*GitHub Repository:* [*https://github.com/SilasKuang/MetforminRNA*](https://github.com/SilasKuang/MetforminRNA)

*All source codes and figures can be found in this repository.*

Three groups of data were analyzed in pairs: SED, AET, AET+MET. We compared data between SED vs. AET, SED vs. AET+MET and AET vs. AET+MET.

First, transcription categories were plotted for each pair. For example, between AET vs. AET+MET, 9581 genes were analyzed. 848 of them showed statistically significant changes in RNA level. 407 were down regulated; 441 were upregulated. Out of the 848 genes, 359 were restrictively significant (p < 0.01).

Chart, bar chart

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Then, volcano plots were made for all the pairs. In these plots, we focus on the top-left corner and the top-right corner. Genes in these corners are the most significantly differentially expressed genes between the two groups of data.

Between AET vs. AET+MET, two genes have shown big differences in transcript levels – Nmrk2 and Tceal5. This finding, particularly Nmrk2, is consistent with that from the Nathan Shock center.

Chart, scatter chart

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Though due to time stringency, I have not yet been able to plot a Venn diagram to show the genes that are commonly affected between SED vs. AET and SED vs. AET+MET. I believe it is certainly worth taking a look, since the direct comparison between AET vs. AET+MET resulted in a different list of differentially expressed genes when compared to that between SED vs. AET and SED vs. AET+MET. For example, between SED vs. AET and SED vs. AET+MET, there is a gene named KY that is differentially expressed in both pairs, but not in the direct comparison between AET vs. AET+MET.

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Finally, enriched pathways were plotted for all 3 pairs.

There were only 4 pathways enriched for the AET vs. AET+MET analysis - PI3K−Akt signaling pathway, Protein digestion and absorption, Focal adhesion, and ECM−receptor interaction. In regard to the number of genes being affected in the pathways, PI3K−Akt signaling pathway and focal adhesion seemed to be the most enriched pathways.

As for the other 2 pairs, many pathways were enriched, including but not limited to the longevity regulation pathways.

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