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Updates in Prenatal Screening and Testing

Myra Wick, MD, PhD

2017 Spring Refresher

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Disclosures

- **Nothing to disclose**

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Objectives

- **Recall relevant background information for prenatal screening and testing.**
- **Summarize current guidelines for cell free DNA (cfDNA, NIPS, NIPT) screening.**
- **Discuss updated recommendations for invasive testing.**

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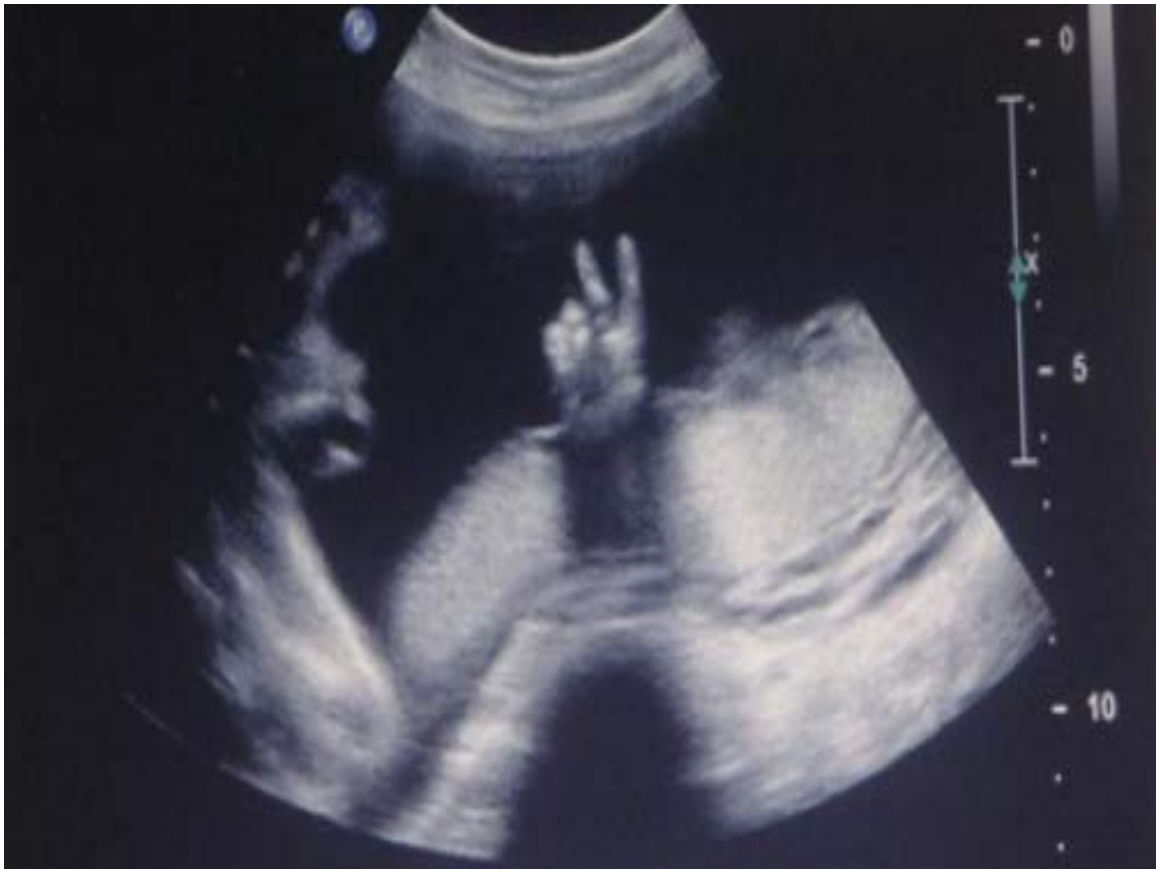
Background:
Prenatal Screening and Testing



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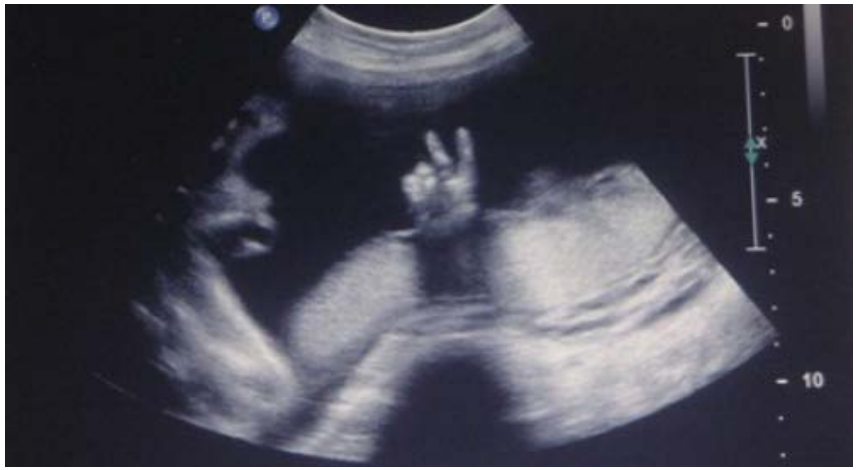
Background:
Prenatal Screening and Testing



What is the distinction between screening and testing?

- **Screening: Non-invasive, not definitive**
- **Testing: Invasive, definitive**

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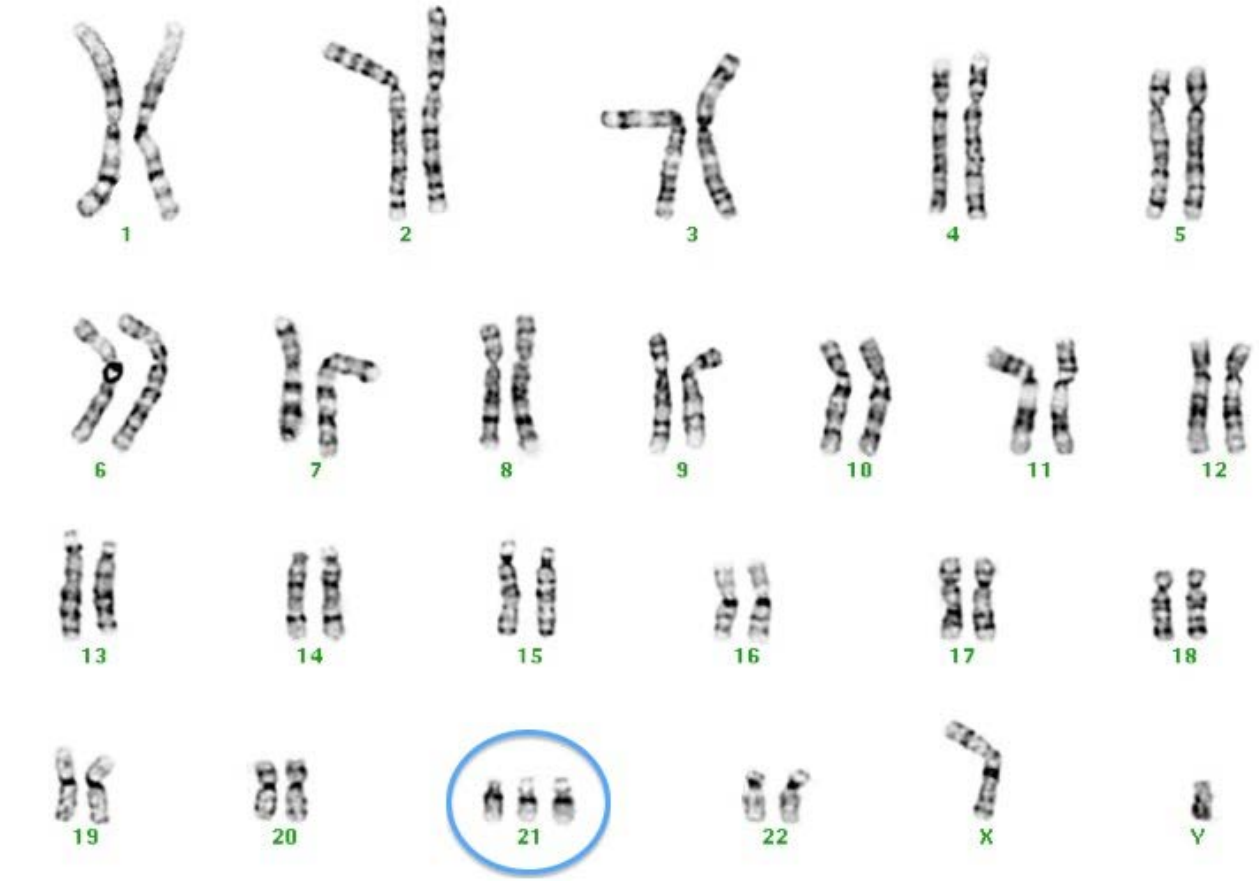
Why do patients request screening?



Down Syndrome

47,XY,+21

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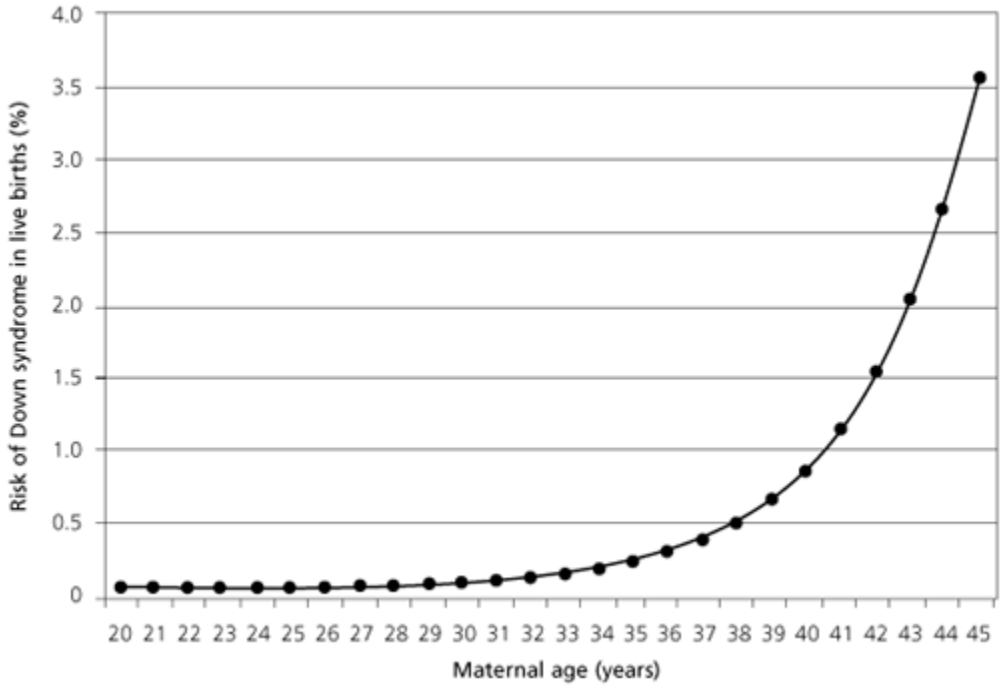
Why do patients request screening?



Maternal age and risk for Down Syndrome

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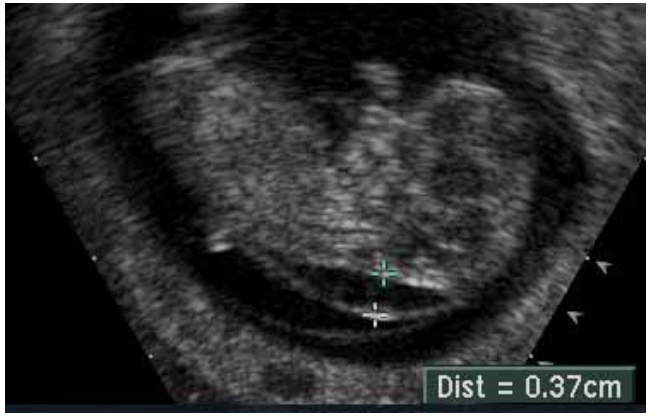
“Traditional” Prenatal Screening

- **Traditional testing**
  - **First trimester screening**
    - **NT + PAPP-A and HCG**
    - **85% detection rate, 5% false positive**
  - **Second Trimester screening (Quad screen)**
    - **HCG, PAPP-A, AFP, estriol**
    - **~81% detection rate, 5% false positive**
  - **Integrated/sequential/contingent screening**
    - **~88-95% detection rate, 5% false positive**
  - **Ultrasound (US) Evaluation at 18-20 weeks**
    - **~50% detection rate for Down Syndrome**



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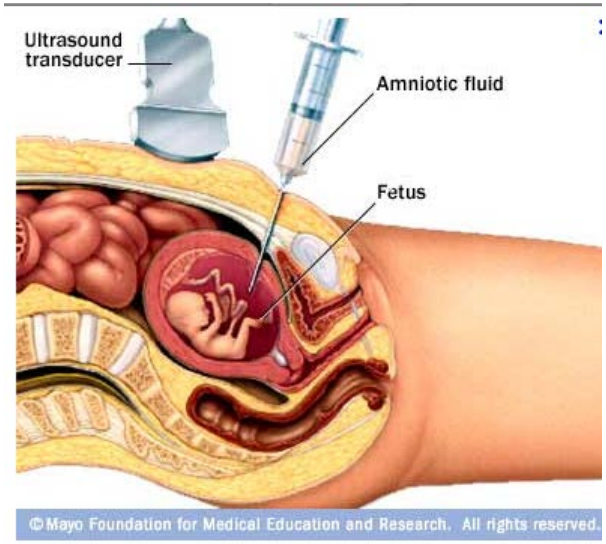
Gold Standard: Invasive Testing

- **Chorionic villus sampling (CVS)**
  - **Performed at 11-14 weeks**
  - **Risk of loss ~0.2-1.3%**
- **Amniocentesis**
  - **Performed >15 weeks**
  - **Risk of loss ~0.1 to 0.9%**



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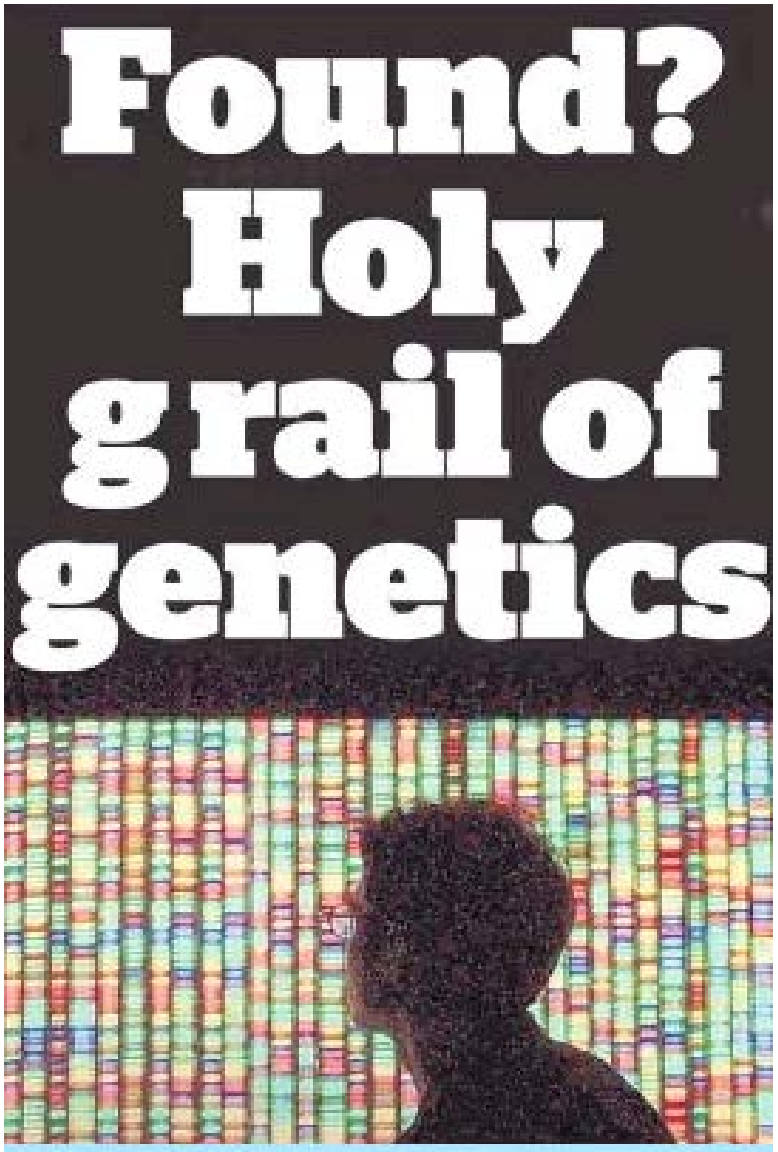
Cell free DNA (cfDNA)



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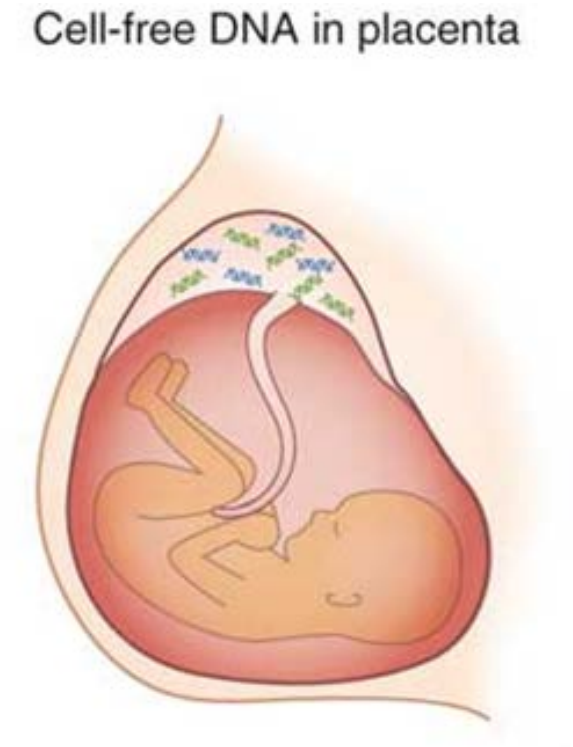
What is “Cell Free DNA”?

- Released through cellular death (apoptosis)
- DNA cleaved into small fragments (150-200bp)
- Released into blood stream as cfDNA
- During pregnancy maternal blood contains both fetal and maternal cfDNA
  - Fetal Fraction
- Most of cell free fetal DNA Is placental in origin.
- Rapidly cleared after delivery



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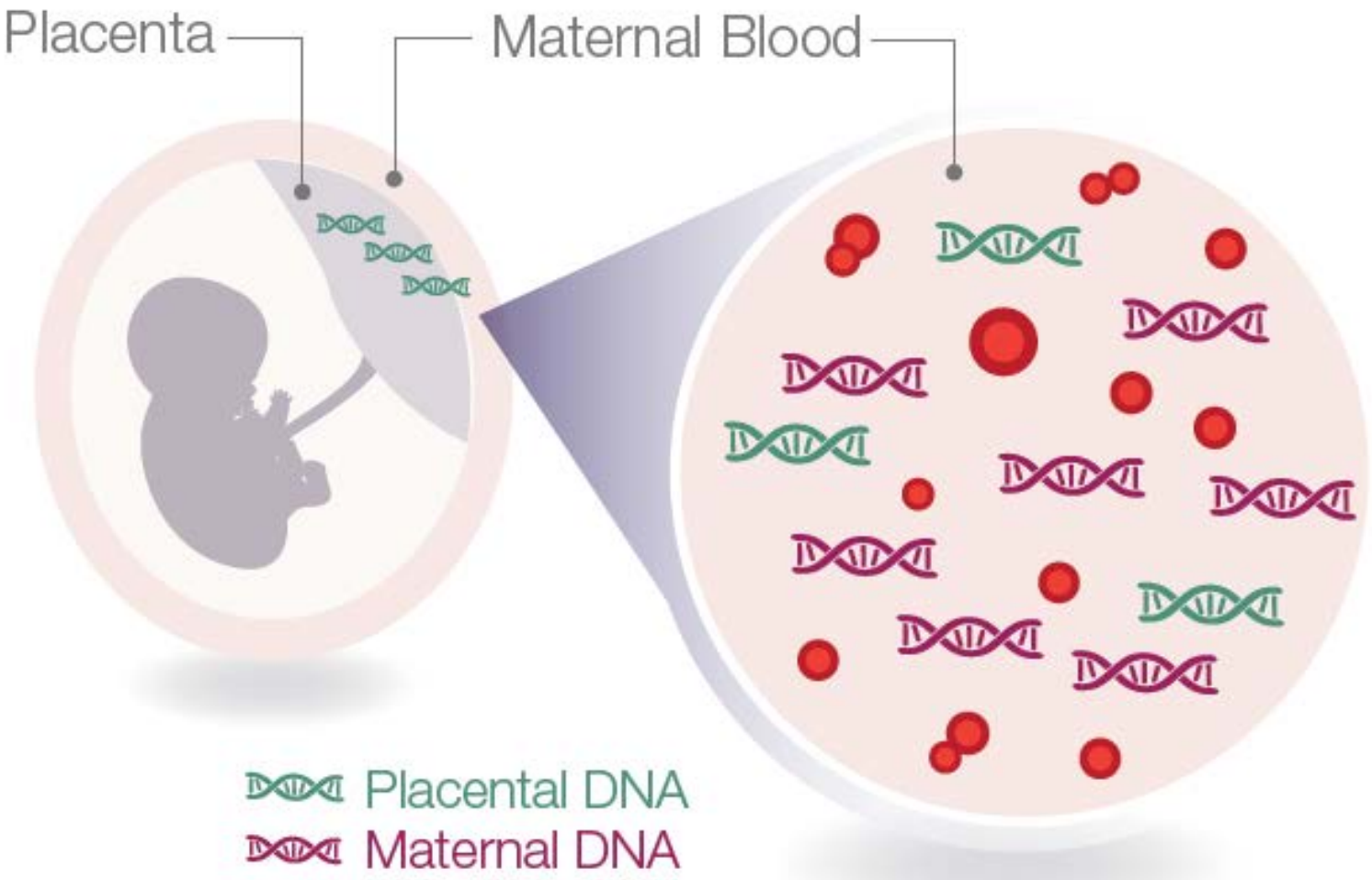
Cell-free DNA; cfDNA (NIPS, NIPT)



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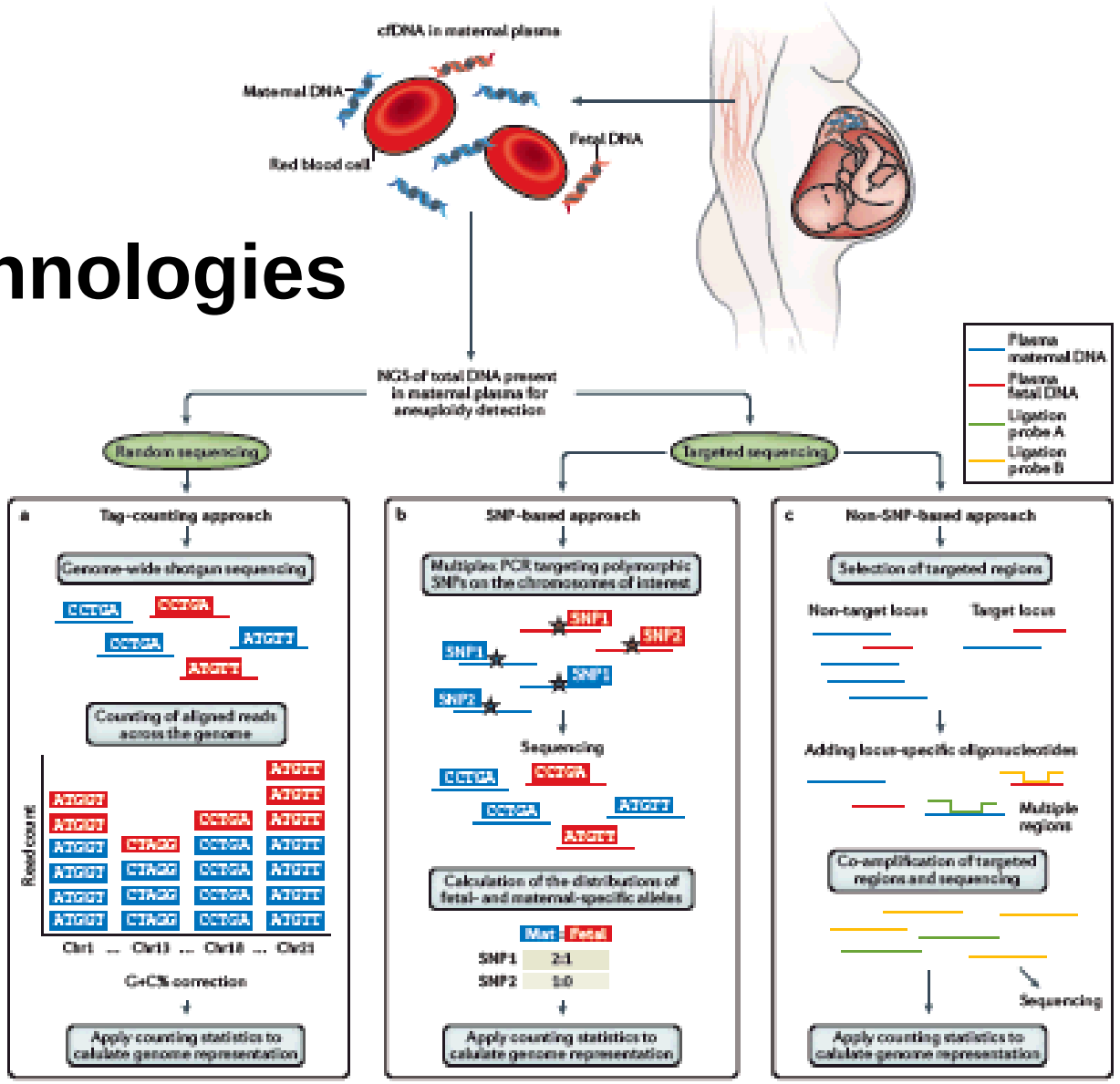
cfDNA Technologies



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Cell Free Fetal DNA and Non-Invasive Prenatal Screening

- 1997: Lo et al -fetal DNA in maternal serum
- 2011: Clinical testing for +21 (high risk pregnancies)
  - Competing commercial laboratories
    - Patents, IP and legal battles
- 2012: Clinical testing for +13, +18
    - ACOG and SMFM: High Risk Pregnancies; (ACMG, SOGC 2013)
- 2013-2014: Sex chromosome aneuploidy and microdeletions
- 2015: cfDNA genome
- 2017: Single gene disorders

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Current Clinical Test Offerings

- **Basic panel: 21, 18, 13 (common aneuploidies), fetal sex**
  - **Extras:**
    - **Trisomy 16 and 22**
    - **Microdeletions**
    - **Sex chromosome aneuploidy**
    - **Triploidy (using SNP technology)**
- **“Genome” (similar to microarray)**
- **Single gene disorders**
  - **Skeletal dysplasias**
  - **Noonan**

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Professional Society Statements on NIPT

★ ACMG	Recommends “informing all pregnant women that NIPS is the most sensitive screening option for traditionally screened aneuploidies”	2016
National Society of Genetic Counselors	“…supports prenatal cell-free DNA (cfDNA) screening, also known as NIPT or NIPS as an option for pregnant patients”	2016
★ The American College of Obstetricians and Gynecologists 1951 • Women's Health Care Physicians •	“any patient may choose cell-free DNA analysis as a screening strategy for common aneuploidies regardless of her risk status”	2015
ASHG	“Different scenarios are possible, including NIPT as an alternative first tier option”	2015
	“The following protocol options are currently considered appropriate: cfDNA screening as a primary test offered to all pregnant women.”	2015

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cfDNA:ACMG Guidelines

ACMG Statement July 2016 doi10.1038/gim.2016.97

- Patient education and pre-test counseling
- Common aneuploidy screening and follow up
- Fetal fraction and no call results
  - Maternal obesity, aneuploidy, gestational age, (Lupus, heparin therapy?)
  - No call due to stretches of homozygosity
- Sex chromosome aneuploidy (SCA)
- Copy number variants (microdeletions, genome wide CNV studies)
  - PPV and NPV have been modeled
- Special considerations
  - Twins/donor oocytes
  - Unanticipated findings
    - Maternal cancer, chromosomal imbalances

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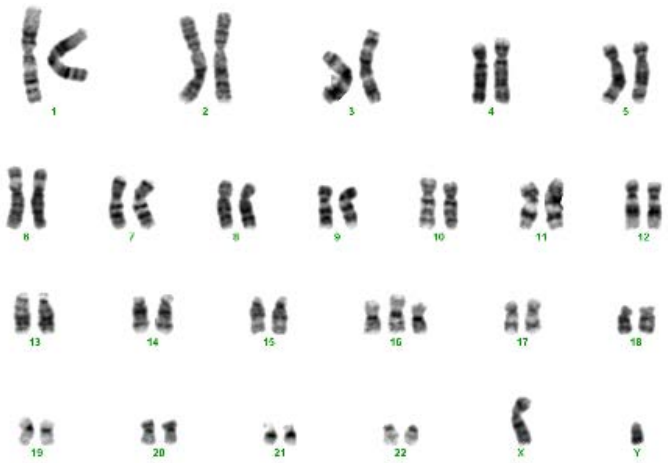
ACMG Statement

- **NIPS and aneuploidy screening**
  - **NIPS: most sensitive screening option for common aneuploidies**
  - **Genetics referral for increased risk of aneuploidy**
  - **Offer diagnostic testing after positive NIPS screen**
  - **Labs to provide DR, SPEC, PPV, NPV**
    - **Do not offer testing if information not available**
  - **Screening should not be offered if this data cannot be provided**
  - **Screen should not include autosomal aneuploidies other than 13, 18, 21**



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ACMG Statement

- **Fetal fraction and no-call results**
  - **5% of patients: non-reportable results**
  - **Factors:**
    - **Maternal weight (BMI >35)**
    - **Gestational age (<10 weeks)**
    - **Twins, triplets etc**
    - **Systemic Lupus Erythematosus**
    - **Aneuploid**
    - **Diagnostic testing after “no-call NIPS”**
      - **… “repeat blood draw NOT appropriate”**
    - **Labs should report fetal fraction**
    - **Offer screening other than NIPS for obese patients**
    - **Lab to report reason for no call**
      - **Genetics referral, offer diagnostic testing**

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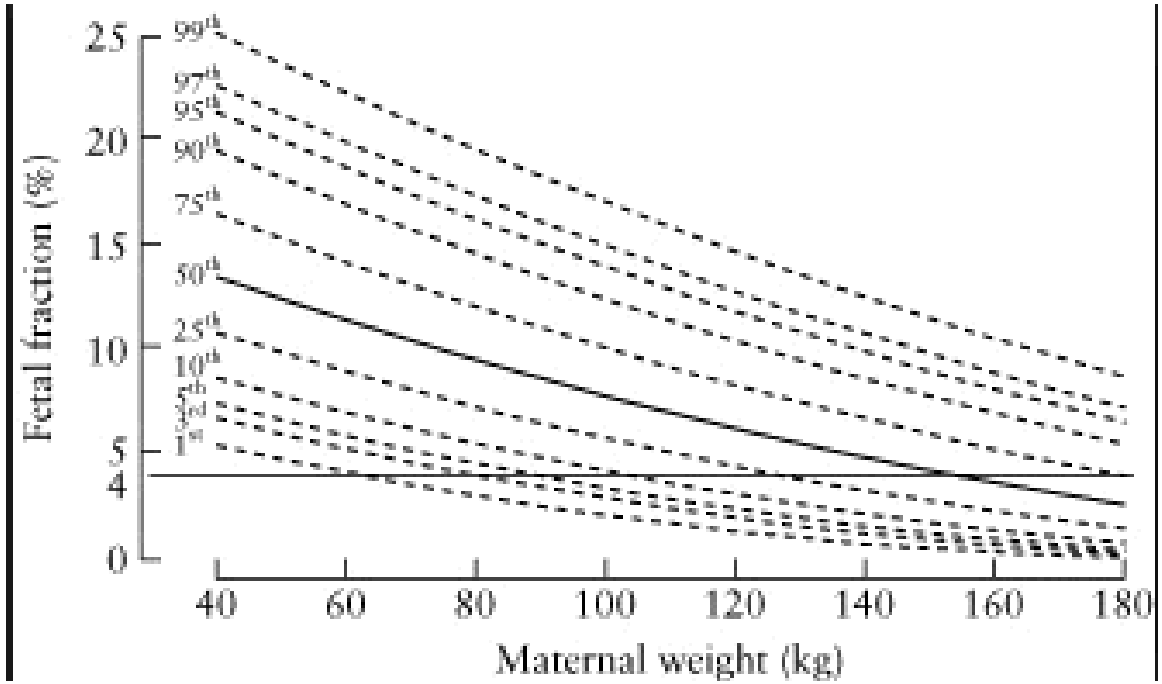
Maternal Weight and Fetal Fraction



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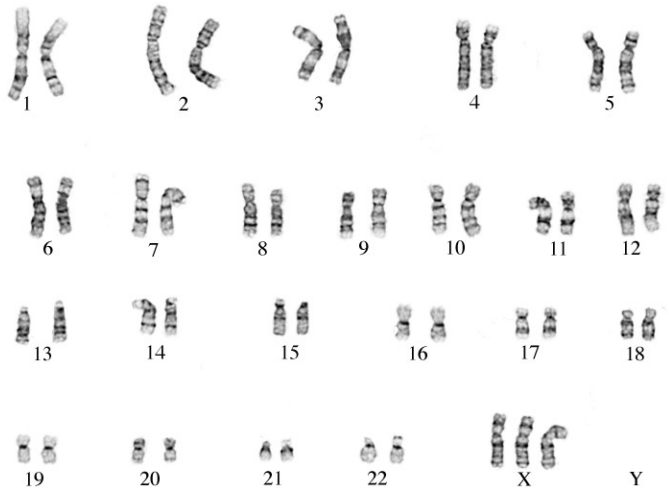
- **Sex chromosome aneuploidy (SCA)**
  - **Discuss availability with all patients**
  - **Discourage use of NIPS for purpose of sex determination**
    - **Pretest counseling (false positives, variable prognosis)**
    - **Genetics referral with high risk NIPS for SCA, offer diagnostic testing**
    - **Educational materials**
    - **Laboratory details regarding DR,SPEC, PPV (48%), NPV**



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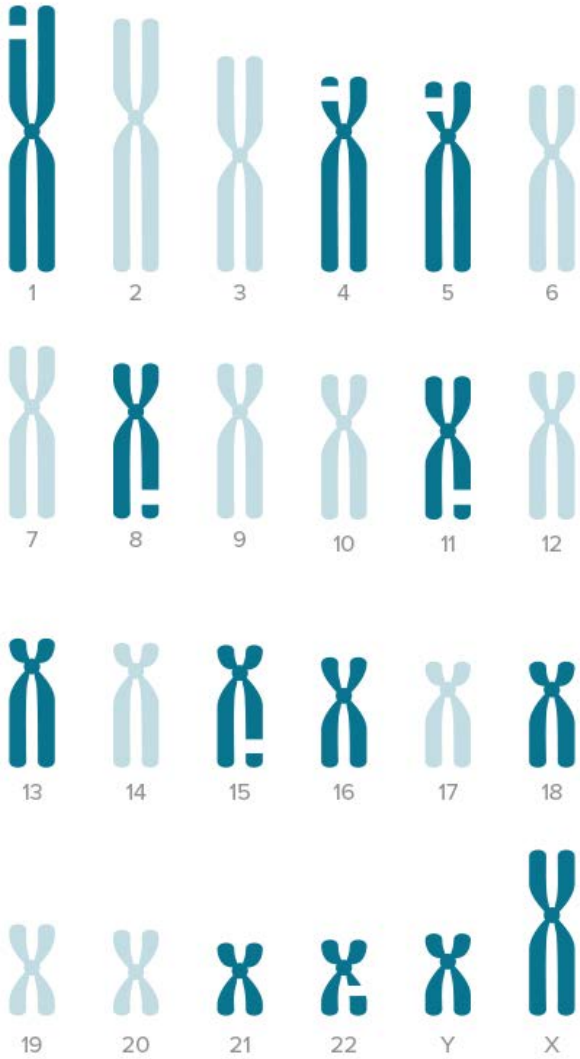
Copy Number Variants



GENERAL OVERVIEW OF SELECT MICRODELETIONS AND TRISOMIES

NAME	SITE OF ANOMALY	FREQUENCY OF LIVE BIRTHS	DESCRIPTION
DIGEORGE SYNDROME[9]	22q11	1 in 4,000	An autosomal dominant condition caused by a submicroscopic deletion of the long arm of chromosome 22. The disorder is characterized by cardiac abnormalities, abnormal facies, thymic aplasia, cleft palate, hypocalcemia (CATCH-22). Most cases are not inherited (*de novo*) but transmission from a parent carrying the 22q11 deletion is seen in ~7% of cases.
1P36 DELETION SYNDROME[10]	1p	1 in 10,000	1p36 deletion syndrome (monosomy 1p36 syndrome) is characterized by a deletion on the short arm of chromosome 1. The disorder is characterized by dysmorphic craniofacial features, developmental delay, brain abnormalities, short feet, congenital heart defects, hypotonia, and brachy/camptodactyly. Most cases are not inherited (*de novo*).
ANGELMAN SYNDROME[11] AND PRADER-WILLI SYNDROMES[12]	15q	1 in 20,000	Both Angelman (AS) and Prader-Willi (PWS) syndromes may be caused by deletions on the long arm of chromosome 15. Maternal deletions lead to AS while paternal deletions result in PWS. Seventy percent of both AS and PWS are caused by deletions on the long arm of chromosome 15. These disorders affect the nervous system and, while both result in developmental delay, each presents with its own unique clinical features.
CRI-DU-CHAT SYNDROME[13]	5p	1 in 50,000	Cri-du-chat (5p minus) is caused by a partial deletion of the short arm of chromosome 5. The disorder is characterized by intellectual disability, developmental delay, microcephaly, hypotonia, distinctive facies, heart defects, and a characteristic cat-like cry. Most cases are not inherited (*de novo*) but transmission from an unaffected parent carrying a balanced translocation is seen in ~10% of cases.
WOLF-HIRSCHHORN SYNDROME[4]	4p	1 in 50,000	Wolf-Hirschhorn is caused by a deletion on the short arm of chromosome 4. It is characterized by a distinctive facial appearance (Greek warrior helmet), developmental delay, intellectual disability, and seizures. Most cases are not inherited (*de novo*).
JACOBSEN SYNDROME[8]	11q	1 in 100,000	Jacobsen syndrome is caused by a deletion in the long arm of chromosome 11. It is characterized by developmental delay, distinctive facies, bleeding disorders and some behavior disorders. Most cases are not inherited (*de novo*).
LANGER-GIEDION SYNDROME[3]	8q	Rare	Langer-Giedion syndrome is caused by a deletion to the long arm of chromosome 8. It is characterized by benign bone tumors (exostoses), short stature, and distinctive facial features. Most cases are not inherited (*de novo*).
TRISOMY 16[14,15]	Chromosome 16	1 in 50,000	Full trisomy 16 is not compatible with life and is the most common cause of miscarriage. Mosaic trisomy 16 may present with intrauterine growth retardation, developmental delay, and congenital heart defects. Most frequent cause of spontaneous miscarriage and IUFD.
TRISOMY 22[16]	Chromosome 22	1 in 40,000	Full trisomy 22 is rarely compatible with life and most individuals die before birth or shortly after. Mosaic trisomy 22 may present with growth retardation, malformations of the head and face, cardiac abnormalities, and developmental delay.

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- **Copy number variants (specific microdeletions, "genome-wide CNVs")**
  - **Discuss availability**
    - **Discuss NIPS versus diagnostic testing**
      - **False positives, false negatives, prognosis**
      - **Referral to genetics for high risk results**
    - **Laboratory specifics (DR, SPEC, PPV, NPV)**
  - **ACMG does not recommend NIPS for genome wide CNVs**
    - **"If this level of information is desired, diagnostic testing…is recommended".**
    - ***Multiple US anomalies and patient declines diagnostic testing.***

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- **Special circumstances**
  - **Twins/donor oocytes**
    - **Contact laboratory regarding validation studies**
  - **Unanticipated findings**
    - **Maternal imbalances**
      - **Maternal cancer**
      - **Maternal chromosomal imbalance**
    - **Patients with history of bone marrow or organ donation from male or sex of donor not known**
    - **Genetics referral for unanticipated findings**

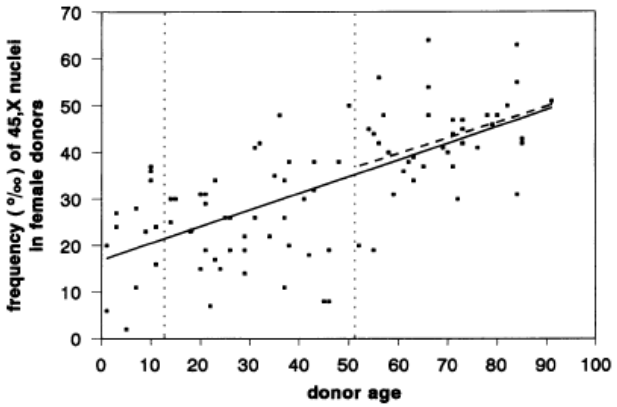

Figure 4 Frequency of X chromosome loss in lymphocyte nuclei of female probands, plotted against donor age. Regression lines for the total group of probands (*bold line*) and for females postmenopause (*broken line*) are shown.

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ACOG/SMFM Recommendations

Practice Bulletin 640, Sept 2015

- **Discuss all options with all patients.**
- **Conventional screening is most appropriate for most women, given current limitations of cfDNA.**
- **Patients choosing cfDNA should understand limitations and benefits.**
- **"The cell free DNA will screen only for the common trisomies, and, if requested, sex chromosome composition.**
- **Diagnostic testing should be recommended after positive cfDNA results.**
- **Multiple screening methods should not be offered.**
- **Management decisions should not be made based on cfCNA results**

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ACOG/SMFM Recommendations

Practice Bulletin 640, Sept 2015

- **Patients with “no call” results should be counseled, and offered comprehensive US and diagnostic testing.**
- **cfDNA should not be used to screen for microdeletions.**
- **cfDNA not recommended for multiple gestations.**
- **Diagnostic testing should be offered after detection of US abnormalities.**
- **Normal cfDNA result does not ensure unaffected pregnancy.**
- **cfDNA does not assess for NTDs or ventral wall defects.**
  - **Offer US or AFP**
- **Patients may decline all screening and testing.**

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cfDNA: What Does it Cost?

Mayo Medical Labs (NIPS)

- **$0 out of pocket for MMSI SELECT/PREMIER**
- **Other insurances - ?**
- **Down syndrome, T18, T13, fetal sex,**
- **sex chromosome aneuploidy**

- **MaterniT21 PLUS**
- **Out of pocket cost is ~$200 *if denied***
- **Down syndrome, T18, T13, fetal sex**
- **SCA is OPTIONAL**

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Table 1. Tests Available for Prenatal Genetic Diagnosis ⇦

Test	Turnaround Time	Conditions Detected	Comments
Conventional karyotype	7–14 days	Chromosomal abnormalities >5–10 Mb	Traditional method for diagnosis of chromosomal abnormalities
FISH—Direct preparation (interphase)	24–48 hours	Rapid assessment of major aneuploidies (chromosomes 13, 18, 21, X, and Y)	FISH with direct testing of cells from CVS is less accurate than with cultured cells from CVS or amniocentesis. Results should be confirmed on cultured cells or have additional clinical features before acting on results.
FISH—Cultured cells (metaphase)	7–14 days	Microdeletions and duplications	Can be used to test for specific abnormalities when clinically suspected
Chromosomal microarray	3–5 days (direct testing); 10–14 days (cultured cells)	Copy number variants >50–200 kb	Whole genome screen for copy number variants. Detects major chromosomal abnormalities except balanced rearrangements and some triploidies. Detection varies with different microarray platforms.
Preimplantation genetic diagnosis	1–2 days	Genetic disorder in which familial mutation has been identified	Because of possibility of error, confirmation with CVS or amniocentesis is recommended
Molecular DNA testing	3–14 days (faster with direct testing than when cultured cells are required)	Genetic mutations previously demonstrated to be present in a family or suspected based on ultrasound or other findings in a fetus	Usually a targeted test focusing on a specific disorder (or category of disorders) suspected to be present in a fetus based on ultrasound findings or family history

Abbreviations: CVS, chorionic villus sampling; FISH, fluorescence in situ hybridization.

ACOG Practice Bulletin 162, May 2016

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Table 2. Characteristics, Advantages, and Disadvantages of Common Screening Tests for Aneuploidy

Screening Test	Approximate Gestational Age Range for Screening (Weeks)	Detection Rate for Down Syndrome (%)	Screen Positive Rate* (%)	Advantages	Disadvantages	Method
First trimester†	10–13 6/7‡	82–87	5	1. Early screening 2. Single test 3. Analyte assessment of other adverse outcome	Lower DR than combined tests NT required	NT+PAPP-A and hCG
Triple screen†	15–22	69	5	1. Single test 2. No specialized US required 3. Also screens for open fetal defects 4. Analyte assessment for other adverse outcomes	Lower DR than with first-trimester or quad screening Lowest accuracy of the single lab tests	hCG, AFP, uE3
Quad screen†	15–22	81	5	1. Single test 2. No specialized US required 3. Also screens for open fetal defects 4. Analyte assessment for other adverse outcomes	Lower DR than combined tests	hCG, AFP, uE3, DIA
Integrated†	10–13 6/7‡, then 15–22	96	5	Highest DR of combined tests Also screens for open fetal defects	Two samples needed before results are known	NT+PAPP-A, then quad screen
Sequential§: Stepwise	10–13 6/7‡, then 15–22	95	5	First-trimester results provided; Comparable performance to integrated, but FTS results provided; also screens for open fetal defects; analyte assessment for other adverse outcomes.	Two samples needed	NT+hCG+ PAPP-A, then quad screen
Contingent screening‖		88–94	5	First-trimester test result: Positive: diagnostic test offered Negative: no further testing Intermediate: second-trimester test offered Final: risk assessment incorporates first- and second-trimester results	Possibly two samples needed	NT+hCG+ PAPP-A, then quad screen
Serum Integrated†	10–13 6/7‡, then 15–22	88	5	1. DR compares favorably with other tests. 2. No need for NT	Two samples needed; no first-trimester results	PAPP-A+quad screen
Cell-free DNA¶	10 to term	99 (In patients who receive a result)	0.5	1. Highest DR for Down syndrome 2. Can be performed at any gestational age after 10 weeks 3. Low false-positive rate in high-risk women (or women at high risk of Down syndrome)	1. NPV and PPV not clearly reported 2. Higher false-positive rate in women at low risk of Down syndrome 3. Limited information about three trisomies and fetal sex 4. Results do not always represent a fetal DNA result	Three roughly equivalent molecular methods
Nuchal translucency†	10–13 6/7‡	64–70	5	Allows individual fetus assessment in multifetal gestations Provides additional screening for fetal anomalies and possibly for twin–twin transfusion syndrome	1. Poor screen in isolation 2. Ultrasound certification necessary	US only

ACOG Bulletin 163, May 2016

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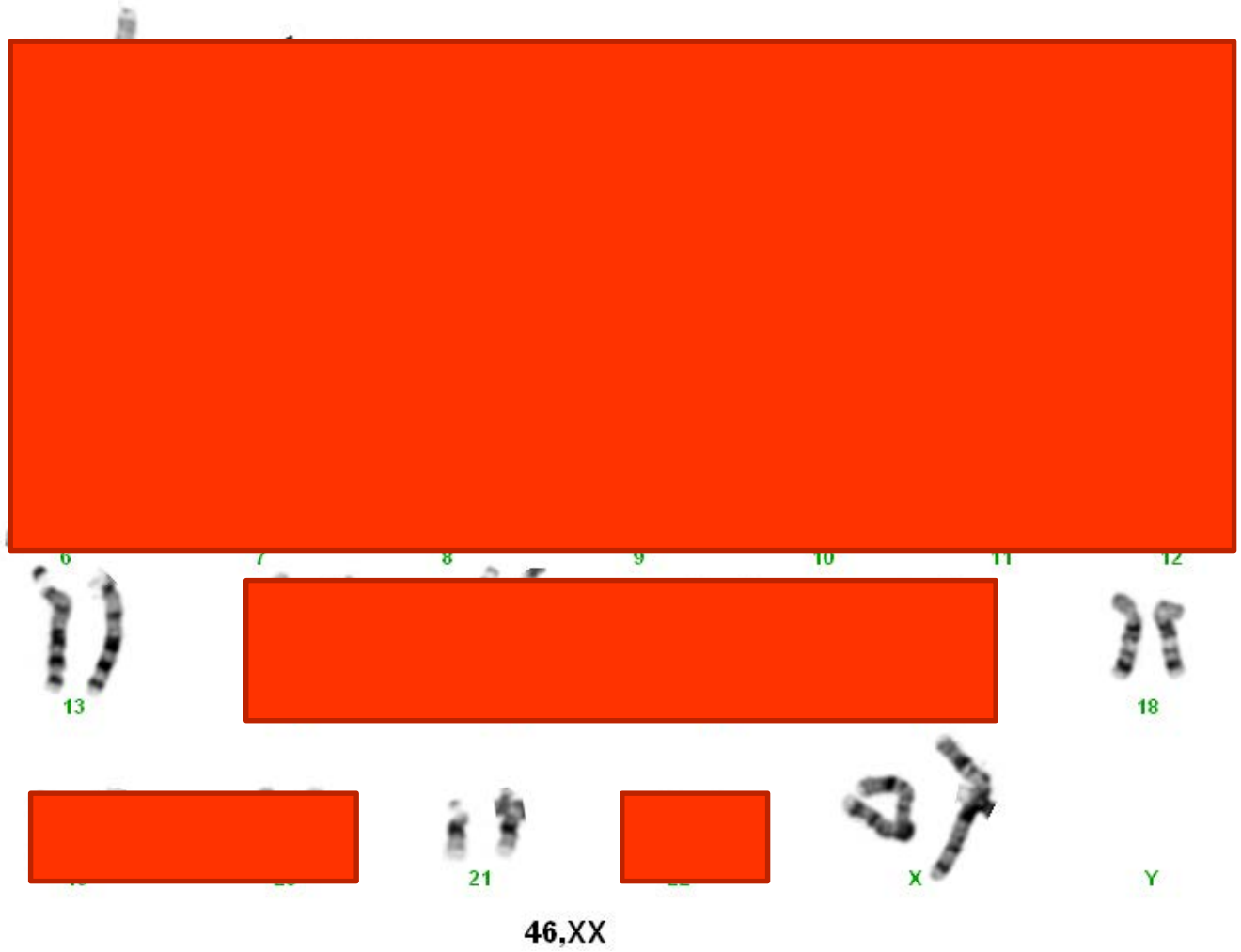
cfDNA and Partial Karyotype…



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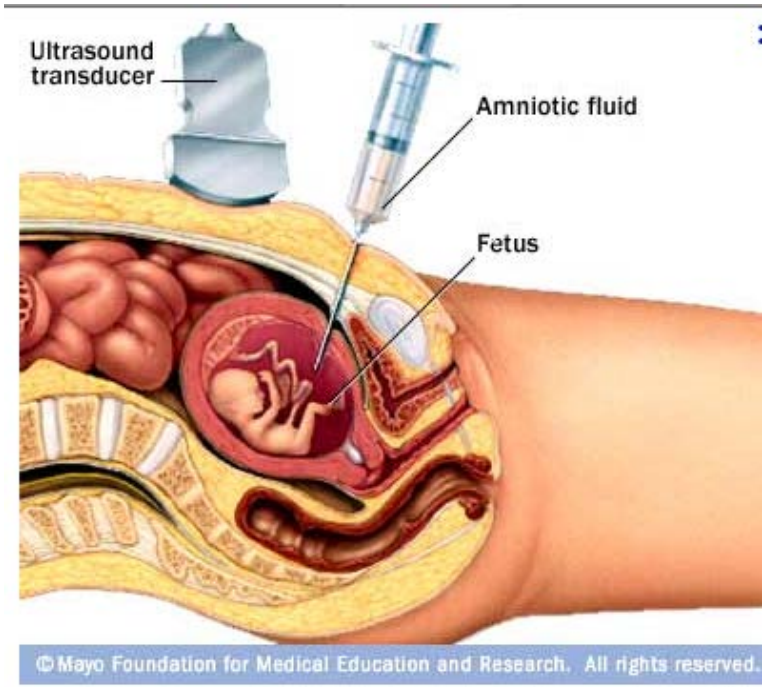
Invasive Testing

- **“When did amnio become so dangerous?”…Dr. David Ledbetter**
- **Testing after invasive procedure?**
- **Chorionic villus sampling (CVS)**
  - **Performed at 11-14 week**
  - **Risk of loss ~0.2-1.3%**
- **Amniocentesis**
  - **Performed >15 weeks**
  - **Risk of loss ~0.1 to 0.9%**



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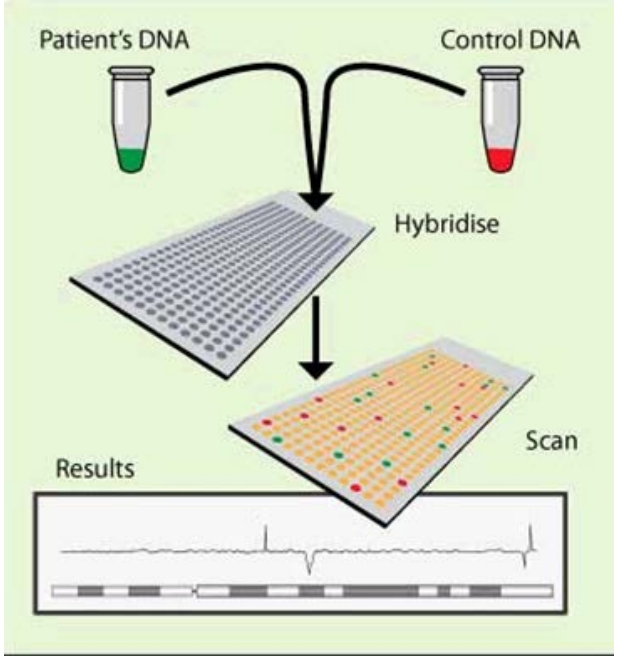
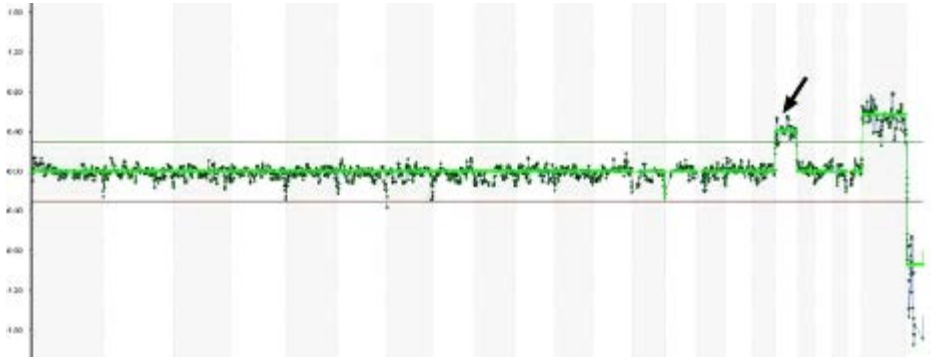
Chromosome Microarray



Trisomy 21



Syndrome	Micro deletion
DiGeorge/Velocardiofacial syndrome	22q11.2
Prader-Willi Syndrome and Angelman syndrome	15q11-ql3
William Syndrome	7q11.23
Miller-Dieker/Lissencephaly syndrome	17p13.3
Tricho-Rhino-Phalangeal (TRP) syndrome	8q24.11-q13
Wolff-Hirschhorn syndrome	4p
Cri-du-chat syndrome	5p15.2
ATR-16 syndrome	16p13.3
1p36 Deletion syndrome	1p36

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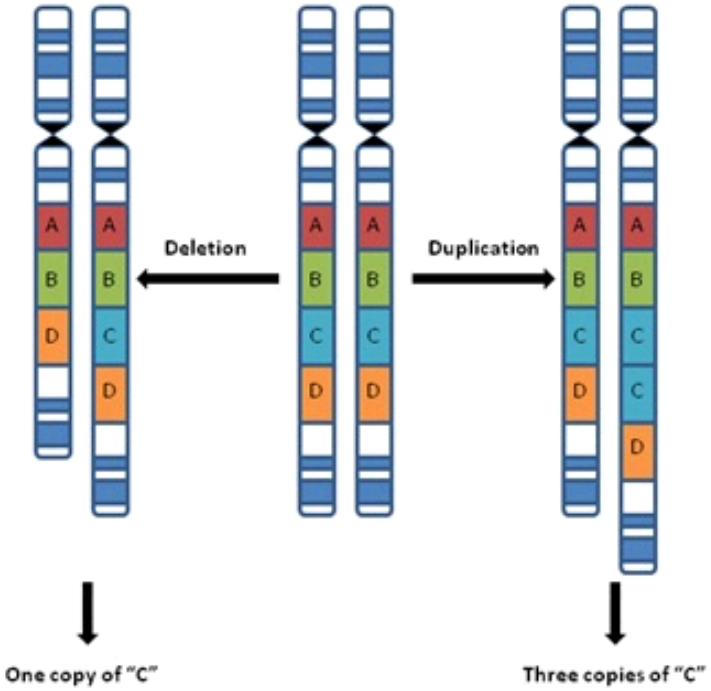
Microarray and Copy Number Variants (CNV)



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Microarray Versus Karyotype
(ACOG Committee Opinion 682, Dec 2016)

- CMA identifies most abnormalities identified by karyotype
  - May identify disorders not identified by karyotype
  - May identify adult onset disorders
  - Doesn't identify
    - Single gene disorders
    - Balanced translocations
- May identify disorders with variable presentation
- May identify consanguinity
- May identify changes (CNV) with unknown disease association
  - Parental studies may help clarify
- Does not require cultures cells

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Prenatal Microarray Versus Karyotype

(ACOG Committee Opinion 682, Dec 2016)

- **After CVS/amnio**
  - **Recommended for fetuses with ≥1 structural abnormality**
    - **Significant finding in 6% of fetuses with normal karyotype**
  - **Consider for structurally normal fetuses**
    - **Significant finding in 1.7% of fetuses with normal karyotype**
  - **CNV in 1.6-3.4% of cases**

	First Trimester Screen	Anatomy Ultrasound	MaterniT21/NIPS cfDNA	CVS	Amnio
Detects	Down syndrome Trisomy 18 NO GENDER	Some chromosome conditions Some birth defects Can sometimes detect gender	Down syndrome Trisomies 13 and 18, Sex chromosome aneuploidy (optional) Microdeletions (optional) Detects gender	All Chromosomes Other genetic testing Detects gender	All chromosomes Other genetic testing Other testing (AFP, antibodies, infections etc) Detects gender
Timing	11-14 weeks	~18-20 weeks	After 10 weeks	11-14 weeks	15-22 weeks
Detection Rate	85% for Down syndrome 80% for Trisomy 18	>95% for neural tube defects, Trisomy 13 & 18 50% for Down syndrome	99% for most 92% for Trisomy 13 60-90% for microdeletions	>99.9%	>99.9%
False Positive Rate	~5% (especially women over 35)	Low	<1%	<1% (mosaicism)	<1%
Procedure	Blood draw + nuchal translucency ultrasound	Ultrasound	Blood draw	Chorionic Villi Sample (placental biopsy)	Amniocentesis
Cost	Usually covered by insurance*	Usually covered by insurance*	$0-200 out of pocket* NIPS covered for MMSI Select and Premier*	Usually covered by insurance*	Usually covered by insurance*

*All insurance plans are different. Each test may be subject to copays and deductibles. Please contact your insurance for any questions about personal coverage

MAYO CLINIC

The Perinatal Exome/Genome

- **Whole Exome/Genome Sequencing in the perinatal setting**
  - **WES/WGS**
  - **Emergency medical genomes**
    - **NICU**
    - **Faster and more cost effective than "genetic odyssey"**
    - **Diagnosis in 20-30% of cases**
- **Prenatal WES/WGS not endorsed by ACOG**

MAYO CLINIC

Thanks for your undivided attention!

Fillmore County History Center