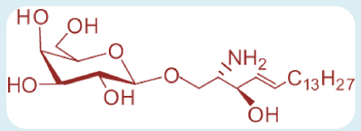
Gaucher & Krabbe disease

Gaucher disease is caused by defects in glucosylceramidase (GBA) which results in increased glucosylceramide (GalCer). A by-product of GalCer accumulation is the lyso form Lyso-Gb1 where the fatty acid moiety is removed.



Lyso-Gb1 is a biomarker for Gaucher disease

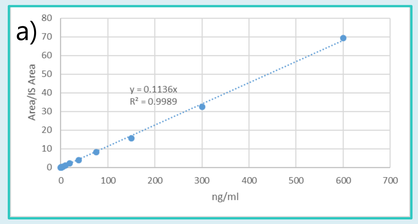
Krabbe disease is caused by defects in galactosylceramidase (GALC) which results in accumulation of galactosylceramide (GalCer). Psychosine is the corresponding lyso-form where the fatty acid moiety is removed and is increased in Krabbe disease.

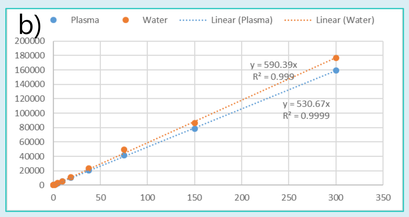


Lyso-Gb1 and psychosine are structural isomers that differ by a single sugar moiety and can be measured combined in one assay as lyso-monhexosylceramide using reverse phase chromatography.

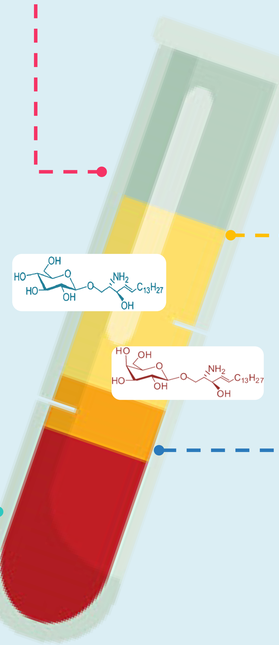
Assay validation

- Matrix effect in plasma =<15%
- Recovery of lyso-gb1 = 80-85%
- Limit of detection = 0.146 ng/ml
- Limit of quantitation = 0.293 ng/ml.
- Intra-batch accuracy CV = 0.86-2.69%
- Precision CV = 3.39-6.35% over 1-100ng/ml spiked QC range.
- Inter-batch accuracy CVs =< 11%
- Linearity up to 600ng/ml (r2>0.99)
- CVs for a
  - low QC of 1 ng/ml <30%.
  - medium QC of 10 ng/ml and high QC of 100 ng/ml were <15%.





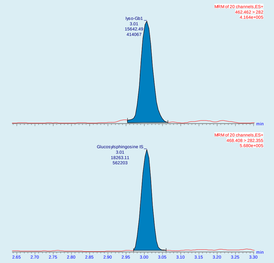
a) calibration curve in plasma shows good linearity up to 600ng/ml. b) calibration curve in water compared with plasma shows minimal matrix effect <15%



LC-MS/MS method

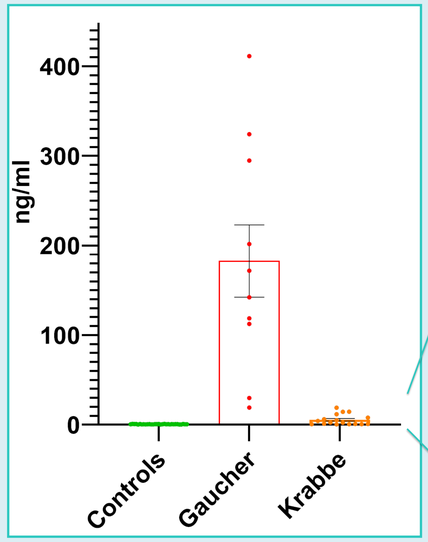
Aim: We developed an assay suitable for clinical use and tested its ability to be used in Gaucher and Krabbe patients

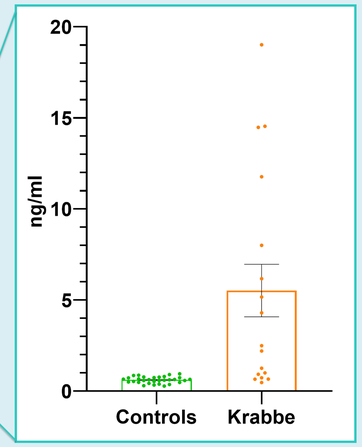
Method: An LC-MS/MS assay was developed using 50µl of plasma extracted using acetone:methanol (1:1) spiked with heavy labelled internal standard. Analysis was performed over a 5 min gradient on a C8 column connected to a Xevo TQ-S



Control	0.279 – 0.948 ng/ml
Gaucher	19.144 – 411.447 ng/ml
Krabbe	0.47-19ng/ml

Application to patient samples





Conclusion: Plasma lyso-gb1 is elevated well above the control range in all Gaucher samples but only 70% of Krabbe patients had levels higher than the control range. This assay has the potential to be suitable for clinical use for Gaucher disease but has limited use for Krabbe disease.

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