Quantitation of Stercobilin in the Fecal Material of Timothy Syndrome Mice as a Putative Biomarker for Autism

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OVERVIEW

Autism Spectrum Disorders (ASD) is a general term for a group of complex disorders that currently affects 1 out of every 68 children in the United States which has risen significantly since 2007 (up from 1 out of every 150 children).[1] In this study, we utilize a highly-controlled animal model of autism using transgenic Timothy Syndrome (TS) mice to determine if stercobilin is a viable biomarker for diagnosing autism.[2] A method to create labelled stercobilin isotopomers for quantitation by mass spectrometry was developed and fine-tuned to test the fecal material from transgenic mice and their littermates.[3] Response factor (RF) calculations were completed to determine the amount of spike that was necessary to quantify the amount of stercobilin in all of our samples. From here we aim to further evaluate the statistical significance of the depletion of stercobilin in "autistic" mice as a potential approach to diagnosing autism.

INTRODUCTION

What is autism?

- Autism spectrum disorder (ASD) and autism are both general terms for a group of complex disorders of brain development. [1]
- ASD can be associated with intellectual disability, difficulties in motor coordination and attention, and physical health issues such as sleep and gastrointestinal disturbances [1]

What is Stercobilin?

- Stercobilin ($C_{33}H_{46}N_4O_6$) is a member of the group of mammalian metabolites known as bilin tetrapyrroles.[4]
- Stercobilin is a product of heme metabolism formed from the degradation of bilirubin.[4]
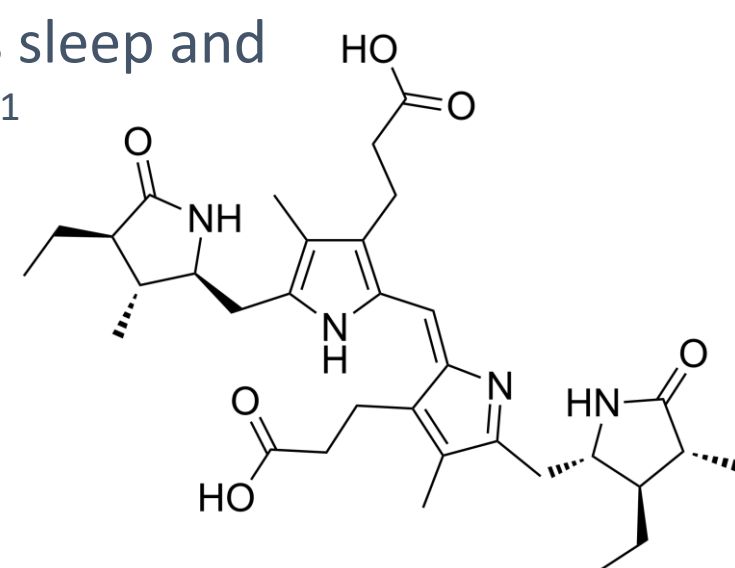
Fig.1: Structure of Stercobilin

METHODS

Timothy Syndrome Mice

- Timothy Syndrome mice have been shown to be accurate behavioral animal models for autism.[2]
- Supernatant from fecal collections are utilized to obtain statistical data of the concentration of stercobilin within each sample.

Fig.2: Control and TS2 mouse

Response Factor (RF) Calculations

- The Standard: Stercobilin in its normal form (MW: 595.34) ranging in concentrations between 5.05×10^{-11} mol to 6.14×10^{-8} mol.

- The Labelled Compound: A 90 µL spike of 2.77×10^{-6} M labelled stercobilin

$$\frac{R_{standard}}{C_{standard}} = RF \frac{R_{labelled}}{C_{labelled}}$$

Where …
$R = response$
$C = Concentration$
$RF = Response\ Factor$

Extraction of Stercobilin from Excrement

- The stercobilin needs to be extracted from the fecal material to be properly analyzed by mass spec.

- The most important part of the process is the removal of large particles and salts, therefore we utilize solid phase extraction (SPE).
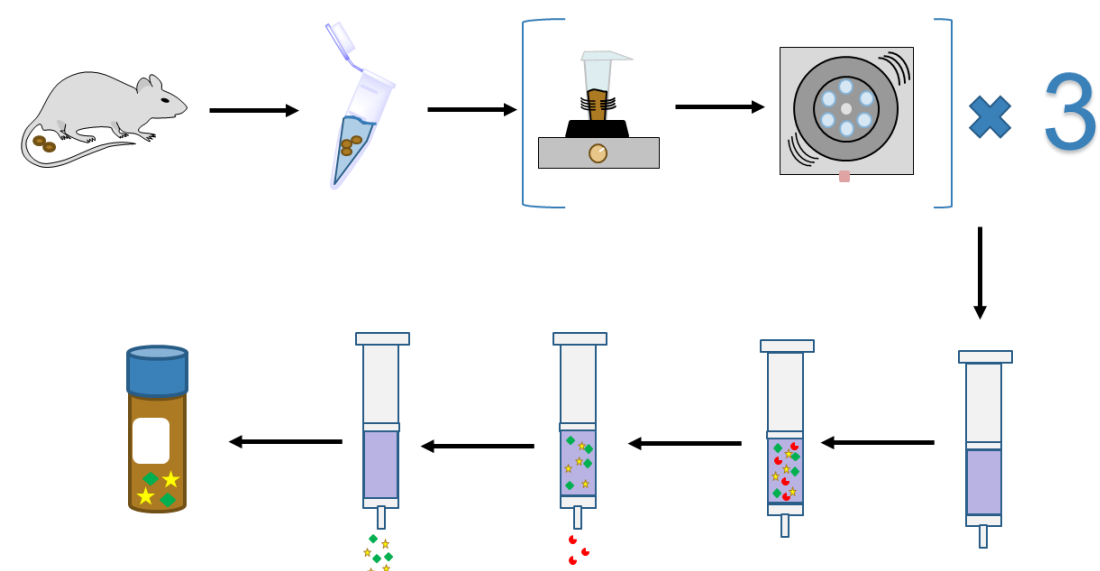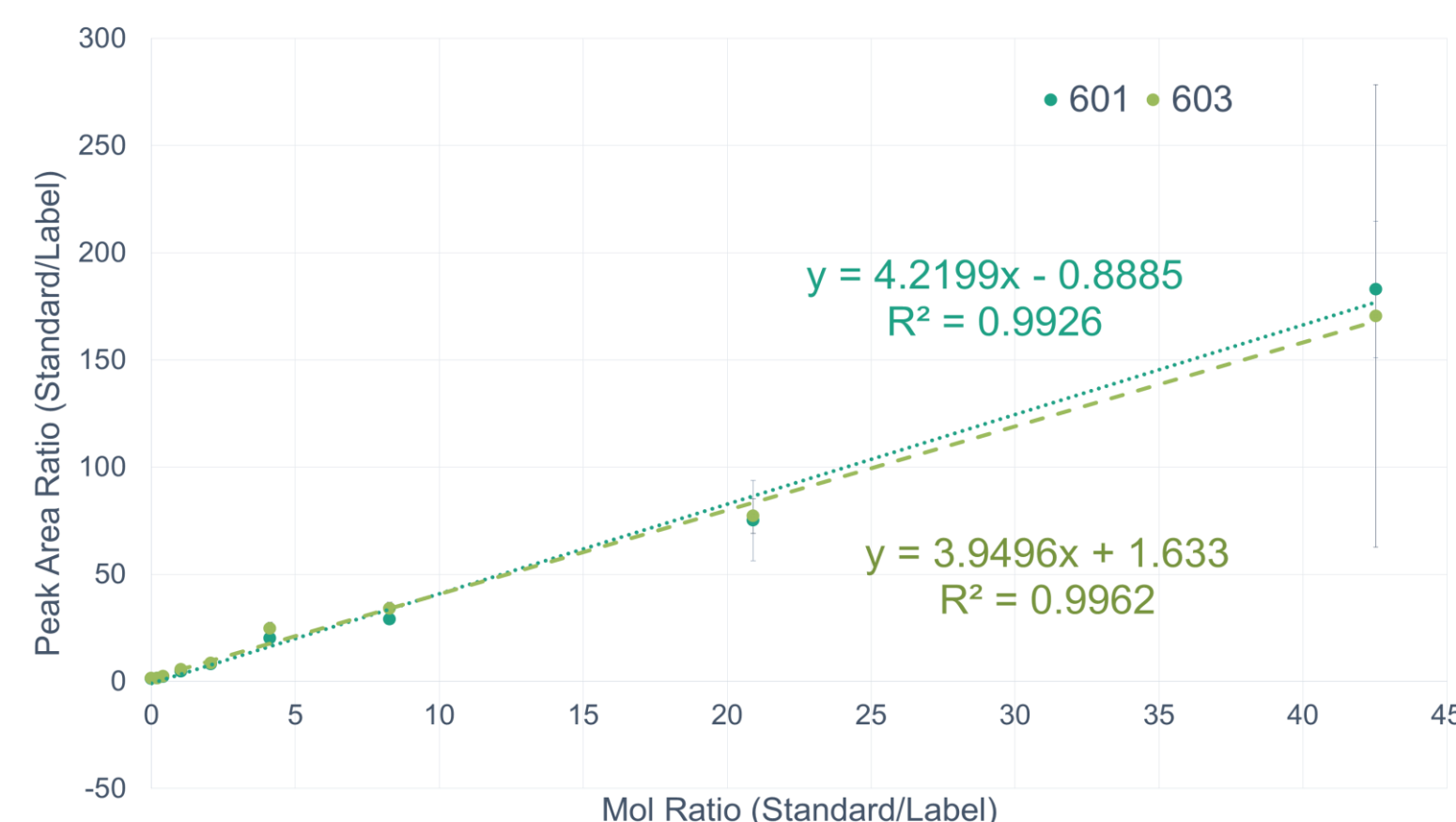
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Fig.3: Illustration of extraction process from mice feces.

Instrumentation

- All analysis is done by a Bruker Daltonics 12T Solarix FT-ICR MS equipped with a SmartBeam II frequency-tripled Nd:YAG MALDI.
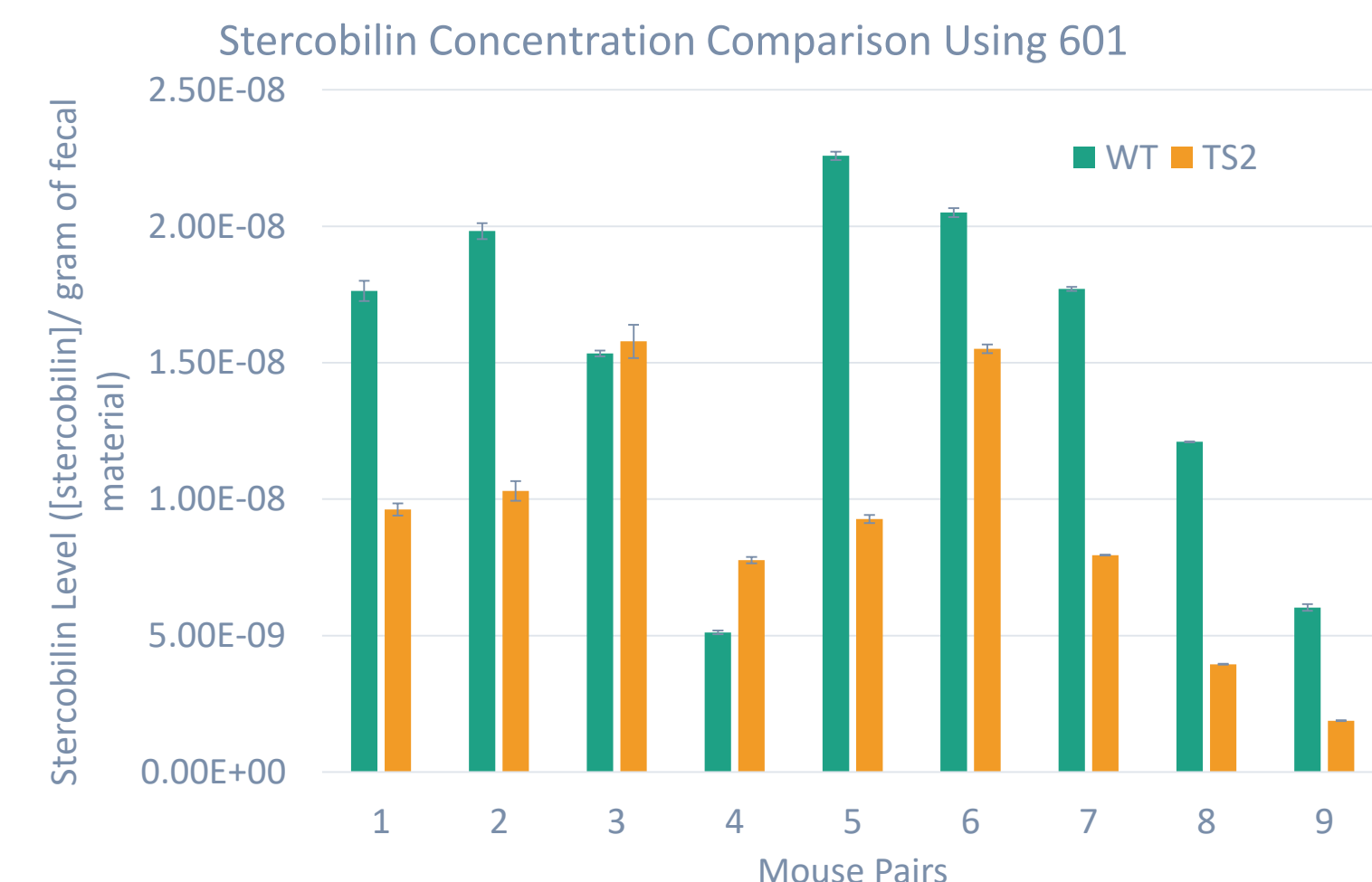
RESULTS

Response Factor Calculations

- 601 • 603

$y = 4.2199x - 0.8885$
$R^2 = 0.9926$

$y = 3.9496x + 1.633$
$R^2 = 0.9962$

Peak Area Ratio (Standard/Label) vs Mol Ratio (Standard/Label)

	601	603
LOD	1.37×10^{-11} mol	1.38×10^{-11} mol
LOQ	5.0×10^{-11} mol	5.0×10^{-11} mol
%CV	5.4- 25.0%	4.9-34.3%

Stercobilin in the Fecal Material of Mice

Stercobilin Concentration Comparison Using 601

WT TS2

	601	603
[WT]	$1.52 \times 10^{-8} \pm 6.2 \times 10^{-9}$ mol	$1.52 \times 10^{-8} \pm 8.4 \times 10^{-9}$ mol
[TS2]	$9.11 \times 10^{-9} \pm 4.60 \times 10^{-9}$ mol	$8.34 \times 10^{-9} \pm 3.90 \times 10^{-9}$ mol

CONCLUSIONS

- Average depletion of stercobilin of 40% and 45% when utilizing the peak areas of 601 and 603 respectively.
- p-Values of 0.03 and 0.04 tell us that at 95% confidence these values are statistically significant and not due to variability within samples; therefore fecal stercobilin depletion has promise as an autism biomarker.

FUTURE WORK

- Testing our hypothesis in human urine.
- Screening the fecal material analyzed for other potential biomarkers.
- Testing TS1 and TS1nn stercobilin concentrations.

REFERENCES

1. Autism Speaks. What Is Autism? https://www.autismspeaks.org/what-autism.
2. P. L. Bader, M. Faizi, et. al.. Mouse model of Timothy syndrome recapitulates triad of autistic traits. *Proc. Natl. Acad. Sci. U. S. A.*, *Early Ed.* **2011**, 108, 15432.
3. H.L. Rudolph, E. R. Sekera, T. D. Wood. Stable 18O-labeling Method for Stercobilin and Other Bilins for Metabolomics.*Rapid Communications in Mass Spectrometry Rapid Commun. Mass Spectrom.* **2016**, 30,1469.
4. E.Kuntz, H.-D. Kuntz. *Hepatology: Textbook and Atlas.* Springer, Wetzlar, **2008**.

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