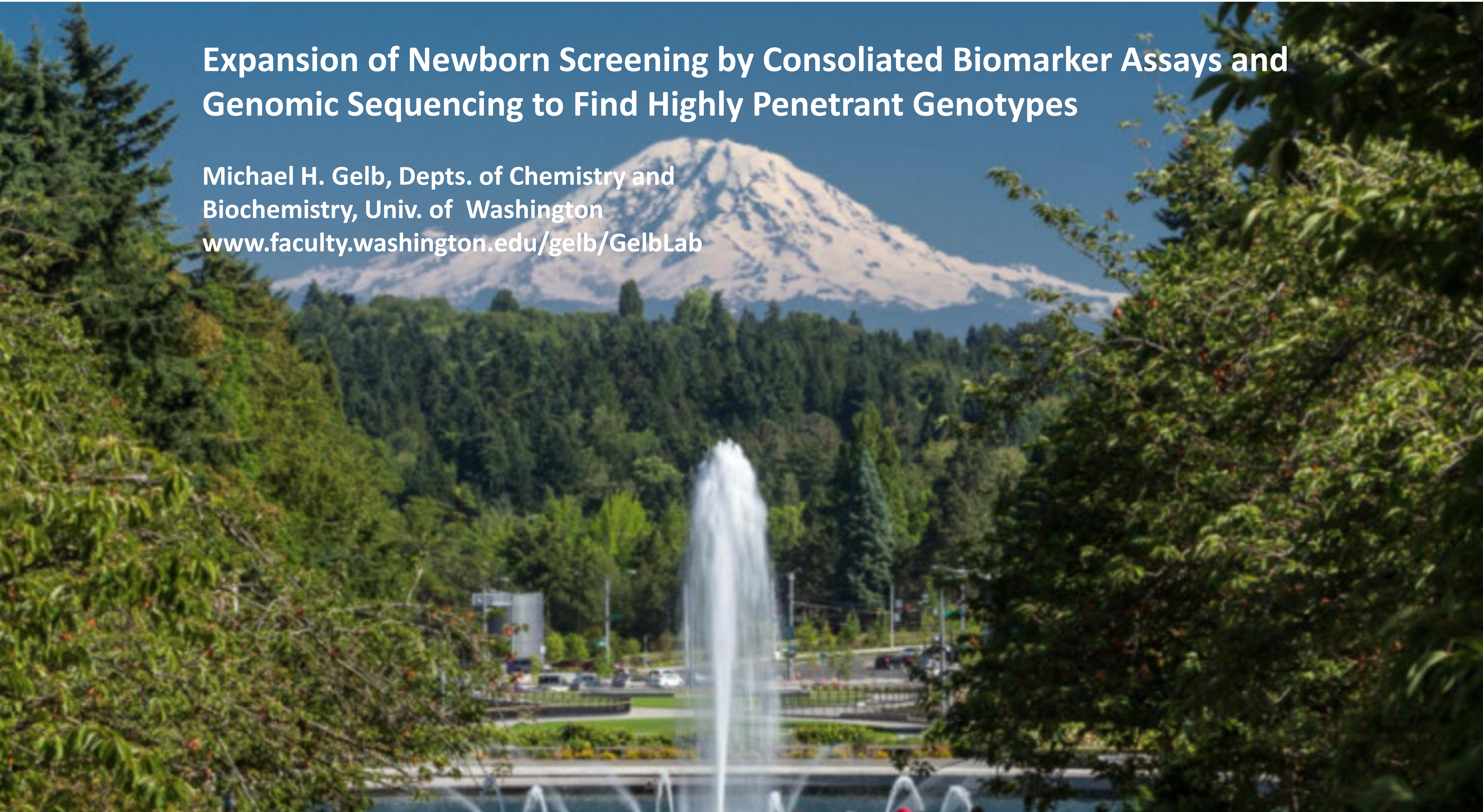

Expansion of Newborn Screening by Consoliated Biomarker Assays and Genomic Sequencing to Find Highly Penetrant Genotypes

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Disclosures

I provide expertise advise to several companies:

Revvity, BioMarin, Ultragenyx, Sentynl, Orchard, Takeda, Forge Biologics, Passagebio, Genomics England

I am a founding member of GelbChem, LLC.

INTRODUCTORY STATEMENTS

1. Biochemical first-tier NBS is usually enormously more precise (low false negative and false positive rates) than first-tier NBSeq

2. NBSeq allows NBS for 100s of actionable pediatric diseases. This level of disease bandwidth is almost certainly not obtainble by biochemical first-tier NBS.

3. Expansion of biochemical first-tier NBS should involve <u>consolidation</u> of as many assays as possible into a minimal number of analytical equipment platforms. This is a form of multiplexing.

4. Of course, biochemical and NBSeq first-tier NBS has to be feasible for population based coverage. A rough price estimate is about $1 per newborn per disease for the actual DBS test. Now we do biochemical NBS on say 60 diseases for about $60 in test costs, and I suppose we can do whole genome for 100s of diseases for a few hundred $ per newborn.

In the next 14 min

I will first show you what is possible now for consolidated biochemical first-tier NBS

I will show you a few examples of how precise it is

I will show you about a new biochemical platform that holds promise for the future

I will show you why the current plan to implement NBSeq is very likely to fail and comment on what we need to do to fix it.

Together these constitute my two cents about how to navigate the future of NBS tests

The Current Collection of Biochemical First-Tier NBS Tests

~40 diseases screened by tandem mass spectromtery (MS/MS)

2-3 diseases screened by quantitative PCR

~4-5 diseases screened by immunoassays

~2 diseases screen by fluorometry

~1-2 diseases screened by electrophoresis methods

Consolidation by MS/MS
(Washington and Illinois NBS lab as examples)

1. MS/MS instrument 1: ~40 conditions (amino acids, organic acids, fatty acids) by flow-injection-MS/MS (sample introduced without a chromatography column)

2. MS/MS instrument 2: 2-7 conditions done (lysosomal storage diseases) by LC-MS/MS (sample introduced with a chromatography column)

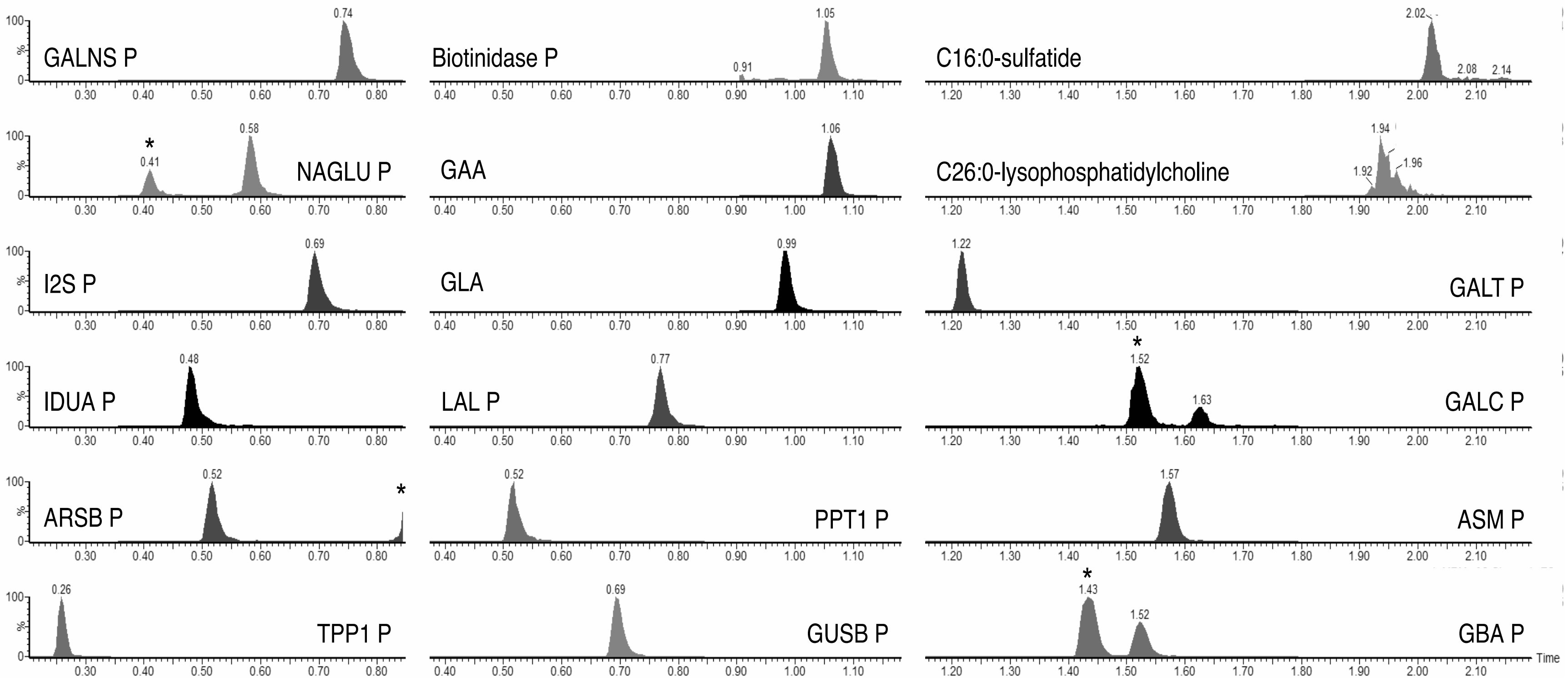
3. MS/MS instrument 3: 1 condition (X-linked adrenoleukodystrophy) by LC-MS/MS

4. Separate fluorimetric instrument: Galactosemia and Biotinidase Deficiency

The key to consolidation is LC-MS/MS. Everything done by fluorescence can be folded into LC-MS/MS, and LC-MS/MS allows many diseases to be done that fluorescence cannot do.

Plan in Illinois and Washington over the next ~1yr: Fold 3 and 4 above into 2. Do everything by 1 flow-injection-MS/MS station and 1 LC-MS/MS station. This will save enormous time and money.

LC-MS/MS for the consolidated quantification of many biomarkers (essentially all are enzymatic products) into 1 assay requiring 2 min per newborn. Currently this is up to 26 diseases.



Current list of 26 conditions: MPS-I, MPS-II, Pompe, Krabbe, X-ALD, Biotinidase Deficiency, Galactosemia, MPS-IVA, MPS-VI, all other MPS subtypes, MLD, CTX, CLN1, CLN2, Niemann-Pick A, B and C, Gaucher, Fabry, Tay-Sachs, Sandhoff, Biotinidase

Red are on the RUSP, Blue are not on the RUSP but done in Taiwan, Black are diseases for which we treatments or treatments on the way but not on the RUSP

NBS by First-Tier Biochemical vs NBSeq

MS/MS-based biomarker analysis will start to run out of options at ~100-200 of diseases over the next decade or so. Metabolomics is a bit of an unknown but I don't think it will add more than ~50 conditions.

I will show you next a new MS/MS technology that is potential a game changer and can extend this by another ~200 diseases.

But NBSeq (whole genome) will surely pass these numbers. It is currently being piloted on ~300-600 diseases, and it covers diseases for which a biochemical NBS assay by any method is unimaginable.

Under development is a potential game changer for biochemical first-tier NBS for actionable, pediatric diseases

Targeted proteomics by LC-MS/MS.

Here we measure the abundance of the protein rather than its functional output.

Wilson disease is a not so rare, highly treatable, early-onset disease caused by deficiency of an intracellular copper transporter. Assays in DBS to measure the protein function are unimaginable, and no biomarkers have emerged after decades of studies.

DBS punch treated with trypsin to generate a signature peptide, capture the signature peptide with a monoclonal antibody, quantify the peptide by LC-MS/MS.

This is not ready for implementation in NBS labs. We are trying to optimize the method to make it appropriate for high-throughput NBS. A pilot study for Wilson disease is in progress in WA state. The next few yrs will be critical to see if this method is appropriate for NBS.

There will be some false negatives in cases where the mutation causes loss of protein function but not lead to protein missfolding/degradation. For Wilson disease ~180 out of 200 confirmed cases are screen positive, so the false negative rate is ~10%. In NBS circles this may be considered taboo, but I remind you that the false negative rate with NBSeq will be massively higher.

Other examples under development: Cystinosis, Primary Immunodeficiencies (XLA, DOCK8, etc), Menkes, TSC. Should be able to do ~50-100 diseaases in 1 x 2 min LC-MS/MS run per newborn.

Sihoun Hahn, Seattle Children’s Research Institute.

Biochemical first-tier NBS is often highly precise (low FP and FN rates)

MPS-I, MPS-II, Krabbe disease. 100,000 newborns measure enzymatic activity by LC-MS/MS gives ~50 FPs per disease. Second-tier analysis using the same DBS by LC-MS/MS reduces the FPs. to 0-2 per disease. The FN rate is ~0. The much higher rates of FPs reported now in some NBS labs is due to the use of second-tier DNA seq. instead of LC-MS/MS.

Should NBS programs do both first- and second-tier LC-MS/MS tests on the same DBS (i.e. as part of NBS)? YES! These two tests are inseparable. Each is like a car with 2 wheels, it only works if you have 4. Should the second-tier test be done in the state NBS lab or contracted out to private labs? I don't care as long as the NBS result is after both tests are done.

Pompe disease: First-tier test is MS/MS, about ~50 FPs per 100,000 newborns. Here we have no good biochemical second-tier test so we go to DNA seq. second-tier. A few turn out to be almost certain infantile-onset disease, and the rest come back as:

~50% FPs
~20% uncertain risk for late-onset disease
~30% completely inconclusive

Note that the DNA is done after 100,000 newborns have been put through the biochemical first-tier filter. Imagine what will happen if we did DNA first-tier on 100,000?

This leads me to the last part of my talk.....

NBSeq (whole genome, first-tier NBS) is a promising approach for tackling 100s of actionable, pediatric disease.

I am a big fan of the efforts and even working hard in this area.

But I am worried that if we don’t think a little bit harder it is going to break in a few years.

The punchline is that we need to focus on the low hanging fruit first. We need to accept very high FN rates to keep FPs rates to a maneable number. With current practices this will not be the case and it will break in a few years.

So I will end this talk with a few minutes of what some might say is my “crystal ball” prediction. No, everything I am going to say next is based on stuff we already know, already published, and already experienced in live NBS programs. And I think many of us know this but nobody does much about it.

Pathogenicity and Penetrance

Most NBSeq pilot programs announce that for autosomal recessive diseases (most diseases) they will call out as first-tier screen positives only those newborns with at least 2 ACMG Pathogenic or Likely-Pathogenic variants, thus they will ignore variants of unknown significance (VOUSs).

Pathogenic means the DNA variant is a very high confidence driver of the disease (a ACMG published list of criteria that must be satisfied). But Pathogenic is not the same as Penetrance.

For autosomal recessive diseases, Penetrance is given to a GENOTYPE, not a variant. It is the fraction of people with a specific GENOTYPE who get the disease.

Pathogenicity versus Penetrance

We take all sources of information reported for genotypes of clinically severe patients and call the variants Early Onset Pathogenic (EOPs). The penetrance of EOP/EOP genotypes are all compressed near 100% so there are no issues.

We take all sources of information reported for genotypes of clinically late onset patients and most of them have an EOP (from the above list) and the other is not an EOPs. We call the non EOP variant a late onset pathogenic variant (LOP). The genotype is EOP/LOP. The penetrance is usually close to 100%, but the age of onset is highly variable and dependent on the degree of residual biochemical function of the LOP.

What about LOP/LOP? Here the penterance is all over the place and the majority are close to 0%.
The LOP/LOP genotype is usually inconclusive, and remember the number of genotypes when you have 100s of variants is enormous (combinatorial problem). The freq. of LOP/LOP in the population is much higher than the freqs of EOP/EOP + EOP/LOP.

LOP/LOP Example: *GALC* variant L634S for Krabbe disease. Listed in ClinVar as Pathogenic. The vast majority of homozygotes do not show signs of KD even as adults.

Pathogenicity and Penetrance

So the plan to call out all Pathogenic/Pathogenic will lead to an implosion for obvious reasons that we all understand. THE FP RATE WILL BE MASSIVE!

Wasserstein says the level of anxiety that families are willing to tolerate may be lower than we anticipated. Tarini says the surveys are biased, and if somehow we knew what the majority of families think the anxiety will be much more widespread. Genomics England said that the majority of families who consented for NBSeq want disease status within the first 30 days of life.

What if we call out only EOP/EOP? The false negative rate willl be like 50% but when applied across 100s of diseases a lot of babies will benefit. Biochemical screeners should not always say that FNs are taboo. This is a paradigm shift in the right direction.

What if we call out also EOP/LOP? Im actually not sure but I am willing to try for a while.

What if we call out also LOP/LOP?. NBSeq will implode in a few years. I am OK calling out LOP/LOP for a small subset of diseases like biotinidase deficiency where the baby takes extra biotin. But for most other diseases the system will implode if you call them out. Note, over time we may learn more about the penetrance of LOP/LOPs but it will take a very long time, and nobody knows how long.

A Useful Model to Facilitate NBS for A Disease Recently Added to the RUSP

Newborn screening for Krabbe disease was recently recommended by the Advisory Comm. on Heritable Disorders in Children and Newborns to be added to the RUSP. We still have more to learn about NBS for Krabbe disease.

The Krabbe disease experts (currently ~30-40 people) came together to form a consortium (facilitated by the Hunter's Hope parent-engaged foundation.

The consortium holds monthly 1 hr online meetings to discuss new cases identified by NBS labs live for Krabbe disease NBS. For most of the states screening for KD, 1-2 members from each state lab participate. The consortium also includes several experts who developed KD NBS and those who see and treat at-risk patients.

The KD Consortium deals with the problem that most pediatricians are not so familar with KD NBS and the pathway to treatment. The key is that representatives of live NBS state labs bring the NBS positives to the attention of the consortium and immediately they get the most informed plan of action.

Arguably, this model is needed for many rare diseases for which NBS is in place or coming. Each disease has its own set of complexities.

I call on all listeners to please read:
https://medium.com/@pouria.sanae/c-956a-g-p-y319c-and-the-case-of-nightmare-carrier-screening-during-our-pregnancy-67d31fd47032

Some closing remarks

The goal is to maximize the number of newborns who benefit from NBS (regardless of the specific disease). Thus, FNs can be tolerated and should no longer be considered taboo.

If we use NBSeq try to reduce the rate of FN to the level that we can achieve with biochemical first-tier NBS, NBSeq will surely break.

It makes no sense now to call out all newborns who are simply Pathogenic/Pathogenic, this should be limited to EOP/EOP and probably EOP/LOP (for some but not all diseases). We should almost never call out LOP/LOP.

For diseases for which we have good first-tier, biochemical NBS (i.e. FN and FP rates close to zero) we should continue to add them to NBS panels in a consolidated way because NBSeq will not achieve the precision of biochemical NBS in the forseable future. Our knowledge of the penetrance of LOP/LOP will improve with time, but it will take a very long time, and nobody knows how long.

Thus, NBSeq will not displace biochemical first-tier NBS in the foreseable future, but the time is right to develop its use for a large number of actionable, neonatal diseases and to focus on highly penetrant genotype. If we call out all newborns with two ACMG Pathogenic variants, NBSeq will surely break soon.