OUTLINES

- Discuss on the ‘a priori’ and ‘post-test’ or ‘residual risk’ after a negative test result
- Implications of the residual risk for pre-test counseling before screening test for common aneuploidies
- Support to women’s decision autonomy
  - Resources
  - Tools
  - Education
The current standard test is the karyotype/CMA on fetal samples:
  • Chorionic villi sampling (CVS): 11-13wg
  • Amniotic fluid sampling (AF): 16-18wg

AF and CVS are carried out for a variety of reasons:
  • fetal US abnormality/ies
  • previous affected fetus/child
  • parent carrier of a chromosome abnormality
  • ...

Main indication: diagnosis of fetal aneuploidies, primarily trisomy 21
MATERNAL AGE AND TRISOMIES

An association between maternal age and trisomies: proneness of older oocytes to maternal meiosis I and II non-disjunction errors.

Screening programs for T21

- Developed starting from 70’s: evolved considerably in the last few decades
- Recent developments in the cfDNA testing: DR ~99%; FPR<0.1%
cfDNA testing performances: a meta-analysis

<table>
<thead>
<tr>
<th>Type of aneuploidy</th>
<th>number of studies n</th>
<th>trisomic cases n</th>
<th>non-trisomic cases n</th>
<th>Singleton pregnancies: weighted pooled</th>
<th>Twin pregnancies: weighted pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DR (95% CI) FPR (95% CI)</td>
<td>DR (95% CI) FPR (95% CI)</td>
</tr>
<tr>
<td>T21</td>
<td>30</td>
<td>1,963</td>
<td>225,032</td>
<td>99.7% (99.1-99.9) 0.04% (0.02-0.08)</td>
<td>100.0% (95.2-100) 0% (0-0.003)</td>
</tr>
<tr>
<td>T18</td>
<td>25</td>
<td>560</td>
<td>212,019</td>
<td>98.2% (95.5-99.2) 0.05% (0.03-0.07)</td>
<td></td>
</tr>
<tr>
<td>T13</td>
<td>18</td>
<td>119</td>
<td>212,883</td>
<td>99.0% (65.8-100) 0.04% (0.02-0.07)</td>
<td></td>
</tr>
<tr>
<td>45,X</td>
<td>23</td>
<td>36</td>
<td>7,677</td>
<td>95.8% (70.3-99.5) 0.14% (0.05-0.38)</td>
<td></td>
</tr>
<tr>
<td>other SCA</td>
<td>11</td>
<td>17</td>
<td>5,383</td>
<td>100.0% (83.6-100) 0.003% (0-0.07)</td>
<td></td>
</tr>
</tbody>
</table>

*peer-review studies reporting on clinical validation or implementation of maternal cfDNA testing in screening for aneuploidies, in which data on pregnancy outcome were provided for more than 85% of the study population (January 2011-31 December 2016)
CfDNA TESTING CANNOT DETECT ALL FETAL CHROMOSOME ABNORMALITIES

CfDNA INFORMED CONSENT DISCLOSURES:

• Many fetal karyotype abnormalities cannot be identified

• Residual risk (RR) still remains

• It is crucial to provide accurate information on the actual rates of karyotype anomalies and RR at all maternal and gestational ages
A priori and residual risk

Any type of test

Risk before test

Test is “negative”

Residual risk after “negative” test

Courtesy: Thomas J Musci
Fetal chromosomal risks from previous studies

- Only for major aneuploidies that are obvious at birth (T21 and 18)
- Inferred the risk for chromosome abnormalities in women <35y at birth
- Not take into account sonography, which is now a routine tool in prenatal care
  - Fetuses with anatomical abnormalities may have been included in these older datasets
  - Skewed risk towards a higher range

Determination of the fetal chromosomal risks stratified according to MA and GA

Enrolled population

- Unbiased retrospective analysis anonymized, database-stored cytogenetic diagnostic results on 129,263 samples of CVS (n=41,782) and AF (n=87,481);

- Indication: MA, anxiety or elective decision (≥35y and <35y)
  - NO other pretest risk factors aside from MA (no increased serum screening, negative family history)
  - NO obvious sonographic abnormalities detected prior to the procedure

- TOMA lab institutional review board approval (#0000015)

Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155
A PRIORI RISK OF A WOMAN TO CONCEIVE A CYTOGENETICALLY ABNORMAL FETUS

Stacked bar plot of the frequency of each chromosomal defect for each maternal age, and gestational age group.

Data on ~90,000 amniocentesis and ~40,000 CVS,

Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155
Effect of MA and GA on the a priori risk for fetal chr abnormalities

- Overall risk for cytogenetic abn at >15GA (including WHITE box)
  - 18y: 1/301
  - 48y: 1/9

<table>
<thead>
<tr>
<th>MA (years)</th>
<th>&lt;15w</th>
<th>1/X</th>
<th>15w</th>
<th>1/X</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>272</td>
<td>282</td>
<td></td>
<td></td>
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<tr>
<td>19</td>
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<td>21</td>
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<td>48</td>
<td>7</td>
<td>9</td>
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</tr>
</tbody>
</table>
Effect of MA and GA on the a priori risk for fetal chr abnormalities

- The risk for common trisomies increases with MA
- In young women: risk is dominated by SCAs and other autosomal unbalanced rearr (red/pink)
- In older women: common trisomies dominate the risk (blue)
Results

Lower frequency of the common trisomies than reported from previous studies, in which sonographic findings were not available.

Frequency of chromosomal aneuploidies is significantly higher in earlier GA.

CVS 2.63% (1100/41782) VS AF 1.82% (1596/87481); OR 0.6873 (95%CI 0.659-0.7428)
Residual risk after a negative screening result

<table>
<thead>
<tr>
<th>MA (years)</th>
<th>Risk for a cytogenetically visible genomic aberration</th>
<th>Proportion of the risk for fetal T21, 18 or 13</th>
<th>Risk for disorders other than T21, 18 and 13</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;15w 1/X</td>
<td>≥15w 1/X</td>
<td>&lt;15w 1/X</td>
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<td>18</td>
<td>272</td>
<td>301</td>
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<td>266</td>
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<td>12.3</td>
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<td>235</td>
<td>263</td>
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<td>85.7</td>
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<td>11</td>
<td>86.8</td>
</tr>
<tr>
<td>48</td>
<td>7</td>
<td>9</td>
<td>87.8</td>
</tr>
</tbody>
</table>
Effect of MA and GA on the residual risk for chr abnormalities not targeted by non-invasive screening strategies

- Clinically significant chromosomal abn *other than* T21,18,13,SCAs at >15GA:
  - 18y: 47% of the a priori risk
  - 48y: 5% of the a priori risk

_Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155_
Newer technologies impact the epidemiology of fetal chr abn:

- pCNV or likely pCNVs prevalence (by CMA) in women with anatomically normal fetuses with normal karyotypes is 1.65% (1/61)
  
  - Clearly pCNVs: 0.5% - 95th% CI 0.2–0.8
  - pCNVs with variable expressivity: 0.6% – 95th% CI 0.3–1.1
  - Likely pVOUS: 0.6% – 95th% CI 0.3–1.1

- Lack of association with MA, serum screening analyte levels or GA
  
  - prevalence fixed at 1.65% (1/61) in all women
A PRIORI RISK OF A WOMAN TO CONCEIVE A CHROMOSOMICALLY ABNORMAL FETUS

Stacked bar plot of the frequency of each genomic defect for each maternal age, and gestational age group (no balanced rearr)
In younger ages, the non-age-dependent pCNVs dominate fetal risk.

CNVs represent the main component of the a priori risk for fetal genomic abnormalities in younger women:

- 80% of the risk in 18y
- 15% of the risk in 48y
Effect of MA and GA on the residual risk for chr abnormalities not targeted by non-invasive screening strategies

- Residual risk for other ‘off-target’ clinically significant genomic abn
  - other than T21,18,13
  - other than T21,18,13, homogeneous SCAs

Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155
After the exclusion of T21,18,13&SCAs, the RR for other pathogenic GENOMIC abnormalities is still consistent

- Not so much different in young (~1/50) and old women (~1/40)

- Even excluding pCNVs with variable expressivity and likely pVOUS, a residual risk of 0.5% (95th% CI 0.2–0.8) for pCNVs with highly penetrant phenotypes still remains: **level of risk to justify offering invasive testing**
‘... Le gestanti che, per scelta personale, in assenza di una indicazione che conferisca loro un rischio “a priori” elevato per le microdelezioni/microduplicazioni, decidano di sottoporsi ad una diagnosi prenatale invasiva, dovrebbero essere informate dell’esistenza del CMA come tecnica di approfondimento diagnostico, ad integrazione del cariotipo fetale. La sua applicazione in questa popolazione dovrebbe rispondere all’obiettivo di ridurre il rischio di sindromi note da microdelezione/microduplicazione associate a fenotipi clinici gravi....’
'Early and noninvasive fetal genetic sequencing is on the horizon. Such expanded prenatal testing could offer patients substantial benefits. But current practices in prenatal screening and the complex nature of genomic science and technology create the risk that these tests will be integrated into care without the robust, evidence-based informed consent processes necessary for respecting women’s autonomy. If that happens, patients will be asked to decide whether to undergo invasive diagnostic testing and then to consider whether to terminate or continue their pregnancy without a full understanding of the results. …'
‘The need for fully informed consent in prenatal screening and testing has never been more urgent. Meeting this need will require adoption of reimbursement policies and professional practice guidelines that support clinicians in breaking with current routine practices, which too often involve dispensing with or failing to adequately carry out an informed consent process. It will also require funding for development of approaches to pretest and posttest education and counseling that empower patients to decide whether to be tested and what to do after receiving their results.’ …

‘Only with these practices and policies in place can women’s decisions about prenatal screening, diagnostic testing, and termination or continuation of pregnancy be truly free and informed.’
HOW TO SUPPORT WOMEN’S AUTONOMY?

Resources:
- Professional societies provide uniform educational materials for providers and women
  - Movies
  - Brochures
  - Slide decks
- Online and residential courses for providers
- Pratice with simulation
- Uniform informed consent (legally revised)

New tools:
- Movies for pretest counseling (@home)
- Apps and softwares to support calculation of RR during pretest counseling
  - Specific MA and GA
- Tele-counseling with recording of the informed consent
- Furum of professional societies on social media

New education strategies:
- Anticipation in preconceptional period
- Social media
- Family doctors
- Teens (reproductive risk education)
HOW TO SUPPORT WOMEN’S AUTONOMY?

Resources:

- GSF and PQF focus on improving the quality of communication regarding prenatal testing options.
- The PQF educates obstetricians to help facilitate quality perinatal patient care. They have developed genetic education modules (GEM) for patients considering prenatal testing to help empower patients to make informed decisions.

https://geneticsupportfoundation.org/  https://www.perinatalquality.org/
HOW TO SUPPORT WOMEN’S AUTONOMY?
HOW TO SUPPORT WOMEN’S AUTONOMY?

ISPD Global Updates (July/August 2017) – Genetic Counseling SIG

QUESTION

CfDNA vs. CVS in the high risk patients?
Increased a-priori risk for genetic abnormalities in pregnancies with U/S abnormalities

<table>
<thead>
<tr>
<th>Type of genomic disorder</th>
<th>Type of test on CVS/AF</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytogenetic abnormalities</td>
<td>Karyotyping</td>
<td>&gt;5-7Mb</td>
</tr>
<tr>
<td>Submicroscopic dels/dups</td>
<td>CMA</td>
<td>Kb--&gt; &lt;5Mb</td>
</tr>
<tr>
<td>Monogenic disorders</td>
<td>WES</td>
<td>bp</td>
</tr>
<tr>
<td>Imprinting disorders</td>
<td>Different molecular tests</td>
<td>Epigenetic</td>
</tr>
</tbody>
</table>
Fetal chromosome abnormalities in pregnancies with U/S abnormalities


* OR 15.58, 95%CI 13.71-17.70
** OR 31.22, 95%CI 24.09-40.46
*** OR 8.98, 95%CI 7.76-10.39
**** OR 21.15, 95%CI 17.16-26.08
Chromosome abnormalities in CVS of pregnancies with U/S abnormalities

- Increased NT (54%)
- CH-Hydrops-Oedema (18%)
- IUGR (2%)
- Omphalocele (2.4%)
- Fetal malformations ndd (19%)

HOMOGENEOUS
- De novo (majority)
- Inherited from a parent carrier of a balanced rearrangement

Patients with U/S abn (387)

- Normal cfDNA result (258; 66.7%)
  - Normal karyotype (229; 87.8%)
  - Abnormal karyotype (29; 11.2%)
    - SCAs (13; 45%)
    - Other chr abn (16; 55%)

- cfDNA analysis for T13,18,21 only and karyotyping in parallel
Increased a-priori risk for genetic abnormalities in pregnancies with U/S abnormalities

<table>
<thead>
<tr>
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<tr>
<td>Imprinting disorders</td>
<td>Different molecular tests</td>
<td>Epigenetic</td>
</tr>
</tbody>
</table>
### INCREMENTAL YIELD BY MICROARRAY WITH NORMAL FETAL KARYOTYPE

- **5.6% (95% CI 4.7-6.6)** structural ultrasound anomaly restricted to one anatomical system and a normal karyotype
- **9.1% (95% CI 7.5-10.8)** poly-malformed fetuses

<table>
<thead>
<tr>
<th>Isolated anomalies</th>
<th>Cardiac</th>
<th>Resp</th>
<th>CNS</th>
<th>Facial</th>
<th>MSK</th>
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</thead>
<tbody>
<tr>
<td><strong>Pooled prevalence (95% CI)</strong></td>
<td>$\frac{22}{476}$</td>
<td>$\frac{5}{81}$</td>
<td>$\frac{35}{563}$</td>
<td>$\frac{6}{113}$</td>
<td>$\frac{24}{305}$</td>
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<tr>
<td></td>
<td>4.6%</td>
<td>6.2%</td>
<td>6.2%</td>
<td>5.3%</td>
<td>7.9%</td>
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<tr>
<td></td>
<td>(2.7-6.5)</td>
<td>(0.9-11.4)</td>
<td>(4.2-8.2)</td>
<td>(1.2-9.4)</td>
<td>(4.8-10.9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Isolated anomalies</th>
<th>GIT</th>
<th>Urogenital</th>
<th>NT &gt;3.5 mm</th>
<th>Cystic hygroma</th>
<th><strong>Total</strong></th>
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</thead>
<tbody>
<tr>
<td><strong>Pooled prevalence (95% CI)</strong></td>
<td>$\frac{7}{105}$</td>
<td>$\frac{9}{153}$</td>
<td>$\frac{5}{162}$</td>
<td>$\frac{12}{262}$</td>
<td>$\frac{125}{2220}$</td>
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<td>6.7%</td>
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<td>(1.9-11.4)</td>
<td>(2.2-9.6)</td>
<td>(0.4-5.7)</td>
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<td>(4.7-6.6)</td>
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</table>
High post-test residual risk for fetal pCNVs

- 5-6 CNVs represent only a portion (~20%) of the overall pCNVs that can affect the fetus

False reassurance to patients – consistent residual risk

Increased a-priori risk for genetic abnormalities in pregnancies with U/S abnormalities

<table>
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<th>Type of genomic disorder</th>
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<td>Different molecular tests</td>
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WHOLE EXOME SEQUENCING IN FETAL ANOMALIES ON U/S – ON THE HORIZON

★ WES examines coding regions (exons) of the genome

---

Aimed to identify the etiology for fetal U/S abnormalities

Actually not recommended outside of the context of clinical trials

Offered on research basis in some labs or for specific clinical indications in other labs (recurrent or lethal fetal anomalies)

Limited published data on prenatal application of WES

Monogenic diseases may be identified in up to 20-30% of fetuses with multiple anomalies suggestive of a genetic disorder for which karyotyping and CMA are normal

Provide options of PGD or early prenatal diagnosis in a future pregnancy
Increased a-priori risk for genetic abnormalities in pregnancies with U/S abnormalities

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<td>Different molecular tests</td>
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</tr>
</tbody>
</table>

Normal fetal karyotype and normal CMA!!
Beckwith–Wiedemann syndrome (BWS) in fetuses with:

- isolated omphalocele
- overgrowth
- polydramnios
- enlarged placenta
- distended abdomen
- visceromegaly
- macroglossia

Imprinting Syndromes and fetal U/S abnormalities

**UPD7mat or other imprinting defects**

Silver Russell syndrome (SRS) in fetuses with:

- IUGR
- Micrognathia
- CHD
- clinodactyly
- Partial or total asymmetry

*Miozzo, Grati et al, Placenta (2001), 22, 813–821; OMIM #180860*
cfDNA TESTING IN FETAL ANOMALIES ON ULTRASOUND

Patients with U/S abn (251)

- Normal cfDNA result (224; 89.2%)
  - Genetic testing not performed (191; 85.3%)
  - Normal genetic testing (21/28; 75%)
  - Abnormal genetic testing (7/28; 25%)
    - Cytogenetic abn. (T13, MX) (28.6%)
    - 2 pCNVs (28.6%)
    - 2 monogenic disorders (28.6%)
    - 1 imprinting disorder (14.3%)

GW cfDNA analysis for all autosomal partial and whole chromosome aneuploidy (no SCAs) \textit{and} karyotyping in parallel

Residual Risk

Increased a priori risk for different types of genetic abnormalities

Any cfDNA testing

Test is “negative”

Residual risk after “negative” test

RR is dependent on:
- detection rate of applied test
- genetic aetiology of the fetal malformation
  - 20-30% RR for cytogenetic imbalances
  - 6-9% pCNVs (submicroscopic)
  - 20-30% monogenic disorders
  - imprinting disorders

Courtesy: Thomas J Musci
Ultrasound abnormality: is there a role for NIPT?

Limited clinical utility in high risk cases

Ultrasound anomaly → NIPT with microdeletion panel

"Positive" NIPT

Invasive test
• Confirmatory testing recommended
• Extremely low PPV for rare conditions

"Negative" NIPT

Invasive test
• Significant residual risk
• Detection rates low or unknown
• Many genetic conditions/microdeletions not addressed by NIPT

Potential for delayed diagnosis, additional cost, and anxiety for patients

Ultrasound abnormality: is there a role for NIPT?

Differential Diagnosis

R/O chromosomal or genetic etiology

Patient counseling
Prognosis
Treatment options

 LV, RV

Courtesy: Thomas J Musci
QUESTION

Which screening strategy?
### COMPARISON OF DIFFERENT SCREENING STRATEGIES FOR THE DETECTION OF THE OVERALL FETAL CYTOGENETIC ABNORMALITIES AT BIRTH

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Screening strategy</th>
<th>First-tier test</th>
<th>Second-tier test</th>
<th>Third-tier test</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTS</td>
<td>Combined first trimester</td>
<td>Combined FTS</td>
<td>Karyotype if risk is ≥1/270</td>
<td>//</td>
</tr>
</tbody>
</table>

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*Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155; Grati et al, manuscript in preparation*
PROPORTION OF CHR DEFECTS DETECTED BY THE DIFFERENT TESTING STRATEGIES

- The distribution and prevalence of the chr abn are different at different MA.
- Although two strategies may show approximately the same overall DR, one may favor the detection of a different subset of chr abn compared with another one.
WHICH SCREENING STRATEGY?

Technological resources
- Detection for large array of off-target chr abn
  At the cost of:
  - a high residual risk for the targets of the test
  - expenses for invasive procedures

Budget
- Low residual risk for the targets of the test
  At the cost of:
  - a very low sensitivity for off-target chr abn
  - expenses for cfDNA tests

Maternal age distribution and stratification

Medical/scientific resources

Grati et al, manuscript in preparation
False positive rate of screenings

Compared with traditional serum±ultrasound screening (TSS), cfDNA tests have a much lower FPR for T21,18,13.

The higher FPR of TSS was often considered a limitation.

Distinct advantage with TSS due to NT’s ability to pick up additional chromosomal abnormalities (‘off-target’) in addition to the higher reflex invasive testing rate.

AIM: present detection rates of all (target and off-target) fetal karyotype abnormalities at birth by different screening strategies including cfDNA test and TSS.

A PRIORI RISK OF A WOMAN TO CONCEIVE A CYTOGENETICALLY ABNORMAL FETUS

Stacked bar plot of the frequency of each chromosomal defect for each maternal age, and gestational age group.

10-15 weeks

15-22 weeks

Common trisomies
NM SCAs
Other severe unbalanced chr abnormalities
Mosaics
balanced structural karyotype anomalies

Data on ~90,000 amniocentesis and ~40,000 CVS.

Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155
PROPORTION OF THE CHROMOSOMAL DEFECTS OCCURRING AT BIRTH DETECTABLE BY DIFFERENT TESTING STRATEGIES

Methods

◆ **Prior risks** for each defect derived from TOMA lab Dataset of ≈130K prenatal dx on CVS (n=43K) and AF (n=87K) with an indication of AMA, anxiety and elective decision (reported by clinicians)

◆ **Fetal loss rate** at birth for T13,18,21°

◆ **Sensitivities and specificities** for common aneuploidies and triploidy abstracted from the published literature:
  
  ◆ **Serum screenings** for T21,18,13, MX, triploids: from prior seminal studies* (5% cumulative FPR)
  
  ◆ **cfDNA testing:** 0.13% cumulative FPR for T21,18,13; 0.273% cumulative FPR for T21,18,13+SCAs°

◆ **Sensitivity** for other karyotype abnormalities correspond to the FPR of TSS or cfDNA tests

◆ **No result rate** with cfDNA testing of 1%: the DR for all chr abnormalities was adjusted downward as a 1% of ‘no result’ cases by cfDNA are actually undetected karyotype abnormalities

THE ROLE OF ‘MATERNAL AGE’ TODAY

Maternal age should have a central role to rationalize resources to obtain the most efficient cost-benefit.
Fetal chromosome abnormalities in pregnancies with U/S abnormalities

Encephalocele → 46,XX,rec(5)dup(5q)inv(5)(p15.2q32)
Fetal chromosome abnormalities in pregnancies with U/S abnormalities

Ventriculomegaly → 47,XY,+15
Fetal chromosome abnormalities in pregnancies with U/S abnormalities

Hydrocephaly $\rightarrow$ 69,XXY
Fetal chromosome abnormalities in pregnancies with U/S abnormalities

Anencephaly → 47,XX,+9
Fetal chromosome abnormalities in pregnancies with U/S abnormalities

Polymalformed fetus (ndd) → 46,XY,r(22)(p11.2q13.3)
Fetuses with CHD ± extracardiac defects (systematic meta-analysis)

- Pooled analysis: 7.0% (95% CI, 5.3–8.6%) incremental yield by CMA (excluding 22q11 microdeletion cases);
- **Incremental yield increases to 12%** (95% CI, 7.6–16%) when 22q11 deletion cases were included
- Stratified analysis: incremental yield
  - 3.4% (95% CI 0.3–6.6%) for isolated CHD
  - 9.3% (95% CI, 6.6–12%) when additional extracardiac malformations were present
ROLE OF CMA IN ANATOMICALLY ABNORMAL FETUSES AND NORMAL KARYOTYPE

- 1 in every 20 anatomically abnormal fetuses with a normal karyotype shows a submicroscopic CNV that explains its phenotype and provides prognostic information

- Professional societies recommend prenatal invasive diagnosis with CMA as first-tier test on AF/CVS in CHD
  - Many different submicroscopic and monogenic causes for CHD
  - Association between CHD and neurodevelopmental delay
PROPORTION OF THE CHROMOSOMAL DEFECTS OCCURRING AT BIRTH DETECTABLE BY DIFFERENT TESTING STRATEGIES

<table>
<thead>
<tr>
<th>MA</th>
<th>FTS</th>
<th>CON</th>
<th>SEQ</th>
<th>cfDNA-T</th>
<th>cfDNA-TXY</th>
<th>QUAD</th>
<th>INT</th>
</tr>
</thead>
<tbody>
<tr>
<td>25y</td>
<td>20%</td>
<td>36%</td>
<td>8%</td>
<td>9%</td>
<td>54%</td>
<td>12%</td>
<td>21%</td>
</tr>
<tr>
<td>35y</td>
<td>44%</td>
<td>43%</td>
<td>37%</td>
<td>41%</td>
<td>69%</td>
<td>37%</td>
<td>47%</td>
</tr>
<tr>
<td>45y</td>
<td>73%</td>
<td>75%</td>
<td>70%</td>
<td>77%</td>
<td>88%</td>
<td>73%</td>
<td>78%</td>
</tr>
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</table>

- **cfDNA-TXY** has the highest DR at all MA;
- **SEQ** has the lowest DR, approximating the **QUAD** only at older MA: with **SEQ** the second-tier cfDNA-T drops down by 40-folds (from 5% to 0.13%) the FPR of the strategy, thereby reducing the likelihood of finding other off-target chr abn
- **CON** is always better than **SEQ** thanks to the larger population performing follow up karyotyping
- Among TSS, **INT** has the highest DR, at the cost of a late GA reporting
- **cfDNA-T** equals or is better than **CON** or **FTS** only at older MA, when trisomies dominate the risk

*Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155; Grati et al, manuscript in preparation*