NONINVASIVE PRENATAL SCREENING FOR ANEUPLOIDY: THE EVIDENCE AND THE SPIN

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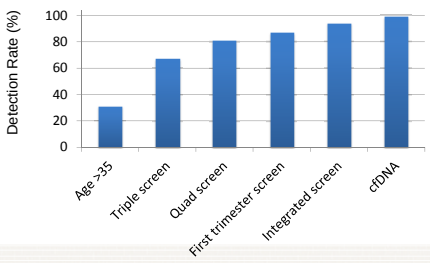
March of Dimes
17th Annual Birth Conference
March 14, 2017

Disclosure

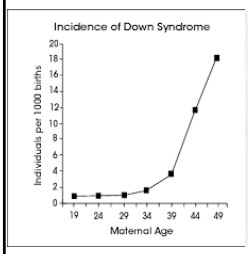
- Research support from Natera
- No other conflicts of interest

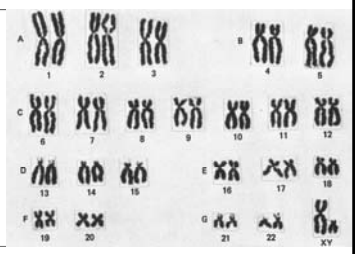
Detection rate of prenatal screening for Down syndrome has improved over time



Why all the focus on Down syndrome?

1979: NICHD Consensus Panel on Amniocentesis





THE LANCET, APRIL 28, 1984

MATERNAL SERUM ALPHA-FETOPROTEIN MEASUREMENT: A SCREENING TEST FOR DOWN SYNDROME

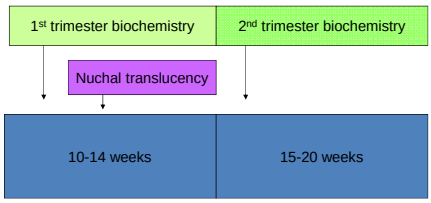
HOWARD S. CUCKLE NICHOLAS J. WALD

Department of Environmental and Preventive Medicine, Medical College of St Bartholomew's Hospital, Charterhouse Square, London ECIM 6BQ

RICHARD H. LINDENBAUM

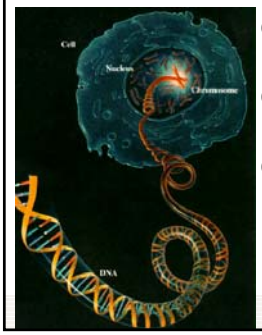
Department of Medical Genetics, Churchill Hospital, Oxford OX3 7LJ

Traditional Serum Screening



Down Syndrome:
93% detection, 4.5% screen positive rate

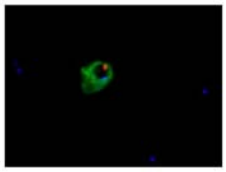
Noninvasive prenatal testing with fetal cells



- Intact (nucleated) cells carry entire genome
- Different cell types have been studied
- Fetal cells exceedingly rare
  - Difficult to extract
  - Fragile
  - Difficult to distinguish from maternal cells

Intact Fetal Cells: Is There a Future?

- Still appeal to this strategy
- Entire fetal genome within each cell



Isolate intact fetal cell(s)
↓
Whole genome amplification
↓
Microarray

1997

THE LANCET
Volume 350, Issue 9076, 16 August 1997, Pages 485-487

THE LANCET

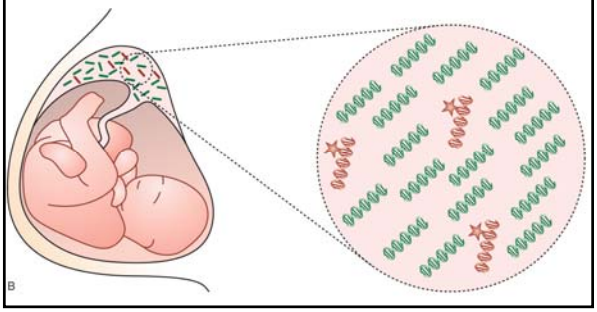
Early report

Presence of fetal DNA in maternal plasma and serum

Y M Dennis Lo, Noemi Corbetta, Paul F Chamberlain, Vik Rai, Ian L Sargent, Christopher W G Redman, James S Wainscoat

Cell free DNA

- Short segments of DNA in maternal plasma
- Primarily placental origin



Noninvasive Prenatal Testing (NIPT) for aneuploidy using cell free DNA (cfDNA)

- Detection requires accurate quantification of DNA from a specific chromosome
- Somewhat different methods are utilized by different laboratories

Next Generation Sequencing

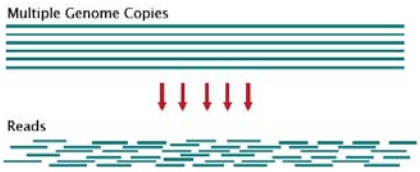


Figure 2: In DNA sequencing, multiple (typically more than a billion) copies of a genome are broken in random locations to generate much shorter reads.

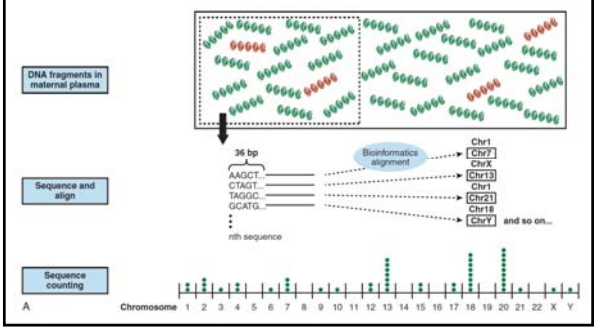
http://gcat.davidson.edu/

Massively Parallel Sequencing (or "shotgun" sequencing = MPSS)

Many cfDNA tests use this approach:

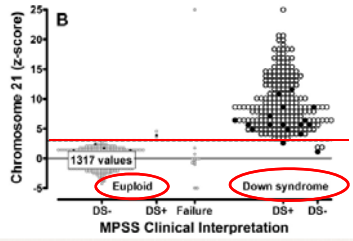
- "Massive": tons of DNA sequencing data
- "Parallel": many pieces of DNA sequenced at the same time
- "Shotgun": it is sequenced randomly

Analysis of cell free DNA



Massively Parallel Shotgun Sequencing (MPSS): *Palomaki et al, 2011*

- Random sampling of cfDNA fragments from all chromosomes
- A z-score value is used as a cut-off for trisomy (z-score of 3)

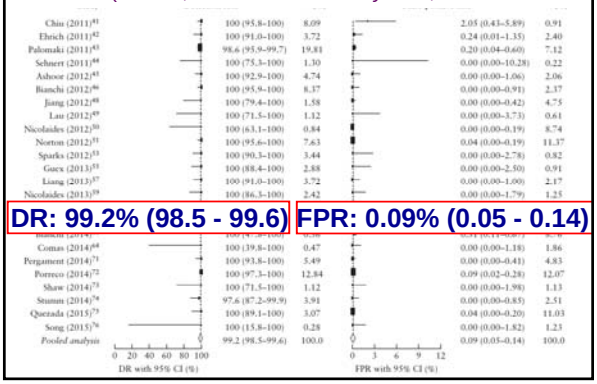


N=1696

Palomaki GE et al. (2011), Genet. Med

cfDNA screening for T21: meta-analysis

(*Gil et al, Ultrasound Obstet Gynecol, 2015*



DR: 99.2% (98.5 - 99.6) **FPR: 0.09% (0.05 - 0.14)**

harmony PRENATAL TEST
NIFTY™
MaterniT21 PLUS
genesis genetics
verifi® prenatal test
percept
panorama natera prenatal screen
the IONA test
progenity
Counsyl

Professional Society Opinions (2012): ACOG; ACMG; International Society of Prenatal Diagnosis; National Society of Genetic Counselors

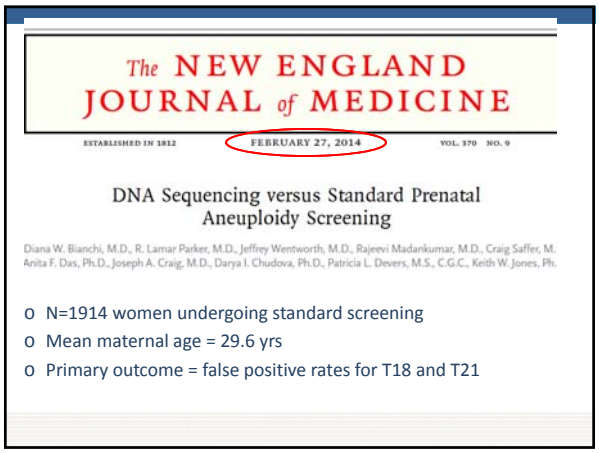
Common themes:

There are recognized benefits, but…

- Not diagnostic
  - Needs confirmation
  - "Advanced screening test"
- Limited detection of trisomies (vs invasive testing)
- Requires comprehensive genetic counseling
- Should only be used in validated groups (eg high risk)
- *Need a low risk study before introducing into general population screening*

Why Did We Need a Low Risk Study?

- The prevalence is lower so the test performance is different?
- The biology of younger, low risk women is different?
- Just to get more data and experience before jumping on the bandwagon



The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 FEBRUARY 27, 2014 VOL. 370 NO. 9

DNA Sequencing versus Standard Prenatal Aneuploidy Screening

Diana W. Bianchi, M.D., R. Lamar Parker, M.D., Jeffrey Wentworth, M.D., Rajeevi Madankumar, M.D., Craig Saffer, M. Anita F. Das, Ph.D., Joseph A. Craig, M.D., Darya I. Chudova, Ph.D., Patricia L. Devers, M.S., C.G.C., Keith W. Jones, Ph.

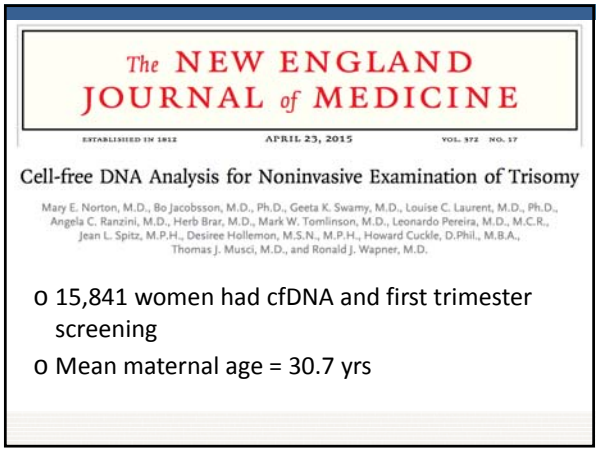
- N=1914 women undergoing standard screening
- Mean maternal age = 29.6 yrs
- Primary outcome = false positive rates for T18 and T21

cfDNA vs Standard Screening

Bianchi et al, NEJM, 2014

	FPR	PPV	
cfDNA	0.3%	45.5%	p<.001
Standard	3.6%	4.2%	

- Only 8 aneuploidy cases in the cohort
  - (T21 (5), T18 (2), and T13(1))
- All were detected



The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 APRIL 23, 2015 VOL. 372 NO. 17

Cell-free DNA Analysis for Noninvasive Examination of Trisomy

Mary E. Norton, M.D., Bo Jacobsson, M.D., Ph.D., Geeta K. Swamy, M.D., Louise C. Laurent, M.D., Ph.D., Angela C. Ranzini, M.D., Herb Brar, M.D., Mark W. Tomlinson, M.D., Leonardo Pereira, M.D., M.C.R., Jean L. Spitz, M.P.H., Desiree Hollemon, M.S.N., M.P.H., Howard Cuckle, D.Phil., M.B.A., Thomas J. Musci, M.D., and Ronald J. Wapner, M.D.

- 15,841 women had cfDNA and first trimester screening
- Mean maternal age = 30.7 yrs

"NEXT" study: 15,841 average risk women

	Cell free DNA screening	First trimester screening	
Detection rate	38/38 (100%)	30/38 (79%)	P=0.008
False positive rate	0.06%	5.4%	P<0.0001
Positive predictive value	81%	3.4%	P<0.0001

Norton et al, NEJM, 2015

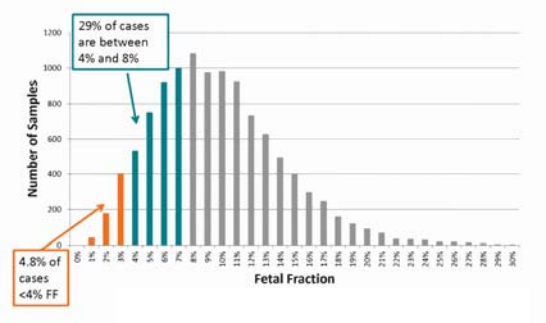
Cell free DNA: Biologic Challenges

False positives:
- Unrecognized or "vanishing" twin
- Placental mosaicism
- Low level maternal mosaicism, esp sex chromosomal
- Maternal genetic variation (copy number variants)
- Maternal malignancy

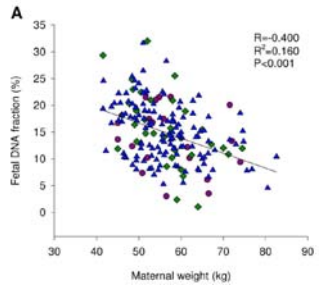
False negatives:
- Low level of fetal DNA
- Placental mosaicism
- Maternal genetic variation (copy number variants)

Failed results:
- Increased BMI
- Low level of fetal DNA
- Fetal aneuploidy

Fraction of cell free DNA that is fetal in origin: "Fetal Fraction"

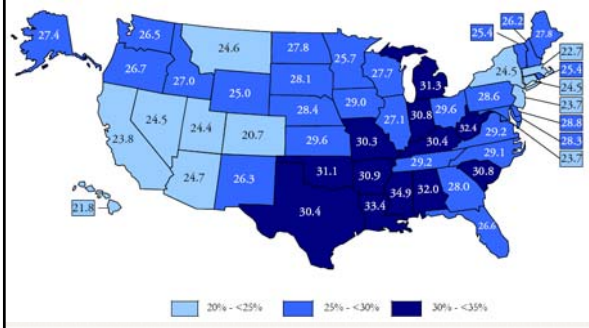


Fetal fraction and maternal weight

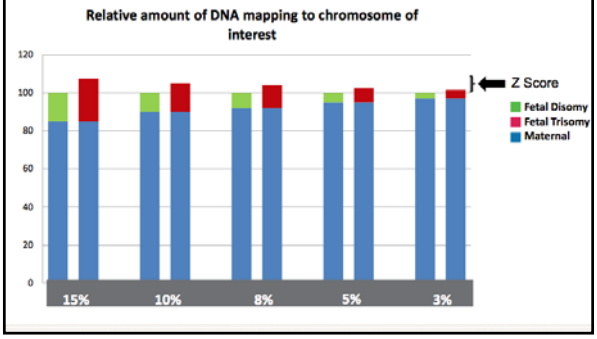


Hudecova I et al, PLoS One, 2014

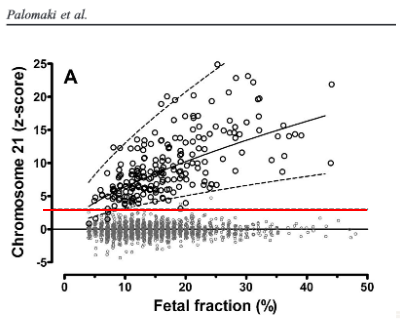
Obesity in US Adults



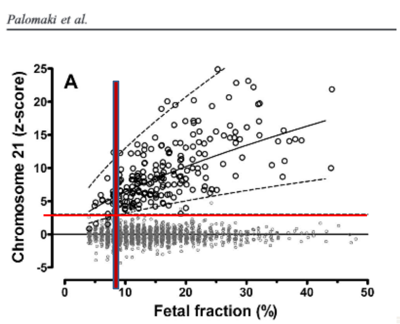
The less fetal DNA, the harder to tell normal from abnormal



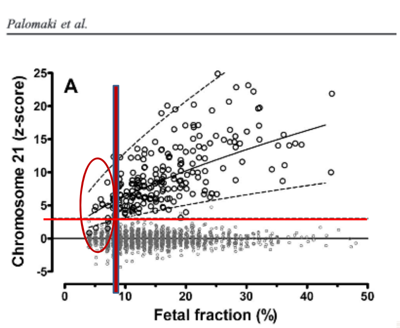
Fetal Fraction



Fetal Fraction



Fetal Fraction



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ESTABLISHED IN 1812 APRIL 23, 2015 VOL. 372 NO. 17

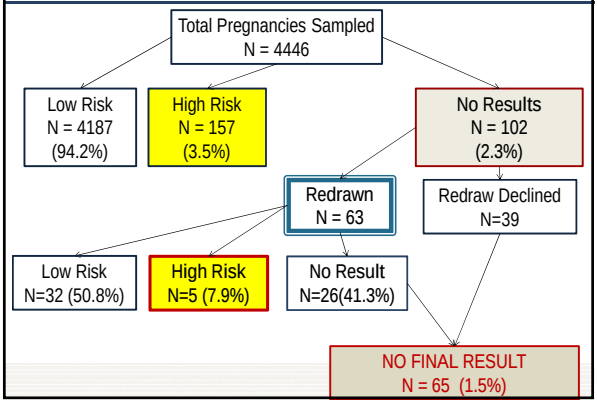
Cell-free DNA Analysis for Noninvasive Examination of Trisomy

"NEXT" study: 15,841 average risk women

- 488 (3%) women had no result
  - Low fetal fraction, failed sequencing, high variance in sequencing
  - Risk of aneuploidy was 1/38 (2.7%)
  - Much higher than 1/236 (0.4%) in cohort

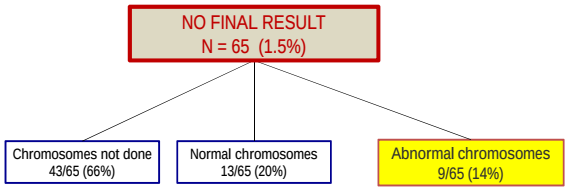
Norton et al, NEJM, 2015

Kaiser Experience: No Results Cases

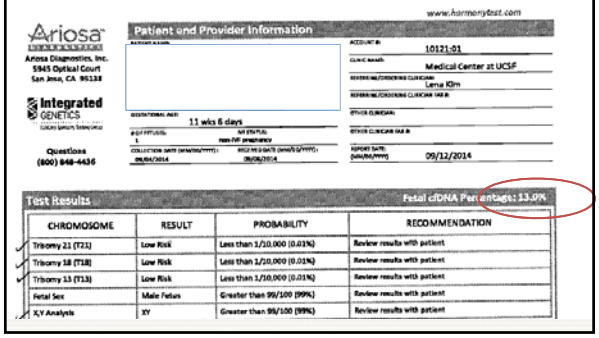


Kaiser Experience: No Results Cases

KPNC 10/29/12 – 6/30/14
Total pregnancies sampled = 4446



Fetal Fraction



Ariosa Diagnostics, Inc.
5945 Optical Court
San Jose, CA 95138

Integrated Genetics

Questions
(800) 848-4436

www.harmonytest.com

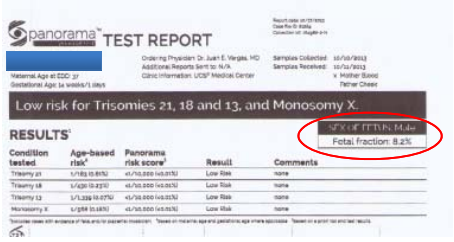
Patient and Provider Information

ACCOUNT #: 10121-01
CLINIC NAME: Medical Center at UCSF
REFERRING/ORDERING CLINICIAN: Lena Kim
GESTATIONAL AGE: 11 wks 6 days
OF FETUSES: 1
ETHNICITY: non-AA
COLLECTION DATE (MM/DD/YYYY): 09/06/2014
REPORT DATE (MM/DD/YYYY): 09/12/2014

Test Results — Fetal cfDNA Percentage: 13.0%

CHROMOSOME	RESULT	PROBABILITY	RECOMMENDATION
Trisomy 21 (T21)	Low Risk	Less than 1/10,000 (0.01%)	Review results with patient
Trisomy 18 (T18)	Low Risk	Less than 1/10,000 (0.01%)	Review results with patient
Trisomy 13 (T13)	Low Risk	Less than 1/10,000 (0.01%)	Review results with patient
Fetal Sex	Male Fetus	Greater than 99/100 (99%)	Review results with patient
X,Y Analysis	XY	Greater than 99/100 (99%)	Review results with patient

Fetal Fraction



panorama™ TEST REPORT

Low risk for Trisomies 21, 18 and 13, and Monosomy X.

Fetal fraction: 8.2%

RESULTS

Condition tested	Age-based risk	Panorama risk score	Result	Comments
Trisomy 21	1/183 (0.55%)	<1/10,000 (<0.01%)	Low Risk	none
Trisomy 18	1/430 (0.23%)	<1/10,000 (<0.01%)	Low Risk	none
Trisomy 13	1/1,339 (0.07%)	<1/10,000 (<0.01%)	Low Risk	none
Monosomy X	1/358 (0.28%)	<1/10,000 (<0.01%)	Low Risk	none

High Risk, Low Risk, and Positive Predictive Value

© American College of Medical Genetics and Genomics

BRIEF REPORT | Genetics inMedicine

Discordant noninvasive prenatal testing and cytogenetic results: a study of 109 consecutive cases

Jia-Chi Wang, MD, PhD[1], Trilochan Sahoo, MD[1,2], Steven Schonberg, PhD[3], Kimberly A. Kopita, MS[1], Leslie Ross, MS[1], Kyla Patek, MS[1] and Charles M. Strom, MD, PhD[1]

Wang et al, *Genetics in Medicine,* 2014

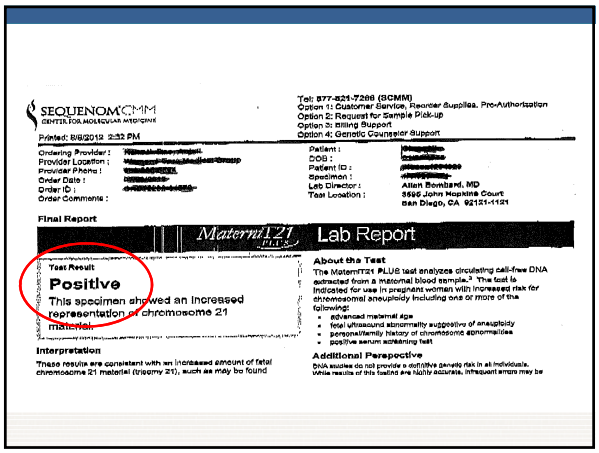
Aneuploidy	No. of positives	No (%) confirmed
T21	41	38/41 (93%)
T18	25	16/25 (64%)
T13	16	7/16 (44%)
45,X	16	6/16 (38%)
Total	**98**	**67 (67%)**

ORIGINAL ARTICLE

Cell-free DNA Analysis for Noninvasive Examination of Trisomy

Mary E. Norton, M.D., Bo Jacobsson, M.D., Ph.D., Geeta K. Swamy, M.D., Louise C. Laurent, M.D., Ph.D., Angela C. Ranzini, M.D., Herb Brar, M.D., Mark W. Tomlinson, M.D., Leonardo Pereira, M.D., M.C.R., Jean L. Spitz, M.P.H., Desiree Hollemon, M.S.N., M.P.H., Howard Cuckle, D.Phil., M.B.A., Thomas J. Musci, M.D., and Ronald J. Wapner, M.D.

Risk Group	Positive predictive value
Entire cohort (mean age 30.7 yrs	81%
Maternal age <35 yo	76%
Low risk serum FTS (<1/270)	50%



SEQUENOM CMM
CENTER FOR MOLECULAR MEDICINE

Tel: 877-624-7266 (SCMM)
Option 1: Customer Service, Reorder Supplies, Pre-Authorization
Option 2: Request for Sample Pick-up
Option 3: Billing Support
Option 4: Genetic Counselor Support

Printed: 8/8/2012 2:32 PM

Ordering Provider :
Provider Location :
Provider Phone :
Order Date :
Order ID :
Order Comments :

Patient :
DOB :
Patient ID :
Specimen :
Lab Director : Allen Bombard, MD
Test Location : 3595 John Hopkins Court
San Diego, CA 92121-1121

Final Report

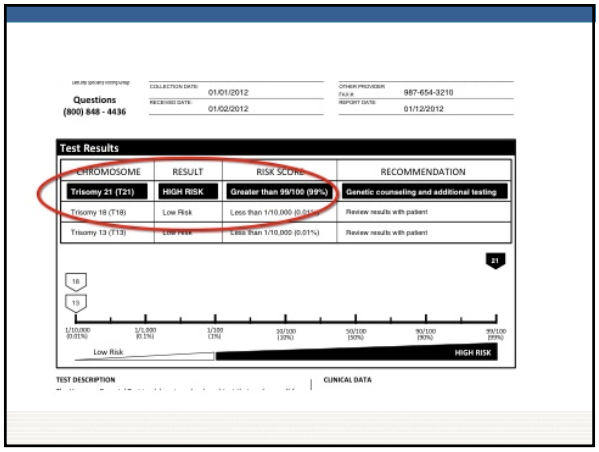
MaterniT21 PLUS Lab Report

Test Result
Positive
This specimen showed an increased representation of chromosome 21 material.

About the Test
The MaterniT21 PLUS test analyzes circulating cell-free DNA extracted from a maternal blood sample.[1] The test is indicated for use in pregnant women with increased risk for chromosomal aneuploidy including one or more of the following:
- advanced maternal age
- fetal ultrasound abnormality suggestive of aneuploidy
- personal/family history of chromosome abnormalities
- positive serum screening test

Interpretation
These results are consistent with an increased amount of fetal chromosome 21 material (trisomy 21), such as may be found

Additional Perspective
DNA studies do not provide a definitive genetic risk in all individuals. While results of this testing are highly accurate, infrequent errors may be



Questions
(800) 848 - 4436

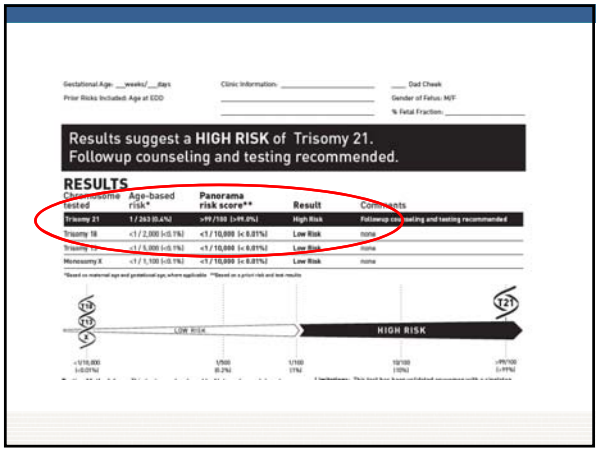
COLLECTION DATE: 01/01/2012
RECEIVED DATE: 01/02/2012
OTHER PROVIDER: 987-654-3210
REPORT DATE: 01/12/2012

Test Results

CHROMOSOME	RESULT	RISK SCORE	RECOMMENDATION
Trisomy 21 (T21)	HIGH RISK	Greater than 99/100 (99%)	Genetic counseling and additional testing
Trisomy 18 (T18)	Low Risk	Less than 1/10,000 (0.01%)	Review results with patient
Trisomy 13 (T13)	Low Risk	Less than 1/10,000 (0.01%)	Review results with patient

Low Risk — HIGH RISK

TEST DESCRIPTION | CLINICAL DATA



Gestational Age: __weeks/__days
Prior Risks Included: Age at EDD
Clinic Information:
__ Dad Cheek
Gender of Fetus: M/F
% Fetal Fraction:

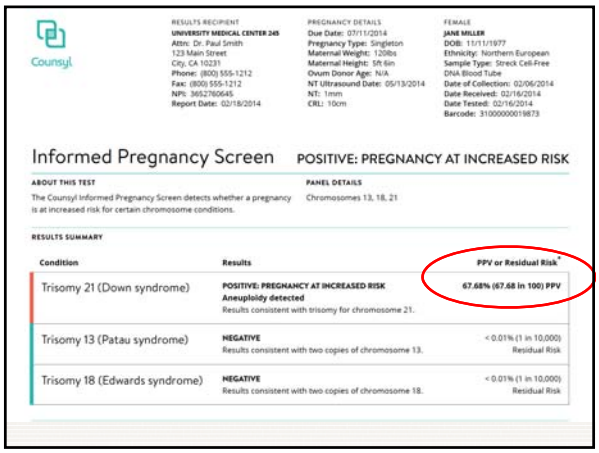
Results suggest a **HIGH RISK** of Trisomy 21.
Followup counseling and testing recommended.

RESULTS

Chromosome tested	Age-based risk*	Panorama risk score**	Result	Comments
Trisomy 21	1 / 263 (0.4%)	>99/100 (>99.0%)	High Risk	Followup counseling and testing recommended
Trisomy 18	<1 / 2,000 (<0.1%)	<1 / 10,000 (< 0.01%)	Low Risk	none
Trisomy 13	<1 / 5,000 (<0.1%)	<1 / 10,000 (< 0.01%)	Low Risk	none
Monosomy X	<1 / 1,100 (<0.1%)	<1 / 10,000 (< 0.01%)	Low Risk	none

*Based on maternal age and gestational age, where applicable. **Based on a priori risk and test results.

LOW RISK — HIGH RISK



Counsyl

RESULTS RECIPIENT
UNIVERSITY MEDICAL CENTER 245
Attn: Dr. Paul Smith
123 Main Street
City, CA 10231
Phone: (800) 555-1212
Fax: (800) 555-1212
NPI: 3652760645
Report Date: 02/18/2014

PREGNANCY DETAILS
Due Date: 07/11/2014
Pregnancy Type: Singleton
Maternal Weight: 120lbs
Maternal Height: 5ft 6in
Ovum Donor Age: N/A
NT Ultrasound Date: 05/13/2014
NT: 1mm
CRL: 10cm

FEMALE
JANE MILLER
DOB: 11/11/1977
Ethnicity: Northern European
Sample Type: Streck Cell-Free DNA Blood Tube
Date of Collection: 02/06/2014
Date Received: 02/16/2014
Date Tested: 02/16/2014
Barcode: 31000000019873

Informed Pregnancy Screen — POSITIVE: PREGNANCY AT INCREASED RISK

ABOUT THIS TEST
The Counsyl Informed Pregnancy Screen detects whether a pregnancy is at increased risk for certain chromosome conditions.

PANEL DETAILS
Chromosomes 13, 18, 21

RESULTS SUMMARY

Condition	Results	PPV or Residual Risk*
Trisomy 21 (Down syndrome)	POSITIVE: PREGNANCY AT INCREASED RISK Aneuploidy detected Results consistent with trisomy for chromosome 21.	67.68% (67.68 in 100) PPV
Trisomy 13 (Patau syndrome)	NEGATIVE Results consistent with two copies of chromosome 13.	<0.01% (1 in 10,000) Residual Risk
Trisomy 18 (Edwards syndrome)	NEGATIVE Results consistent with two copies of chromosome 18.	<0.01% (1 in 10,000) Residual Risk

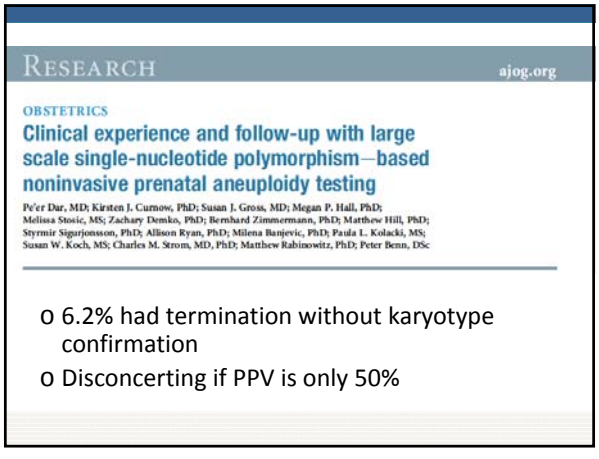
The poorly understood PPV

The Boston Globe — Metro

NEWS | METRO | ARTS | BUSINESS | SPORTS | OPINION | POLITICS | LIFESTYLE | MAGAZINE | INSIDERS

Oversold prenatal tests spur some to choose abortions

Stacie and Lincoln Chapman's healthy son was born in November 2013.



RESEARCH — ajog.org

OBSTETRICS

Clinical experience and follow-up with large scale single-nucleotide polymorphism–based noninvasive prenatal aneuploidy testing

Pe'er Dar, MD; Kirsten J. Curnow, PhD; Susan J. Gross, MD; Megan P. Hall, PhD; Melissa Stosic, MS; Zachary Demko, PhD; Bernhard Zimmermann, PhD; Matthew Hill, PhD; Styrmir Sigurjonsson, PhD; Allison Ryan, PhD; Milena Banjevic, PhD; Paula L. Kolacki, MS; Susan W. Koch, MS; Charles M. Strom, MD, PhD; Matthew Rabinowitz, PhD; Peter Benn, DSc

- 6.2% had termination without karyotype confirmation
- Disconcerting if PPV is only 50%

Consequences of false positive results

cfDNA

N=100,000
↓ 1% false positives
1000 abnormal results
↓ 6.2% TAB w/o confirmation
62 TAB
↓ ↘ 50% PPV
31 TP 31 FP
↓
31 TAB of normal fetuses

Consequences of false positive results

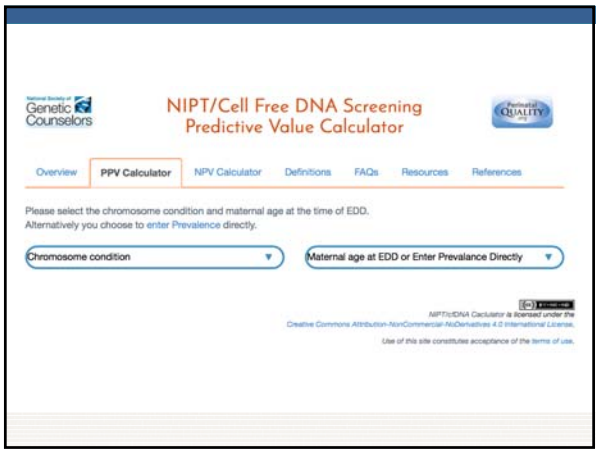
cfDNA

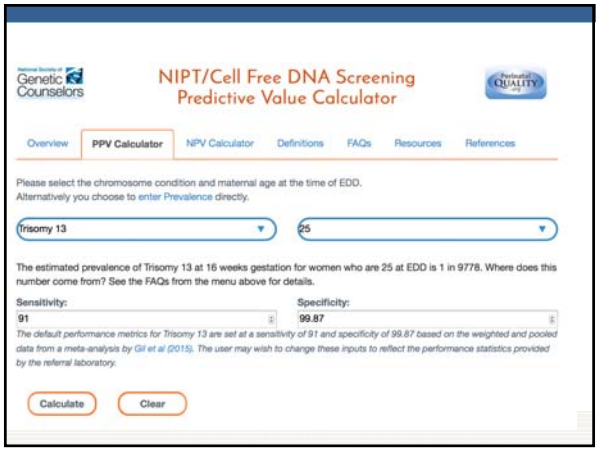
N=100,000
↓ 1% false positives
1000 abnormal results
↓ 6.2% TAB w/o confirmation
62 TAB
↓ ↘ 50% PPV
31 TP 31 FP
↓
31 TAB of normal fetuses

Serum Screening

N=100,000
↓ 5% false positives
5000 abnormal results
↓ 0.1% loss rate (amnio)
5 losses of normal fetuses

PPV Calculator: www.perinatalquality.org


National Society of Genetic Counselors
NIPT/Cell Free DNA Screening
Predictive Value Calculator
Perinatal Quality
Overview
PPV Calculator
NPV Calculator
Definitions
FAQs
Resources
References
Please select the chromosome condition and maternal age at the time of EDD.
Alternatively you choose to enter Prevalence directly.
Chromosome condition
Maternal age at EDD or Enter Prevalance Directly
NIPTcfDNA Calculator is licensed under the
Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.
Use of this site constitutes acceptance of the terms of use.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Perinatal Quality
Overview
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NPV Calculator
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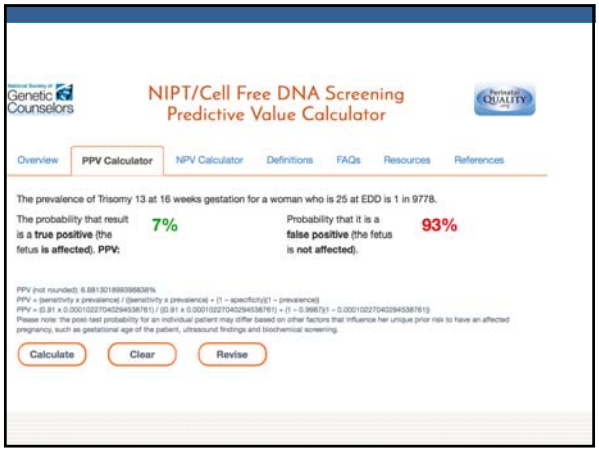
FAQs
Resources
References
Please select the chromosome condition and maternal age at the time of EDD.
Alternatively you choose to enter Prevalence directly.
Trisomy 13
25
The estimated prevalence of Trisomy 13 at 16 weeks gestation for women who are 25 at EDD is 1 in 9778. Where does this number come from? See the FAQs from the menu above for details.
Sensitivity:
91
Specificity:
99.87
The default performance metrics for Trisomy 13 are set at a sensitivity of 91 and specificity of 99.87 based on the weighted and pooled data from a meta-analysis by Gil et al (2015). The user may wish to change these inputs to reflect the performance statistics provided by the referral laboratory.
Calculate
Clear


National Society of Genetic Counselors
NIPT/Cell Free DNA Screening
Predictive Value Calculator
Perinatal Quality
Overview
PPV Calculator
NPV Calculator
Definitions
FAQs
Resources
References
The prevalence of Trisomy 13 at 16 weeks gestation for a woman who is 25 at EDD is 1 in 9778.
The probability that result is a true positive (the fetus is affected). PPV:
7%
Probability that it is a false positive (the fetus is not affected).
93%
Calculate
Clear
Revise

University of North Carolina at Chapel Hill

Positive Predictive Value of Cell Free DNA Calculator

Baseline Risk
(•) Age-related risk ( ) A priori risk

Maternal Age (20)

20 22 24 26 28 30 32 34 36 38 40 42 44

Gestational Age in Weeks (10)

10 12 14 16 18 20

Test
(•) Harmony® ( ) Materniti 21® ( ) Panorama® ( ) Verifi®

	Trisomy 21	Trisomy 18	Trisomy 13
Age-related risk	1:804	1:1993	1:6347
Test Sensitivity	99	98	80
Test Specificity	99.97	99.93	99.9
PPV	80%	41%	11%

DOWN SYNDROME!!

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 APRIL 23, 2015 VOL. 372 NO. 17

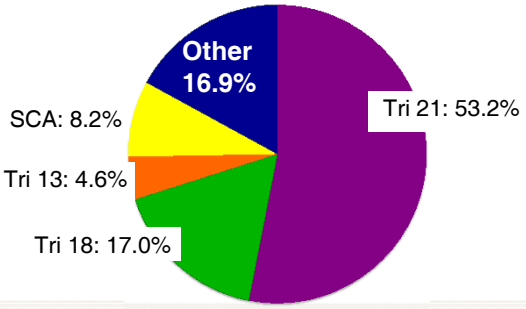
Cell-free DNA Analysis for Noninvasive Examination of Trisomy

"NEXT" study: 15,841 average risk women

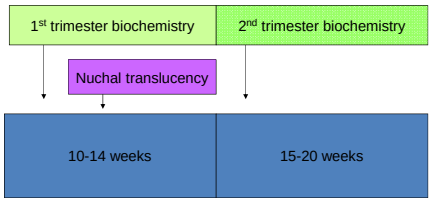
- Total of 68 significant aneuploidies in the cohort
  - 57/68 (84%) were detected

Norton et al, NEJM, 2015

Trisomies 13,18 and 21 comprise 75% of aneuploidies

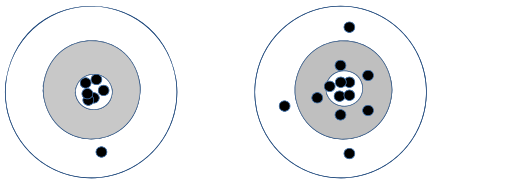


Traditional Serum Screening



Down Syndrome:
93% detection, 4.5% screen positive rate

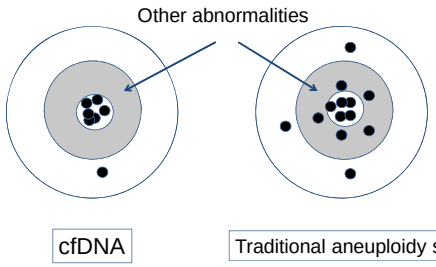
cfDNA is more precise for T13, 18, 21



cfDNA

Traditional aneuploidy screen

cfDNA is more precise for T13, 18, 21



Disorder	Prevalence
Common trisomies (13,18,21)	0.2%
Other chromosome abnormalities	0.4%
Microdeletions and duplications	1.5%
Mendelian Genetic Disorders	0.4%
Congenital heart defects	0.3%
Other structural defects	3%
Adverse OB outcomes	15-20%
Total	~25%

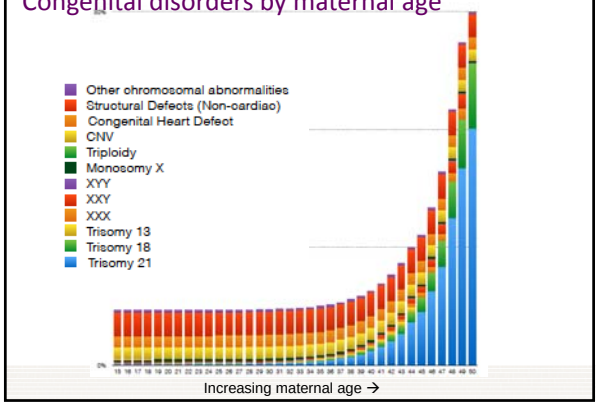
Causes of Birth Defects and Other Adverse Perinatal Outcomes: *It's Not All Down Syndrome*

Table. Risk of Down's Syndrome and Chromosomal Abnormalities at Live Birth, According to Maternal Age.*

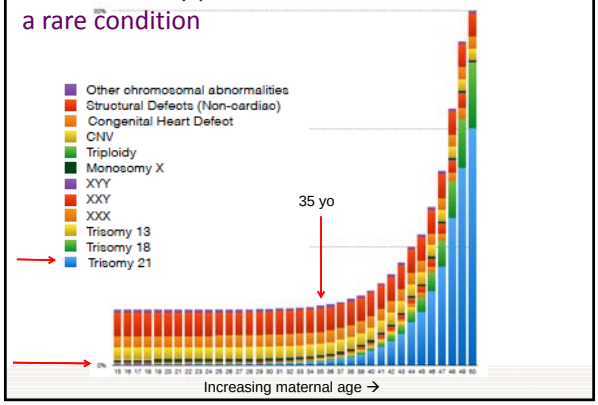
Maternal Age at Delivery (yr)	Risk of Down's Syndrome	Risk of Any Chromosomal Abnormality
20	1/1667	1/526
25	1/1200	1/476
30	1/952	1/385
35	1/378	1/192
40	1/106	1/66
45	1/30	1/21

* Modified from Hook et al.[3]

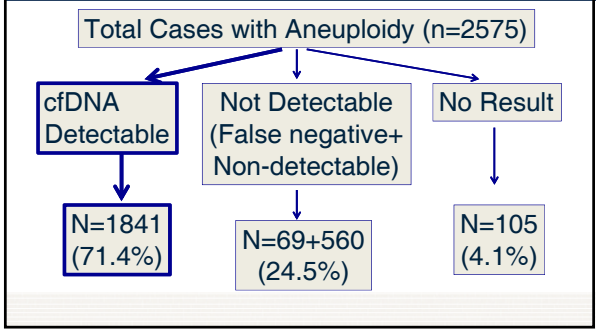
Congenital disorders by maternal age



cfDNA is a very precise test for a rare condition



cfDNA Detection Rate n=452,901 patients screened in CA



Chromosomal Microarray (CMA) for Prenatal Diagnosis

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 DECEMBER 6, 2012 VOL. 367 NO. 23

Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis

Ronald J. Wapner, M.D., Christa Lese Martin, Ph.D., Brynn Levy, M.Sc.(Med.), Ph.D., Blake C. Ballif, Ph.D., Christine M. Eng, M.D., Julia M. Zachary, Melissa Savage, M.S., Lawrence D. Platt, M.D., Daniel Saltzman, M.D., William A. Grobman, M.D., M.B.A., Susan Klugman, M.D., Thomas Scholl, Ph.D., Joe Leigh Simpson, M.D., Kimberly McCall, B.S., Vimla S. Aggarwal, M.B., B.S., Brian Bunke, B.S., Odelia Nahum, M.Sc., Ankita Patel, Ph.D., Allen N. Lamb, Ph.D., Elizabeth A. Thom, Ph.D., Arthur L. Beaudet, M.D., David H. Ledbetter, Ph.D., Lisa G. Shaffer, Ph.D., and Laird Jackson, M.D.

Diagnostic Yield of Chromosomal Microarray in Cases with Normal Karyotype

Indication for Testing	Clinically Relevant (N=96)
U/S Anomaly *N=755*	**6.0%**
AMA *N=1,966*	**1.7%**
Positive Screen *N=729*	**1.7%**
Other *N=372*	**1.3%**

New "menu" in prenatal testing

Screening test for common aneuploidies (cfDNA) (1/500)

VS

Invasive diagnostic testing with CMA (1/60)

cfDNA and chromosomal microarray

IF:

CMA detects an abnormality in 1.7% of cases (about 1/60)

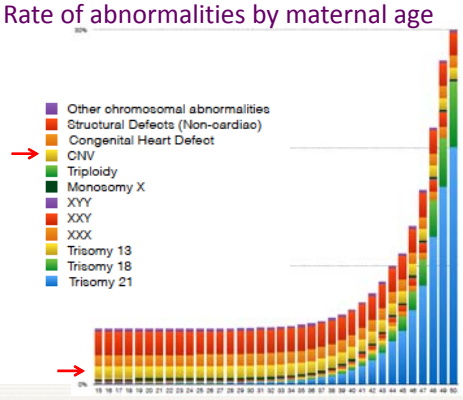
AND:

cfDNA detects T13,18, 21 – about 1/500 pregnancies

THEN:

- If cfDNA is the routine screening test, it will detect only about **12%** of diagnosable chromosomal abnormalities

Rate of abnormalities by maternal age



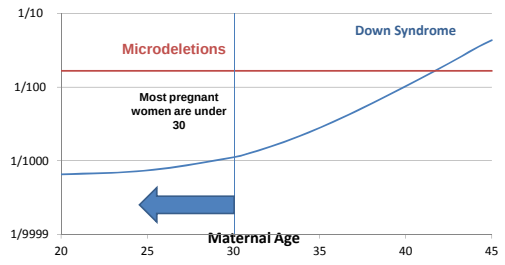
Increasing maternal age →

Expanded panels

- Trisomies 9, 16 and 22
  - Rarely seen in viable pregnancies except as mosaics
  - Common causes of confined placental mosaicism
    - Much more common in CVS samples than amniocentesis
  - Even complete trisomy in the placenta often associated with a normal fetus
- Microdeletions (22q, 1q36, 5p-, 4p-, 15q11-13)
  - Also 8q-, 11q-
- MaterniTGenome

Should all women be offered screening for microdeletions?

Microdeletions are More Common Than Down Syndrome for Women Under 40

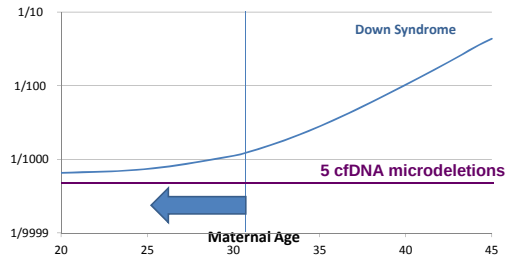


Adapted from: Snijders, et al. *Ultrasound Obstet Gynecol 1999;13:167–170*

Microdeletion syndromes are rare

Syndrome	Frequency	Features
22q11.2 (DiGeorge)	1/4,000	Varies: cardiac, palatal, immune, intellectual disability
1q36	1/10,000	Severe intellectual disability (ID), +/- obvious structural anomalies
Angelman	1/20,000	Severe ID, seizures, speech delay
Prader-Willi	1/30,000	Obesity, ID, behavioral problems
Cri-du-chat	1/50,000	Microcephaly, ID, +/- CHD
Wolf-Hirshhorn	1/50,000	ID, seizures, +/- CL/CP
Total	**1/2500**	

Microdeletions are More Common Than Down Syndrome for Women Under 40



Adapted from: Snijders, et al. *Ultrasound Obstet Gynecol 1999;13:167–170*

cfDNA for microdeletions is NOT a noninvasive microarray

Prevalence of 5 microdeletion syndromes: ~1/2500

Prevalence of CNV by microarray: 1/60

Detection rate of cfDNA for all pathogenic CNV: 4.2%

The false positives add up….

Trisomy	Detection Rate	False Positive Rate
Trisomy 21	99%	0.1%
Trisomy 18	97%	0.3%
Trisomy 13	87%	0.6%
Sex chromosomes	86%	0.6%
Total		**1.6%**

The false positives add up….

Trisomy	Detection Rate	False Positive Rate
Trisomy 21	99%	0.1%
Trisomy 18	97%	0.3%
Trisomy 13	87%	0.6%
Sex chromosomes	86%	0.6%
Microdeletions	??	1.0%
Total		**2.6%**

The future: Whole genome sequencing

BRIEF REPORT

Clinical Diagnosis by Whole-Genome Sequencing of a Prenatal Sample

Michael E. Talkowski, Ph.D., Zehra Ordulu, M.D., Vamsee Pillalamarri, M.S.,

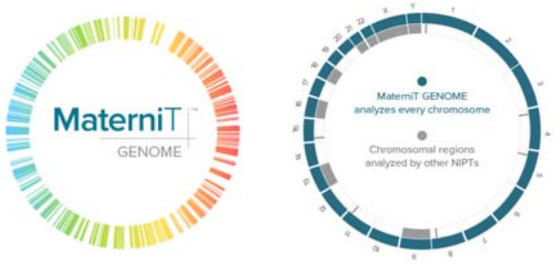
ARTICLE

doi:10.1038/nature11251

Non-invasive prenatal measurement of the fetal genome

H. Christina Fan[1]†*, Wei Gu[1]*, Jianbin Wang[1], Yair J. Blumenfeld[2], Yasser Y. El-Sayed[2] & Stephen R. Quake[1,3,4]

"Non-invasive Genome"



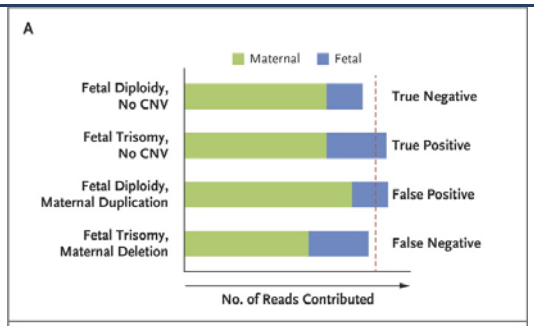
NATURE | COMMENT

Pregnancy: Prepare for unexpected prenatal test results

Diana W. Bianchi

Women are learning about their own health problems through fetal screening. Revise consent forms and raise awareness, urges Diana W. Bianchi.

Maternal genetic variants and cfDNA



Snyder et al, NEJM, 2015

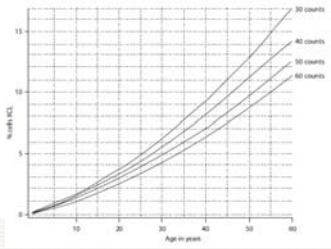
Natural X Chromosome Loss

Cytogenetic and Genome Research (2007)

X chromosome loss and ageing

L.M. Russell[a] P. Strike[b] C.E. Browne[a] P.A. Jacobs[b]

- 665 women (0-80yrs)
- Lymphocyte cultures on 19,650 cells
- G-banding analysis for presence of 1 or 2 X chromosomes



Preliminary Communication

Noninvasive Prenatal Testing and Incidental Detection of Occult Maternal Malignancies

Diana W. Bianchi, MD; Darya Chudova, PhD; Amy J. Sehnert, MD; Sucheta Bhatt, MD; Kathryn Murray, MS; Tracy L. Prosen, MD; Judy E. Garber, MD; Louise Wilkins-Haug, MD, PhD; Neeta L. Vora, MD; Stephen Warsof, MD; James Goldberg, MD; Tina Ziainia, MD; Meredith Halks-Miller, MD

JAMA. 2015;314(2):162-169. doi:10.1001/jama.2015.7120
Published online July 13, 2015.

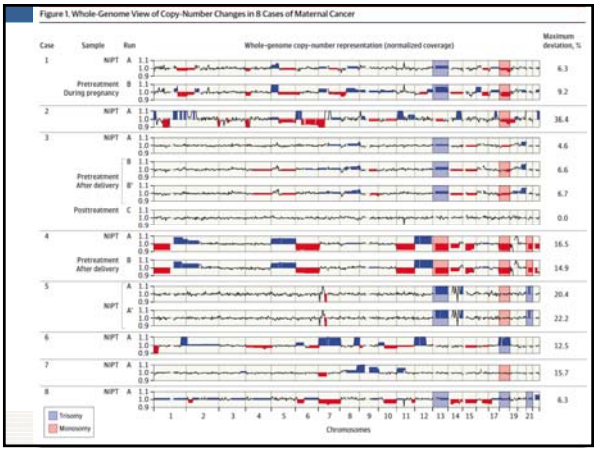
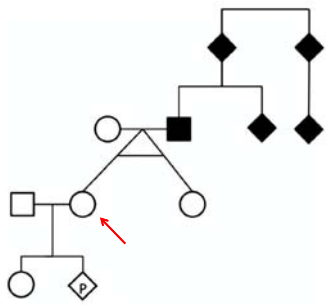


Table 2. Association of Maternal Cancers With Different Types of Aneuploidies Detected at Noninvasive Prenatal Testing

Type of Aneuploidy Detected by NIPT	Total No. of Samples	No. of Known Maternal Cancers (%) [95% CI]
Single trisomy[a]	2650	2 (0.08) [0-0.27]
Single SCA[b]	950	0 (0) [0-0.39]
Single trisomy + SCA	30	0 (0) [0-11.5]
Single monosomy	88	1 (1.14) [0-6.1]
Multiple aneuploidy[c]	39	7 (17.9) [7.5-33.5]
Total abnormal NIPT	3757	10 (0.26) [0.12-0.48]



Meschino et al, Prenatal Diagn, 2016

cfDNA screening

- Trisomy 13, 18, 21
- Sex chromosomes
- +/- microdeletions
- Maternal cancer
- Maternal CNV
- Maternal sex chromosomal aneuploidy

Traditional screening

- Trisomy 18, 21, +/-13
- Other chromosomal
- Early dx fetal anomalies, including cardiac (NT)
- Spina bifida and ventral wall defects (MSAFP)
- Adverse obstetric outcomes
  - Preeclampsia, PTB, FGR

The Public Health Evidence for FDA Oversight of Laboratory Developed Tests: 20 Case Studies

Office of Public Health Strategy and Analysis
Office of the Commissioner
Food and Drug Administration

November 16, 2015

The Public Health Evidence for FDA Oversight of Laboratory Developed Tests: 20 Case Studies

"…these products may have caused or have caused actual harm to patients."

Office of Public Health Strategy and Analysis
Office of the Commissioner
Food and Drug Administration

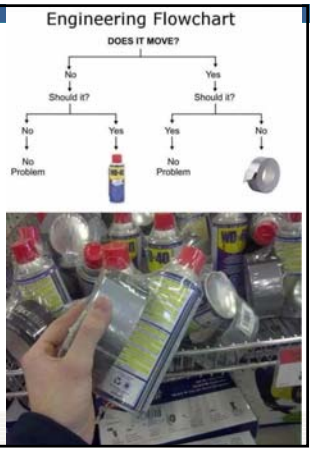
November 16, 2015

C. Tests with the Potential to Yield both Many False-Positive and False-Negative Results

i. Noninvasive Prenatal Testing (A.K.A. cell-free DNA testing)

Category	LDT Characteristics
LDT Name	Noninvasive prenatal cell-free DNA testing (NIPT, or cfDNA)
Description	Blood test to identify traces of fetal chromosomes in maternal blood
Purpose	To detect a range of fetal chromosomal abnormalities
Target Population	Pregnant women concerned about a fetal chromosomal abnormality
Alternatives	Invasive testing, including amniocentesis and chorionic villi sampling; "quad testing" of multiple substances combined with ultrasound imaging
LDT Problem 1	Lack of clinical validation that tests detect and predict fetal abnormalities at an appropriate rate
LDT Problem 2	Many false-positive results when used in the general population
Clinical Consequence	Women with false-positive results may abort a normal pregnancy; women with false-negative results may deliver a child with an unanticipated genetic syndrome
Potential Impact of FDA Oversight	Assurance the test meets minimum performance standards; evaluation of manufacturer claims
Cost Impact of Inaccuracy	Not estimated

If only it were this simple…



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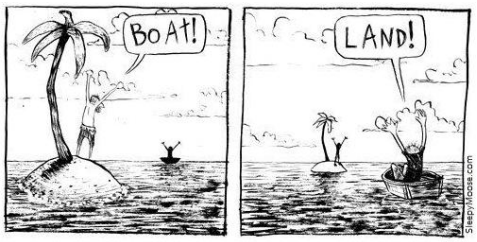
BIOLOGY

We Need More Proof That Prenatal Gene Screens Are Beneficial

Blood tests are safer for pregnant women but do not tell the whole truth

By The Editors on February 1, 2016



perspective...

The American College of Obstetricians and Gynecologists
WOMEN'S HEALTH CARE PHYSICIANS

Society for Maternal-Fetal Medicine

(Published Electronically Ahead of Print on June 26, 2015)

COMMITTEE OPINION

Number 640 • September 2015 *(This Committee Opinion Replaces Committee Opinion Number 545)*

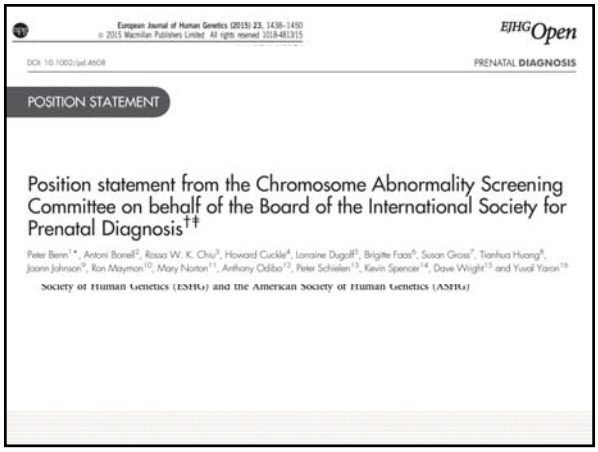
Committee on Genetics
Society for Maternal–Fetal Medicine

This document reflects emerging clinical and scientific advances as of the date issued and is subject to change. The information should not be construed as dictating an exclusive course of treatment or procedure to be followed.

Cell-free DNA Screening for Fetal Aneuploidy

ACOG/SMFM September 2015

- Conventional screening is most appropriate first line screen for most patients
- Ethically any patient may choose cfDNA screening, but should be counseled regarding limitations and benefits
- Diagnostic testing is required to confirm abnormal results before irreversible decisions
- Testing for microdeletions and in twins should not be performed


European Journal of Human Genetics (2015) 23, 1438–1450
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EJHG Open

DOI: 10.1002/pd.4608

PRENATAL DIAGNOSIS

POSITION STATEMENT

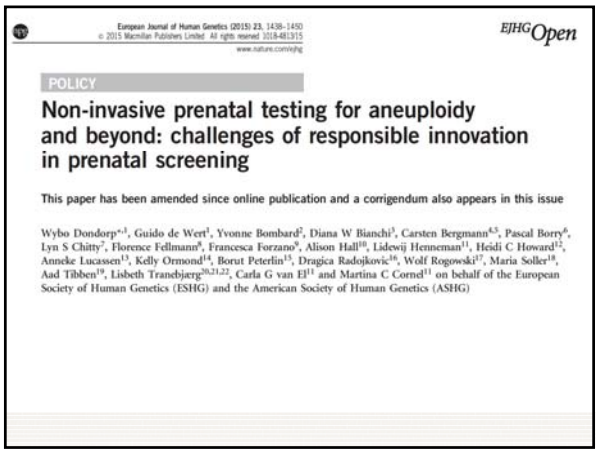
Position statement from the Chromosome Abnormality Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis†‡

Peter Benn[1]*, Antoni Borrell[2], Rossa W. K. Chiu[3], Howard Cuckle[4], Lorraine Dugoff[5], Brigitte Faas[6], Susan Gross[7], Tianhua Huang[8], Joann Johnson[9], Ron Mayman[10], Mary Norton[11], Anthony Odibo[12], Peter Schielen[13], Kevin Spencer[14], Dave Wright[15] and Yuval Yaron[16]

Society of Human Genetics (ESHG) and the American Society of Human Genetics (ASHG)

International Society for Prenatal Diagnosis, 2015

- Appropriate protocols include:
  - cfDNA as a primary, secondary or contingent test
  - traditional first and/or second trimester multiple marker screening approaches
- Not recommended:
  - Maternal age only as a screening test
  - NT only
- Microdeletion screening should only be offered for clinically significant, severe disorders



European Journal of Human Genetics (2015) 23, 1438–1450
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www.nature.com/ejhg

EJHG Open

POLICY

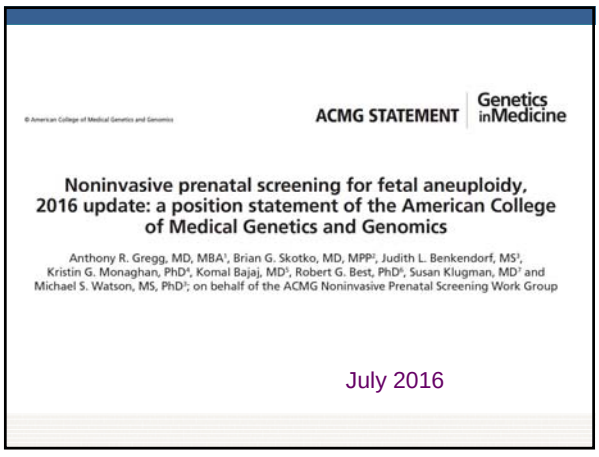
Non-invasive prenatal testing for aneuploidy and beyond: challenges of responsible innovation in prenatal screening

This paper has been amended since online publication and a corrigendum also appears in this issue

Wybo Dondorp[*,1], Guido de Wert[1], Yvonne Bombard[2], Diana W Bianchi[3], Carsten Bergmann[4,5], Pascal Borry[6], Lyn S Chitty[7], Florence Fellmann[8], Francesca Forzano[9], Alison Hall[10], Lidewij Henneman[11], Heidi C Howard[12], Anneke Lucassen[13], Kelly Ormond[14], Borut Peterlin[15], Dragica Radojkovic[16], Wolf Rogowski[17], Maria Soller[18], Aad Tibben[19], Lisbeth Tranebjærg[20,21,22], Carla G van El[11] and Martina C Cornel[11] on behalf of the European Society of Human Genetics (ESHG) and the American Society of Human Genetics (ASHG)

ESHG and ASHG Position Statement, 2015

- NIPT is more accurate than traditional screening, but is not diagnostic
- Should be a clear policy for dealing with secondary and incidental findings
- NIPT for sex chromosomal aneuploidy and microdeletions is not recommended
- Also consideration of pretest counseling, informed choice, and the goals and scope of prenatal screening



ACMG STATEMENT | Genetics inMedicine

Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics

Anthony R. Gregg, MD, MBA[1], Brian G. Skotko, MD, MPP[2], Judith L. Benkendorf, MS[3], Kristin G. Monaghan, PhD[4], Komal Bajaj, MD[5], Robert G. Best, PhD[6], Susan Klugman, MD[7] and Michael S. Watson, MS, PhD[5]; on behalf of the ACMG Noninvasive Prenatal Screening Work Group

July 2016

American College of Medical Genetics, 2016

Recommends that ALL pregnant women should be:

- Offered the option of either screening or diagnostic testing
- Offered NIPS for sex chromosomal aneuploidies
- Informed of availability of NIPS for microdeletions

American College of Medical Genetics, 2016

Laboratory Guidance

- Should work with public health officials, policy makers, and insurers to make NIPS available to all women
- Provide detection rate, FP rate, PPV and NPV on reports
- Report fetal fraction

American College of Medical Genetics, 2016

Recommends AGAINST:

- NIPS for aneuploidies other than T13, 18, 21 (eg trisomy 9, 16, 22)
- NIPS for genome wide CNVs

American College of Medical Genetics, 2016

Other issues addressed:

- Diagnostic testing should be offered for a "no call" result, rather than repeat testing
- Other options should be suggested for women with significant obesity
- Referral to genetics professional with positive NIPS, no call results other than FF

Summary

- cfDNA is a better test for Down syndrome than current screening – but it is screening!
- Patients need to be carefully counseled about the trade-offs
- Adding microdeletion screening has minimal impact on detection, but potential to significantly increase the false positive rate
- With expanding panels, incidental findings will increase


Thank You!