COLLEGE OF SCIENCE
THE UNIVERSITY OF ALABAMA IN HUNTSVILLE

Research and Creative Experience for Undergraduates Program (RCEU) Summer 2016

Analyzing Urine in Mice with Tyrosinemia Type I Using NMR Spectroscopy

Emily Olsen, Gordon MacGregor and Bernhard Vogler,
Department of Biological Sciences and Department of Chemistry

Background

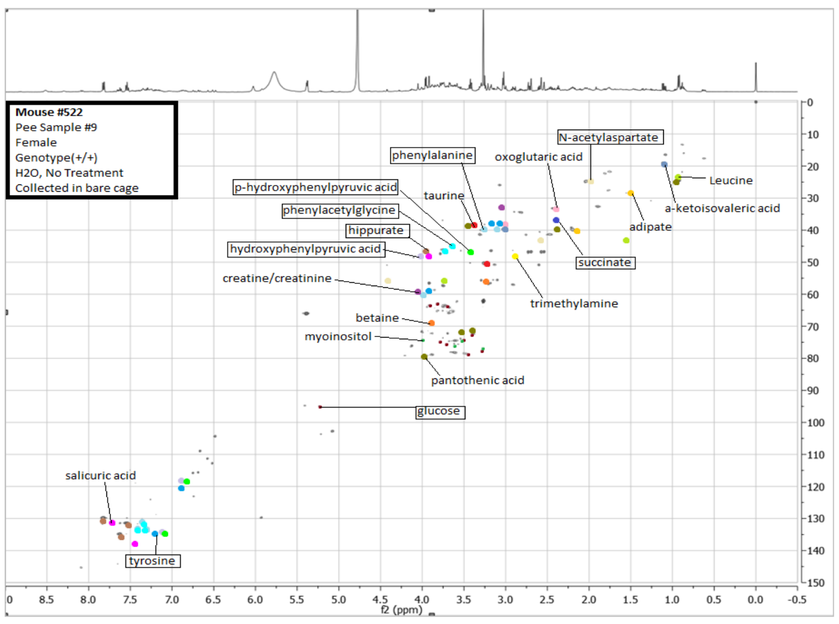
Tyrosinemia Type I is a rare autosomal recessive disorder that occurs on the FAH gene, which codes for the enzyme fumarylacetoacetase in the tyrosine metabolism. It is treated using the drug nitisinone (NTBC), helping to prevent the buildup of harmful metabolites, which can cause liver damage and altered behavior[1]. Urine was collected in order to be analyzed using 2D heteronuclear single quantum coherence (HSQC) nuclear magnetic resonance (NMR) spectroscopy. Different databases were accessed in order to create a library of metabolites with known properties to compare with the urine mix.

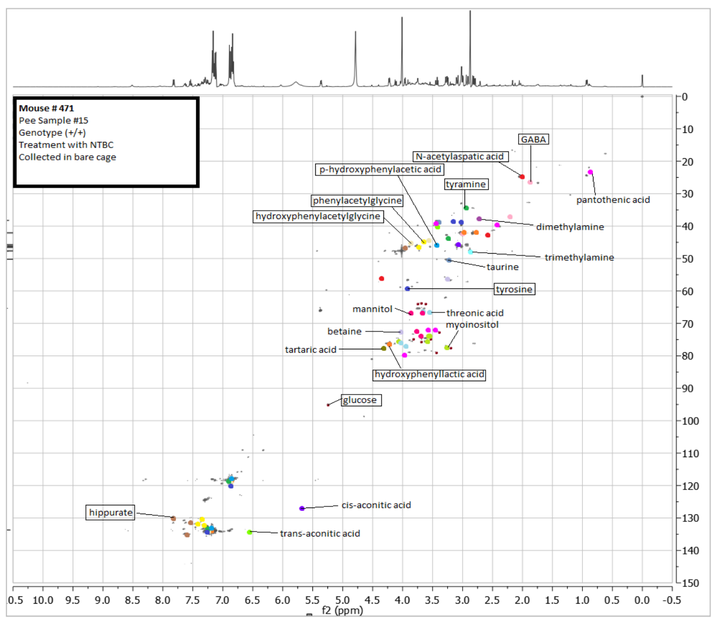
Methods

Once the urine was collected, it was frozen until prepared for the Varian 500 Hz NMR machine. The spectra produced by the urine were processed with Mnova, and then the peak lists were transferred to the MetaboMiner[2] software for identification of metabolites. The “Pee Library” in MetaboMiner was developed from the known properties of different compounds on HMDB[3] and BMRB[4]. These knowns were compared to the complex urine mix in an attempt to identify unknown peaks using the specific uniqueness values of the library and the known data from HMDB and BMRB.

Results

The spectra of the diseased mice *only* contained traces of hydrocinnamic acid. Both the disease mice and the mice with NTBC treatment contained tyramine, a metabolite of tyrosine, in the urine. In general, water mice contained weaker aromatic peaks than the other two types.





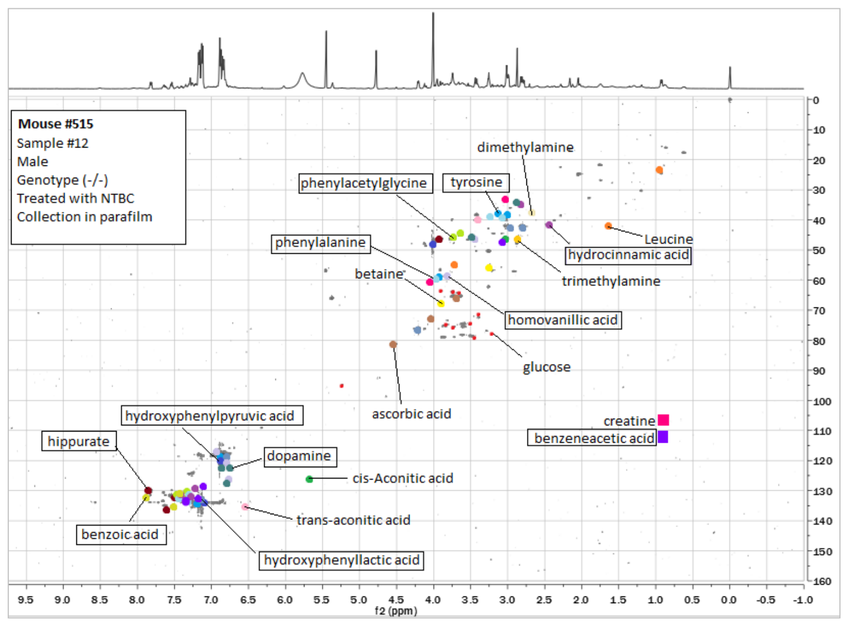


Figure 1: (Right) is the urine spectrum of a water mouse. *Figure 2*: (Middle) shows the urine spectrum of a non-diseased mouse with NTBC treatment. *Figure 3*: (Left) shows the spectrum of a diseased mouse treated with NTBC.

Conclusions

Metabolomics is a complex new area of chemistry, and the amount of data needed to gain a well-informed insight into metabolism is overwhelming to work with. The HMDB alone contains data for over 40,000 different compounds, with only a few of these having known NMR spectra. The number of metabolites of interest was approximately 200, but only 47 of them were able to be added to the MetaboMiner software. It is considered an accomplishment to identify the 21 metabolites of interest and an additional 32 metabolites from the software. The advantage of using HSQC is the dispersion of overlapped peaks from a normal proton spectrum. However, since the machine only identifies with carbon 13, this can also be a challenge to identify compounds. It should also be noted that urine is a complex biological sample, and “pulling apart” compounds is difficult when they are so similar to each other.

References

1. Hillgartner, M. H., Coker, S. B., Koenig, A., Moore, M. E., Barnby, E., MacGregor, G. G. Tyrosinemia type I and not treatment with NTBC causes slower learning and altered behavior in mice. *Journal of Inherited Metabolic Disease* [Online]. **2016**. 10.1007/s10545-016-9949-6 http://link.springer.com/article/10.1007%2Fs10545-016-9949-6 (accessed August 5, 2016).
2. *MetaboMiner*, version 2008. Jianguo Xia, Trent C Bjorndahl, Peter Tang and David S Wishart. Alberta, CA. (Accessed August 5, 2016).
3. *Human Metabolome Database* [Online], version 3.6. http://www.hmdb.ca/ (Accessed August 5, 2016).
4. *Biological Megnetic Reference Data Bank* [Online]. July 12, 2016. The Board of Regents of the University of Wisconsin System, http://www.bmrb.wisc.edu/ (Accessed August 5, 2016).

Acknowledgements

Acknowledgements go to David Cook of the RCEU program, UAH Office of the Provost, UAH Office of the Vice President for Research and Economic Development and the Alabama Space Grant Consortium.