Cell-free DNA Testing and the Newborn Screening Program

What we're doing now and future directions

Conflicts of Interest

- No conflicts to disclose

Workshop Objectives

- Review cell-free DNA technology
  - Discuss benefits and limitations of the current uses of this technology
  - Discuss benefits and limitations of proposed future directions
- Review the NYS newborn screening program
  - Discuss benefits and limitations compared to other newborn screening programs
  - Discuss benefits and limitations of proposed future directions

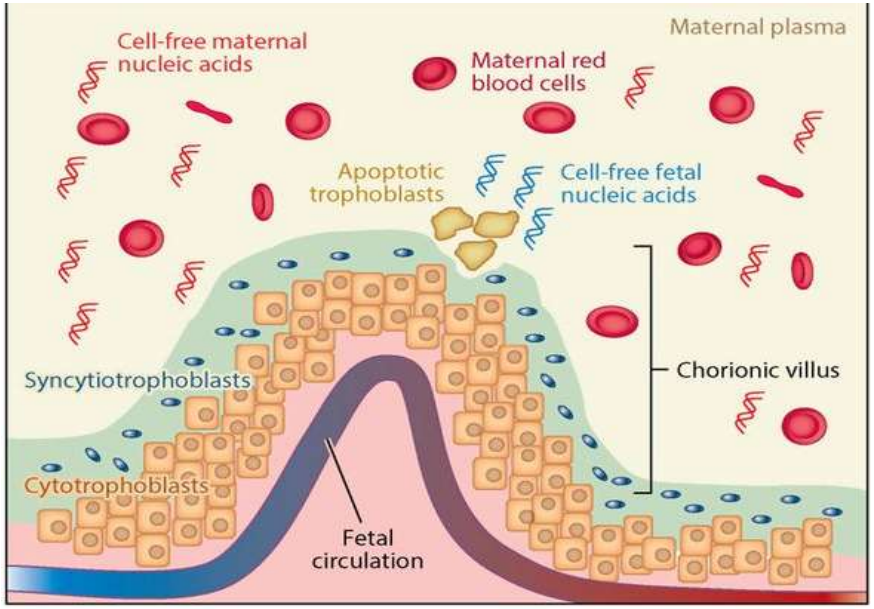
Cell-free DNA testing

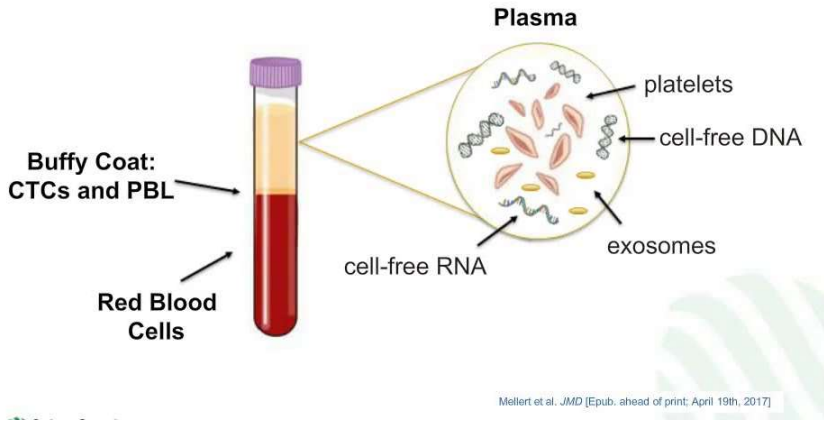
- The many names of cell-free DNA screening/testing
  - Non-invasive prenatal screening (NIPS)
    - No. Just….no
  - Non-invasive prenatal testing (NIPT)
  - Non-invasive prenatal diagnosis (NIPD)
    - Problematic
  - Any/All brand names
  - Anyone aware of/using others?

Indications for cell-free DNA testing

- Cell-free DNA screening is currently covered by most insurers for “high risk” patients
  - Women over 35 at delivery
  - Women with a history suggestive of an increased risk for aneuploidy
    - Previously affected child
    - NOT a second cousin twice removed with Down syndrome
  - Women with an ultrasound finding associated with aneuploidy
    - Basically any ultrasound finding would fall under this category
  - Women with positive maternal serum screening

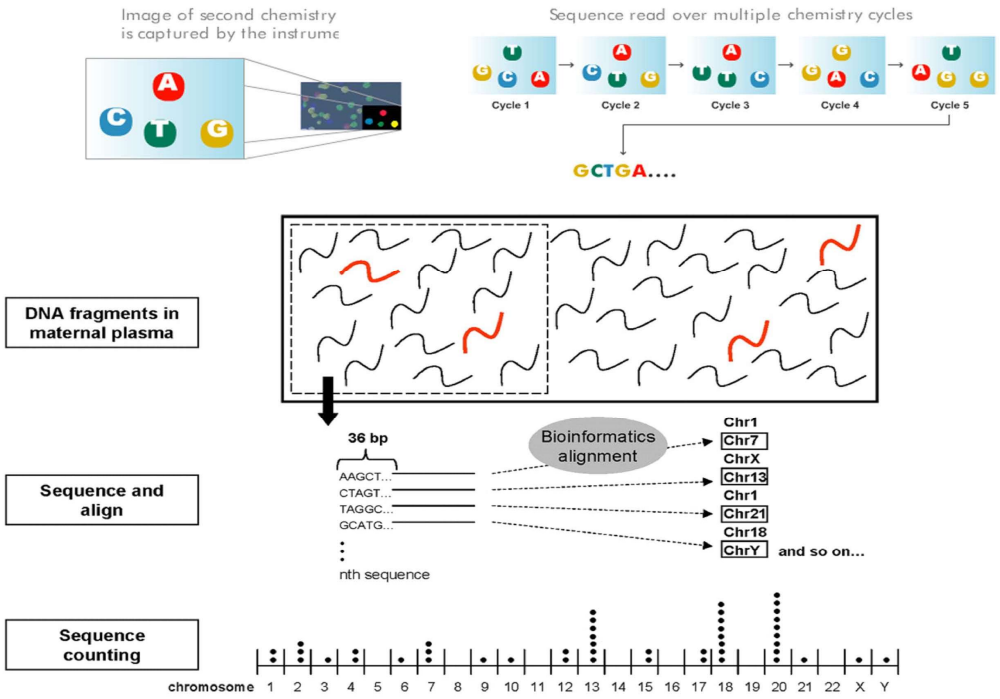
Where Does Cell-Free DNA Come From?





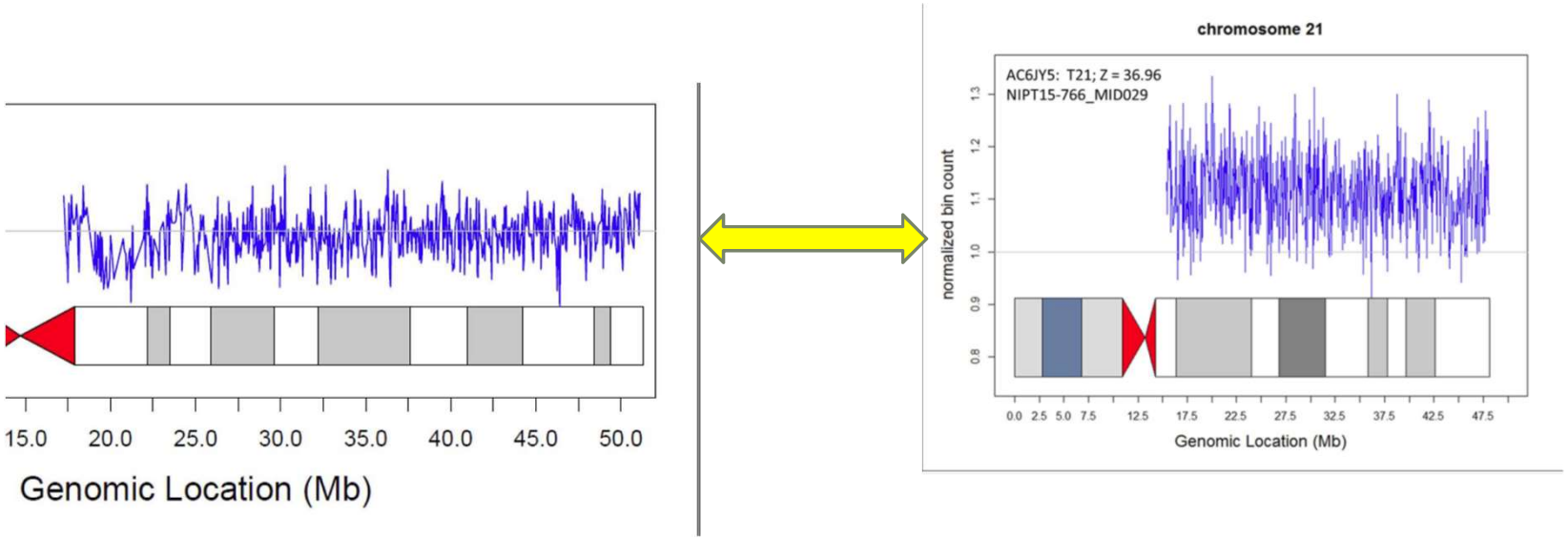
Wong FCK, Lo YMD. 2016.
Annu. Rev. Med. 67:419–32

How Does it Work? Massively Parallel Shotgun Sequencing (MPSS)



Chiu RWK et al. PNAS 2008;105:20458-20463

Data Output

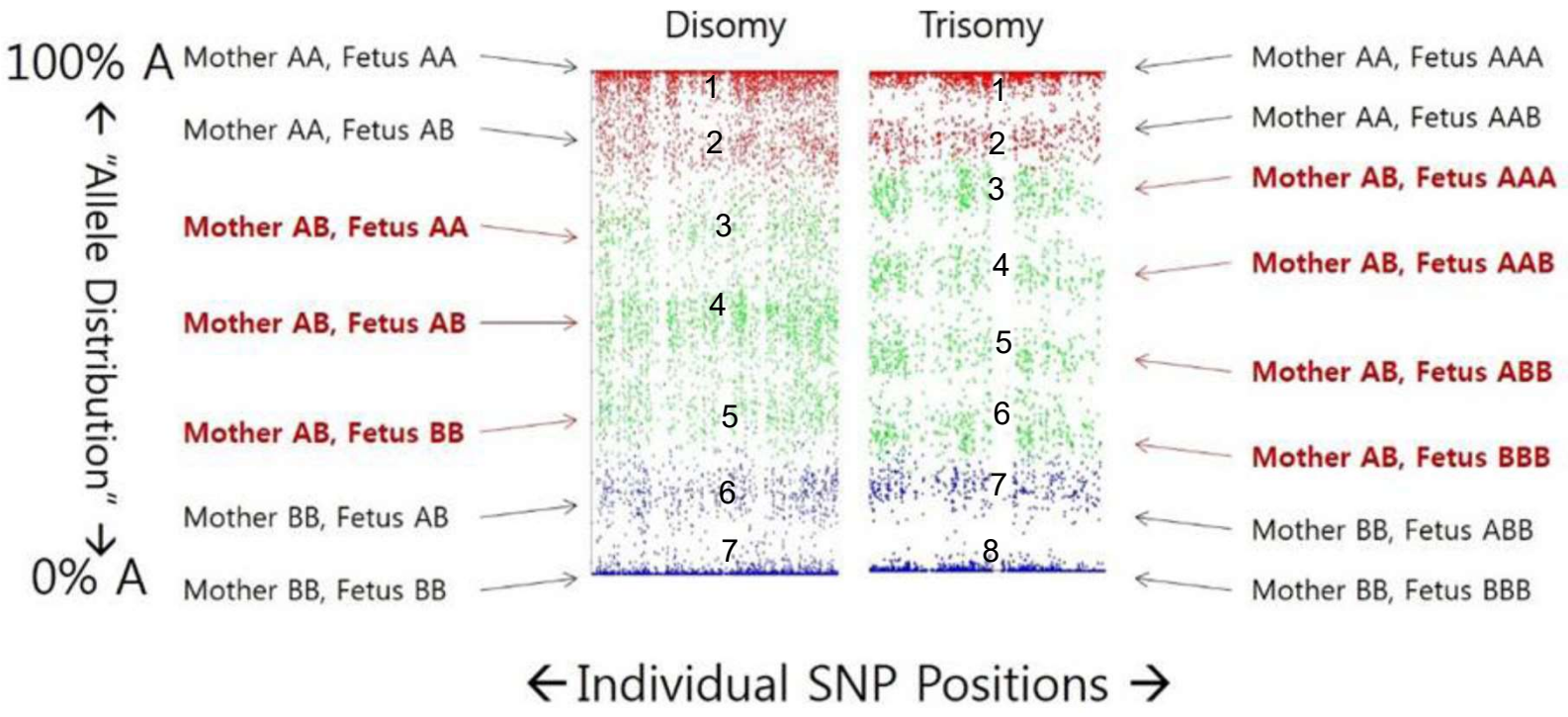


PLoS One. 2017 Mar 1;12(3):e0167130. doi: 10.1371/journal.pone.0167130. eCollection 2017.

Show of Hands

- Who is using Natera's Panorama for cell-free DNA testing?
  - This lab uses a completely different testing methodology (sorry!)

SNP-Based Cell-free DNA testing



J Genet Med 2015; 12(2): 66-71.

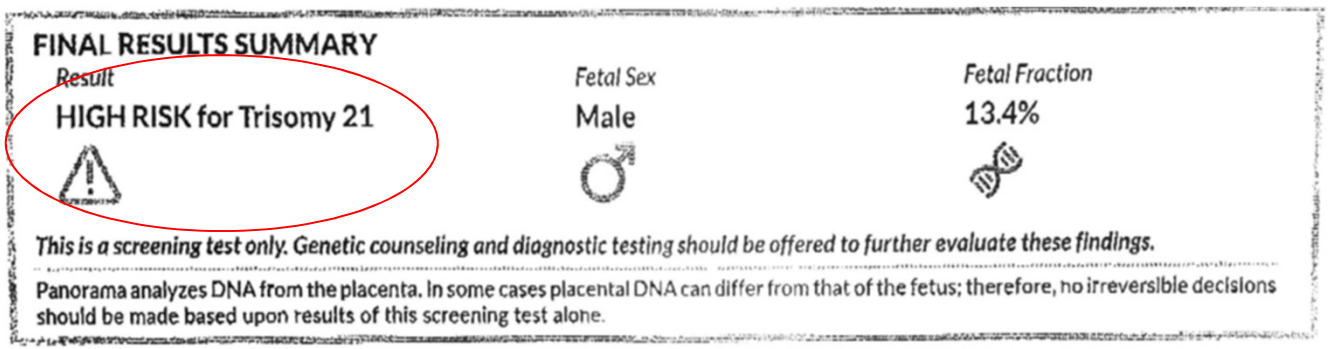
Comparing and Contrasting Technologies

	MPSS	SNP-based
Egg Donors	√	✗
Triploidy detection	✗	√
Twins*	√	√
Zygosity of twins	✗	√

Conditions Screened

- Trisomy 21
- Trisomy 18
- Trisomy 13
- Sex Chromosome aneuploidies
  - Limitations in twin pregnancies
- Triploidy*
  - Lab dependent
  - Not associated with advanced maternal age
- Some microdeletion disorders
  - Not associated with advanced maternal age

Sample Report



FINAL RESULTS SUMMARY

Result	*Fetal Sex*	*Fetal Fraction*
HIGH RISK for Trisomy 21	Male	13.4%

This is a screening test only. Genetic counseling and diagnostic testing should be offered to further evaluate these findings.

Panorama analyzes DNA from the placenta. In some cases placental DNA can differ from that of the fetus; therefore, no irreversible decisions should be made based upon results of this screening test alone.

RESULT DETAILS: ANEUPLOIDIES

Condition tested[1]	*Result*	*Risk Before Test*[2]	*Risk After Test*[3]
Trisomy 21	High Risk	1/38	9/10
Trisomy 18	Low Risk	1/89	<1/10,000
Trisomy 13	Low Risk	1/280	<1/10,000
Monosomy X	Low Risk	1/255	<1/10,000
Triploidy	Low Risk		

RESULT DETAILS: MICRODELETIONS

Repeat To Yourself: It's Just a Screening Test

- Not all positive results are equally concerning.
- The positive predictive values are highest for Down syndrome and lowest for monosomy X and trisomy 13.
- We don't even have consistent positive predictive values for the microdeletion syndromes
  - And sometimes you identify a **maternal** microdeletion incidentally.

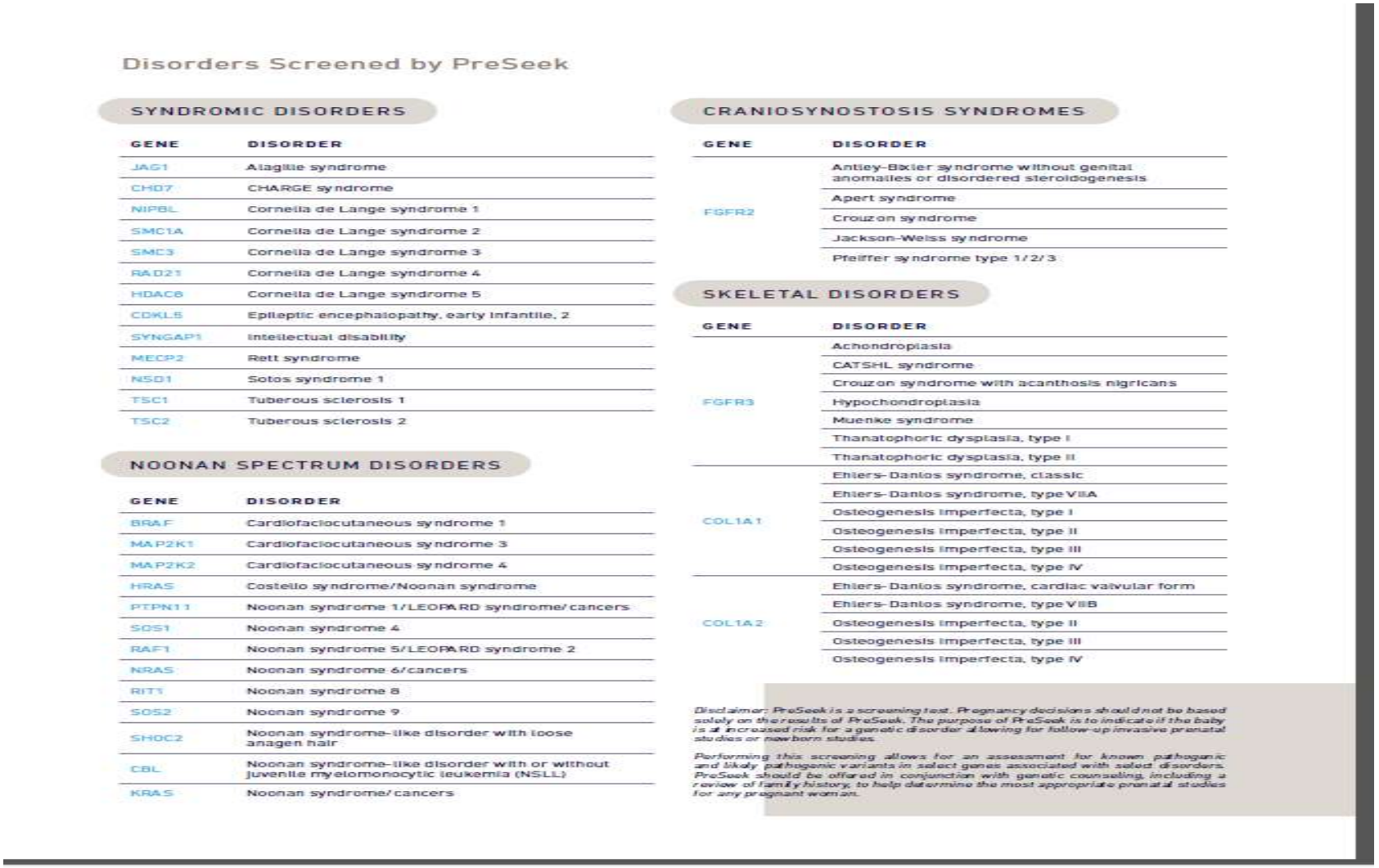
Now that we're all experts….

- What does the future look like for cell-free DNA testing?
  - Single-gene cell-free DNA testing
    - This is actually available now
  - Whole exome/genome cell-free DNA testing
    - This will likely be coming soon
  - And to everyone's horror:
    - Direct-to-consumer cell-free DNA testing
      - Probably only a matter of time given what is currently available by DTC testing.

Who is single-gene cell-free testing for?

- Advanced Paternal Age
  - Men over 40 at conception have an up to 1% chance to have a child with a dominant genetic condition caused by a de novo mutation.
  - Currently there is no recommended screening/testing for these APA risks aside from ultrasound evaluation.
- Ultrasound Anomalies
  - When aneuploidy screening is reassuring
- Patients who want to know “everything”
- Patients who would otherwise decline invasive testing but desire additional information

What testing is available?



Disorders Screened by PreSeek

SYNDROMIC DISORDERS

GENE	DISORDER
JAG1	Alagille syndrome
CHD7	CHARGE syndrome
NIPBL	Cornelia de Lange syndrome 1
SMC1A	Cornelia de Lange syndrome 2
SMC3	Cornelia de Lange syndrome 3
RAD21	Cornelia de Lange syndrome 4
HDAC8	Cornelia de Lange syndrome 5
CDKL5	Epileptic encephalopathy, early infantile, 2
SYNGAP1	Intellectual disability
MECP2	Rett syndrome
NSD1	Sotos syndrome 1
TSC1	Tuberous sclerosis 1
TSC2	Tuberous sclerosis 2

NOONAN SPECTRUM DISORDERS

GENE	DISORDER
BRAF	Cardiofaciocutaneous syndrome 1
MAP2K1	Cardiofaciocutaneous syndrome 3
MAP2K2	Cardiofaciocutaneous syndrome 4
HRAS	Costello syndrome/Noonan syndrome
PTPN11	Noonan syndrome 1/LEOPARD syndrome/cancers
SOS1	Noonan syndrome 4
RAF1	Noonan syndrome 5/LEOPARD syndrome 2
NRAS	Noonan syndrome 6/cancers
RIT1	Noonan syndrome 8
SOS2	Noonan syndrome 9
SHOC2	Noonan syndrome-like disorder with loose anagen hair
CBL	Noonan syndrome-like disorder with or without juvenile myelomonocytic leukemia (NSLL)
KRAS	Noonan syndrome/cancers

CRANIOSYNOSTOSIS SYNDROMES

GENE	DISORDER
FGFR2	Antley-Bixler syndrome without genital anomalies or disordered steroidogenesis
	Apert syndrome
	Crouzon syndrome
	Jackson-Weiss syndrome
	Pfeiffer syndrome type 1/2/3

SKELETAL DISORDERS

GENE	DISORDER
FGFR3	Achondroplasia
	CATSHL syndrome
	Crouzon syndrome with acanthosis nigricans
	Hypochondroplasia
	Muenke syndrome
	Thanatophoric dysplasia, type I
	Thanatophoric dysplasia, type II
COL1A1	Ehlers-Danlos syndrome, classic
	Ehlers-Danlos syndrome, type VIIA
	Osteogenesis imperfecta, type I
	Osteogenesis imperfecta, type II
	Osteogenesis imperfecta, type III
	Osteogenesis imperfecta, type IV
COL1A2	Ehlers-Danlos syndrome, cardiac valvular form
	Ehlers-Danlos syndrome, type VIIB
	Osteogenesis imperfecta, type II
	Osteogenesis imperfecta, type III
	Osteogenesis imperfecta, type IV

Disclaimer: PreSeek is a screening test. Pregnancy decisions should not be based solely on the results of PreSeek. The purpose of PreSeek is to indicate if the baby is at increased risk for a genetic disorder allowing for follow-up invasive prenatal studies or newborn studies.

Performing this screening allows for an assessment for known pathogenic and likely pathogenic variants in select genes associated with select disorders. PreSeek should be offered in conjunction with genetic counseling, including a review of family history, to help determine the most appropriate prenatal studies for any pregnant woman.

Expanded screening Issues

- Sometimes a maternal and paternal sample is required
  - Paternal samples are not always available
  - Could identify non-paternity in those instances where a paternal sample is required.
- Would still recommend diagnostic testing confirmation
- Not available if mom is affected with a testable condition
- Not available in twin pregnancies
- We're already finding unexpected incidental diagnoses with limited testing
  - Maternal malignancies
- Insurance coverage?
  - Unlikely

What is whole exome sequencing?

- Exons are the coding parts of the genome
- Because most known mutations that cause disease occur in exons, whole exome sequencing is thought to be an efficient method to identify possible disease-causing mutations.

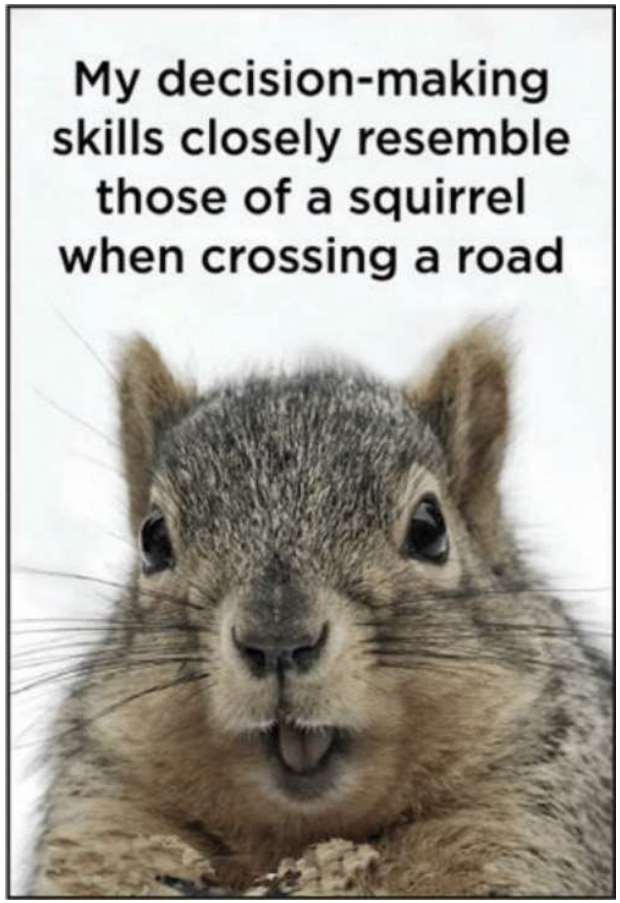
Why would we want whole exome testing during a pregnancy?

- Ultrasound findings can be very non-specific
  - Can lead genetics professionals down a rabbit hole of single-gene testing
    - And we may still not arrive at an answer
- “Wait and see” approach is not very palatable to patients
  - Sometimes additional ultrasound evaluations are suggested as a pregnancy progresses to give practitioners clues as to what other genetic testing may be indicated
    - This prolonged time to a potential diagnosis can be very difficult for our patients.

Barriers and Discussion Points

- Still facing significant challenges
  - Turn-around-time
    - Prenatal exomes on amniotic fluid currently take 8-12 weeks.
  - Cost
    - Likely to be a deal-breaker for most patients at this time
  - Variant classification
    - Trying to differentiate a de novo mutation from “noise” introduced during the sequencing process
- Exclusion of variants
  - We will be hugely in need of a consensus committee on the exclusion of adult-onset disorders
    - The last thing the intended parents of a child with multiple congenital anomalies needs is to be given a prenatal diagnosis of a cancer predisposition syndrome
- Variant reclassification
  - What happens when a VUS becomes a pathogenic variant?

The push-pull of additional information using uncertain technology



Before we move on…

- My personal/professional predictions?
  - Microdeletion testing will become more commonplace, especially for ultrasound anomalies, followed rapidly by general population screening
  - Single-gene testing will be sparsely used for another 5 years before becoming more wide-spread
  - Whole exome testing will be available to order within the next 5 years, but will have sparse uptake at first.
- Question or comments regarding cell-free DNA testing?

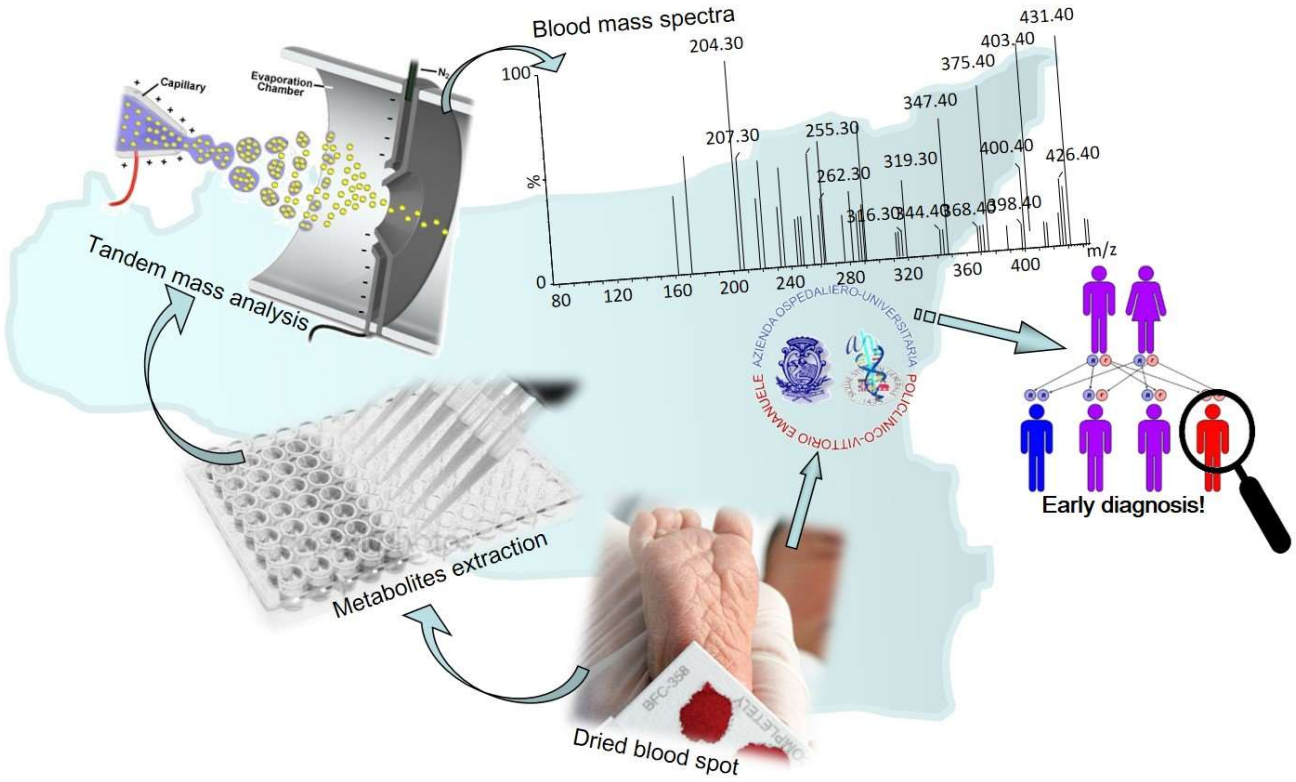
NYS Newborn Screening Program

- Newborn screening is process by which neonates are screened for a select number of conditions for which early detection has been shown to improve outcomes.
- This testing is required for all newborns born in New York State unless the parents confirm, in writing, that they have a religious objection.

From the NYS Department of Health:

- A small blood sample is collected from the newborn's heel usually 1-2 days after birth.
- The blood is used to screen for 50 different disorders.
  - Most are genetic
- There is no charge for this service.
- Most newborns will not have one of these disorders.
- Newborns with one of these disorders may look healthy at birth, which is why the testing must be performed to find those with a disorder. The earlier treatment is started, the better the outcome is for the newborn.
- Screening is designed to identify all newborns with the potential for one of these disorders. *Further testing is then required to verify whether or not your newborn has the disorder.*

How is newborn screening performed



Int. J. Neonatal Screen. **2018**, *4*(2), 12

Are all newborn screening programs created equal?

- NO!
  - The Department of Health and Human Services determines the Recommended Universal Screening Panel (RUSP)
  - Disorders on the RUSP are chosen based on evidence that supports the potential net benefit of screening, the ability of states to screen for the disorder, and the availability of effective treatments. It is recommended that every newborn be screened for all disorders on the RUSP.
- States ultimately determine what disorders their NBS program will screen for.

2014

STATE	Recommended Uniform Screening Panel (RUSP) – 31 Core Conditions[1] - Continued on p. 2																			
	Metabolic conditions detectable by Tandem Mass Spectrometry (MS/MS)																			
	Fatty Acid Disorders					Organic Acid Disorders									Amino Acid Disorders					
	CUD	LCHAD	MCAD	TFP	VLCAD	GA-I	HMG	IVA	3-MCC	Cbl-A,B	BKT	MUT	PROP	MCD	ASA	CIT	HCY	MSUD	PKU	TYR-I
Alabama	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Alaska	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Arizona	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Arkansas	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
California	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Colorado	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Connecticut	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
D. of Columbia	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Delaware	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Florida	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Georgia	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Hawaii	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Idaho	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Illinois	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Indiana	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Iowa	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Kansas	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Kentucky	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Louisiana	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Maine	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Maryland	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Massachusetts	●	●	●	D	●	●	●	●	D	●	●	●	●	D	●	●	●	●	●	●
Michigan	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Minnesota	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Mississippi	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Missouri	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
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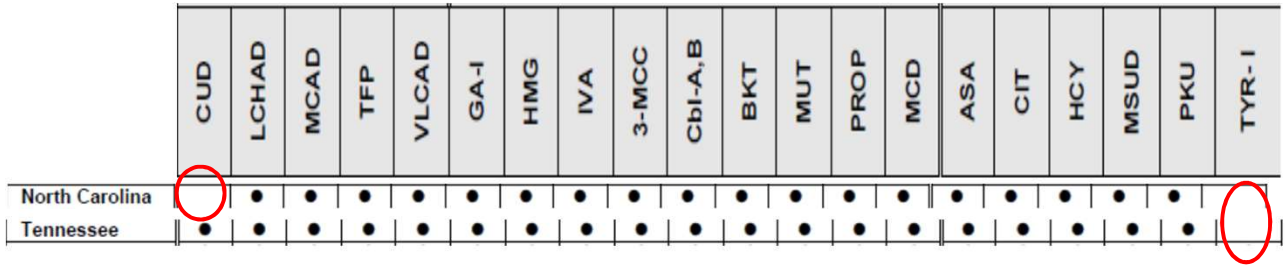
Nebraska	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Nevada	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
New Hampshire	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
New Jersey	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
New Mexico	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
New York	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
North Carolina		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	
North Dakota	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Ohio	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Oklahoma	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Oregon	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Pennsylvania	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Rhode Island	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
South Carolina	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
South Dakota	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Tennessee	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	
Texas	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Utah	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Vermont	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Virginia	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Washington	●	●	●	●	●	●	●	●	D	●	●	●	●	●	●	●	●	●	●	●
West Virginia	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Wisconsin	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Wyoming	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

Are there states that really don't screen for the RUSP disorders?

- As of the most recently compiled data in 2014, yes.



- Neither NC or TN performed screening for Tyrosinemia Type I
  - Symptoms usually appear in the first few months of life and include failure to thrive, diarrhea, vomiting, jaundice, cabbage-like odor, and increased tendency to bleed (particularly nosebleeds). Tyrosinemia type I can lead to liver and kidney failure, softening and weakening of the bones, problems affecting the nervous system, and an increased risk of liver cancer
- NC additionally did not screen for carnitine update defect
  - Typically, initial signs and symptoms of this disorder occur during infancy or early childhood and often include encephalopathy, cardiomyopathy, confusion, vomiting, muscle weakness, and hypoglycemia. Serious complications such as heart failure, liver problems, coma, and sudden unexpected death are also a risk. Severe illness due to CUD can be triggered by periods of fasting or illnesses such as viral infections, particularly when eating is reduced.

A round of applause for NYS

- The NYS NBS program screens for all 35 RUSP disorders plus 15 others.
- Are there states that screen for others?
  - You betcha
    - This means that being born in a neighboring state can drastically influence the likelihood of an early diagnosis.

How do conditions get added to the RUSP?

- First, a condition is nominated
  - Cover letter by the lead nominator that identifies all multi-disciplinary team members and their organizational affiliation(s), if applicable;
  - Letters of support (from multi-disciplinary team members), if applicable;
  - Completed COI forms
  - Responses to the Nomination Form
  - Supporting data and scientific/clinical references to substantiate all responses to Nomination Form questions.
- Then, a workgroup reviews the package and compiles a summary for Committee consideration and votes to assign (or not) the condition to the external Condition Review Workgroup.
- The Condition Review Workgroup completes an evidence-based review, provides updates, and presents a final report to the Committee on the assigned conditions.
- The Committee discusses and deliberates on the evidence and uses a decision matrix to guide the final decision. Then the Committee votes to recommend (or not) adding the nominated condition to the RUSP.
- A final decision is made by the Secretary for Health and Human Services.

The Decision Matrix

ACHDNC
Secretary's Advisory Committee on Heritable Disorders in Newborns and Children

NET BENEFIT/ CERTAINTY			READINESS			FEASIBILITY	
			Ready	Developmental	Unprepared		
SIGNIFICANT Benefit	Certainty	HIGH	**A1** Screening for the condition has a high certainty of significant net benefits, screening has high or moderate feasibility. Most public health departments are ready to screen.	**A2** Screening for the condition has a high certainty of significant net benefits and screening has high or moderate feasibility. Public health departments have only developmental readiness.	**A3** Screening for the condition has a high certainty of significant net benefits and screening has high or moderate feasibility. Public health departments are unprepared for screening.	Feasibility	HIGH or MODERATE
			A4 There is high certainty that screening would have a significant benefit; however, most health departments have low feasibility of implementing population screening.				LOW
		MOD	**B 1-4** There is moderate certainty that screening would have a significant benefit.			---	
Small to ZERO Benefit	Certainty	MOD/HIGH	**C 1-4** There is high or moderate certainty that adoption of screening for the targeted condition would have a small to zero net benefit.			---	
NEG Benefit	Certainty	MOD/HIGH	**D 1-4** There is high or moderate certainty that adoption of screening for the targeted condition would have a negative net benefit.			---	
---	Certainty	LOW	**L 1-4** There is low certainty regarding the potential net benefit from screening.			---	

Example of a condition not recommended

- Krabbe
  - Huge push for this to be added to the newborn screening panel due largely to the influence of former Buffalo Bills player, Jim Kelly.
  - Committee ruled that there was insufficient evidence to determine there was a consensus on the definition of infantile onset Krabbe, insufficient evidence regarding the testing algorithm to determine cost effectiveness, and additional information needed on the benefits of HSCT as treatment for the condition
  - NYS added to the condition to their NBS

What do you do if you're planning on delivering in a state whose NBS doesn't satisfy you?

- Private-pay expanded NBS
  - Several commercial laboratories offer expanded newborn screening options.
    - PerkinElmer offers an expanded newborn screening panel with 1,722 genes at a cost of $850 and a turnaround time of 3 weeks.
      - There is no data in the medical literature that screening for additional disorders markedly improves outcomes for those disorders.

Is more screening always better screening?

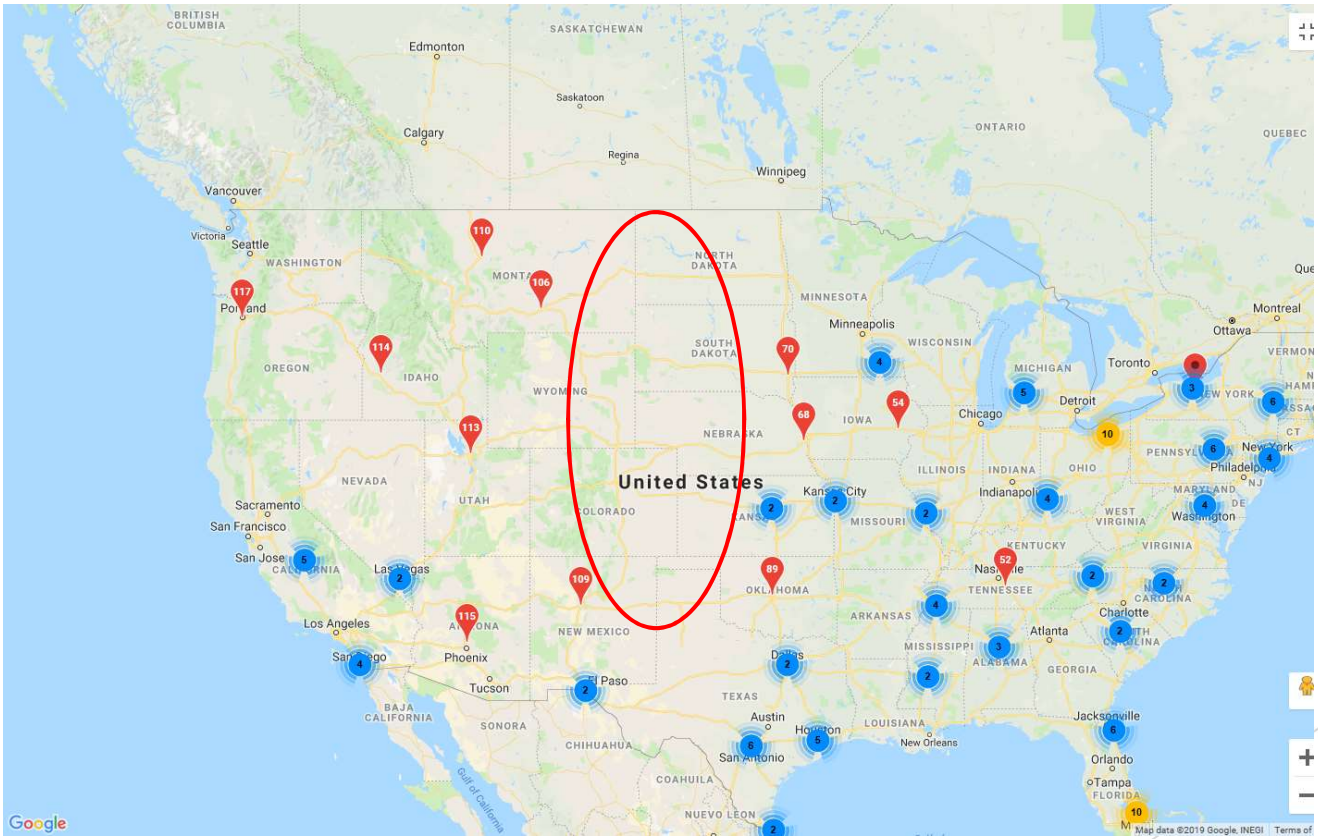
- X-linked adrenoleukodystrophy (X-ALD) was recently added to the RUSP list
  - Anyone seen the movie Lorenzo's Oil?
- X-ALD has variable expressivity (symptoms of each person with X-ALD can differ, even within the same family).
  - For example, some boys may have the childhood cerebral form of X-ALD, while other members of the same family may have the adrenal insufficiency-only type (Addison disease)
- Recommended surveillance after positive NBS?
  - MRI every 6 months beginning from age 3-10
  - Wait and see approach

Spinal Muscular Atrophy

- SMA was also recently added to the RUSP panel because of emerging treatment protocols
  - Many states are still getting their programs up and running
- Spinraza is FDA approved for the treatment of SMA
  - Intrathecal injection series
    - Initial dose
    - 14 days later, second dose
    - 14 days later, third dose
    - 30 days later, fourth dose
  - Maintenance every 4 months for life
  - Price tag of $750,000 for the first year and $375,000/year every year after.

Spiranza Treatment Centers

Notice anything?



Is the treatment effective?

- Published outcomes look very good.
  - Treated asymptomatic infants are achieving motor milestones not seen in untreated infants
- For those who can withstand the long-term treatment and can get to a treatment center, this is huge advantage

Whole Exome Newborn Screening

- Just like whole exome cell-free DNA testing, this is likely coming soon.
- Major issues in WES Newborn screening including the current lack of informed consent in the NBS process
- NBS samples can be stored for decades without explicit consent
- Public education regarding NBS is poor
  - Many people are unaware NBS is being completed
- But those issues aside…..

WES Consensus Needed

- Just like with cell-free DNA testing, consensus guidelines will be necessary
- Likely will exclude adult-onset conditions
- But do we exclude something like Tay-Sachs disease?
  - Is there any utility in informing parents of a newborn that their child has a lethal, untreatable genetic disease?
    - In the prenatal setting, offering this information make more sense as it can help intended parents make decisions regarding continuation vs termination of pregnancy
- How about Duchenne Muscular Dystrophy?
  - Mean age at diagnosis is 3 years
  - Many parents could have another child before the first is diagnosed and miss an opportunity to utilize assisted reproductive technologies.

WES can of worms

- And what about variants of uncertain significance?
  - Sequence changes that have uncertain clinical impact
- There are already concerns regarding state cut-off values which may be set too conservatively to identify all affected children
  - A positive screen in one state might be a negative screen in a different state.
- Do we “treat” children with a VUS just in case it turns out to be a pathogenic variant?
- How would we notify parents if a VUS classification is changed to pathogenic?


WHAT WE'VE GOT HERE
IS A VERY COMPLICATED SITUATION
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My predictions?

- The RUSP will continue rigorous review of conditions to be added to the panel by nomination
- Commercial labs will begin to advertise more effectively for their expanded newborn screening.
  - I expect it will look a lot like advertisements for cord blood banking.
- Whole exome sequencing will be offered in the next decade.
  - Early retirement?

Questions or comments?