Current advances in the application of genome-wide NIPT

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## Percept® NIPT (VCGS assay)

<table>
<thead>
<tr>
<th>Year</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 2015</td>
<td>Illumina WGS NIPT tech transfer (13, 18, 21, X, Y)</td>
</tr>
<tr>
<td>2016-2017</td>
<td>WISECONDOR. Rare aneuploidy, parental translocations, mosaicism estimation, co-twin demise, triplets</td>
</tr>
<tr>
<td>2018</td>
<td>Segmental aneuploidies (as additional findings)</td>
</tr>
</tbody>
</table>

- NATA/RCPA accredited to NPAAC Standards and ISO 15189
- > 65,000 pregnancies screened (primary screen); 90% at 10-12 weeks; primarily within State of Victoria, Australia.
- Fully integrated clinical genetics service, liaise closely with cytogenetics lab
Rare autosomal aneuploidy

Known parental rearrangements

Genome-wide NIPT

Segmental aneuploidy

Maternal malignancy
Origin of cfDNA and relationship to CVS

Rare autosomal aneuploidy

Trisomies other than 13, 18, 21
• Lethal when non-mosaic

Increased risk for feto placental disease
• Miscarriage, true fetal mosaicism, UPD, CPM, IUGR
• Normal pregnancy outcomes are also common
VCGS approach for RAA screening

- WGS - obtain sequencing data on all chromosomes
  - Calculate fetal fraction (proportion of placental cfDNA in sample)
  - Calculate trisomic fraction (proportion of trisomic cfDNA in sample)
  - Estimate ratio of trisomic cfDNA to placental cfDNA (mosaicism)
  - Determine distribution of counts (segmental vs whole; mosaic vs non-mosaic)
Rare autosomal trisomies, revealed by maternal plasma DNA sequencing, suggest increased risk of feto-placental disease

Mark D. Pertile,1,2a Meredith Halks-Miller,3,4a Nicola Flowers,1 Catalin Barbacioru,4 Sarah L. Kinnings,3 Darcy Vavrek,3 William K. Seltzer,3 Diana W. Bianchi2,6a


- TF:FF ratio is highest in pregnancies with rare trisomy affected by miscarriage, TFM, UPD and IUGR
- Can be used to predict pregnancies at most risk for complications
<table>
<thead>
<tr>
<th>Study</th>
<th>Study size no.</th>
<th>Rare trisomy no. (%)</th>
<th>Most common rare trisomies</th>
<th>Population screened</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lau et al. (2014)</td>
<td>1,982</td>
<td>7 (0.35)</td>
<td>22</td>
<td>Elevated and average risk</td>
</tr>
<tr>
<td>Pescia et al. (2016)</td>
<td>6,388</td>
<td>50 (0.78)</td>
<td>7, 8, 22, 16</td>
<td>Elevated and average risk</td>
</tr>
<tr>
<td>Pertile et al. (2017) [Cohort 1]*</td>
<td>72,932</td>
<td>246 (0.34)</td>
<td>7, 15, 16, 22</td>
<td>Not determined</td>
</tr>
<tr>
<td>Pertile et al. (2017) [Cohort 2]</td>
<td>16,885</td>
<td>60 (0.36)</td>
<td>15, 7, 16, 22</td>
<td>Elevated and average risk</td>
</tr>
<tr>
<td>Fiorentino et al. (2017)</td>
<td>12,078</td>
<td>17 (0.14)</td>
<td>22, 7, 15</td>
<td>Elevated and average risk</td>
</tr>
<tr>
<td>Ehrich et al. (2017)</td>
<td>10,000</td>
<td>78 (0.78)</td>
<td>16, 7, 3</td>
<td>Elevated risk</td>
</tr>
<tr>
<td>Van Opstal et al. (2018)^</td>
<td>2,527</td>
<td>24 (0.95)</td>
<td>16, 7, 9</td>
<td>Elevated risk</td>
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<tr>
<td>Brison et al. (2018)</td>
<td>19,735</td>
<td>58 (0.29)</td>
<td>7, 16, 22</td>
<td>Elevated and average risk</td>
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<tr>
<td>Scott et al. (2018)</td>
<td>23,388</td>
<td>28 (0.12)</td>
<td>7, 16, 22</td>
<td>Elevated and average risk</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>165,915</strong></td>
<td><strong>568 (0.34)</strong></td>
<td></td>
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</tr>
</tbody>
</table>

*outcome data not available


^ cFTS >1:200; 9/24 (38%) cases T16 (low PAPP-A)
VCGS RAA frequency
n=141/40,310 (0.35%)

Similar to CVS rare trisomy distribution in cytotrophoblast

- Miscarriage
- UPD
- Fetal growth restriction
Miscarriage – RAA outcomes
n=75/182 (41%) [includes ongoing pregnancies]

- 54,000 pregnancies
- Chromosome dependent
- Gestation dependent
- Prior USS dependent
- Most represent missed Ab

T14 \(6/7\) (86%)
T15 \(39/44\) (89%)
T16 \(8/27\) (30%)
T22 \(14/24\) (58%)

\(67/102\) (66%)

UPD – RAA outcomes

Ongoing pregnancies n=16/68* (24%)

*16/68 (23.5%) UPD

Ongoing pregnancies tested using SNP CMA

28 trisomic conceptions (min)
- 3 pathogenic UPD
- 2/5 UPD15mat Prader-Willi syn.
- 1/1 UPD14mat Temple syn.
- 0/11 UPD7

1 in 3,375 pregnancies

<table>
<thead>
<tr>
<th>Case</th>
<th>CPM</th>
<th>TFM</th>
<th>UPD</th>
<th>IUGR</th>
<th>Error</th>
<th>TF/FF ratio</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>1</td>
<td>yes</td>
<td></td>
<td>severe</td>
<td></td>
<td>meiosis I</td>
<td>0.9</td>
<td>Preterm 34/40, IUGR</td>
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<tr>
<td>2</td>
<td>yes</td>
<td></td>
<td></td>
<td></td>
<td>mitotic</td>
<td>0.2</td>
<td>Normal livebirth</td>
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<tr>
<td>3</td>
<td>yes</td>
<td></td>
<td>severe</td>
<td></td>
<td>mitotic</td>
<td>0.9</td>
<td>IUFD 17/40, IUGR, MCA</td>
</tr>
<tr>
<td>4</td>
<td>yes</td>
<td>yes</td>
<td></td>
<td></td>
<td>meiosis I</td>
<td>0.7</td>
<td>TOP, TFM, UPD2</td>
</tr>
<tr>
<td>5</td>
<td>yes</td>
<td>yes</td>
<td></td>
<td></td>
<td>meiosis II</td>
<td>0.8</td>
<td>TOP, TFM, UPD2</td>
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<tr>
<td>6</td>
<td>yes</td>
<td></td>
<td>placental</td>
<td>severe</td>
<td>mitotic</td>
<td>1.0</td>
<td>Preterm 32/40, IUGR</td>
</tr>
<tr>
<td>7</td>
<td>yes</td>
<td></td>
<td>NA</td>
<td>severe*</td>
<td>NA</td>
<td>1.0</td>
<td>TOP, IUGR</td>
</tr>
</tbody>
</table>

* Including disproportionally short long bones <1st centile

IUGR, intrauterine growth restriction; IUFD, intrauterine fetal death; MCA, multiple congenital abnormalities; NA, not available; TOP, termination of pregnancy; TFM, true fetal mosaicism; UPD, uniparental disomy.


- Advise increased ultrasound surveillance
e.g. Trisomy 2 (100%) NIPT

NIPT

Amnio normal
SNP CMA

Maternal SNP
CMA where
relevant

Whole CV SNP CMA – Trisomy 2

Pregnancy outcome data
Plac biopsy mos isoUPD2. Severe IUGR 1st centile, Preterm 32/40

CVS 100% T2, anhydramnios, FDIU at 17/40 (CPM) placental insufficiency

Amnio TFM T2 (15%) MI, biparental disomy, severe IUGR <1st centile, Preterm 34/40

Amnio TFM T2 (15%) MII, UPD2 (TOP)
Summary of VCGS RAA experience

182/54,000 (0.34%) single RAA reported (19 awaiting outcomes)

- 163 known outcomes
  - 75/163 miscarriage (46%) (USS vs no USS; primarily missed misc.)
- 88 continued pregnancies with known outcome
  - True fetal mosaicism (TFM) 17/88 (19%)
  - Uniparental disomy (UPD) 16/88 (18%) [min estimate; includes cases w/o SNP data]
  - IUGR < 3–5th centile 20/88 (23%)
  - Preterm delivery <37 weeks 15/88 (17%)
  - Congenital abnormalities 9/88 (10%)
  - Uncomplicated outcome 26/88 (30%)
Known parental rearrangements

- Reciprocal translocations
  - Known carriers (low to high risk)

- Inversions
  - Known carriers (low to high risk)

- Hx of de novo rearrangement
  - Low recurrence risk
Advanced NIPT & unbalanced translocations

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What are chromosomal translocations?

Unbalanced translocations and prenatal diagnosis

Figure 1. Balanced translocation between one chromosome 4 and one chromosome 16. The carrier has a history of recurrent miscarriage.

However, carriers of balanced translocations are at risk of passing on an unbalanced form of the chromosomal rearrangement at conception, resulting in genetic material.

prenatal NIPT provides a non-invasive option

In March 2017, VCGS gained NATA/RCPA accreditation to use NIPT in pregnancies at increased risk for unbalanced...
Known parental rearrangements

Reciprocal translocation analysis (n=115)

- 90% high risk unbalanced translocation
- 10% low risk

- Karyotype assessment prior to screening (min 15 Mb segment); prefer CMA coordinates
- Earlier USS (confirm singleton, exclude co-twin demise)
- Screen from 10-11 weeks
- 12 high risk (8/8 confirmed; 1x FDIU, 1x formalin, 2x no test)
- 49/103 low risk confirmed, remainder ongoing/outcome sought
Known parental rearrangements – case example

Maternal carrier t(3;15)(p23;q26.1), G15P3
NIPT at 11 weeks gestation

NIPT: gain 3p26.3p24.1 gain (27 Mb)
CVS CMA: 3p26.3p24.1(66,894-27,120,082)x3,
15q26.2q26.3(97,535,118-102,397,836)x1

der(15)t(3;15)(p23;q26.1)
CVS karyotype
VCGS NIPT experience for known rearrangements

- High demand
- Patients at very low risk are often offered PND. Reluctant if Hx is poor. High demand for screening previous de novo findings.
- Genome-wide NIPT provides an alternative
Segmental aneuploidy

Partial chromosomal anomalies associated with pathogenic disease. Widens clinical utility of NIPT.

Sensitivity influenced by fetal fraction, CNV size and read depth

- De novo deletions and duplications (non-recurrent)
- Inherited and de novo unbalanced translocations and other rearrangements
- Isochromosomes (9p, 12p, 18p)

Biological factors do affect FPR (e.g. CPM, maternal mosaicism)
### VCGS validation study results

#### Segmental aneuploidy (>10 Mb)

<table>
<thead>
<tr>
<th>Screening test result</th>
<th>Number</th>
<th>Sensitivity 78.6% (49.2-95.3%)</th>
<th>Specificity 99.9% (99.9-100%)</th>
<th>PPV* = 61.1%      NPV = 99.9%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cases</td>
<td>15,600</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test positive</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test negative</td>
<td>15,582</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>True positive</td>
<td>11 (~1 in 1400)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>True negative</td>
<td>15,579</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>False positive</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>False negative</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>False positive rate (FP/FP+TN)</td>
<td>1 in 2,227 (0.04%)</td>
<td></td>
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</tbody>
</table>

* prospective screening PPV is 31% (9/29 from ~18,000 referrals)
Segmental aneuploidy examples

Wolf-Hirschhorn syndrome (4p-)

Cri du chat syndrome (5p-)

Miller Dieker Lissencephaly syndrome (17p-)

Pallister-Killian syndrome (tetrasomy 12p)

Biological factors affecting accuracy

- False positive: Confined placental mosaicism
- False positive: Maternal mosaicism
- False negative: Placental mosaicism for normal cell line
Segmental aneuploidy – maternal mosaicism

Maternal karyotype 8% cells r(18)(p11.2q22.2)

Maternal karyotype 23% cells i(8)(q24.12q24.3) [24 Mb 8q tetrasomy]

Maternal SNP CMA mos dup 8q24.12q24.3
Segmental aneuploidy – false negative

CVS for NT 7.3mm at 13+1

- Mosaic deletion/isodisomy 11q in cytotrophoblast (16 Mb)
- Deletion 11q in mesenchyme/fetus
- Deleted chromosome 11q repaired from non-deleted homologue

NIPT at 10+3 Low risk (FF 6%)
Maternal malignancy

10/125,426 NIPT referrals with malignancy reported to lab
- Neuroendocrine, Non-Hodgkin (B-cell) lymphoma, colorectal, acute T-cell lymphoblastic leukaemia, anal

3/4,000 NIPT referrals with genome-wide imbalance
- Ovarian, follicular lymphoma, Hodgkin lymphoma
VCGS known maternal malignancy cases

- 15C200407: Melanoma, Liver metastases
- 17C615173: Hodgkin lymphoma
- 18C602095: Cervical cancer
- 18C609583: Breast Cancer
- 18C614613: Metastasised colorectal cancer
- 18C617667: Liver metastases, Colon cancer
- 18C609713: Mediastinal large B cell lymphoma
- 19C604723: Liver metastasis, Rectal cancer

8/~60,000 samples
Frequency ~1 in 7,500 samples
All unknown prior to NIPT
Maternal malignancy

NIPT, mat age 27yrs, 2x QC failure, 10+2 and 11+2, data outside of expected range

rectal cancer with solitary liver metastasis
Maternal malignancy

NIPT, mat age 38yrs, 3x QC failure, 15+3, 16+3, 27+6, data outside of expected range

Metastatic breast carcinoma involving 2 lymph nodes, HER2 positive
Maternal malignancy

Genome-wide NIPT can be very specific when identifying copy number profiles that might be indicative of maternal cancer

- Up to 1 in 1,000 women might be falsely alarmed about a risk for cancer using standard NIPT. Even worse if genome wide screening is included (Benn et al., Prenat Diagn. 2019 Apr;39(5):339-343). [ISPD debate 2018]

- General consensus these results should be reported
- VCGS has reported 8 suspected cases (6 confirmed) in 65,000 referrals
- Multiple whole and partial chromosome copy number abnormalities raise strong suspicion
- Always test two independent samples to replicate result
WGS approach expands the clinical utility of NIPT
- Identifies pathogenic conditions not detectable using standard NIPT, for a low additional false positive rate
- Other findings may aid pregnancy management and surveillance

Laboratory accreditation is important
- Labs must work to high quality standards and understand biology of cfDNA screening results
- For patients, informed consent and genetic counselling support is key
- Evidence that informed patients have a preference for wider screening
Summary

- More clinical validation work is needed
  - VCGS NIPT includes genome-wide screening as standard
  - Our clinicians see value in wider screening, although this is not without its problems. Good collaborative approach between clinicians and lab/genetics team
- Education and training is important
  - Healthcare professionals and other users (benefits and limitations)
Acknowledgements

NIPT scientific and technical staff

Genetic counselling team

Cytogenetics team
Thank you and questions