NSIGHT NBSeq: Evaluating the Potential of Exome Sequencing for Newborn Screening

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on behalf of the NBSeq Team

Disclosures

I have no financial interests to disclose

NBSeq, UCSF and Collaborators

- Could exome sequencing replace the current mass spectrometry (MS/MS) performed by public health laboratories as a newborn screen for inborn errors of metabolism (IEMs)?

- Could sequencing augment the information from current newborn screening and improve case resolution and public health outcomes?

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LETTERS

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The role of exome sequencing in newborn screening for inborn errors of metabolism

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Public health newborn screening (NBS) programs provide population-scale ascertainment of rare, treatable conditions that require urgent intervention. Tandem mass spectrometry (MS/MS) is currently used to screen newborns for a panel of rare inborn errors of metabolism (IEMs)[1–4]. The NBSeq project evaluated whole-exome sequencing (WES) as an innovative methodology for NBS. We obtained archived residual dried blood spots and data for nearly all IEM cases from the 4.5 million infants born in California between mid-2005 and 2013

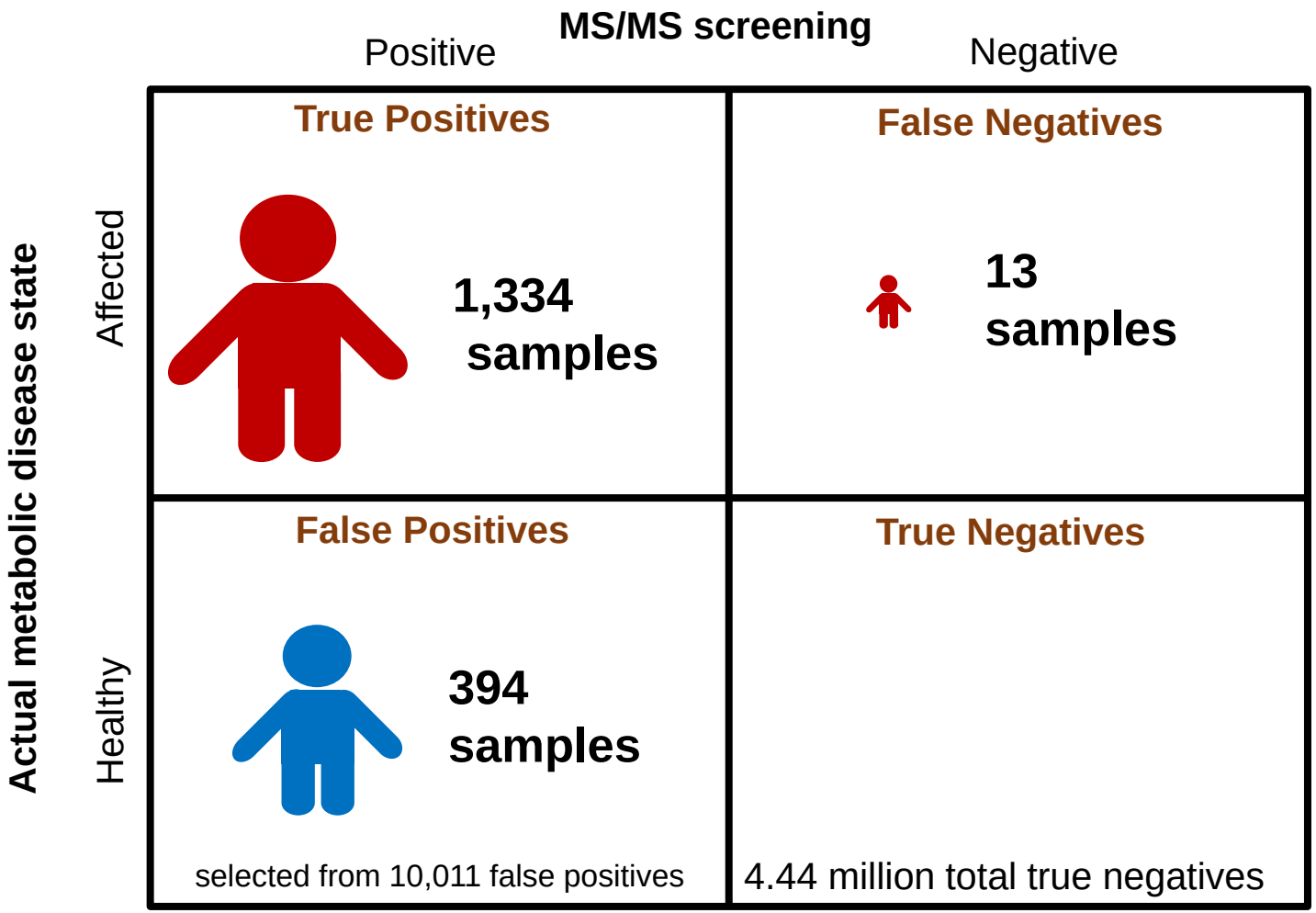
newborns[12]. Yet, population-scale studies to establish performance characteristics of sequencing for NBS have not been reported. NBS IEMs provide an ideal model for evaluating the role of sequencing in population screening because most are Mendelian disorders affecting well-understood biochemical pathways and many have been studied extensively. Moreover, sensitivity and specificity of sequence-based detection of IEMs can be directly compared to those of current MS/MS screening. Studying WES to identify IEMs already included in NBS can also suggest its potential utility for fur-

Findings from the NBSeq study have been published recently

Whole exome sequencing of dried blood spots from every affected child for 8.5 years in CA

Primary question:

Can sequencing replace or augment current MS/MS based newborn screening?



CPDH data from 7 July 2005 to 31 December 2013

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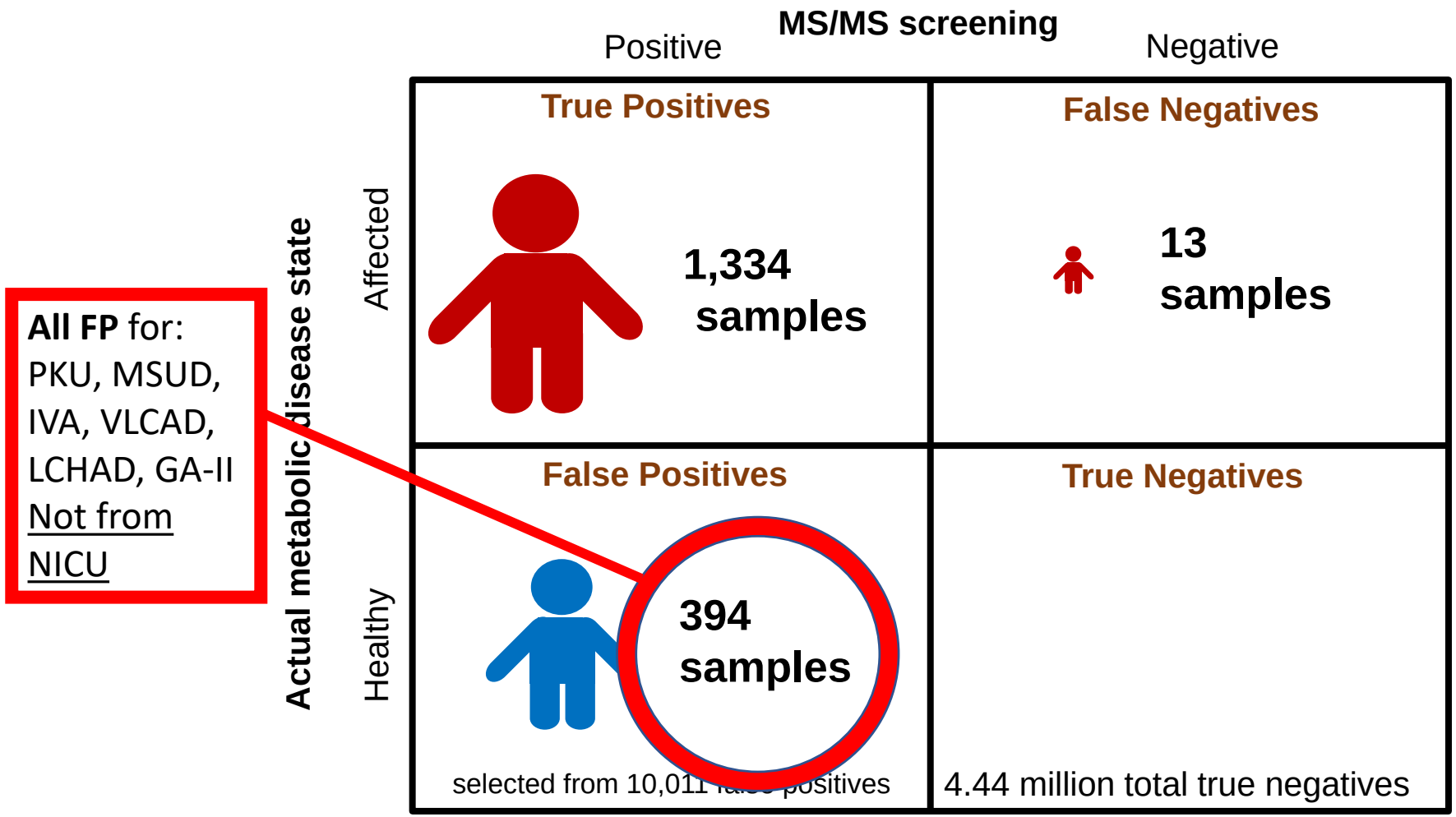
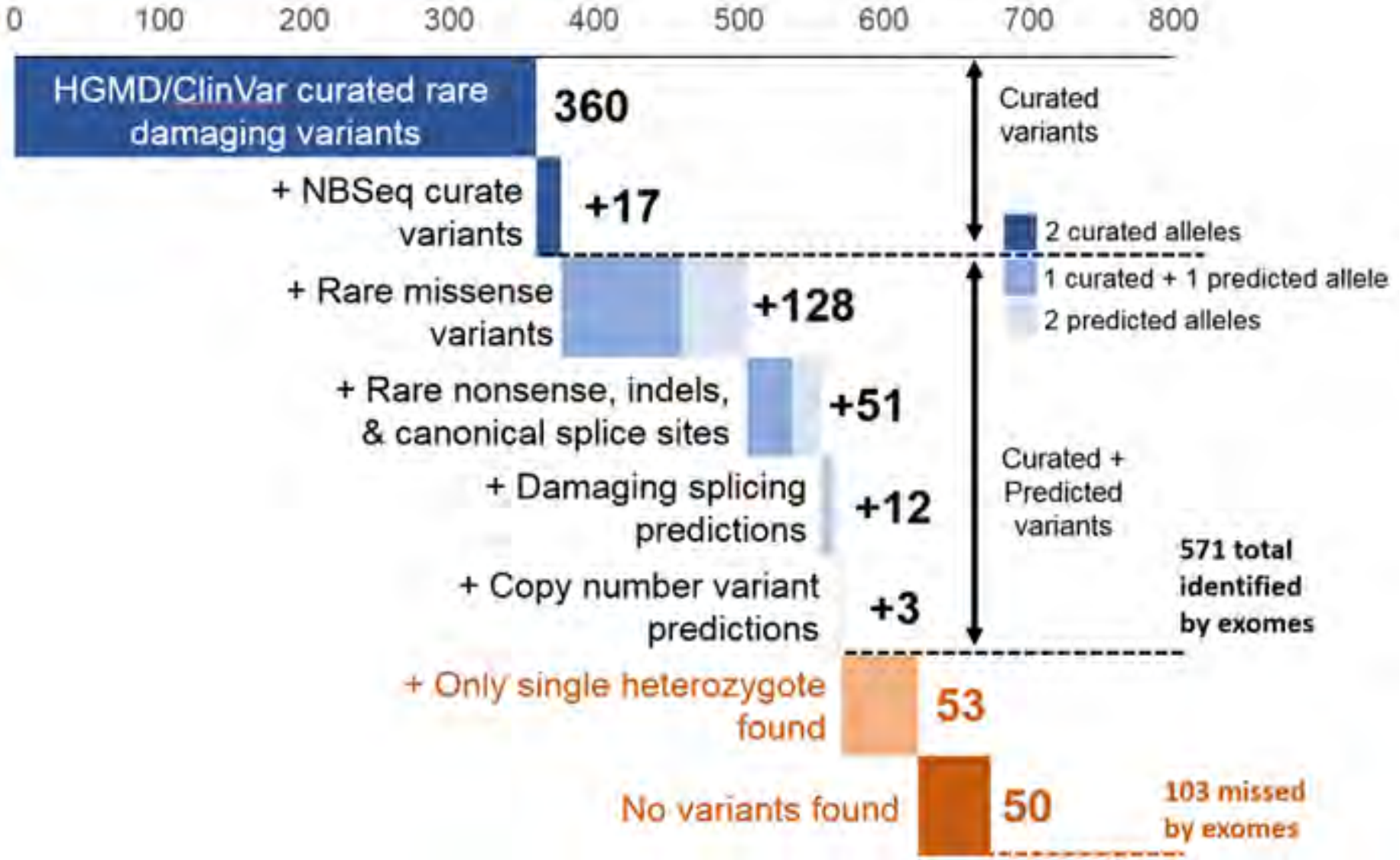


CPDH data from 7 July 2005 to 31 December 2013

NBSeq DBS and Exomes

- Largest-to-date WES study of unbiased IEM-affected cases, a benchmark for capabilities of WES for NBS.
- 1,728 DBS requested
  - 1,334 with IEM (including 9 affected cases missed by MS/MS).
  - 394 false positives.
- 538 of these omitted
  - 338 not sequenced due to limited funds (caucasian SCAD, 3MCC, PAH) or 26 not having an IEM in the core disease panel.
  - 200 WES attempted; exomes did not pass quality control.
- **1,190 exomes analyzed** (DNA from DBS good for WES)
  - 805 with an IEM, 385 false positives by MS/MS.
  - 178 = validation set, to develop screening analysis pipeline.
  - 1, 012 = test set: 674 with an IEM.

Performance of Sequencing for Identification of Affected Individuals (n=674)

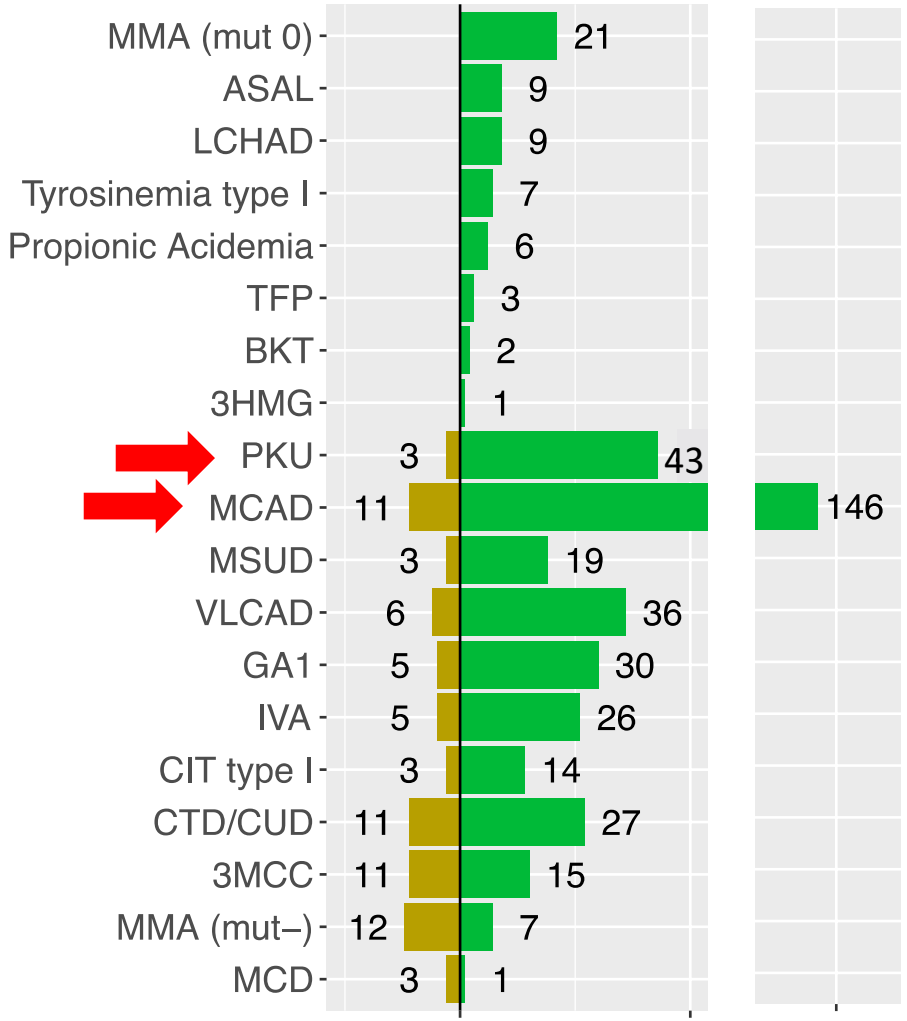


Summary of Outcome

- Sensitivity for predicting disease from genetic variants had an overall weighted average sensitivity of 88% and specificity of 95%.
- Sensitivity varied across IEMs
  - PKU:93%, 3-MCC:71%, citrullinemia type1:77%
- Current NBS by MS/MS is 99.3% sensitive and 99.7% specific; therefore, DNA sequencing alone cannot currently replace MS/MS screening for these disorders.
- A pipeline using only curated variants achieved 99.6% specificity and 58% sensitivity.

Exome Slice Sensitivity By Disorder

Core Metabolic Disorders

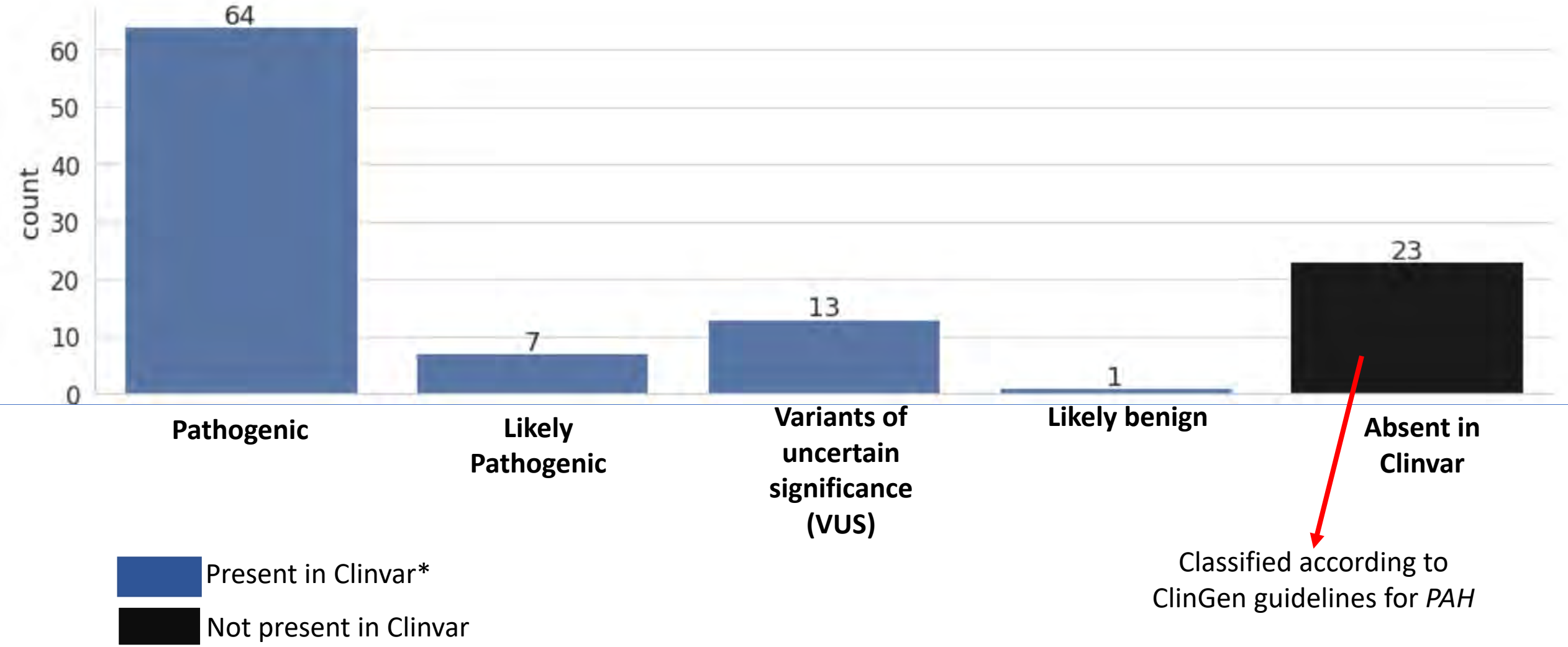


Overall Exome Sensitivity 88%

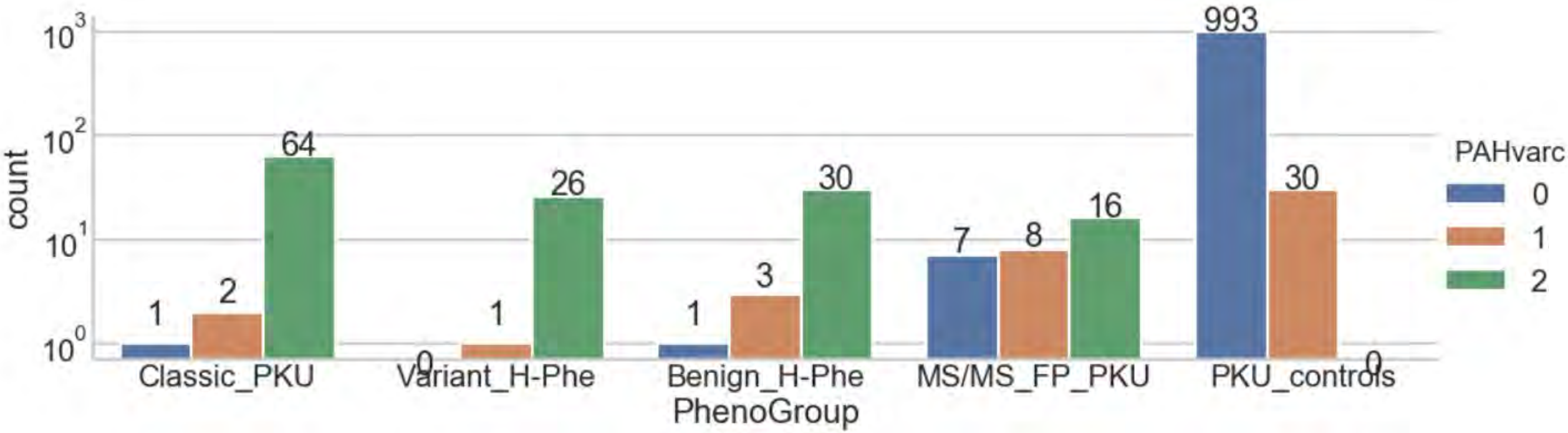
Exome identified IEM

Exome missed IEM

108 different PAH variants flagged by exome screening pipeline



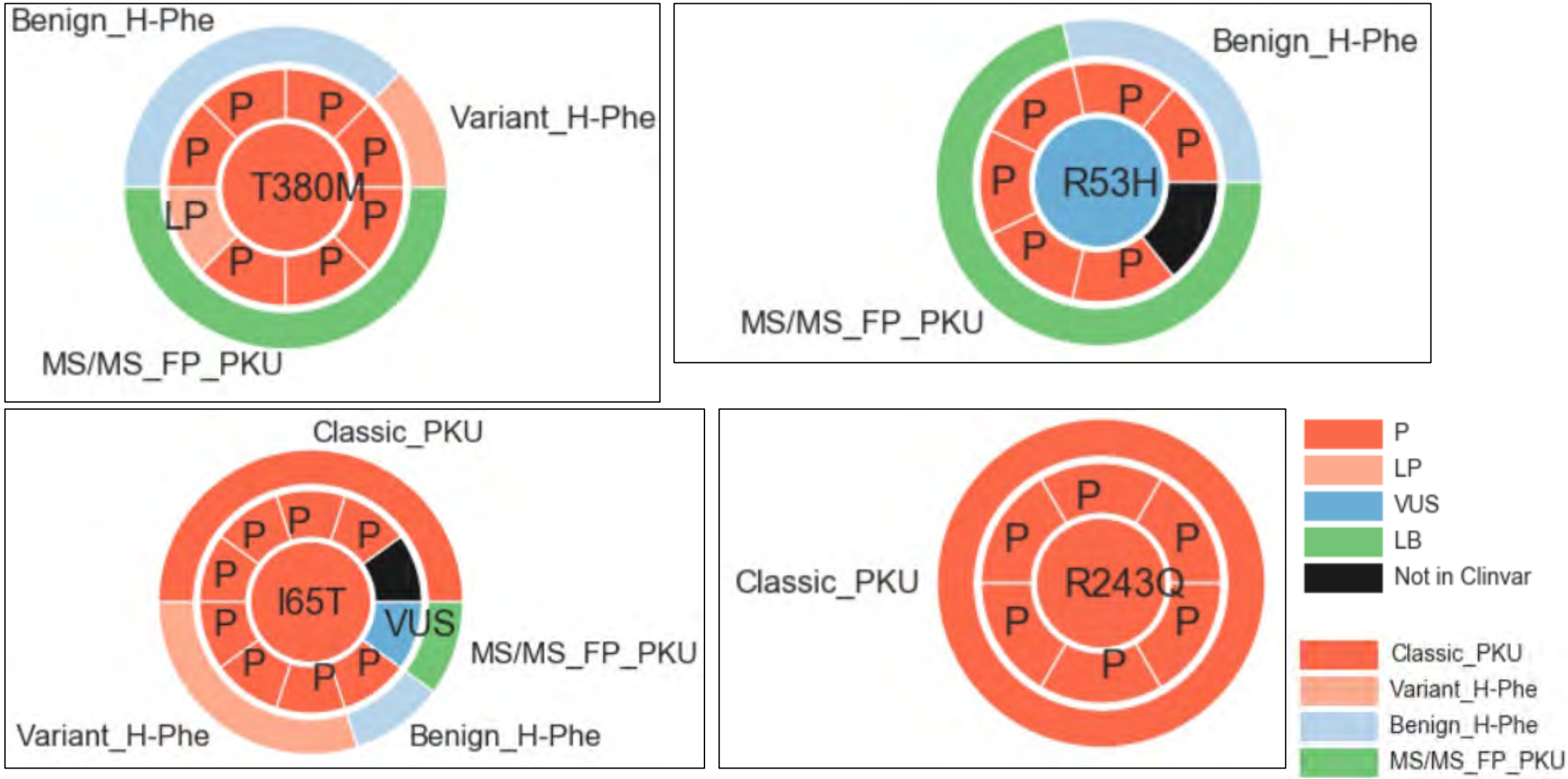
NBSeq PKU phenotypic groups stratified by number of PAH alleles flagged per sample by screening pipeline



Diplotype pathogenicity assertions in samples with two PAH variants

Phenotype Group	Total number	Number with 2 PAH alleles	Annotations of the two PAH alleles					
			P,P	P,LP	P,VUS	P,LB	LP, VUS	VUS,VUS
Classic PKU	67	64	49	3	11	1	0	0
Variant H-PHE	27	26	22	0	3	0	1	0
Benign H-PHE	34	30	19	2	5	0	2	2
FP PKU Screen	31	16	5	1	8	0	0	2

P – Pathogenic, LP – Likely pathogenic, VUS – Variant of uncertain significance, LB – Likely benign


Benign_H-Phe
Variant_H-Phe
T380M
P
LP
MS/MS_FP_PKU
Benign_H-Phe
R53H
MS/MS_FP_PKU
Classic_PKU
I65T
VUS
MS/MS_FP_PKU
Variant_H-Phe
Benign_H-Phe
Classic_PKU
R243Q
P
LP
VUS
LB
Not in Clinvar
Classic_PKU
Variant_H-Phe
Benign_H-Phe
MS/MS_FP_PKU

What Did NBSeq Learn about WES Applied to NBS?

- Genetic tests do not guarantee that one can identify disease perfectly.
- 1/3 of variants had not been seen before, so it was hard to predict what effect they would have.
- WES analysis was insufficient to identify pathogenic variants in populations with diverse ethnic backgrounds.
- The notion of variants being pathogenic is an oversimplification for autosomal recessive disorders such as most IEMs. Need combined prediction, diplotypes/genotypes.

NBSeq: Exome Sequencing for Newborn Screening

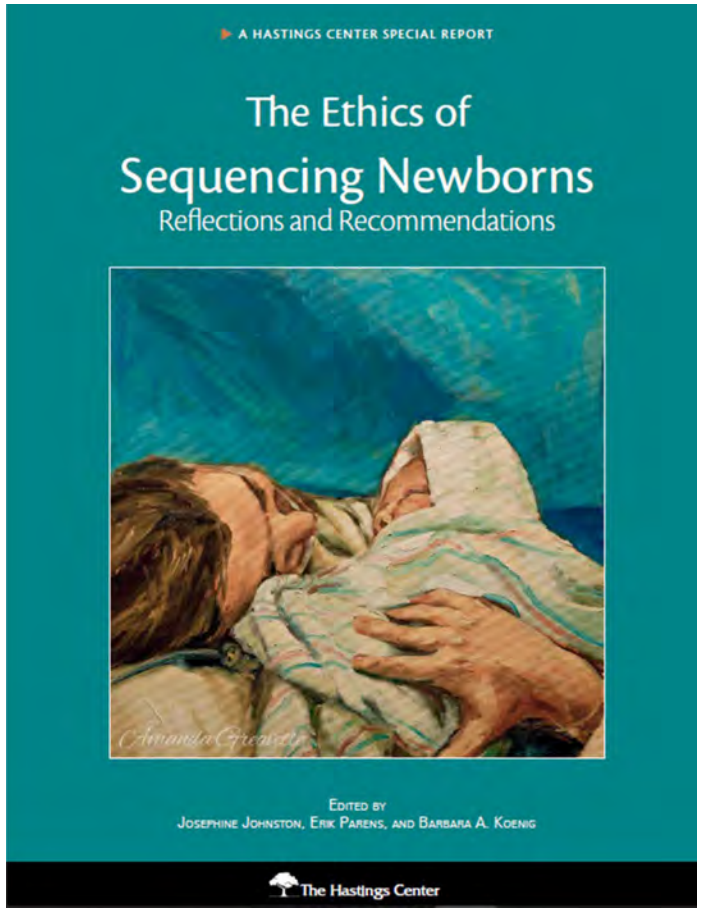
At this time, WES alone is unsuitable as a sole newborn screening modality for inborn errors of metabolism.

For selected disorders, sequencing was as good as MS/MS.

Sequence information from WES could reduce false positive results and facilitate an accurate and timely case resolution.

How will sequencing enhance, challenge, or transform traditional state-mandated NBS?

Working Toward Nuance: Public Health



- Do not use targeted or whole-genome sequencing as sole screen
  - Cannot detect everything
  - Concerns over storage of results
  - Concerns over discrimination or insurance uses
  - Potential for results to generate unnecessary distress
  - Potential for results to require counseling and generate unneeded follow-up care and monitoring
- OK to use targeted sequencing
  - As a secondary test following a positive screen
  - As a primary screen to detect conditions that meet all screening criteria

NBSeq: Sequencing of Newborn Blood Spot DNA to Improve Newborn Screening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TATA TATA Consultancy Services

Consortium on Law, Science & Health Policy

NIH Eunice Kennedy Shriver National Institute of Child Health and Human Development

National Human Genome Research Institute