Prenatal screening for Down syndrome using cell free (cf)DNA: Current issues

Clinical Laboratory Improvement Advisory Committee
November 18, 2015

Glenn E. Palomaki, PhD
Associate Professor
Department of Pathology and Laboratory Medicine
Women & Infants Hospital
Alpert Medical School at Brown University
Providence, Rhode Island USA

Women & Infants'

BROWN

Disclosures

- **Employer**: Women & Infants Hospital of Rhode Island. All grants/contracts, honorarium and consultant fees through WIH
- **Grant / Research Support**:
  2008/12: Co-PI of a Sequenom supported independent study
  2013/15: PI of a Natera supported independent study
- **Salary / Consultant Fees**:
  Beckman Coulter, PerkinElmer, Ansh Labs, Celula, LabCorp
- **Honorarium / Expenses**:
  Natera, Celula, PerkinElmer
- **Stocks / Bonds**: None
- **IP / Royalty**: None

Outline

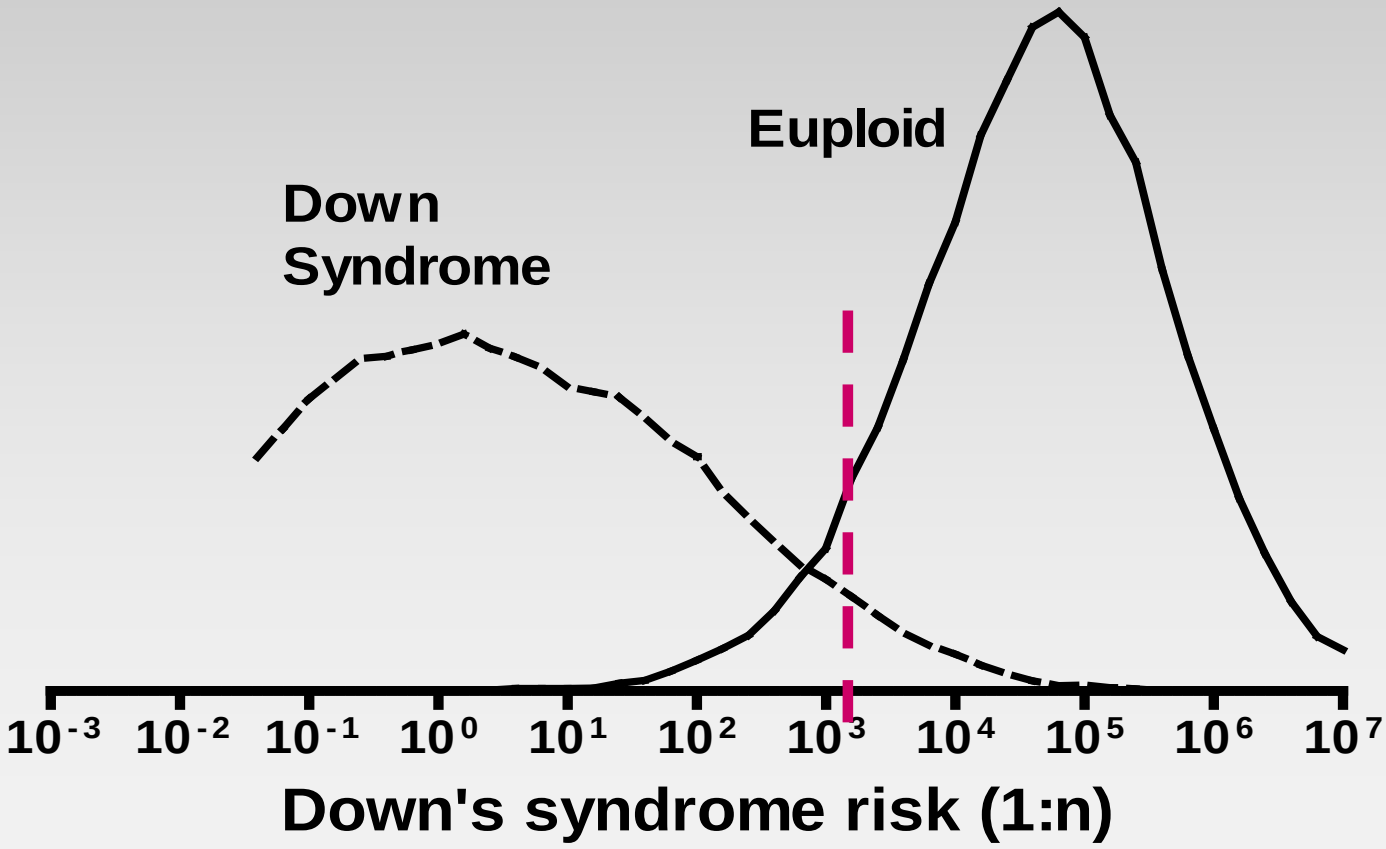
- cfDNA test failure rates
- Down syndrome detection and false positive rates
- Screening for trisomy 18 and trisomy 13
- Reasons for false positive and false negative results
- Additional issues
  - Sex chromosome abnormalities and fetal sex
  - Positive predictive value
  - Screening twin pregnancies
  - Risk in test failures
  - Select microdeletions and ‘Genome’ screening
  - Proficiency testing and laboratory oversight (FDA)
  - Screening in the general pregnancy population
  - Billing issues

Terminology and concepts

- Cell-free ‘placental’ DNA is more correct
- NIPD, NIPT, NIPS versus cell-free DNA
- Screening test not a diagnostic test
- A screening test implies there is a better test (*e.g.,* diagnostic)
- Shotgun versus Targeted sequencing
- Counting versus SNP-based methods
- Fetal fraction (placental / total cfDNA)
- Test failure - no clinical useful result returned for a suitable sample that undergoes the testing procedure

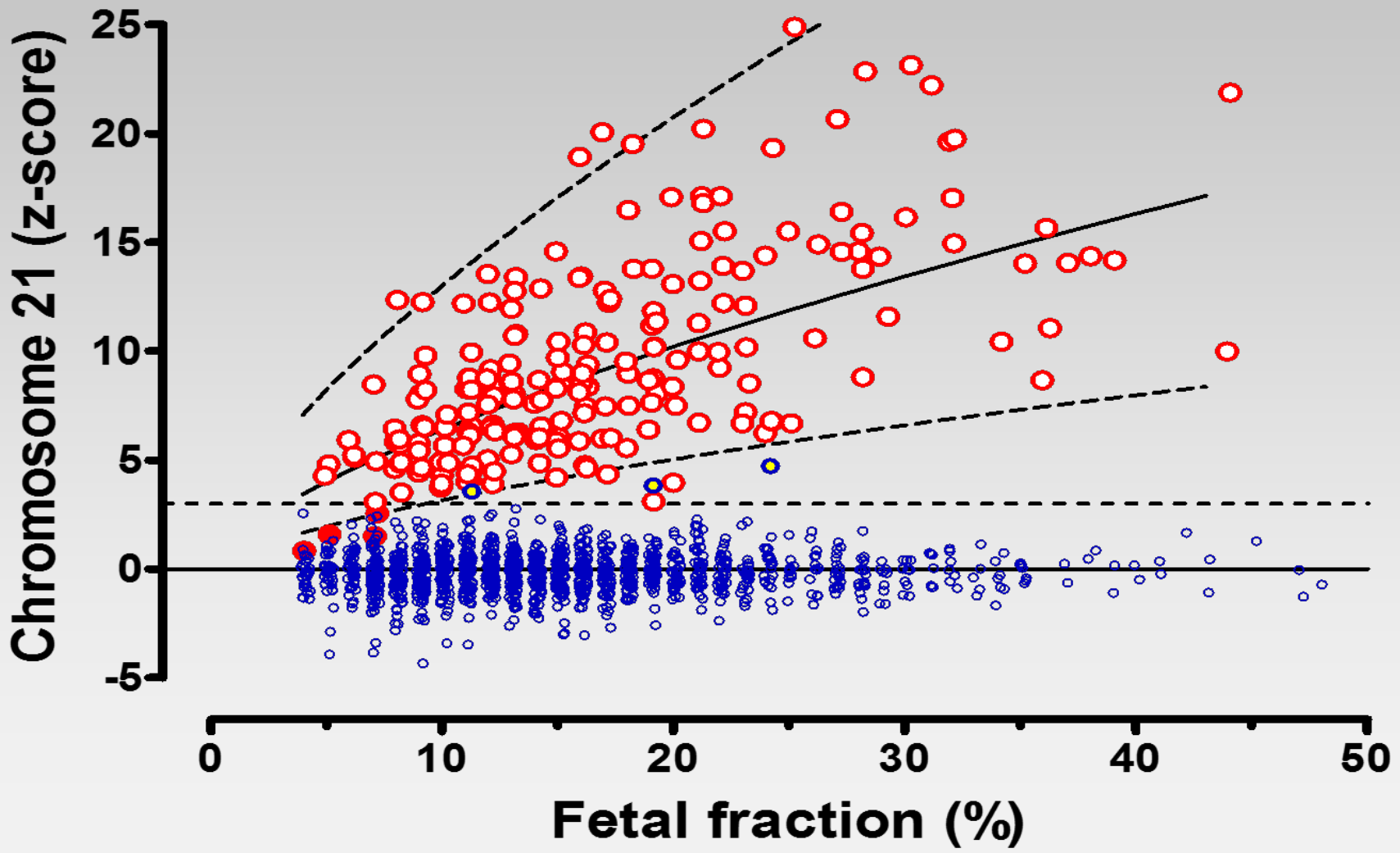
Down syndrome screening: Integrated

- ‘combined’ + ‘quadruple’ = ‘integrated’ test
- At a 1:100 risk cut-off
  - 90% DR
  - 2% FPR



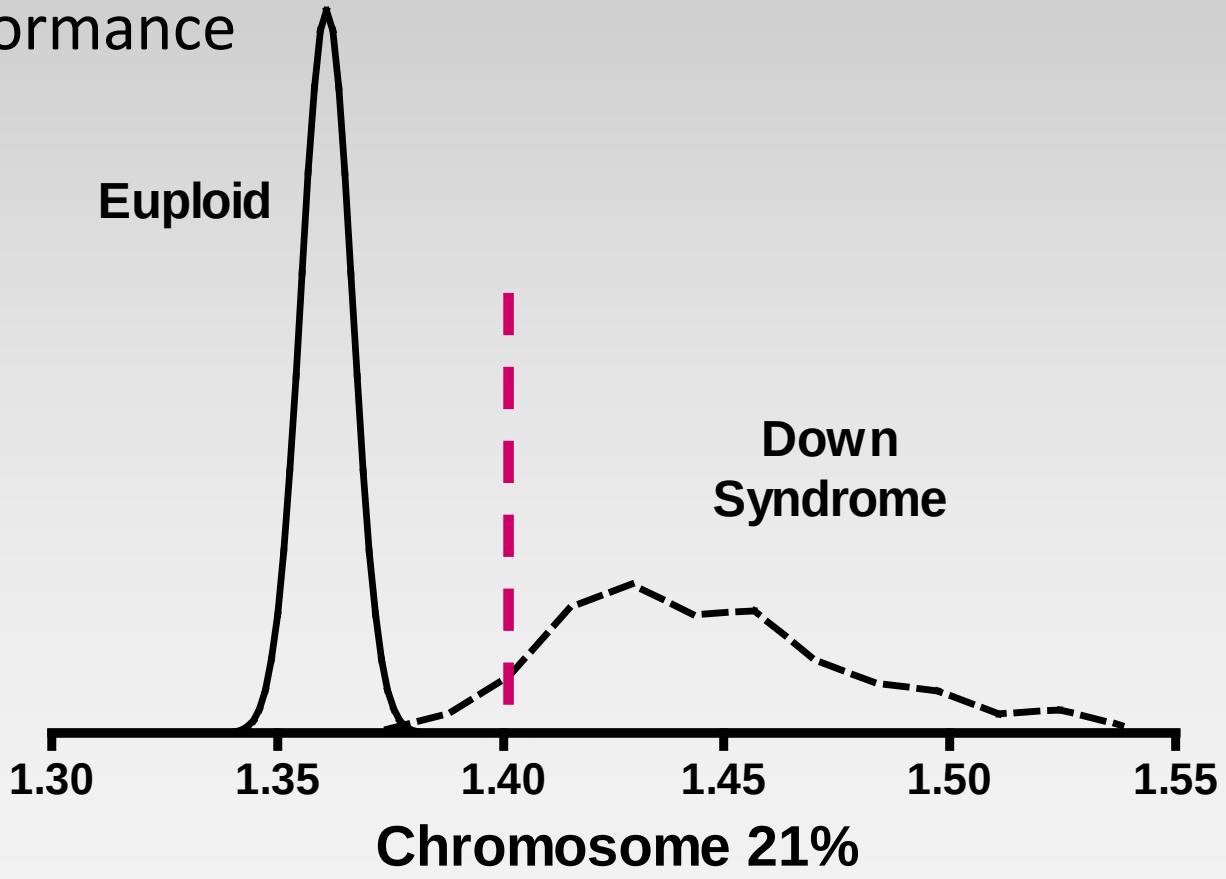
External clinical validation study: T21

(GE Palomaki et al., GIM 2011)



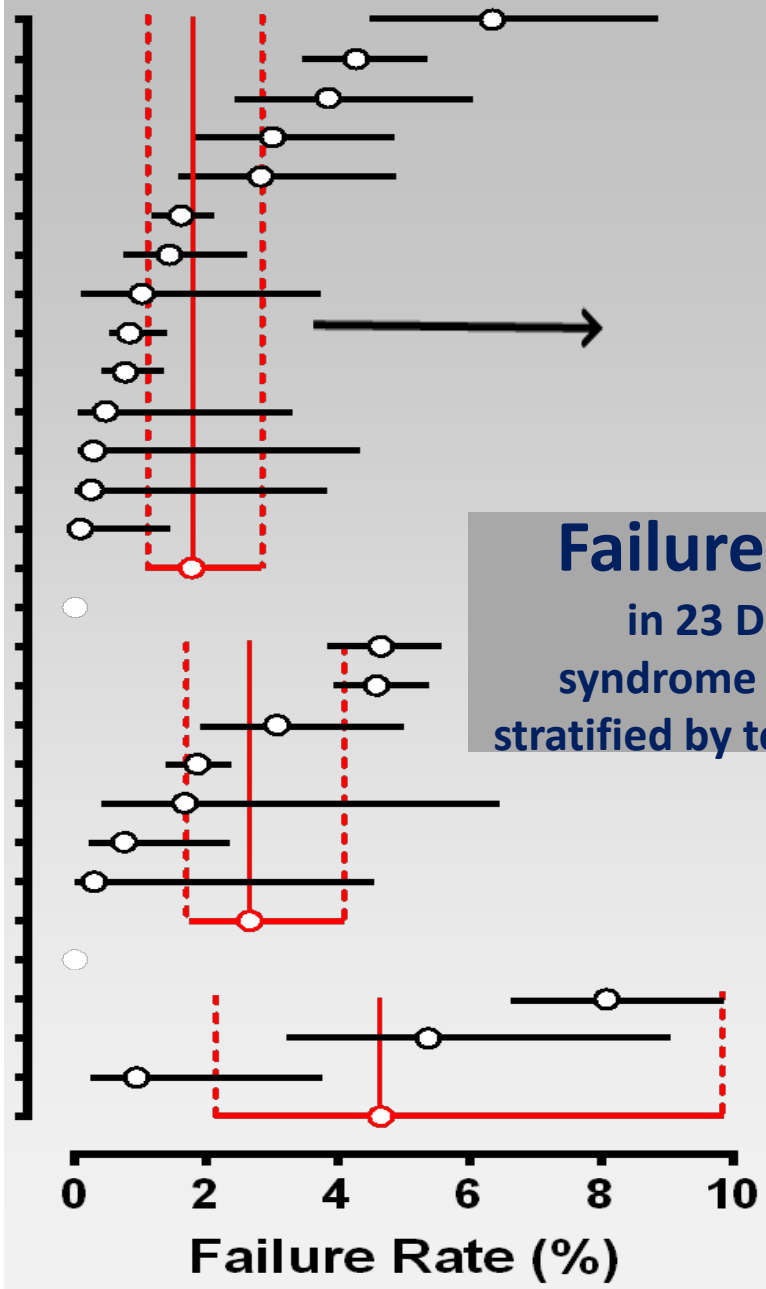
Down syndrome screening: cfDNA

- cfDNA testing of maternal plasma
- Tests involve next generation sequencing (NGS)
- Very high performance
  - 98-99% DR
  - < 0.5% FPR



Study	Fail/Total	Rate	95% CI	
Stumm 2014	32 / 504	6.4%	4.5	8.8
Song 2013	82 / 1916	4.3%	3.5	5.3
Ehrich 2011	18 / 467	3.8%	2.4	6.0
Bianchi 2012	16 / 532	3.0%	1.9	4.9
Liang 2013	12 / 424	2.8%	1.6	4.9
Porreco 2014	54 / 3340	1.6%	1.2	2.1
Chiu 2011	11 / 764	1.4%	0.8	2.6
Sehnert 2011	0 / 48	1.0%	0.06	14.3
Bianchi 2014	17 / 2042	0.8%	0.5	1.3
Palomaki 2011	13 / 1696	0.7%	0.5	1.3
Song 2015	1 / 213	0.4%	0.07	3.4
Guex 2013	0 / 176	0.3%	0.02	4.4
Shaw 2014	0 / 200	0.3%	0.02	3.9
Lau 2012	0 / 567	0.1%	0.01	1.4
Shotgun, counting		**1.77%**	**1.12**	**2.81**
Nicolaides 2012	100 / 2149	4.7%	3.8	5.6
Norton 2012	148 / 3228	4.6%	3.9	5.4
Verweij 2013	16 / 520	3.1%	1.9	5.0
Quezada 2015	54 / 2905	1.9%	1.4	2.4
Comas 2014a	2 / 120	1.7%	0.4	6.4
Ashoor 2012	3 / 400	0.7%	0.2	2.3
Sparks 2012	0 / 167	0.3%	0.02	4.6
Targeted, counting		**2.66%**	**1.73**	**4.07**
Pergament 2014	85 / 1051	8.1%	6.6	9.9
Nicolaides 2013	13 / 242	5.4%	3.1	9.0
Comas 2014b	2 / 213	0.9%	0.2	3.7
SNPs		**4.65%**	**2.14**	**9.82**

Heterogeneity high in all subgroups (I^2>88%)
Test for reduction in heterogeneity, p=0.097



Failure Rate

in 23 Down syndrome studies, stratified by test method

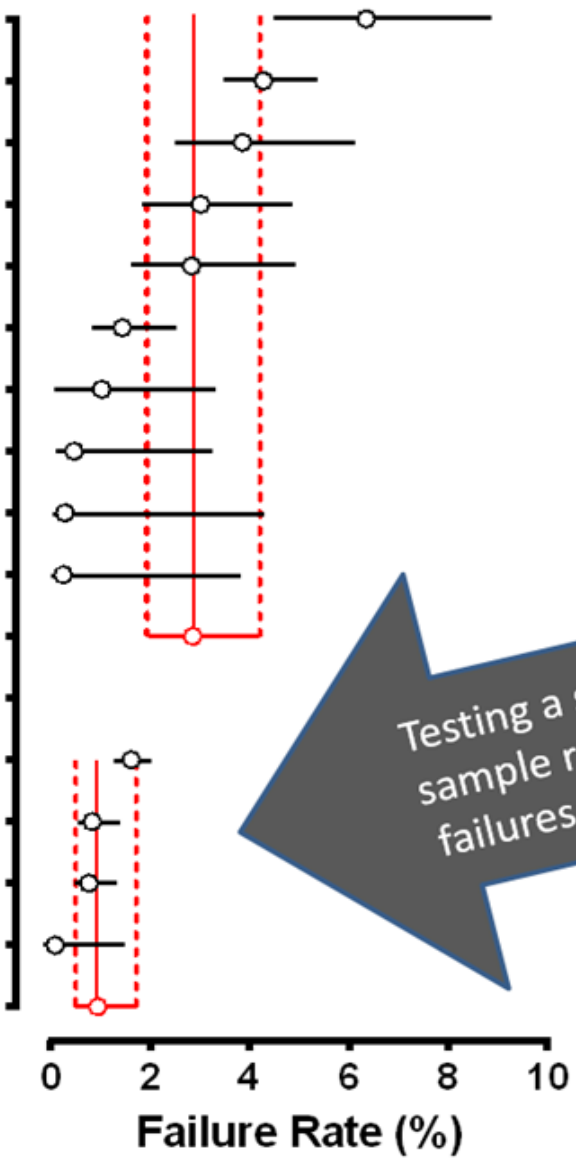
Heterogeneity between studies? An example

One study explored how SNP testing performed prior to 10 weeks' gestation (Pergament 2014)

Gestational Ageand	Samples tested	Number Failed	Failure Rate
Any	1,051	85	8.1%
<9,0	95	26	27%
9,0 to 9,6	56	10	18%
≥10,0	900	49	5.4%

Failure Rates in 13 studies using the Shotgun method

Study	Fail/Total	Rate	95% CI	
No duplicate sample available				
Stumm 2014	32 / 504	6.3%	4.5	8.8
Song 2013	82 / 1916	4.3%	3.5	5.3
Ehrich 2011	18 / 467	3.9%	2.4	6.0
Bianchi 2012	16 / 532	3.0%	1.9	4.9
Liang 2013	12 / 424	2.8%	1.6	4.9
Chiu 2011	11 / 764	1.4%	0.8	2.6
Sehnert 2012	0 / 48	1.0%	0.1	14.3
Song 2015	1 / 213	0.5%	0.1	3.3
Guex 2013	0 / 176	0.3%	0.0	4.3
Shaw 2014	0 / 200	0.2%	0.0	3.8
		2.86%	1.9	4.2
Duplicate sample tested				
Porreco 2014	54 / 3340	1.6%	1.2	2.1
Bianchi 2014	17 / 2042	0.8%	0.5	1.3
Palomaki 2011	13 / 1696	0.8%	0.4	1.3
Lau 2012	0 / 567	0.1%	0.0	1.4
		0.94%	0.5	1.7



Testing a second sample reduced failures by 67%

Heterogeneity high in all subgroups (I^2<75%)
Test for reduction in heterogeneity, p=0.002

Screening performance for autosomal aneuploidies

Insufficient evidence to conclude whether the DR/FPR for Down syndrome, T18 or T13 differ by test methodology. Reasonable performance estimates are:

Disorder	DR (%)	FPR (%)
Down syndrome	98.6	1.01[1]
Trisomy 18	94.9	0.12
Trisomy 13	91.3	0.12
All	97.0[2]	1.25[3]

[1] Includes failures that do not resolve after repeat testing

[2] Weighted 6:2:1 (T21/T18/T13)

[3] Sum of the individual FPRs

Sex chromosome testing

- Labs have the ability to interpret sex chromosomes
  - Sex chromosome aneuploidies (monosomy X, 47XXY, 47XXX and 47XYY) and fetal sex
- Most report the results in some way on all samples
- Issues with sex chromosome aneuploidy (SCA) testing
  - Disorders are considered less serious
  - Higher test failure rates (5-10%)
  - Less sensitive and specific leading to lower PPVs
  - Potential social issues with earlier identification of fetal sex
  - But, many SCA's are undiagnosed, when there is potential for beneficial treatments

False positive and false negative results

- False positive results
  - Confined placental mosaicism (placenta abnormal, fetus normal)
  - Demised twin (remaining placenta sheds DNA)
  - Maternal mosaicism (mother is assumed euploid)
  - Maternal cancer (multiple trisomy / monosomy findings)
  - Maternal duplication/deletion
  - Transplant recipient (kidney from male may cause fetal sex error)
  - Chance / technical issues
- False negative results
  - Confined placental mosaicism (fetus abnormal, placenta normal)
  - Low but acceptable fetal fraction
  - Technical issues such as GC content
  - Chance

Positive predictive values / patient risks

- Individual patient risks were the common currency of prenatal screening (e.g., 1:50 for Down syndrome)
- For serum screening, risks have been validated (reported risks: 1 in 50 are close to actual odds: 2 in 120)
- Some labs report results; others do not
- cfDNA testing does not result in a wide range of risks as most are capped (e.g., > upper limit or < lower limit)
- What risks caps are reasonable?
  - Several labs report >99 in 100, while their own data suggest the risk is less than 9 in 10 (90% PPV)
  - PPV will be lower in the general population: 50% or 1 in 2

cfDNA screening of twin pregnancies

- Monozygotic twins (~1/3 of all twins)
  - Not distinguishable from a singleton by any methodology
  - Total fetal fraction higher than singleton
  - Screening performance at least as good as in singletons
- Dizygotic twins (~2/3 of all twins)
  - SNP method can identify dizygotic twins but can't interpret results (inappropriate sample)
  - Zygosity testing is routine for some SNP-based methods
  - Fetal fraction for each twin is lower than for a singleton
  - Although overall fetal fraction may be acceptable, the contributions from one fetus may fall below 4%
- Professional groups recommend against testing twins

Results of cfDNA testing in twin pregnancies

	Shotgun	Targeted	All	
Fetal Fraction[1]	Total > 4%	Smallest > 4%		
Studies	4	2	6	
T21 DR	17/ 17	20/ 22	37/ 39	95%
T18 DR	1/ 2	5/ 5	6/ 7	86%
T13 DR	1/ 1	1/ 1	2/ 2	100%
FPR	0/226	0/504	0/730	0%
Failure rate (%)	0%	3.9%		

[1] The fetal fraction for shotgun methods used total fetal DNA, while targeted methods assigned a fetal fraction to each fetus, when dizygotic.

Microdeletion/duplication syndromes

Disorder / syndromeSNPs	Prevalence (per 100,000)	Size (Mbases)	Shotgun	SNPs
22q11.2 DiGeorge	25 to 50	1.5 to 3	X	X
5p Cri-du-chat	2 to 5	30	X	X
15q Prader-Willi	3 to 10	5.9	X	X
15q Angelman	2 to 8	5.9	X	X
1p36 deletion	10 to 20	10	X	X
4p16.3 Wolf-Hirschhorn	2	1 to 10	X	
8q Langer-Giedion	Rare	1 to 25	X	
11q Jacobsen	1	7 to 20	X	
Trisomy 16	2	90	X	
Trisomy 22	2	51	X	

Implementation issues with microdeletion testing

- Disorders chosen by size, not by need
- Detection rates are not yet well defined
- Low prevalence for some disorders (1:10,000 or less)
- Estimated DR/PPV based mainly on artificial samples
- Some disorders have variable penetrance (DiGeorge)
- Add costs to both test methodologies (maintain deeper sequencing or add additional SNPs)
- Questionable clinical utility (e.g., trisomy 22)

- Recommended against by professional organizations

‘Whole genome screening’

- Shotgun methods already collect sequencing data across the genome, but have restricted analysis to selected chromosomes (i.e., 21, 18, 13, X and Y)
- Rather than putting fragment counts into ‘bins’ defined by the chromosome, but them into equal-sized bins across the genome (e.g., 3000 1Mb bins)
- Then can look for 3000 aneuploidies!
- The laboratory claims to detect dup/del of 7Mb or larger

- Remember – cfDNA testing is based on **PLACENTAL** DNA
- What is the clinical utility of these findings?
- Some findings may be variants of unknown significance

External oversight

- Currently, all labs are CLIA certified and CAP accredited
- All tests are laboratory-developed (LDTs)
- Potential for FDA oversight of LDTs?
- CAP has relevant 'checklists' used by on-site inspectors
  - General laboratory requirements
  - Requirement for laboratories using sequencing
  - Specific requirement for laboratories performing cell free DNA test for aneuploidy
    - Information on laboratory requisition
    - Reporting of results
    - Monitoring of results (e.g., failure rate, screen positive rate)
- External PT is challenging due to variations in LDT methodologies (e.g., SNPs, fragment length)

Testing in ‘low risk’ pregnancies

- Two high-profile studies
  - One concluded the false positive rate is lower with cfDNA testing (0.2% versus 5%, Bianchi et al., NEJM 2014)
  - Another concluded the Down syndrome detection rate is higher with cfDNA testing (100% versus 79%, Norton et al., NEJM 2015)
- Both were observational
  - testing protocols were not those used in practice (no repeats)
  - no documentation of decision-making
  - many subjects lost to follow-up

Three real differences in cfDNA testing: high risk vs. general pregnancy population

- Counseling/education
  - High risk often has genetic counseling while the general population is too large for this activity, so education would need to come from primary care providers
- Positive (Negative) predictive values
  - High risk population has few false positives compared to true positives, while in the general population, most positives are false positives (even less predictive for other trisomies and sex aneuploidies and microdeletions)
- Impact on test failures / no calls
  - High risk can still be offered diagnostic testing, while the general population would need other options

Billing issues

From: Prenatal SIG Discussion [support@list.nsgc.org]
Sent: Wednesday, March 11, 2015 2:13 AM
To: PrenatalSIGForum@list.nsgc.org
Subject: cost of cfDNA testing

I just got a call from a patient with billing problem from xxx and thought I would share:

xxx billed her insurance $5,478.00. The insurance paid xxx $3,451.41 and they wrote off $557.87. She got a bill for $1500.00.

I called an yyy Rep. He said the list price for yyy is $2,495.
The contracted rate with their biggest insurance providers is $695.

Take home messages

- cfDNA screening for Down syndrome is better than current tests
- For other disorders (e.g., T18/T13), it is at least as good
- Tests for sex chromosome defects are possible, but more difficult
- Improving technology allows for more disorders to be identified (microdeletions), but more data are needed
- Restricting use in the general population is due to implementation issues (e.g., education, handling failures, PPV)
- Perhaps due to the dominance of commercial labs, tests are being introduced before validation data are published (e.g., genome)
- Due to the highly competitive nature of cfDNA testing, misinformation and mistrust is high
- Who pays and how much is paid is a barrier to routine implementation

References

- Lo YMD *et al.,* Presence of fetal DNA in maternal plasma and serum. *Lancet* 1997;**350**:485-87
- Chiu RWK *et al.,* Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. *PNAS* 2009;**105**:20458-63
- Palomaki GE *et al.,* DNA sequencing of maternal plasma to detect Down syndrome: An international clinical validation study *Genet Med* 2011;**13**:913-20
- Bianchi DW et al., Integration of noninvasive DNA testing or aneuploidy into prenatal care: What has happened since the rubber met the road? *Clin Chem* 2014;**60**:78-87
- Bevilacqua E *et al.,* Performance of screening for aneuploidies by cell-free DNA analysis of maternal blood in twin pregnancies. *Ultrasound Obstet Gynecol* 2015;**45**:61-6
- ACOG/SMFM Committee Opinion #640: Cell-free DNA screening for aneuploidy. *Am J Obstet Gynecol* 2015;126:691-2
- Gil MM *et al.,* Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis. *Ultrasound Obstet Gynecol* 2015;**45**:249-66