Non-Invasive Prenatal Screening as a Clinical Service: Benefits, Limitations and Challenges

Urvashi Surti, PhD

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NIPT by next generation sequencing

DNA fragments in maternal plasma

Fragments sequencing and alignment

Sequence counting

Chromosomes

Bioinformatics and chromosome aneuploidy determination
PLAN

• Review our experience at Magee Womens Hospital with the clinical cases that were sent out for NIPT from 2012 to the present

• Review some of the investigational cases involving NIPT performed at the academic laboratory, Magee Womens Research Institute by Dr. David Peters, PhD and his team
NIPT send outs

- 2012: 97
- 2013: 559
- 2014: 876
- 2015: 849
- 2016: 434
Test statistics

NIPT

Karyotype
NIPT detection rate

2012: No results
2013: No results
2014: No results
2015: No results

No results, inconclusive, Sex chr, Trisomy 13, Trisomy 18, Trisomy 21
High false positive rate for sex chromosome abnormalities

NIPT concordance

- 6/20 (30%) - false positive
- suspected trisomy -10
- Negative - 9
- Not tested - 1
**NIPT false negative/positive results**

<table>
<thead>
<tr>
<th></th>
<th>T21</th>
<th>T18</th>
<th>T13</th>
<th>Sex chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>False positive</strong></td>
<td>0 (3)</td>
<td>0 (6)</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td><strong>False negative</strong></td>
<td>1</td>
<td>?</td>
<td>?</td>
<td>2</td>
</tr>
<tr>
<td><strong>Sex discordant</strong></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>
**NIPT positive results**

<table>
<thead>
<tr>
<th>NIPT positive</th>
<th>Prenatal confirmation</th>
<th>Postnatal confirmation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>82 (3.8%)</td>
<td>30 (36%)</td>
<td>14 (17%)</td>
<td>44 (54%)</td>
</tr>
</tbody>
</table>

**NIPT negative results**

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Prenatal studies CVS/Am</th>
<th>Postnatal studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIPT negative</td>
<td>2046</td>
<td>8</td>
<td>?</td>
</tr>
<tr>
<td>US abnormal</td>
<td>490</td>
<td>22 (4%)</td>
<td>?</td>
</tr>
</tbody>
</table>
What are we missing?

<table>
<thead>
<tr>
<th></th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal MA</td>
<td>13.5%</td>
<td>16%</td>
<td>16.5%</td>
<td>19.4%</td>
</tr>
</tbody>
</table>
Cell free DNA based microdeletion detection screening and the importance of maternal genetic counseling
Case 1 Clinical Presentation

- 40 yr. old G6P2 underwent NIPT for AMA
- She also enrolled in a research study and her blood sample was banked
- PMHx: Chronic hypertension
- **Clinical Test was positive for 22q deletion DiGeorge syndrome**
- Referred to Magee fetal anatomy and echocardiography. Testing was within normal limits, except for growth restriction
- Pt declined diagnostic genetic testing during pregnancy.
- Maternal Echocardiogram did not show abnormalities and she did not have stigmata of DiGeorge syndrome.
Reference lab report

Test Result for Chromosomes 21, 18 and 13

<table>
<thead>
<tr>
<th>Negative</th>
<th>This specimen showed an expected representation of chromosome 21, 18 and 13 material. Clinical correlation is suggested.</th>
</tr>
</thead>
</table>

Test Result for Y Chromosome

<table>
<thead>
<tr>
<th>Y chromosomal material detected</th>
<th>Consistent with a male fetus.</th>
</tr>
</thead>
</table>

Additional Findings: Decreased representation of chromosome 22q.

These findings are suggestive of a 22q deletion, affecting the region associated with DiGeorge, and several phenotypically overlapping syndromes.

22q11 (DiGeorge, Velo-cardio-facial, Shprintzen, Sadelakova, and conotruncal anomaly face syndromes) is an autosomal dominant condition caused by a submicroscopic deletion of the long arm of chromosome 22. The disorder is characterized by Cardiac Abnormalities, Abnormal Facial Features, Thymic Aplasia, Cleft Palate, Hypocalcemia (CATCH-22). Incidence is ~1 in 4000 births. Most cases are not inherited (de novo), but transmission from a parent carrying the 22q11 deletion is seen in ~7% of cases.¹

Performance

The performance characteristics of the MaterniT21 PLUS laboratory-developed test (LDT) have been determined in a clinical validation study with pregnant women at increased risk for fetal chromosomal aneuploidy.²³

<table>
<thead>
<tr>
<th>Intended Use</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Confidence Interval (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy 21</td>
<td>99.1%</td>
<td>99.9%</td>
<td>96.3 - 99.8%</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>&gt;99.0%</td>
<td>99.6%</td>
<td>99.2 - 99.8%</td>
</tr>
<tr>
<td>Trisomy 13</td>
<td>91.7%</td>
<td>99.7%</td>
<td>99.3 - 99.8%</td>
</tr>
<tr>
<td>Y chromosome</td>
<td>99.4%</td>
<td>99.0%</td>
<td>99.0 - 99.6%</td>
</tr>
</tbody>
</table>

Limitations of the Test

DNA test results do not provide a definitive genetic risk in all individuals. Cell-free fetal DNA does not replace the accuracy and precision of prenatal diagnosis with CVS or amniocentesis. A patient with a positive test result should be referred for genetic counseling and offered invasive prenatal diagnosis for confirmation of test results. A negative test result does not ensure an unaffected pregnancy. The absence of an Additional Finding does not indicate a negative result. While results of this testing are highly accurate, not all chromosomal abnormalities may be detected due to placental, maternal or fetal mosaicism, or other causes. Sex chromosomal aneuploidies are not reportable for known multiple gestations. The health care provider is responsible for the use of this information in the management of their patient.
Clinical Presentation

- Patient developed severe preeclampsia
- Baby boy was born at 32 5/7 weeks by C section

Physical Exam: non-dysmorphic male, no webbing, no abnormal genitalia.
Postnatal baby echocardiogram: structurally normal heart

- Karyotype and FISH for DiGeorge were performed
Chromosome analysis

Idic Y?
FISH analysis

Commercial TUPLE (HIRA)/ARSA probe Vysis

Commercial SRY/CEPX probe Vysis

Negative DiGeorge/VCFS

Positive for Isodicentric Y

Yatsenko 2015 | Genetics in medicine
Microarray analysis

Positive for Isodicentric chromosome Y

Chromosome Y

Yatsenko 2015 | Genetics in medicine
DiGeorge Critical region

2.6 Mb Standard A-D Deletion

0.8 Mb Atypical B-D deletion

Deletion in the patient
Chromosome 22q11.2 Deletion Syndrome

• Typical DGS/VCFS
  • 1:4000 births
  • Developmental delay
  • Craniofacial abnormality/CP
  • Immune deficiency
  • Cardiac anomalies
  • Renal and eyes anomalies
  • Hypoparathyroidism
  • 7% of parents have 22q11.2 deletion

• Atypical DGS/VCFS
  • Rare with milder phenotype
  • Developmental delay
  • Facial dysmorphism
  • Cardiac anomalies
  • 50% of parents have 22q11.2 deletion
• Whole Genome sequencing of maternal plasma DNA obtained at 11 wks gestation was performed in MWRI academic lab and showed that coordinates of the deleted region matched closely with the deletion detected by microarray. Lab providing the NIPT results should provide the coordinates.

• Evaluation of the trio of fetus, mother and the father is critical.
A. DiGeorge Critical region

B. 2.6 Mb Standard A-D Deletion

C. 0.8 Mb Atypical B-D deletion

D. Folds Difference from Expected

Yatsenko 2015 | Genetics in medicine
Case 2 normal NIPT results and abnormal cytogenetics
• 19-year-old gravida 1 para 0

• Ultrasound at 19 weeks: echogenic bowel noted - only abnormality. Some structures (including cardiac) not evaluated due to sub optimal visualization. Order to repeat US

• Ultrasound at 20 wks 5 days: placentomegaly, thickened nuchal fold, small fetal stomach, possible right clubbed foot, areas of echogenic bowel, bilateral foot abnormalities.

• Refused any invasive testing but agreed to NIPT and CF carrier screening and CMV infection screening (due to echogenic bowel).

• CMV IGM test negative

• CF carrier screen test negative for mutations and variants.

• NIPT: results consistent with diploid chromosomes for 21, 13, 18, and diploid XY sex chromosomes
Ultrasound at 25 weeks: fetus large for gestational age, polyhydramnios, thickened placenta, multiple anomalies as noted earlier.

Maternal blood pressure was elevated 140/94 therefore she was evaluated for pre-eclampsia.

Delivered premature male newborn at 25 weeks 4 days. Baby sent to Children’s Hospital; however, due to multiple congenital abnormalities, resulted in neonatal death.

Obtained peripheral blood from neonate

G-banded results: 69,XXY

CGH + SNP microarray on newborn blood -> could be consistent with the presence of a triploid cell line but did not display the typical triploid pattern.

Possibly diploid/triploid mosaicism
Pathology report on placenta “consistent with placental mesenchymal dysplasia”

- placenta >90 percentile
- absence of trophoblastic hyperplasia
- multiple cystic areas scattered throughout placenta

FISH for 18/X/Y on paraffin placental section:

**Triploid XXY**
(Some cells with XY and some XX most likely due to physical truncation of paraffin section)

Extracted DNA from “cystic” and “normal” appearing areas of the placenta for **microarray analysis**.
(Villi from these areas failed to grow in culture)
Genome view of the “cystic” appearing villi

Whole genome appears to be **triploid XXY**.
arr(1-22)x3,(X)x2,(Y)x1

Case was ran with a male control
Whole genome view for the “normal” appearing villi

Appears to be $46,XX$, **dup(5)(q21.2q35.3)**.
arr[GRCh38] 5q21.2q35.3(104299800_180684501)x1

Case was run with a male control
Chromosome 5 showing large duplication (76.4Mb) on the q-arm (5q21.2q35.3)
Paternal Origin of Triploidy
STR Analysis: D5S818(5q21-q31)

Maternal

Newborn Blood

Paternal

No (<5%) Maternal Contamination
CGH+SNP microarray analysis on mom’s blood was normal

CGH+SNP microarray analysis on dad’s blood was normal

Follow-up: contacted dad about blood for karyotypic analysis to look for balanced rearrangement involving 5q ------ patient declined
Prenatal detection of fetal triploidy from cell-free DNA testing in maternal blood.
Nicolaides KH¹, Syngelaki A, del Mar Gil M, Quezada MS, Zinevich Y.

METHODS:
Plasma and buffy coat samples obtained at 11-13 weeks' gestation from singleton pregnancies with diandric triploidy (n=4), digynic triploidy (n=4), euploid fetuses (n=48) were sent to Natera, Inc. (San Carlos, Calif., USA) for cfDNA testing. Multiplex polymerase chain reaction amplification of cfDNA followed by sequencing of single nucleotide polymorphic loci covering chromosomes 13, 18, 21, X, and Y was performed. Sequencing data were analyzed using the NATUS algorithm which identifies copy number for each of the five chromosomes.

NATUS—Next-generation Aneuploidy Testing Using SNPs
CONCLUSIONS:
cfDNA testing by targeted sequencing and allelic ratio analysis of single nucleotide polymorphisms covering chromosomes 21, 18, 13, X, and Y can detect diandric triploidy and raise the suspicion of digynic triploidy.
Case 3

40yo G3 P1011 at 11 weeks gestation.
PMH: Hepatitis C
Family history: unremarkable

Referred for AMA

Chose cfDNA
“Reliable NIPT evaluation not possible”
(did not meet quality control thresholds)
Repeat…..same result

Declined amnio
MMS: DS risk 1:187; Tri18 risk 1:1071
Ultrasound at 20 weeks

Large Left Adnexal Mass
(concerning for malignancy)

Ascites

Possible liver mass
CT:
Possible mass in transverse colon
Probable metastases to lung, liver and lymph nodes
Needle biopsy of liver mass:
Metastatic Adenocarcinoma
Probable GI origin
She recently delivered a normal baby
Case 4 and Case 5

- Next 2 prenatal cases were investigated in the academic laboratory as part of the research study to determine if known small deletions could be identified by NIPT
  - 4.2Mb deletion on 12p
  - 318.46Kb deletion on 5p15

46, XY, del(12)(p11.22p12.1)
Asperger’s Syndrome
Facial dysmorphia
Brachydactyly
Short stature

46, XX, del(12)(p11.22p12.1)
Failure to thrive
Developmental delay
Dysmorphic features

46, XY, del(12)(p11.22p12.1)
G-band karyotype

12p12.1

4.2 Mb deletion
Case 4

- Amniocentesis was performed at 21 wks of gestation and the microarray analysis showed the same 4.2 Mb deletion.
- Maternal sample was drawn at 35 wks of gestation and plasma DNA was extracted.
- Fetal DNA fraction was determined to be 5.7%.
- HiSeq2000 DNA seq was compared to 7 normal maternal plasma samples.
- All 7 pairwise comparisons detected 4.2 Mb deletion.

NEJM 2011 10;365(19):1847
Case 5

- G8P3 patient seen at 19wks gestation with increased risk for Down syndrome (1:42) and open neural tube defect (1:160) by multiple marker screen
- Amnio was performed, club feet and echogenic bowel noted
- 46,XX karyotype; CGH+SNP array revealed 318.4kb loss of 5p15.33
Array CGH profile showing an interstitial deletion in the short arm of chromosome 5. Top: Idiogram of chromosome 5. The deleted 5p15.33 region is indicated by a red rectangle. Below: A magnified view of the 5p subtelomeric region. [GRCh37/hg19] Shaded blue area indicates a loss in DNA copy number detected by 22 oligonucleotide probes (blue dots), located in the interval chr5:174,979-493,441 and encompassing an approximately 318 kb segment.
Case 5

• Subsequently CVS was performed on the next pregnancy
• MA analysis showed the same 5p deletion that was paternally inherited
• Plasma sample was obtained at 11 wks gestation
• 5p deletion could not be identified in plasma samples from both the pregnancies that were sequenced
http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0153182
Case 5

• This sample was part of the study that examined Genomics Variants Frequency (GVF) for each 50kb region in the human genome. Whole genome seq of 15 normal maternal plasma and six CVS controls was performed. Coef of variation of relative read counts was determined.

Chu T PLOS ONE June 2016
Case 5

• Extremely high sample to sample normal variation in the deleted region of 5p was noted in the normal control population resulting in failure to detect it in the plasma sample of this patient

• This represents a serious challenge to the application of NIPT technology for the detection of microdeletions and microduplications
Conclusions

• NIPT (NIPS) is a screening test
• False positive and negative cases should be investigated to determine the biological basis using the fetal, maternal and paternal samples in a commercial as well as an academic setting
• Precise genomic coordinates for the microdeletions and duplications should be available for proper genetic counseling
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