Next-Gen DNA Sequencing in Prenatal Screening for Down Syndrome: *How is it best used?*

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Objectives

- Briefly review the technology behind Next-Gen DNA Sequencing
- Learn about the clinical applications of Next-Gen DNA Sequencing in the prenatal setting
  - What patient population may be appropriate for testing?
  - Where does the test fit into currently available testing modalities?
    - Logistics:
      - Gestational age?
      - Cost/ insurance issues
  - Clinical cases

Disclosures

- I have no financial relationship with a commercial entity producing health-care related products and/or services

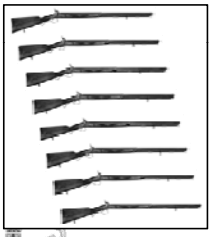
Changing the course of prenatal diagnosis

TIME

The New York Times

ABC NEWS

Shotgun Sequencing
Massively Parallel Sequencing
Massively Parallel Shotgun Sequencing
Massively Parallel Genomic Sequencing
Next Gen Sequencing



Massively:
"Many, many pieces of DNA…

Parallel: *Tens of millions*
…are sequenced at the same time."

Shotgun:
To emphasize the incomplete nature of the sequencing in this type of test.

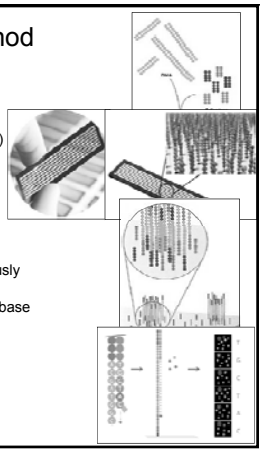
www.dna-dude.com

The Method

Library preparation:
- Take samples of maternal plasma
- Isolate free DNA fragments
- Add adapters to fragments (for binding and ID)
- Attach DNA to flow cell surface

Cluster generation:
- Amplify millions of fragments simultaneously

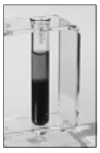
Sequencing:
- Each fragment cluster sequenced simultaneously
- First 36 bases of each fragment sequenced
- Sequences compared to human genome database
- Chromosome assignment



Why all the fuss over MPSS for screening?

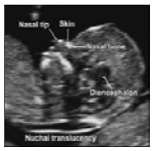
- Earlier screening than traditional serum screening
- Much better Detection Rate for common trisomies
  - Nearly as good as diagnostic testing
- Much lower False Positive Rate
- Why was there "negative press" early on?

Current Prenatal Screening



Determine risk based on multiple markers

- maternal age
  (the older, the higher the risk)
- maternal serum markers
  (in 1st and 2nd trimesters)
- fetal ultrasound marker
  (nuchal translucency)



If found to be high risk:

- offer invasive diagnostic procedure & karyotype
- risk of miscarriage 1 in 100 – 1 in 200 (maybe lower)

Current Screening Performance

The best we can do today for Down Syndrome Screening:

- Detection Rate = 90% (*i.e.,* 10% of cases are missed)
- False Positive Rate = 2-5% (many women called positive)
- 1 in 20 pregnancies at high risk pregnancies by current screening will have a Down syndrome fetus.
- If all high risk have invasive diagnosis:
  19 of the 20 will not have Down syndrome, but because of diagnostic methods, are at increased risk of having a miscarriage.

Question:

How to reduce the number of women who must consider invasive testing?

Introduce a secondary screening method that has close to 100% detection while eliminating most of the false positives.

Prenatal Screening Using Noninvasive Prenatal Diagnosis with MPSS

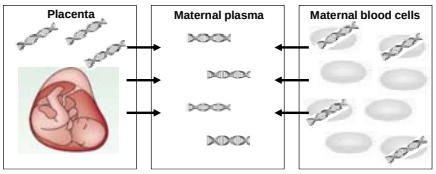
Cell-free **Fetal** DNA in maternal plasma

1997 Fetal DNA is present in maternal plasma and serum. *Lo et al, Lancet*

1999 After delivery, fetal DNA clears the maternal plasma very rapidly; half-life is about 15 minutes. *Lo et al, Am J Hum Genet*

2010 Entire fetal and maternal genome is found in maternal plasma. *Lo et al, Science Transl Med*

YM Dennis Lo, FRS
Chinese University of Hong Kong



- Both cell-free ***fetal*** and cell-free ***maternal*** DNA circulate in maternal plasma.
- Fetal DNA comes primarily from the placenta.
- Maternal DNA comes primarily from maternal blood cells.
- Cell-free fetal and maternal DNA circulate in maternal plasma as relatively short fragments (150-200 base pairs) and represent the entire genome.
- Fetal DNA is 5-25% (~10% on average) of the total cell-free DNA in maternal plasma.

Method to determine fetal Down syndrome using free DNA fragments in maternal plasma

In late 2008, two 'proof of concept' papers in *PNAS*:

Fan HC, *et al.* Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. *PNAS* 2008;105:15255.

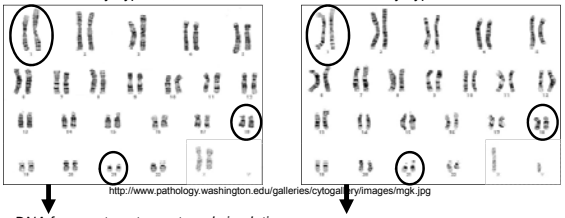
Chiu RWK, *et al.* Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massive parallel genomic sequencing of DNA in maternal plasma. *PNAS* 2008;105:20458.

The results were almost perfect!

Why Does It Work?

Mother's karyotype: 46,XX

Fetus' karyotype: 46,XY



http://www.pathology.washington.edu/galleries/cytogallery/images/mgk.jpg

- *DNA fragments enter maternal circulation.*
- *Sequencing method does not differentiate maternal from fetal DNA.*
- *Each unique fragment is assigned to a specific chromosome.*
- *Each chromosome represents a fixed percentage of total.*

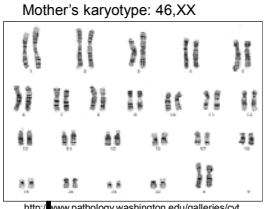
chrom1 = 8.5%

chrom18 = 2.8%

chrom21 = 1.3%

Why Does It Work?

Mother's karyotype: 46,XX



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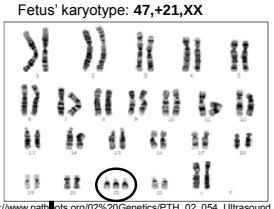
Fetus' karyotype: **47,+21,XX**



http://www.pathbuts.org/02%20Genetics/PTH_02_054_Ultrasound_Scanning_and_Cytogenetics/d/ELFH_Session/442/tab_679.html

- *A trisomy 21 fetus contributes 50% more chromosome 21 fragments.*
- *This adds to the 1.3% contributed by the mother.*
- *Therefore, the % chrom21 measured in an affected pregnancy must be higher than 1.3%.*

Why does it work?

- *If the fetus is euploid, the % chrom 21 is always constant, no matter what the fetal DNA contribution is.*
- *When the fetus has trisomy 21, the higher % chrom21 measured in the maternal circulation is proportional to the contribution of fetal DNA to total DNA.*

Identifying Down syndrome using MPSS

- Establish a reference range for *% chrom 21* (mean ± SD).
- Compare an individual patient's *% chrom 21* to the reference range by calculating the **z-score**.
- The z-score tells you how many SDs the patient's value is from the mean (at the mean, z=0).
- Set a z-score cut-off to identify fetal Down syndrome: ≥ +3 for example.

Three Studies in 2011

In early 2011, three studies using essentially the same method:

Chiu RW, *et al. BMJ 2011;342:c7401.*
Down syndrome 86 DR = 100%
euploids: 146 FPR = 2.1%

Ehrich M, *et al. Am J Obstet Gynecol* 2011;204:183-5.
Down syndrome 39 DR = 100%
euploids: 410 FPR = 0.2%

Sehnert AJ et al. *Clin Chem* 2011;57:1042-9.
Down syndrome: 13 DR = 100%
euploids: 34 FPR = 0.0%

A Fourth Study in 2011

Palomaki GE, *et al*. DNA sequencing of maternal plasma to detect Down syndrome: An international clinical validation study. *Genet in Med* 2011;13:913-20.

- Document the performance (sensitivity and specificity) of a laboratory-developed test (LDT) to identify Down syndrome in early pregnancy based on massively parallel shotgun sequencing of free DNA in maternal plasma.
- Establish a sample bank to allow for documentation of subsequent improvements in the LDT, including the identification of other aneuploidies (e.g., trisomy 18).

Study funded by Sequenom, Inc., San Diego, CA


Genetics inMedicine | ORIGINAL RESEARCH ARTICLE Open

DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study

Glenn E. Palomaki, PhD[1], Cosmin Deciu, MS[2], Edward M. Kloza, MS[1], Geralyn M. Lambert-Messerlian, PhD[1], James E. Haddow, MD[1], Louis M. Neveux, BA[1], Mathias Ehrich, MD[3], Dirk van den Boom, PhD[3], Allan T. Bombard MD, MBA[4–6], Wayne W. Grody, MD, PhD[5–7], Stanley F. Nelson, MD[5,7,8] and Jacob A. Canick, PhD[1]

Clinical Validity for all three trisomies

- 99.1% of samples were interpreted
  - 17 failures (3 trisomy 18 and 14 euploid)
- *98.9% detection rate for trisomies (281/284)*
  - 210/212 (99.1%) for Down syndrome
  - 59/59 (100%) for trisomy 18
  - 12/13 (91.7%) for trisomy 13
- 1.3% false positive rate among euploids
  - 1/1,688 (0.1%) for Down syndrome
  - 5/1,688 (0.3%) for trisomy 18
  - 16/1,688 (1.0%) for trisomy 13

Palomaki et al, 2012

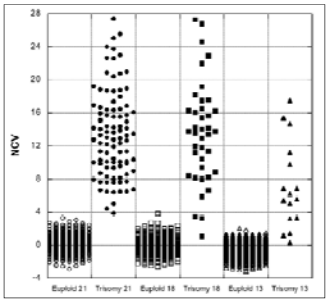
How do patients understand this?

- Very high performance
  - 98-99% DR; 0.2% FPR
- Low uninformative rate (<1%) if 2 samples/person.
- In other words…
  - Will be positive in 99/100 Down syndrome pregnancies
  - Will be mistakenly positive in 1 out of 500 even though the fetus is unaffected
  - Test will not give an answer in 1 out of 125 women

More excellent results!

- **Feb 23** (Epub ahead): Bianchi, et al, Genome-Wide Fetal Aneuploidy Detection by Maternal Plasma DNA Sequencing, *Obstetrics and Gynecology*, Vol 119, No. 5, May 2012.

Results: Autosomes



Bianchi, et al, *Obstetrics and Gynecology*, Vol 119, No. 5, May 2012, e-pub ahead

Who could/should be offered MPSS prenatal screening?

- Women who are thinking about diagnostic testing
  - Chorionic villus sampling
  - Amniocentesis
- However, they must be considered "high risk" as defined by:
  - Advanced maternal age
  - Abnormal ultrasound finding
  - Personal or family history of aneuploidy
  - Positive 1st or 2nd trimester screening test

What about multiples?

- Samples from twins are accepted
- No higher order multiples
- Multiples paper is now in press

Who cannot be offered the test currently?

- No biological reason why the test should be less sensitive in the "low risk" population
  - Sequenom and Verinata are not accepting samples from "low risk" women
  - No studies in low risk population
    - Stay tuned!

Should MPPS be offered as a primary screening test for all women?

Positive Attributes

- Very high DR: 98-99%
- Very low FPR: 0.2%

Negative Attributes

- High cost
- Infrastructure not in place (yet)
- Turnaround time
  - Unacceptable?
- No published data in low risk population

Where do I see this fitting into the current testing schema?

- If someone is AMA at early GA ,offer MPSS vs. other forms of screening
  - Higher detection rate than integrated and other forms of screening
  - Second trimester targeted scan still recommended due to increased risk of structural abnormalities
  - msAFP also recommended as MPSS does not give risk for neural tube defects

Where do I see this fitting into the current testing schema?

- If not AMA, offer routine serum screening
  - First trimester, second trimester or integrated serum test
  - If routine screening returns positive, then offer MPSS vs. diagnostic testing (CVS or amniocentesis)
  - If U/S reveals abnormality, offer MPSS vs. amniocentesis/CVS
  - Can be offered at any GA, beginning at 10 weeks

What is an abnormal U/S?

- U/S unfortunately holds a lot of weight but is a poor screen
  - Especially for Down Syndrome
- False positive rate 11-12%
- 50% detection rate
  - But… it's everyone's favorite test

What is an abnormal U/S?

- Easy to agree that major structural abnormalities are considered abnormal
  - CHD, brain malformations, clefts, limb defects
- But what about soft markers?
  - These can be normal variants in the low risk population with normal serum screening

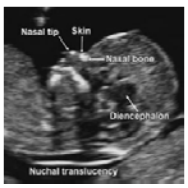
Must consider the entire clinical scenario. If you consider the patient to be at high risk for aneuloidy based on age, ultrasound findings and/or prior serum screening or personal history of aneuploidy, and would offer her diagnostic testing, then do the same with MPSS.

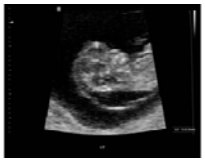
What if I get a positive MPSS test result?

- Diagnostic testing currently recommended
  - CVS: 10-12+ wks
  - Amniocentesis: 15 weeks onward
- May want to consider going straight to diagnostic testing in the patient at later GA who would consider termination
  - Consider turnaround time

Does my patient still need an NT?

- Provides additional information especially in the setting of a cystic hygroma
- MPS is not informative for Turner syndrome, one of the most common causes for cystic hygroma
- Provides information on congenital heart abnormalities





What about an amniocentesis or CVS?

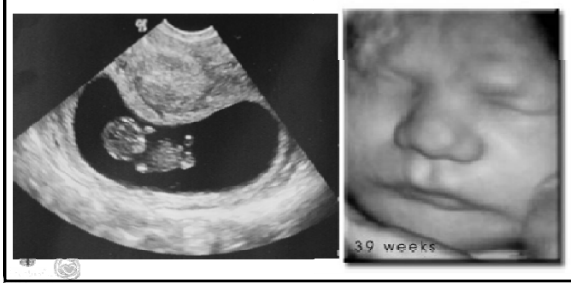
- MPS detects only certain aneuploidies
- Other genetic syndromes may go undetected in cases of an increased NT or cystic hygroma
  - Noonan syndrome, and many others
- May want to consider invasive testing with karyotype and reflex to microarray in this setting

What about an msAFP and a level II?

- All patients should be offered msAFP
  - To aid in the detection of ONTD and abdominal wall defects
- AMA:
  - offer level II secondary to an independent risk of structural abnormalities with advanced maternal age

What gestational age can I draw the test?

- 10w +0 days to term



What is the turnaround time?

– 8 to 10 business days from the time the sample arrives at Sequenom or Verinata.

What does ACOG say about MPPS?

- Nothing yet
- But we know the ACOG statement in Nov, 2011:
- "All women, **regardless of age**, should have maternal age of 35 years alone should no longer be used as a cutoff to determine who is offered screening versus who is offered invasive testing."

Prenatal Detection of Down Syndrome using Massively Parallel Sequencing (MPS): a rapid response position statement from a committee on behalf of the Board of the International Society for Prenatal Diagnosis, 24 October 2011

- ISPD statement generally follows the guidelines that I am providing today

Cost

- Pricing varies based on payer and perhaps lab.
- Without coverage the test is on the order of $1900
- Many commercial PPOs have a copay of $235
- Comercial HMOs, Medicaid, and other insurances may have a copay of $475.
- Payment plan may be an option

Who is offering this clinically?

- 2 CLIA approved labs are currently offering MPPS testing:
  - Sequenom CMM
  - Verinata
- Technology is same
- Method of testing and method of expressing is different
- Published data indicate that the tests are probably about the same in terms of performance

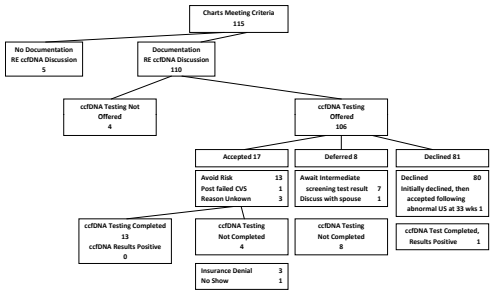
WIH PDC Experience During First 10 weeks of Offering MPSS

- After IRB approval, we examined eligible medical records of women attending our PDC during the first 10 weeks of implementation Nov 4, 2011 to Jan 20, 2012
- Eligibility
  - AMA, abnormal serum screen, family histroy, or suspicious ultrasound finding

WIH PDC Experience During First 10 weeks of Offering MPSS

Indications for Referral N (%)	
Maternal Age	61 (58%)
Maternal Age +Other Indication	20 (19%)
Positive Serum Screen	17 (16%)
Abnormal Ultrasound	8 (7%)
Total	106 (100%)

WIH PDC Experience During First 10 weeks of Offering MPSS



WIH PDC Experience During First 10 weeks of Offering MPSS

ccfDNA Testing by Indication and Insurance Coverage			
Indication	**PPO** Women Accepting/ Total Covered	**Non-PPO** Women Accepting/ Total Covered	Total
Maternal Age	6/41(15%)	0/18 (0%)	6/59 (10%)
Maternal Age +Other Indication	3/9(33%)	1/11 (10%)	4/20 (20%)
Positive Serum Screen	2/9 (22%)	3*/8 (38%)	5/17 (29%)
Abnormal Ultrasound	2/6 (33%)	0/4 (0%)	2/10 (20%)
Total Accepted (p=0.19)	13/65 (20%)	4/41 (10%)	17/106 (16%)
Total Completed (p=0.01)	12/65 (18%)	1/41 (2%)	13/106 (12%)

WIH PDC Experience During First 10 weeks of Offering MPSS

Reasons for Declining ccfDNA N(%)	
No Further Testing Desired/Wouldn't change anything	30 (37%)
Opted for CVS/Amniocentesis	20 (24%)
Follow-Up Screen Negative	15 (19%)
Cost/insurance issues	8 (10%)
Undocumented	8 (10%)
Total	81(100%)

Conclusions

- MPSS testing will certaintly play an important role in prenatal diagnosis
- It is an exciting, early, non-invasive, accurate method for the detection of T21, T18, and T13 (and sex chromosome abnormalities) in high risk patients
- Patients must be accurately informed about the benefits and limitations of the test prior to consenting