



NIPT

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Disclosure

- Faculty: William Ehman MD
- Relationships with commercial interests:
 - No pharmaceutical interests
 - I am a consultant & facilitator with the MORE^{OB} Program for which I sometimes receive honoraria
 - I am a self employed Family Physician
- I will not recommend off-label drugs/devices

Objectives

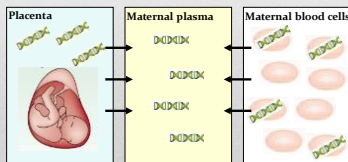
- What is NIPT?
- How does it work?
- What are the applications?
- NIPT in clinical practice
 - Where does it fit in?
 - PEGASUS
- Other issues
 - Ethical, Legal and Social

Terminology

- **cffDNA**: Cell free fetal DNA
- NIPD: Non-invasive prenatal diagnosis
- **NIPT**: Non-invasive prenatal testing
- NIPS: Non-invasive prenatal screening
- NGS: Next generation sequencing
- MPS: Massively parallel sequencing

Cell free DNA (cfDNA) in Maternal Circulation

- Both the mother and the fetus* produce cell-free DNA (cfDNA)
- Maternal (cfmDNA): Breakdown of hematopoietic cells
- Fetal (cffDNA): Apoptosis placenta (syncytiotrophoblast)



*Lo et al, Lancet 1997

cfDNA In Maternal Plasma



- Fragments of extracellular cffDNA detectable by 4 wks
- cffDNA ↑ with gestation
- Sufficient (10% total cfDNA) by 7-10wk
- up to 50% by term

- Rapid clearance Post Partum (~1-2 h)
- “Real-time snapshot of fetal genetic status”

Prenatal screening/diagnostic applications of cfDNA

- Fetal autosomal aneuploidies
- Fetal sex determination
 - X-linked disorders, CAH
- Rhesus typing
- Single gene disorders
 - Huntingtons, achondroplasia, MD
- Microdeletion syndromes
- Sex-chromosome aneuploidy
- Whole fetal genome sequencing



Chromosomal Risk by Age

Table 4: Risk of Down Syndrome and Other Chromosome Abnormalities in Live Births by Maternal Age

Maternal Age (At Term)	Down Syndrome	Total Chromosome Abnormality	Maternal Age (At Term)	Down Syndrome	Total Chromosome Abnormality	Maternal Age (At Term)	Down Syndrome	Total Chromosome Abnormality
25	1 in 1,250	1 in 476	32	1 in 637	1 in 323	39	1 in 125	1 in 81
26	1 in 1,190	1 in 476	33	1 in 535	1 in 286	40	1 in 94	1 in 63
27	1 in 1,111	1 in 455	34	1 in 441	1 in 234	41	1 in 70	1 in 49
28	1 in 1,031	1 in 435	35	1 in 356	1 in 179	42	1 in 52	1 in 39
29	1 in 955	1 in 417	36	1 in 281	1 in 149	43	1 in 40	1 in 31
30	1 in 840	1 in 385	37	1 in 217	1 in 123	44	1 in 30	1 in 21
31	1 in 741	1 in 365	38	1 in 166	1 in 105	>45	>1 in 24	>1 in 19

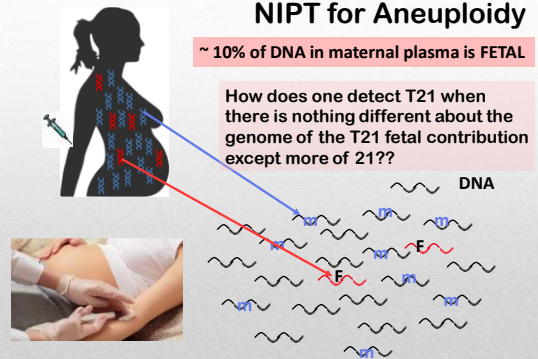
Source: Hedt CA and Hook EB. 1996

History of Aneuploidy Screening

- 70's & 80's: AMA(>35 yrs): < 1/3 of DS detected
 - 2% abnormal ~ 0.5-1.0% fetal loss from procedure
- 90's: MSS (double, triple, quad marker screen)
 - 4% screen positive had abnormal chromosomes
- Late 90's: 1st TM + 2nd TM (combined) +/- NT
 - 1 in 30+ = aneuploidy; 90% of DS detected
 - Amniocentesis fetal loss: 0.5 -1% (1 loss for 5 DS)
- 1997: cffDNA discovered in maternal circulation
 - "Cataclysmal change" for screening

NIPT for Aneuploidy

~ 10% of DNA in maternal plasma is FETAL

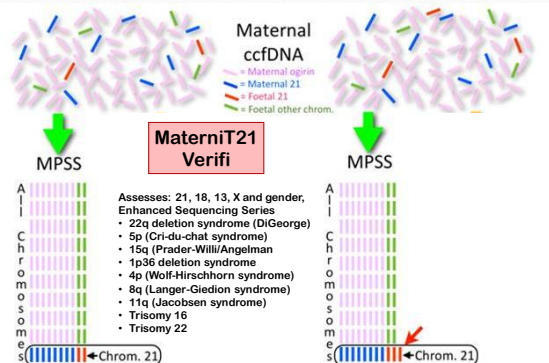


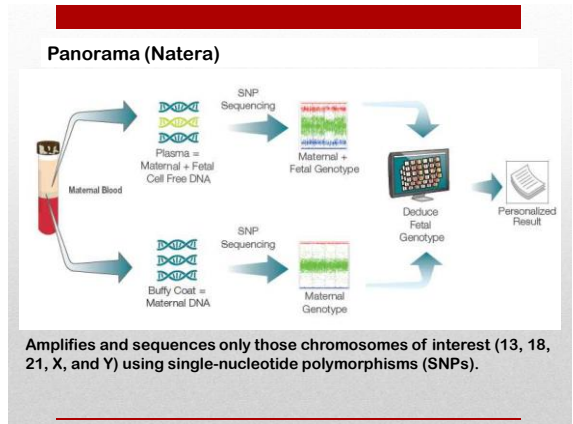
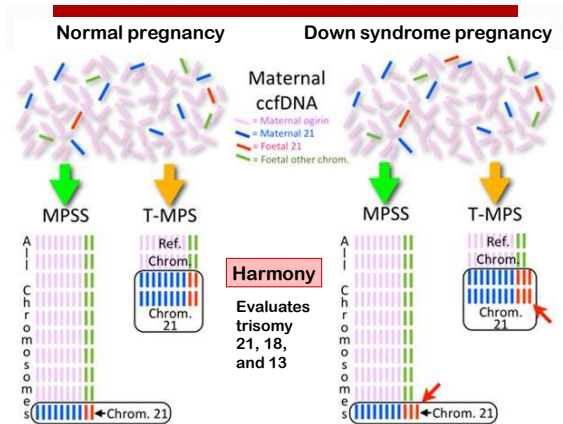
NIPT Methods of Detecting Aneuploidy

- Shotgun massively parallel sequencing (s-MPS)
 - Millions of DNA fragments are mapped to corresponding genome; aneuploidy will have excess or deficit of chromosome in question
- Targeted massively parallel sequencing (t-MPS)
 - Selectively amplifies the chromosomes of interest (21, 18, 13)
- Maternal DNA "subtracted out"
 - fetal DNA is sequenced.

Normal pregnancy

Down syndrome pregnancy





Availability of NIPT

- Panorama™ (LifeLabs) - no twins**
 - 21,18,13,X,Y & triploidy, gender (\$795)
 - + DiGeorge (22q11.2) syndrome (\$1050)
 - + Cri-du-chat, 1p36 Deletion, Angelman, Prader-Willi (\$1100)
- Harmony™ - twins ok**
 - 21,18,13 (\$795)
 - +Y, gender (\$815)
 - + X (X0, XXY, XYY, XXXY) (\$815)
- Verifi™ - twins ok**
 - 21,18,13 (\$795)
 - +9,16, (22q11.2) syndrome, X,Y, Cri-du-chat, 1p36 Deletion, Angelman, Prader-Willi, 5p Cri du Chat, 4p Wolf-Hirschhorn (\$1050)

*Cost estimate in 2014

Ontario: Criteria for NIPT Coverage

- Section A: for 21, 18 & 13 aneuploidy**
 - Singleton and any of the following
 - Maternal multiple marker +
 - Age ≥ 40
 - NT ≥ 3.5 mm
 - Previous pregnancy with aneuploidy
- Section B (need specialist consult)**
 - Ultrasound anomalies suggestive of trisomy
 - Risk of aneuploidy > +Mat. Mult. Marker alone:
 - E.g soft markers
 - For Sex chromosome determination with:
 - Risk of sex limited or sex chromosome disorder

NIPT vs Amniocentesis(B.C.)

	NIPT Blood Test	Amniocentesis
Detection Rate		
Down syndrome	>99%	100%
Trisomy 18	>97%	100%
Trisomy 13	Approx. 80% *	100%
False Positive Rate (FPR)	<0.5% **	0
Risk to Pregnancy	0	1 in 200 pregnancy loss
Failure Rate	2 to 4%	< 1 in 1000
Result Turn-Around Time	Approx. 14 days	Approx. 14 days ***
Cost	\$795	Covered by Province
Serum Screen Eligibility*	Screen+ DS, T18, >40yr, prev. trisomy, IVF/ICSI	Screen+ DS, T18, or spina bifida

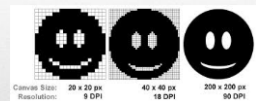
* Limited Data

** positive NIPT results need amniocentesis for confirmation.

*** Rapid test for 21, 18, 13, X, Y within 48 - 72 hrs for high-risk or late gestation

Factors that influence accuracy and scope of NIPT

- Sequencing depth
- GC base content
- Fetal fraction



% GC Content by Chromosome



- GC = guanine-cytosine content
- DNA with high GC-content is more stable
- FF = cell-free fetal DNA
- Total (mat. & fetal) cfDNA
- 4% minimum to perform test

Factors that affect fetal fraction

- **Maternal weight (-)**
 - Obesity lowers fetal DNA
- **Gestational age/CRL (+)**
 - 0.1% /w to 21w, then 1% /w to term
- **Fetal karyotype**
 - T21 (+), T18, 13 (-)
- **Serum PAPP-A, fb-HCG (+)**
- **Mosaicism (-)**
- **Multiple pregnancy**
- **NOT affected by:**
 - Maternal age
 - fetal gender, NT

NIPT: Other issues

- 10-14 day turn-around
- Up to 5% of cases do not generate a result, leads to delayed diagnosis
- All +ve NIPT results require confirmatory invasive testing

Uses: Fetal Sex Determination

- Presence of Y-chromosome
- 1st clinical application of NIPT
- Helpful for
 - fetal genital ambiguity
 - Serious x-linked conditions
 - Congenital adrenal hyperplasia

Uses: Non-invasive fetal blood group genotyping

- Determination of fetal RH D, c, E, Kell status using maternal blood sample
- Used to guide prenatal management in sensitized patients*
- Selective prophylaxis in unsensitized patients**



*In Alberta, samples sent to Bristol, (£250) covered by Canadian Blood services
Offered routinely for fetal D antigen status in Denmark and Netherlands at 25/28w

Uses: Single gene disorders

Paternally inherited or de novo autosomal dominant disorders

- Achondroplasia
- Apert syndrome
- Early onset primary dystonia I
- Thanatophoric dysplasia
- Huntington's disease

Autosomal recessive conditions where parents carry the same altered allele

- Sickle cell anaemia
- β -thalassaemia
- X-linked conditions
- Haemophilia

Autosomal recessive conditions where parents carry different altered alleles

- α -thalassaemia
- β -thalassaemia
- congenital adrenal hyperplasia
- Cystic fibrosis
- Leber congenital amaurosis
- Propionic acidemia
- Frasers syndrome
- Autosomal recessive polycystic
- kidney disease

Sex chromosomal aneuploidy (SCA)

- SCA 1:400 LB (> autosomal trisomy)
- Detection rate for Turner syndrome (45,X): 90 – 95%, usually US findings
- Too few cases to know the detection rate of 47,XXX, 47,XXY, 47,XYY
- Counseling complex!

Performance of NIPT in twin pregnancies

- Only published data is from using Harmony:
 - results could be reported in 93% of cases
 - DR for T21 - ~92%

NIPT: Advantages and Disadvantages

Advantages	Disadvantages
Convenient	Expensive \$ 800.00-1200
Early results	Complex
No risk of miscarriage	Significant test failure rate (2-4%)
>99% accurate T21	Turn-around time (10-14 d)
Peace of mind	Limited scope
Capabilities "moving target"	Is still a screening test; invasive testing required for confirmation
Improved detection rate (9%), decreased FPR (5.0 to 0.2%.) Hui et al 2013	False +/- s - Placental source, similar to CVS - Non-fetal sources of aneuploid xm's can cause false +
Potentially cost-effective	Loss of other benefits of screening

NIPT

Where does NIPT testing for aneuploidy fit within Canadian Prenatal Maternal Screening?



PEGASUS - Activities



1. Compare MPSS vs T-MPS done in Canada
2. Cost effectiveness of NIPT algorithms
3. Ethical, legal & social issues
4. Develop tools for health care end users

PEGASUS:

Personalized Genomics for prenatal Aneuploidy Screening USING maternal blood

- Background:
 - 450,000 Canadian women pregnant/year
 - For Down's syndrome screening: 10,000 will have amniocentesis
 - 350 will have Down Syndrome
 - 70 normal pregnancies will be lost 2° to amniocentesis
 - NIPT could avoid 9700 amniocentesis & 70 losses
- Purpose: Research evidence based, cost effective approach to implementation of NIPT

PEGASUS - Arms



PEGASUS ARMS

**High Risk:
N=3600**

1. +screen; US, etc.
2. To exclude aneuploidy & avoid amnio/ CVS
3. Compare with reference standard

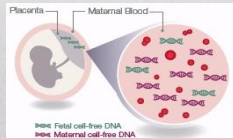
**Low Risk
N=2000**

1. Will be informed
2. To assess cost-effectiveness and utility of primary and contingent algorithms
3. Validated with industry standard

Differential diagnosis of “False Positives” (0.5%) Cases

- Discordant cases: multiple biological explanations

- Confined placental mosaicism
- Twin demise
- Maternal sex chromosome abnormality
- Maternal Tumor
- Lab error



Ethical and Social Issues

- Ease of access and test safety may lead to routinization: compromising informed decision making and reproductive autonomy
- Pressure to test:
 - By caregiver to avoid wrongful birth suit
 - Social pressure, leading to notion that women are responsible for bearing a child with a disability
- May increase the number of terminations
- May stigmatize people with disabilities
- Non-medical use: e.g. fetal gender; paternity
- Increasing need for counselling

Physician Liability and NIPT

- Are physicians obligated to disclose its availability to eligible patients as part of the discussion about prenatal screening/diagnosis?
- If discussed, it is important to disclose limitations with respect to accuracy and number of disorders detected compared with invasive options
- Failure to disclose could lead to false assurances raising consent and liability issues, particularly if the child is disabled

Toews, Caulfield, JOGC 2014;36(10):907-914

Mitigating the Ethical, Legal and Social Issues

- “these challenges can be overcome with approaches to counselling that support parents to make informed choices by providing a clear discussion on the implication of results and allowing time for reflection”
- “Regulation and guidelines... and strategies for the training and education of health professionals to facilitate best practice are essential.”

Daley, Arch Dis Child Fetal Neonatal Ed 2014

SOGC: NIPT for Trisomy 21,18,&13, using cff DNA

- “should be an option available to women at increased risk in lieu of amniocentesis. Pretest counselling... should include a discussion of the limitations...”
- “No irrevocable obstetrical decision should be made in pregnancies with a positive non-invasive prenatal testing result without confirmatory invasive diagnostic testing”
- “studies in average-risk pregnancies and a significant reduction in the cost of the technology are needed before this can replace the current maternal screening approach using biochemical serum markers with or without fetal nuchal translucency ultrasound”

SOGC Committee Opinion No 287, Feb. 2013

NIPT: Take home messages

1. NIPT uses next generation sequencing to directly measure fetal DNA in the maternal circulation
2. Aneuploid and non-aneuploid applications
3. Higher sensitivity/specificity than current screening tests
4. The commercial tests have similar performance
5. Potential to replace fetal karyotyping
6. cfDNA reflects the placenta; most FP's have underlying biological abnormalities
7. Currently recommended as 2nd tier test
8. All NIPT positives need to be confirmed with invasive techniques
9. **Pre and Post test counselling essential**