PREDICTIVE MODELING AND CHARACTERIZATION OF ALTERED METABOLIC ACTIVITY IN PROPIONIC ACIDEMIA

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BACKGROUND

Propionic Acidemia (PA) and other Inborn errors of metabolism give rise to global alterations in metabolic function. Many of these alterations cannot be predicted from enzyme function *per se*, due to the high degree of interconnectivity between metabolic reactions. However, these emergent changes in metabolism can be studied using genome-scale metabolic models (GEMs). Constraint-based and topological analysis of metabolic networks can yield insights into disease-related perturbations that occur in cellular metabolite uptake, production, and internal utilization. Thus, these models provide a platform for predicting potential biomarkers and therapies for inborn errors of metabolism, as well as a wide variety of other diseases affecting metabolic function. Furthermore, experimental data (e.g., transcriptomic data) can be integrated into the analysis of GEMs to improve the accuracy of the predictions they generate.

APPROACH

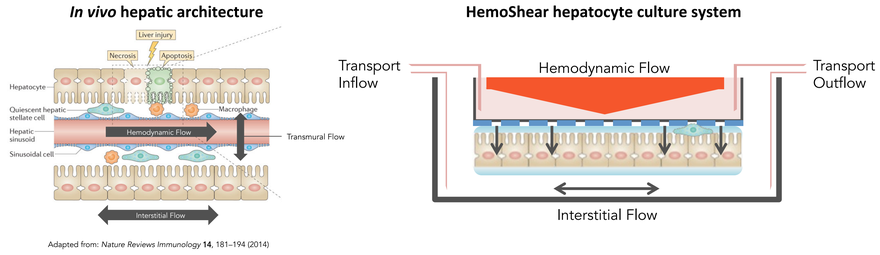
We utilized an *in vitro* organotypic cell culture system to grow hepatocytes from a donor with PA, as well as five normal donors. RNA-seq was used to generate transcriptomic profiles for each experimental group. Differential mRNA expression analysis was performed and integrated into a state-of-the-art hepatocyte-specific GEM. Three approaches were used to interpret the differential expression results: (1) gene set tests based on metabolic subsystems annotated within the GEM; (2) topological association of metabolites and differentially expressed enzyme mRNAs through connections annotated in the GEM; and (3) constraint-based modeling of normal and PA flux distributions.

CONCLUSIONS

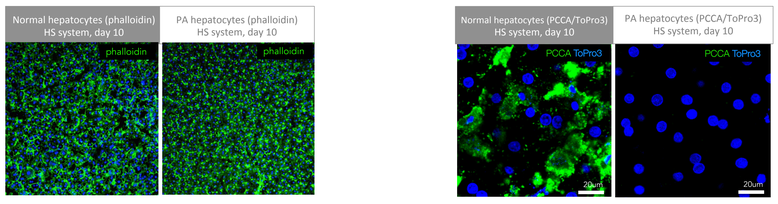
We show that we are able to infer relevant, known disease biology associated with PA by utilizing the GEM as a platform for analyzing transcriptomic data. Some examples of system-level perturbations that we observed include alterations to amino acid metabolism, energy production, and pyruvate utilization. In addition, we present novel hypotheses regarding altered immune function, and a potential disruption of normal glycosylation activity. To our knowledge, this is the first analysis of transcriptomic data from a PA patient utilizing a genome-scale metabolic reconstruction.

EXPERIMENTAL SYSTEM & STUDY DESIGN

- The HemoShear liver system technology is based on the microarchitecture of the liver sinusoids and creates a flow-based culture system that recapitulates transmural perfusion, nutrient gradients, and interstitial fluid movement. Details of the system design and function are described by Dash et al. [1].

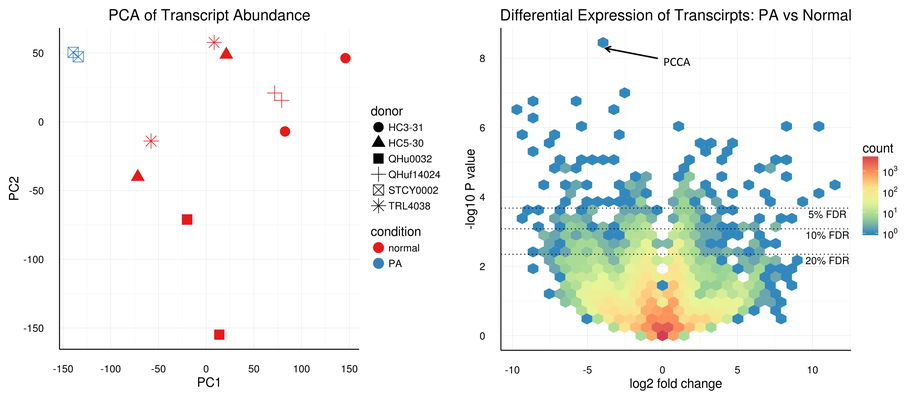


Adapted from: *Nature Reviews Immunology* **14**, 181–194 (2014)



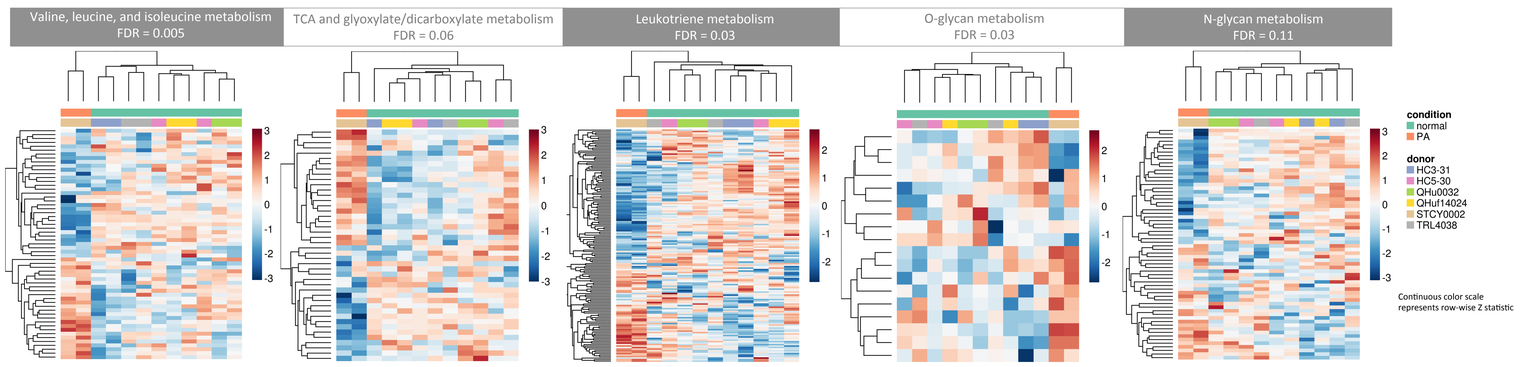
- Transcriptomic data was generated from hepatocytes grown for 10 days in the HemoShear system.
- Hepatocyte donors included 5 normal donors and one PA patient with c.973C>T/c.973C>T; p.Arg313Stop/p.Arg313Stop nonsense mutations in PCCA.
- Transcriptomic data was integrated with a modified version of the iHepatocytes2322 genome-scale metabolic reconstruction published by Mardinoglu et al. in 2014 [2].

SUMMARY OF THE PA VS NORMAL DIFFERENTIAL TRANSCRIPTOMIC SIGNAL



- Principal component analysis of global transcript abundance shows PA and normal samples tend to separate along the 1st principal component.
- Transcriptomic differences are robust: 231 differentially expressed transcripts at an FDR threshold of 10%
- PCCA shows the most significant differential abundance

REGULATION OF METABOLIC SUBSYSTEMS AND METABOLITE-CENTRIC ENZYMATIC NEIGHBORHOODS

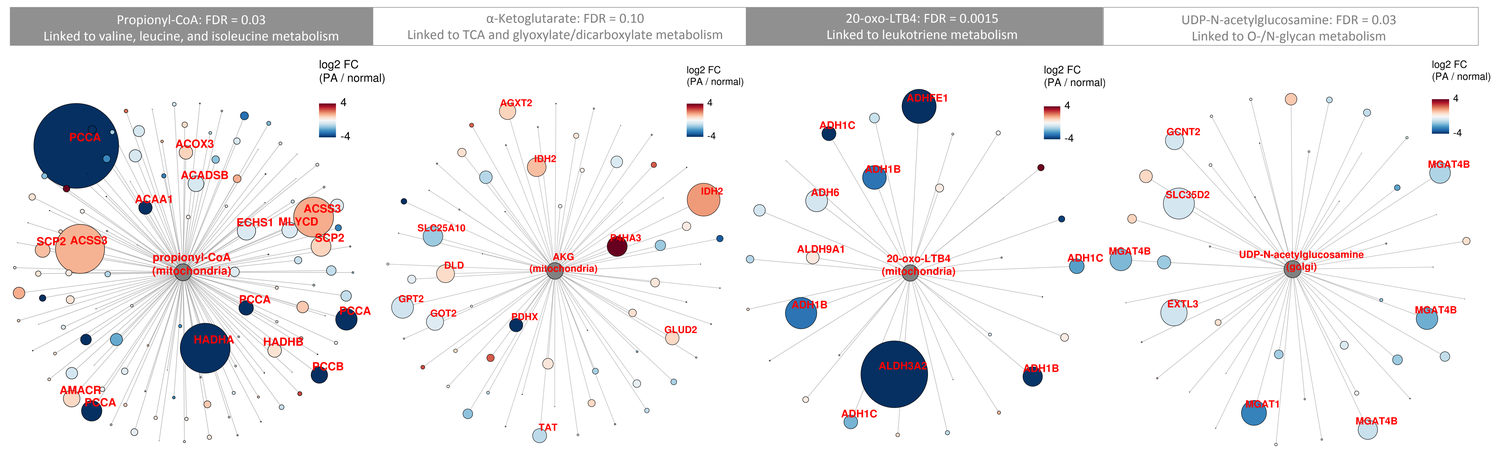


Differential regulation of metabolic subsystems (above)

- Transcript abundance heatmaps of a selection of metabolic subsystems significantly enriched for differentially expressed transcripts.
- PA samples cluster separately from normal samples.
- Includes expected subsystems: valine, leucine and isoleucine metabolism and TCA cycle
- Transcriptional regulation of leukotriene metabolism is altered, suggesting possible immune dysregulation.
- O- and N-glycan metabolism also shows evidence of transcriptional regulation, suggesting alterations in glycosylation and Golgi function.

Regulation of metabolite centric enzymatic neighborhoods (below)

- Networks show the connectivity of enzymes to specific metabolites (central nodes)
- Each network is significantly enriched for differentially expressed transcripts.
- Each metabolite plays an important role in the subsystems above.
- Color of nodes represent log2 fold change (PA/normal)
- Size of transcript nodes are proportional to the statistical significance of the PA vs normal differential expression.
- Transcript nodes with labels have a raw P value ≤ 0.05.
- Metabolite-centric differential expression enrichment is consistent with subsystem-level differential expression



CONSTRAINT-BASED MODEL OF METABOLIC FLUX IN NORMAL AND DISEASE STATES

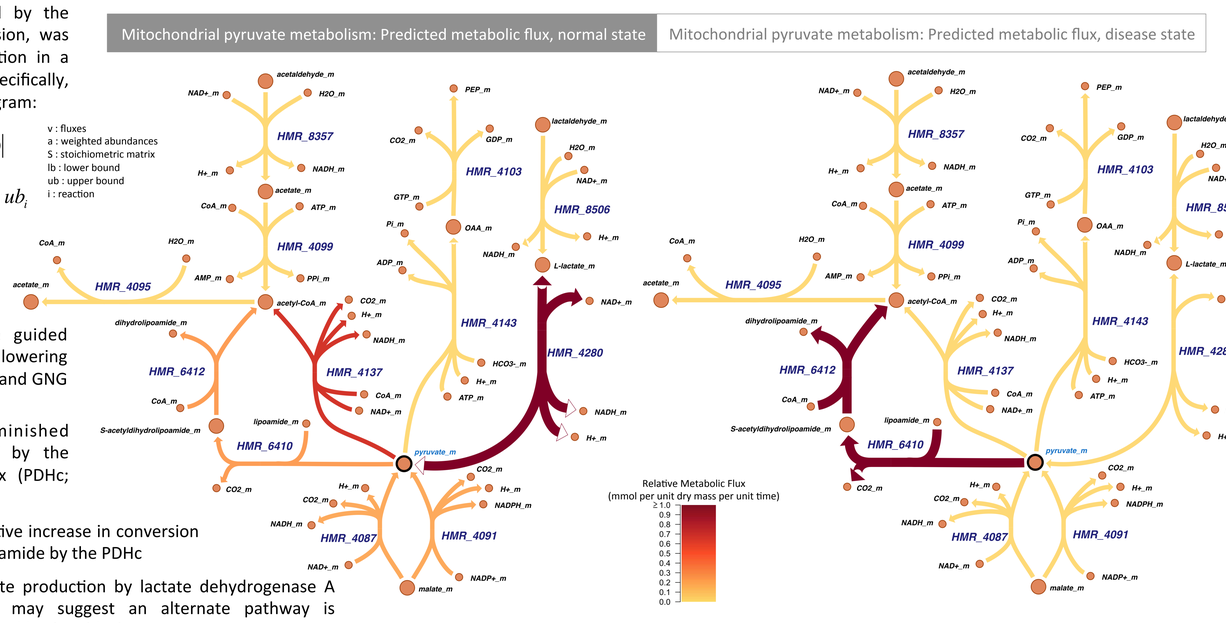
- Gene expression data, weighted by the probability of differential expression, was used to calculate a flux distribution in a manner similar to Lee et al. [3]; specifically, we solved the following linear program:

Minimize: $C = \sum_i |\log(v_i) - \log(a_i)|$

Subject to: $S \cdot \vec{v} = \vec{0}$ and $lb_i \le v_i \le ub_i$

v : fluxes
a : weighted abundances
S : stoichiometric matrix
lb : lower bound
ub : upper bound
i : reaction

- Normal and PA models based on transcriptomic states. PA model also includes a full KO of the PCCA/PCCB reaction
- In addition, the models were guided toward a gluconeogenic state by lowering the relative cost of glucose export and GNG reactions.
- Model correctly predicts diminished decarboxylation of pyruvate of by the pyruvate dehydrogenase complex (PDHc; reaction coded as HMR_4137).



- Solutions suggest a potential relative increase in conversion of pyruvate to S-acetyldihydrolipoamide by the PDHc
- The predicted decrease in L-lactate production by lactate dehydrogenase A (reaction coded as HMR_4280) may suggest an alternate pathway is responsible for the clinically observed lactic acidosis

REFERENCES

[1] Dash A., et al. Hemodynamic flow improves rat hepatocyte morphology, function and metabolic activity in vitro. *Am J Physiol.* 2013.
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[3] Lee et al. Improving metabolic flux predictions using absolute gene expression data. *BMC Syst Biol*. 2012.

ACKNOWLEDGEMENTS

The authors would like acknowledge Jason Papin for useful discussions regarding GEM analysis, and the technical assistance of Christin Hamilton, Crystal Passmore, Andy Pryor, Nathan Day, Josh Thomas, Morgan Donovan, Sandi Walton and Diana Berry.

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