Sequence coverage of genes for inborn errors of metabolism by DNA prepared from residual newborn screening dried blood spots

An update on the NBSeq Project
within the NSIGHT Consortium

Bob Currier, PhD
Genetic Disease Screening Program
California Department of Public Health

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Project 1: Sequencing in NBS

- 1570 dried blood spots from the CDPH Biobank
  - 1357 true positive metabolic disorder cases
  - 203 false positive and false negative metabolic disorder cases
- Extract and sequence DNA
- Annotate variants in a set of ~90 primary metabolic genes and additional genes identified through pathway analysis
- Identify variants associated with metabolic disorders. Compare with variants found in false positive and false negatives

Project 1: Sequencing in NBS
Analytical Plan

- Analysis 1: Call variants using no information other than the sequence data.
  - (This models the performance of sequencing as primary population screening.)
  - Determine sensitivity, specificity, and positive predictive value.
- Analysis 2: Call variants using results of standard newborn screening, in particular, MS/MS profile.
  - (This analysis models the performance of sequencing as a second tier test.)
- Analysis 3: After unblinding diagnosis, analyze sequence variants for associations with clinical history.

DNA Isolation Using Autogen965 DNA Extractor

- 96-well deep plates
- One 3 mm DBS punch per well, 2 punches per sample
- Method: ProK digestion, organic extraction, alcohol ppt, resuspension
- Yield by Nanodrop OD260: 200 – 2000 ng DNA per punch (mean 650 ng)
- 260/280 mean 1.8

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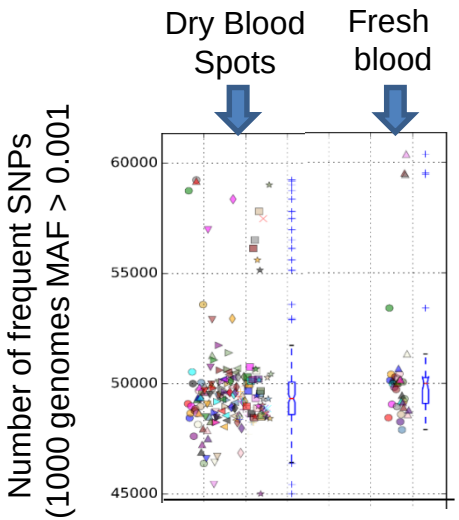
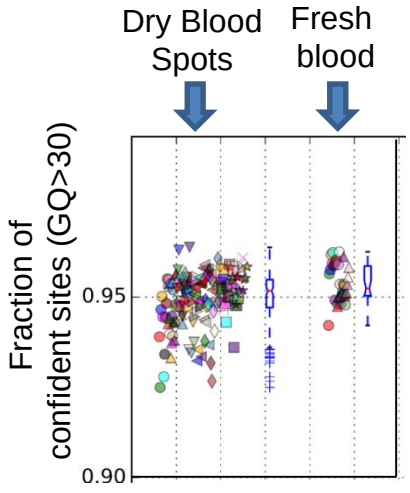
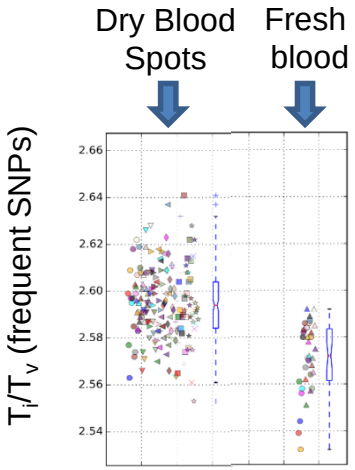
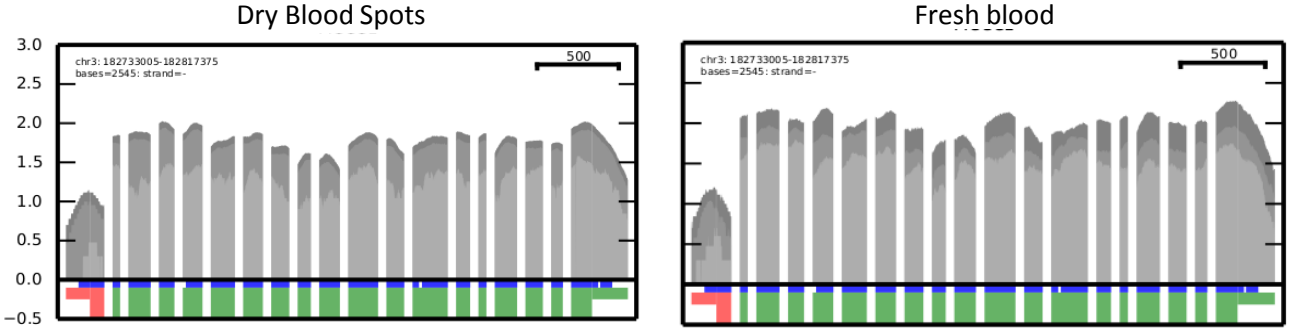
Exome sequence quality from DBS comparable to that from fresh blood







Gene Coverage for a metabolic disorder gene (MCCC1)



Only 5/188 blood spot samples had poor quality metrics

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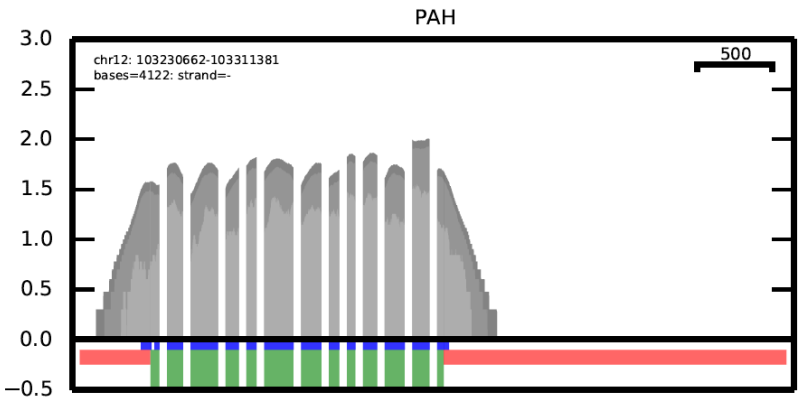
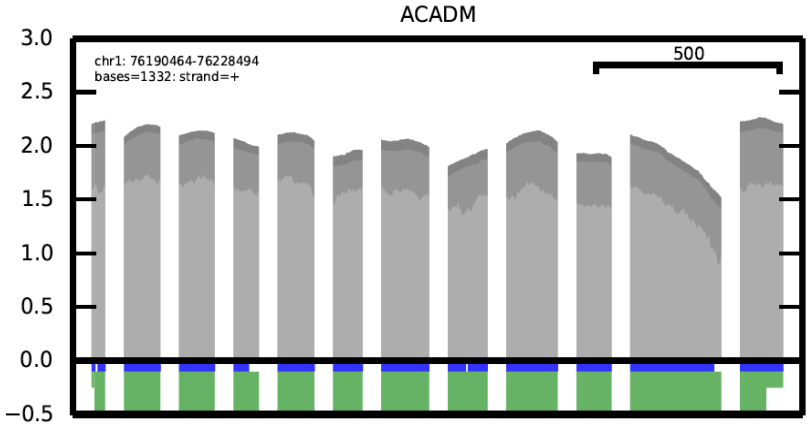
Coverage Plots

PAH (PKU)



ACADM (MCADD)

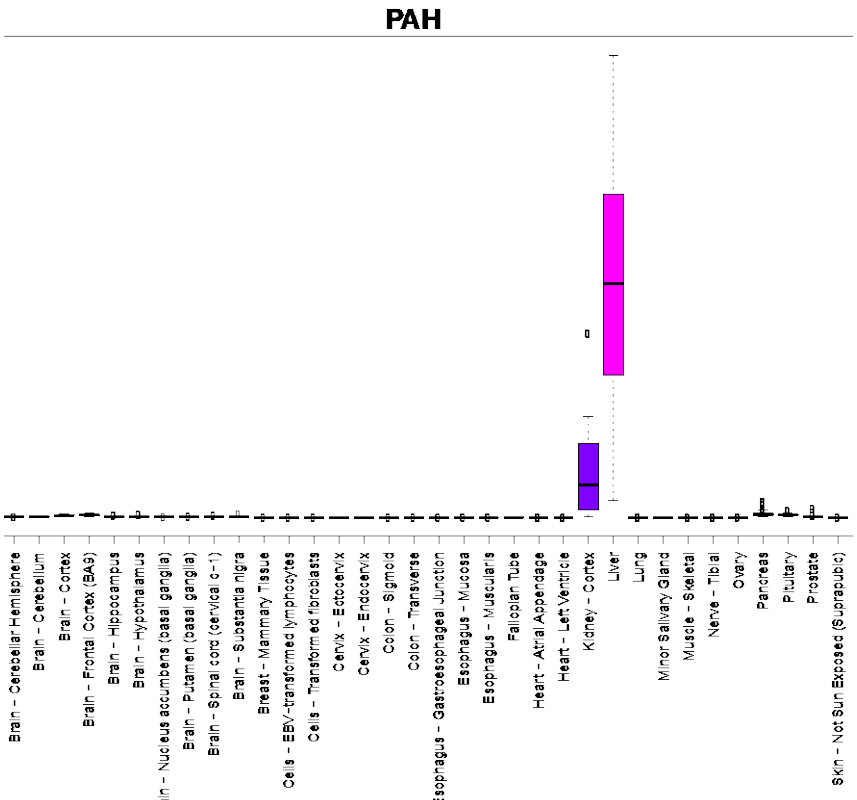


Exons are denoted by the green bars, and UTR by the surrounding red bars. Introns are not according to scale. The capture region (exons plus some of the UTRs) is in blue. The vertical scale is $\log_{10}$(coverage). The top of the darkest grey represents the median coverage, the medium grey is the 20th percentile, and the lightest grey is the least well covered sample.

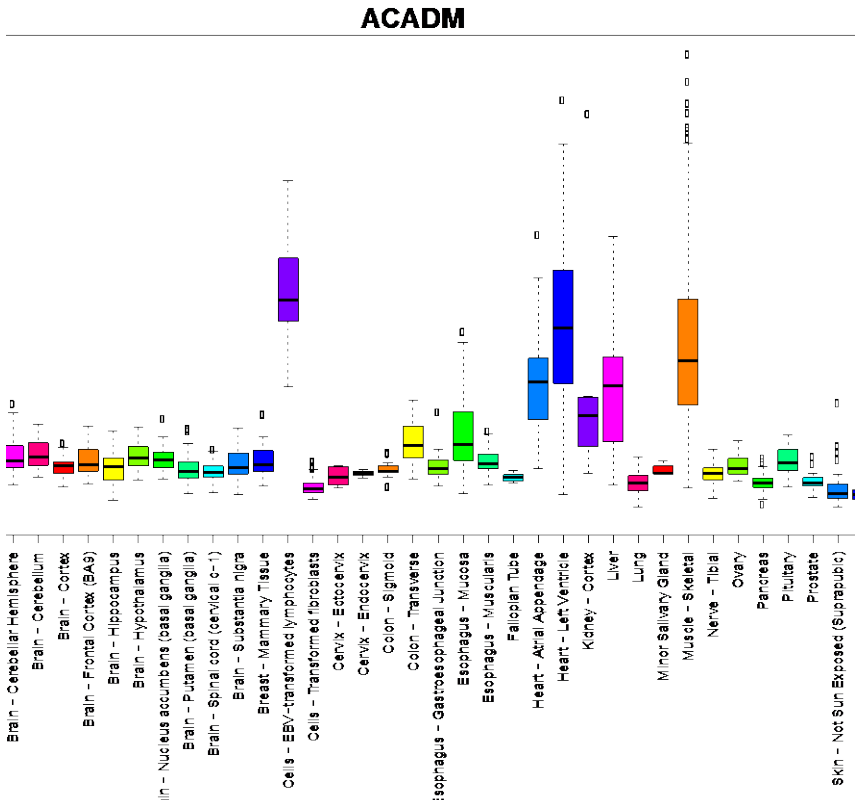
Gene Expression

For PAH, the essential tissue is the liver. For ACADM, many tissues are involved. Different transcripts are expressed differently in different tissues, making variant interpretation more complex.

PAH (PKU)



ACADM (MCADD)



Variant Calling (the easy part)

- Variants that completely interrupt the formation of the resulting protein are easy:
  - Addition/deletion of Stop Codons
  - Addition/deletion of Splice Sites
  - Frame shifts

Variant Calling (the hard part)

- Non-synonymous variants.
  - What is the impact on protein synthesis?
  - What is the impact on enzyme function?
  - What is the impact on enzyme stability?
- Large scale copy number variants
  - Essentially invisible

Specific Issues

PAH (PKU)

- PKU is characterized by residual function < 1% of normal.

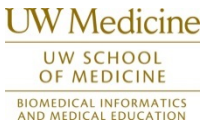
ACADM (MCADD)

- One mutation is too common to be responsible for a “rare disease”.
  - 985A>G (Lys304Glu)

Conclusions

- Sufficient high quality DNA can be prepared from two 3 mm punches from a DBS to produce exome sequence data.
- Variant assessment for disease determination remains very complex, with challenges at every level of interpretation.

Thanks to Our Collaborators and Funders



UCSF	Laia Bars	**UC Hastings Law**	**NBSTRN**
Jennifer Puck	Mark Kvale	Jaime King	Amy Brower
Barbara Koenig	Neil Risch	Monica Smith	
Pui-Yan Kwok	Richard Lao		**ACMG**
Alan Nguyen		**The Hastings Center**	Irina Butler
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Carol Fraser-Browne	Aashish Adhikari		Tiina Urv
Diya Vaka	Yangyun Zou	**TCS**	
Eunice Wan		Uma Sunderam	**NIH/NHGRI**
		Ajithavalli Chellappan	
Flavia Chen	**CDPH**		Anastasia Wise
Galen Joseph	Bob Currier	Kunal Kundu	Brenda Iglesias
George Freedman		Rajgopal Srinivasan	Joy Boyer
Joseph Shieh	**UW**	Sadhna Rana	
Julie Harris-Wai	Sean Mooney		