USING ARRAYS FOR PRENATAL DIAGNOSIS

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Is chromosome microarray appropriate for prenatal diagnosis (PND)?
Is microarray appropriate for PND

The Context

- G-banded Karyotype in Australia for decades
- Fetal anomalies on ultrasound scan
- Increased risk on a screening test
- Maternal age and the risk of aneuploidy
- Maternal anxiety/ maternal choice
Is microarray appropriate for PND

- What is the benefit of microarray for PND?
The benefit of microarray for PND – diagnostic

- Identify copy number variations < 5-10 Mb
- Causing or associated with:
  - congenital anomalies
  - micro-deletion/ micro-duplication syndromes
  - intellectual disability
  - autism spectrum disorders
  - single gene disorders
- Qualify/ Quantify the significance of rearrangements detected by g-banding
The benefit of microarray for PND - diagnostic

Microdeletion Syndromes

- DiGeorge syndrome
- Williams-Beuren syndrome
- Prader-Willi syndrome
- Angelman syndrome
- Miller-Dieker syndrome
- Smith-Magenis syndrome
- Wolf-Hirschhorn syndrome
- Cri du Chat syndrome
- Langer-Giedion syndrome
- DiGeorge Syndrome 2

OVERALL – occurs 1/1600 deliveries
The benefit of microarray for PND - diagnostic

- T2 scan Tetralogy of Fallot
- Amniocentesis 46,XY,t(5;6)
  - balanced vs suspicious?
  - Call for parental bloods
  - Microarray on backup culture
15.5Mb deletion
6q21q22.31
The benefit of microarray for PND - diagnostic

- T1 scan nuchal translucency 6.7mm
- Amniocentesis 46, XY
cryptic translocation - der(18)t(3;18)(p26;p11)

arr 3p26.3p26.1(56,668-8,616,087)x3 - 8.6Mb duplication

arr 18p11.32p11.31(50,739-4,990,626)x1 - 4.9Mb deletion
The benefit of microarray for PND

T1CS 1 in 3 : (46,XY)

19/40 USS
+ diaphragmatic hernia
+ Cleft lip

microarray

- Amniotic band syndrome
- Craniofrontonasal dysplasia
- Fetal alcohol syndrome
- Fryns syndrome
- Oculo-auriculo-vertebral dysplasia
- Postaxial acrofacial dysostosis syndrome
- Absent / hypoplastic tibia, polydactyly, arachnoid cyst
- Bartsocas-Papas syndrome
- Cantrell's pentalogy
- Fetal mycophenolate mofetil syndrome
- Ivemark syndrome
- Lethal multiple pterygium syndrome
- Simpson-Golabi-Behmel syndrome
- Spondylothoracic dysplasia
- Thoracoabdominal schisis, limb defects
The benefit of microarray for PND diagnostic - single gene disorder

↑ T1CS 1 in 3 + CDH + Cleft lip: (46,XY)
- 1.3Mb deletion on Xq26.2, Deletion incl GPC3 gene
  Simpson-Golabi-Behmel syndrome
The benefit of microarray for PND – genetic counselling

Classical Dandy-Walker malformation
The benefit of microarray for PND
Benefit of microarray specific genetic counselling?

**FOXCI is required for normal cerebellar development and is a major contributor to chromosome 6p25.3 Dandy-Walker malformation**

Kimberly A Aldinger¹, Ordan J Lehmnn², Louanne Hudgins³, Victor V Chizhikov⁴, Alexander G Bassuk⁵, Lesley C Ades⁶, Ian D Krantz⁷, William B Dohyln⁸ & Kathleen J Milken⁹,¹⁰

*Nature Genetics* Volume 41 | Number 9 | September 2009

1037
The benefit of microarray for PND - detection

Additional Detection of chromosome anomaly
Microarray vs standard karyotype
- Hillman et al (2011) UOG - Systematic review

Overall results for all clinical indications

- Bi et al. (2008)\textsuperscript{13}
- Shaffer et al. (2008)\textsuperscript{14}
- Sahoo et al. (2006)\textsuperscript{12}
- Le Caignec et al. (2005)\textsuperscript{17}
- Tyreman et al. (2009)\textsuperscript{18}
- Coppinger et al. (TG) (2009)\textsuperscript{9}
- Coppinger et al. (WG) (2009)\textsuperscript{9}
- Vialard et al. (2009)\textsuperscript{20}
- Kleeman et al. (2009)\textsuperscript{19}

Total

- 8\% (95\% CI, 4–13\%)
- 2\% (95\% CI, 2–2\%)
- 3\% (95\% CI, 3–4\%)
- 8\% (95\% CI, 6–11\%)
- 22\% (95\% CI, 18–26\%)
- 0\% (95\% CI, 0–0\%)
- 3\% (95\% CI, 3–4\%)
- 11\% (95\% CI, 8–15\%)
- 2\% (95\% CI, 2–3\%)

4\% (95\% CI, 2–9\%)
The benefit of microarray for PND - detection

Additional Detection of chromosome anomaly
Microarray vs standard karyotype
- Hillman et al (2011) UOG - Systematic review

Abnormal ultrasound scan

- Bi et al. (2008)
- Shaffer et al. (2008)
- Le Caignec et al. (2005)
- Tyreman et al. (2009)
- Vialard et al. (2009)
- Kleeman et al. (2009)

Total

Percentage

2% (95% CI, 1–6%)
2% (95% CI, 2–2%)
8% (95% CI, 6–11%)
22% (95% CI, 18–26%)
11% (95% CI, 8–15%)
2% (95% CI, 2–3%)
5% (95% CI, 2–14%)
The benefit of microarray for PND - detection

The NICHD Prenatal Microarray Study Group

Multicentered, prospective, blinded comparison of cytogenetic results obtained by karyotype with those achieved by chromosomal microarray analysis

PI Ronald Wapner

Successful CMA result was achieved in 98.8%

N=4340
Array = Karyotype for aneuploidies
Normal G-banded karyotype,
microarray adds relevant information

- Structural Anomalies = 6.0% of cases
- Screen Positive (n=729) = 1.6% of cases
  AMA (n=1966) = 1.7% of cases
Heart anomaly and normal g-banding
- $N = 123$
- 17% had a significant CNV on array

Of the 17%,
- 60% were not detected by 22q11.2 FISH
The benefit of microarray for PND - detection

NICHD trial

- Conclusion
  - CMA should be transitioned to become the first tier test for invasive prenatal cytogenetic diagnosis”

The benefit of microarray for PND - detection

- NICHD 6.0%
- Fiorentino 6.1%
- Rosenfeld/Shaffer 6.6%
- Schwartz 5.7%

Is microarray appropriate for PND

What is the benefit of microarray for PND?

- Better detection of chromosomal anomalies
- Specific findings: genotype phenotype
- Better information: genetic counselling

There is a clear benefit
Is microarray appropriate for PND

- Is there a benefit in using high density genome-wide oligonucleotide microarray for PND?
Is high density array appropriate for PND

Shaffer et al (Prenat Diag 2012)
prenatal samples by any array (historical)
- for any indication: detection 5.3%
- abnormal ultrasound: detection 6.5%
Prenatal samples by oligonucleotide array
- for any indication: detection 6.5%
- abnormal ultrasound: detection 7.6%
Is high density array appropriate for PND

<table>
<thead>
<tr>
<th>Author</th>
<th>Breman</th>
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## Is high density array appropriate for PND

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<th>Tyreman</th>
<th>Evang.</th>
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Is high density array appropriate for PND

- T2 scan
  - Unilateral mild ventriculomegaly
  - Intraventricular thrombus?
- Repeat scan normal?
- Fetal MRI abnormal
  - Microlissencephaly
  - Cerebellar hypoplasia?
Is high density array appropriate for PND

200Kb dup of 1p32
- 200Kb dup in 1p32 (maternal)
- Assuming the dup is a tandem dup
  - disrupts a gene in a pathway expressed in migrating cerebral and cerebellar neurons
- Currently sequencing paternal allele
Is microarray appropriate for PND

- Is there a benefit in using high density genome-wide oligonucleotide microarray for PND?
  - Better detection of small significant CNVs
  - Detect moderate CNVs with better clarity
  - More accurate identification of extent and gene content of CNVs

- There is a benefit
Is microarray appropriate for PND

- Is there a benefit in using SNP microarray for PND?
The benefit of SNP microarray for PND?

Improved sensitivity for small deletions
The benefit of SNP microarray for PND?

Diagnose Triploidy

Log R neutral
The benefit of SNP microarray for PND? Diagnose uniparental isodisomy disorders

- Paternal UP isodisomy 14 – lethal in childhood
- Fetal club hand, club foot, short long bones, abdominal wall defect

Log R neutral
ΔΔ: Low level mosaicism dup 11p15 or mosaicism for isodisomy 11p15 in a newborn with Beckwith syndrome

The benefit of SNP microarray for PND?
Segmental UP isodisomy imprinting disorders
Is microarray appropriate for PND

- Is there a benefit in using **SNP** microarray for PND?
  - Increased sensitivity for small deletions
  - Diagnose triploidy
  - Diagnose uniparental isodisomy
  - Diagnose lower levels of mosaicism (7%)
    - Bruno et al, J Med Genet 2011
- There is a **clear benefit**
Is it appropriate to use chromosome microarray for prenatal diagnosis?

- What is the risk of using microarray for prenatal diagnosis
The risk of microarray for PND

Technical:

- Not detect balanced changes
  - e.g. translocation (0.6% of ID pts)
- No positional information
  - translocation T21 – FISH with microarray
  - “Duplication” as a result of a parental insertion
- Only as sensitive/ specific as its probe coverage
- Cost?
The risk of microarray for PND

The main risk is CNVs of uncertain significance

- Novel de novo CNVs
- Predisposition CNVs
- Incidental findings

- This is especially so if the array is performed in the absence of fetal anomaly
The risk of microarray for PND

CNVs of uncertain significance

We know that uncertainty about results causes anxiety and distress

There is concern about “unnecessary termination”

“All events >1 Mb were observed in only 1 or 2 normal individuals…”

“Large variants are generally deleterious wrt size and gene content.”

The majority of people harbor CNVs >100 kb
The risk of microarray for PND

- Determining pathogenicity of novel CNVs
  - Size
  - Gene content
  - Gene function studies
  - Reports of cases / databases
  - Inheritance – i.e. wait for parental bloods
- NB Experience and access to data from others!

The risk of microarray for PND

**NICHD trial**

CNVs of uncertain significance

- 2007 Guidelines : VOUS 2.5%
- 2012 Guidelines : VOUS 1.5%

The risk of microarray for PND

Shaffer et al, Prenatal Diagnosis 2012
2004 to 2011 (n= 5003) prenatal diagnosis MA

- findings with unclear clinical significance
  - 4.2%
  - reduce to 0.39% if only de novo CNVs considered
The risk of microarray for PND

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The risk of microarray for PND

The reported prevalence of uncertain CNVs is influenced by:

- The manner in which significance is determined
  - e.g. what weight is placed on inheritance from a normal parent.
- And reporting policies regarding predisposition CNVs
The risk of microarray for PND

- Predisposition CNV’s: risk factors for
  - Intellectual disability
  - Autism
  - Psychiatric illness
  - Epilepsy

- We do not understand the genetic modifiers
- We do not know the carrier frequency and significance in different ethnic groups
The risk of microarray for PND

- **Penetrance** for CNVs associated with schizophrenia
  - Vassos et al Hum Mol Genet 2012

- 15q13.3  1q21.1  15q11.2  17p12  2p16.3  16p13.1  16p11.2

- Penetrance estimated from case controlled studies to be 2-7%

- No figures for risk with prenatal ascertainment.
The risk of microarray for PND

Girirajan et al NEJM 2012

CNVs of uncertain significance

- Children with a second large CNV more likely to have symptoms as a result of a “predisposition CNV”
- Children with ≥ 2 large CNVs = 8x risk of Dev delay
- Males gender is a risk factor for penetrance
The risk of microarray for PND

Girirajan et al NEJM 2012

CNVs of uncertain significance

- We may give accurate risks in future based on gene content.

- **Caution** warranted when using data for prenatal counselling
Genetic Modifiers of CNV penetrance

440kb del 1q21.1 pat in TAR syndrome region
Compound inheritance of a low-frequency regulatory SNP and a rare null mutation in exon-junction complex subunit RBM8A causes TAR syndrome
Albers et al Nature Genetics 2012
Is it appropriate to use high density microarray for PND

- What is the risk of using high density microarray for prenatal diagnosis
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Ganesamoorthy 2013- All detectable by standard resolution arrays
Is it appropriate to use high density microarray for PND

- What is the risk of high density microarray for prenatal diagnosis
  - There may be a risk of increased detection of uncertain variants that needs to be managed
  - Underlines the importance of consent/counselling
Is it appropriate to use microarray for PND

- What is the risk of SNP microarray for prenatal diagnosis
The risk of SNP microarray for PND

- Long continuous stretches of homozygosity (LCSH)
- Diagnostic in 1%
- Otherwise not a problem

multiple LSCH
=> consanguinity
The main risk is the detection of:

- non-paternity with parental samples
- Parental relatedness
- Close parental relatedness
The risk of SNP microarray for PND

- Non-paternity and consanguinity have not been a practical problem for VCGS clinicians

- Institutions need policies and procedures for the management of very close parental relatedness.
Ethically correct approach:

**CNVs of uncertain significance**

We know that uncertainty about results causes anxiety and distress:

- We need to manage clients distress rather than limiting access to testing

There is concern about “unnecessary termination”

- We need to give women choices- autonomy

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McGillivray et al (Prenatal Diagnosis 2012)
Yes, microarray is appropriate for PND!

– Offer the best test for detecting pathogenic CNVs (High density SNP microarray)
– through a Cytogenetics laboratory with experience/high turnover
– as part of a suite of tests (e.g., banded karyotype, targetted array, NIPD)
– With pre- and post-test counselling/consent
– and access to genetic counsellors for distress
Acknowledgement

RWH/ VCGS Genetics staff
VCGS Pathology Cytogenetics staff
RWH Ultrasound Department staff
RWH Fetal Medicine Unit staff
RCH Fetal Radiology staff