Prenatal Genetic Testing

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Prenatal Genetic Screening

- Primary purpose is screening for Down syndrome and neural tube defects
- Can also detect other birth defects and abnormalities

Overview of Prenatal Aneuploidy Testing

Screening

- Current status, including serum and ultrasound screening

Diagnostic Testing

- Amniocentesis and CVS

Non-invasive Prenatal Testing (NIPT)

- Cell free fetal DNA
- Review of the technique(s)

MIT Technology Review

Prenatal DNA Sequencing

Reading the DNA of fetuses is the next frontier of the genome revolution. Do you really want to know the genetic destiny of your unborn child?

By Antonio Regalado on April 23, 2013

Prenatal Testing

Screening tests

- Maternal age
- Biochemical screening (maternal blood or serum)
- Cell free DNA testing
- Ultrasound (e.g. nuchal translucency)

Diagnostic tests

- Amniocentesis
- Chorionic villus sampling (CVS)
- Preimplantation genetic diagnosis

Both/either

- Ultrasound

Integrated Screening

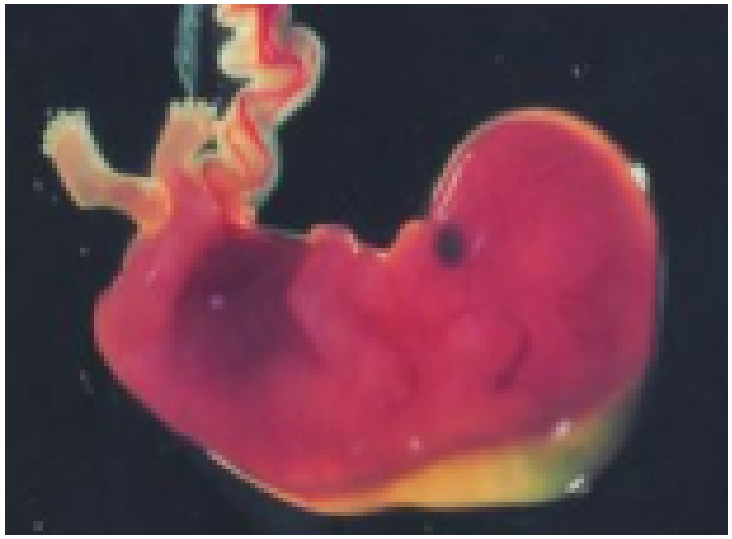
First Trimester (First trimester combined screening)

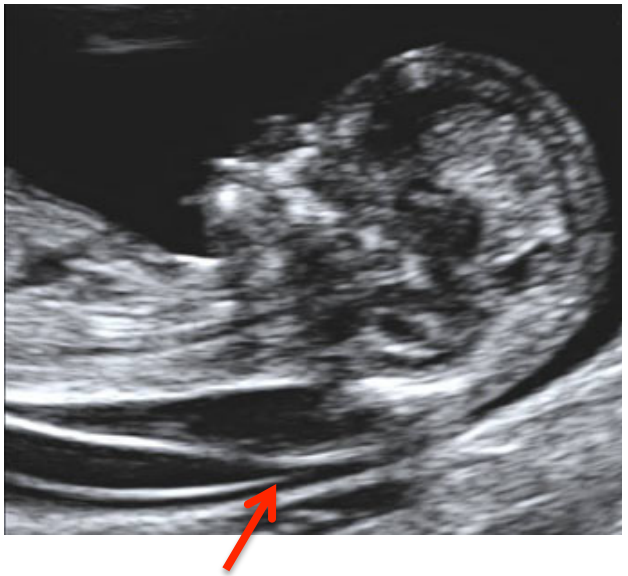
- Pregnancy-Associated Plasma Protein-A (PAPP-A)
- Human chorionic gonadotropin (hCG)
- Nuchal translucency ultrasound

Second trimester (Quad screen)

- Alpha-fetoprotein (AFP)
- Human chorionic gonadotropin (hCG)
- Unconjugated estriol (uE3)
- Inhibin A

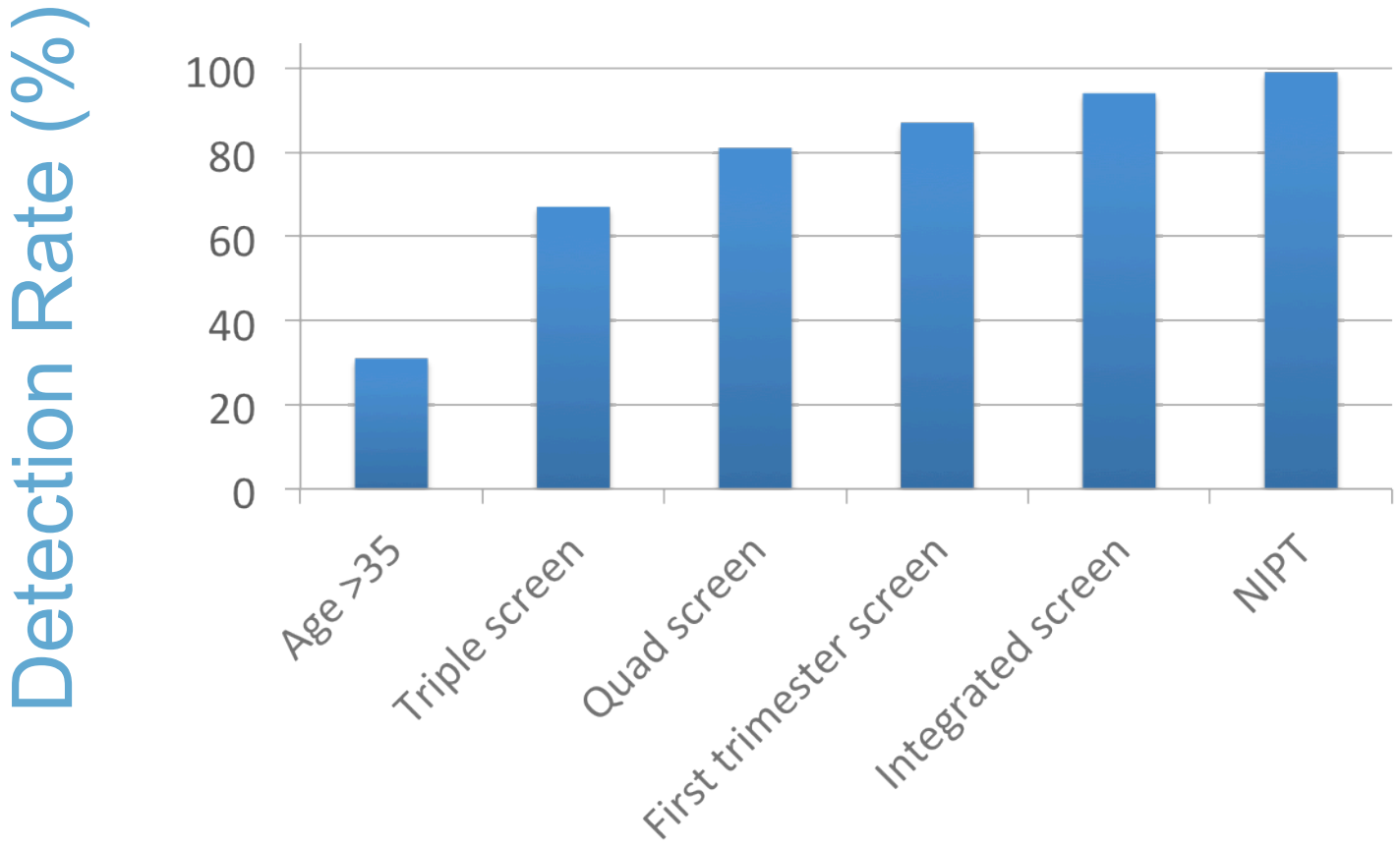
Nuchal translucency ultrasound





"Nuchal translucency" refers to fluid behind the fetal neck in the first trimester of pregnancy (11-14 wks)

Detection Rate of Prenatal Screening for Down syndrome has improved over time



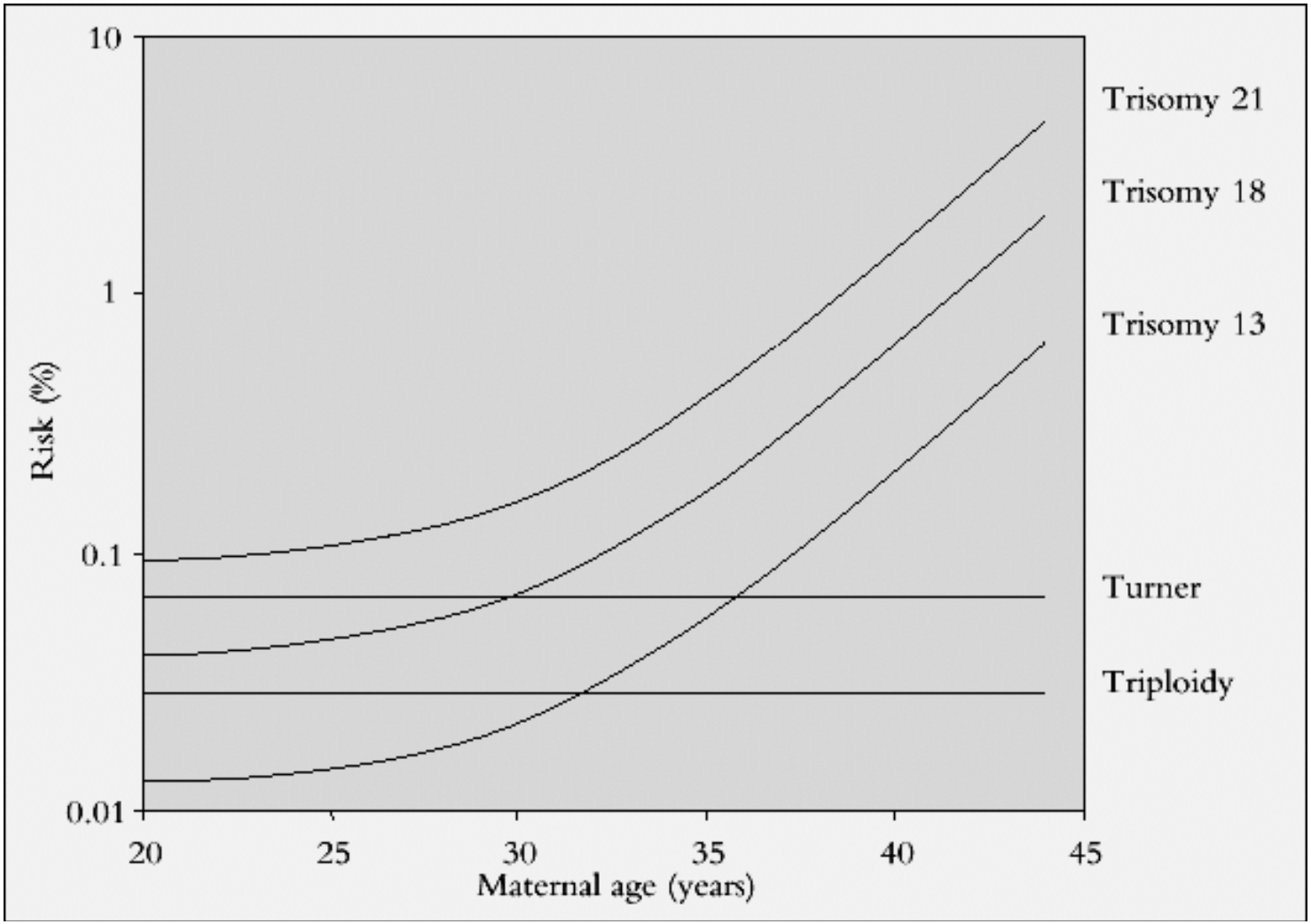
Prenatal Diagnostic Testing

- Amniocentesis
- Chorionic villus sampling (CVS)

Indications for Prenatal Diagnosis

- Increased risk of aneuploidy
  - Maternal age
  - Abnormal serum screening
  - Abnormal cell free DNA results
  - Ultrasound abnormalities
  - Parent carries chromosomal translocation
- Elevated MSAFP
  - Increased risk for open fetal defects (ventral wall and neural tube)
- Increased risk of genetic disease
  - Family history
  - Carrier of genetic disorder by screening
- Maternal request

Maternal age and chromosomal abnormalities



ACOG
PRACTICE
BULLETIN

CLINICAL MANAGEMENT GUIDELINES FOR
OBSTETRICIAN–GYNECOLOGISTS

NUMBER 77, JANUARY 2007

Replaces Practice Bulletin Number 27, May 2001, and Committee Opinion Number 296, July 2004

Screening for Fetal Chromosomal Abnormalities

This Practice Bulletin was developed by the ACOG Committee on Practice Bulletins—Obstetrics, the ACOG Committee on Genetics, and the Society for Maternal–Fetal Medicine Publications Committee with the assistance of Ray Bahado-Singh, MD, and Deborah Driscoll, MD. The information is designed to aid practitioners in making decisions

In the past decade, numerous markers and strategies for Down syndrome screening have been developed. Algorithms that combine ultrasound and serum markers in the first and second trimesters have been evaluated. Furthermore, the practice of using age cutoffs to determine whether women should be offered screening or invasive diagnostic testing has been challenged. The purpose of this document is to 1) present and evaluate the best available evidence for the use of ultrasonographic and serum markers for selected aneuploidy screening

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CLINICAL MANAGEMENT GUIDELINES FOR
OBSTETRICIAN–GYNECOLOGISTS

NUMBER 88, DECEMBER 2007

Invasive Prenatal Testing for Aneuploidy

This Practice Bulletin was developed by the ACOG Committee on Practice Bulletins—Obstetrics and the Committee on Genetics with the assistance of James Goldberg, MD. The information is designed to aid practitioners in making decisions about appropriate obstetric and gynecologic care. These

Prenatal diagnosis of fetal chromosomal abnormalities is the most common indication for invasive prenatal testing. The prevalence of chromosomal abnormalities in clinically recognized early pregnancy loss is greater than 50% (1). Fetuses with aneuploidy account for 6–11% of all stillbirths and neonatal deaths (2). Chromosomal abnormalities that are compatible with life but cause considerable morbidity occur in 0.65% of newborns, and structural chromosomal rearrangements that will eventually affect reproduction occur in 0.2% of newborns (3). Consequently, screening and diagnostic programs to detect the

ACOG Recommendations, January and December 2007

- "Invasive diagnostic testing for aneuploidy should be available to all women, regardless of maternal age."
- "Pretest counseling should include…risks and benefits…screening tests…screen positive rate…detection rate…aneuploidies other than DS…"
- "Maternal age 35 alone should no longer be used to determine who is offered…invasive testing."

Maternal age screening

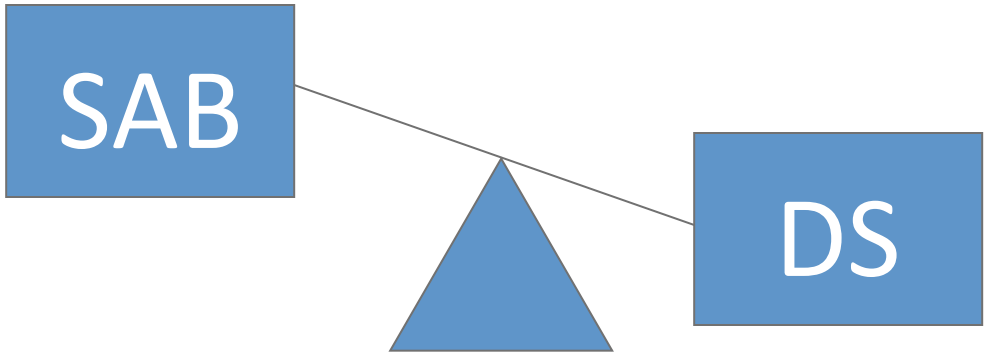
- 35 y.o. “standard” age to offer invasive prenatal diagnosis (1979)

- Risk of miscarriage from procedure = risk of Down syndrome

Clin Pediatr 1979 Aug;18(8):454-62.

The 35 year old cut-off

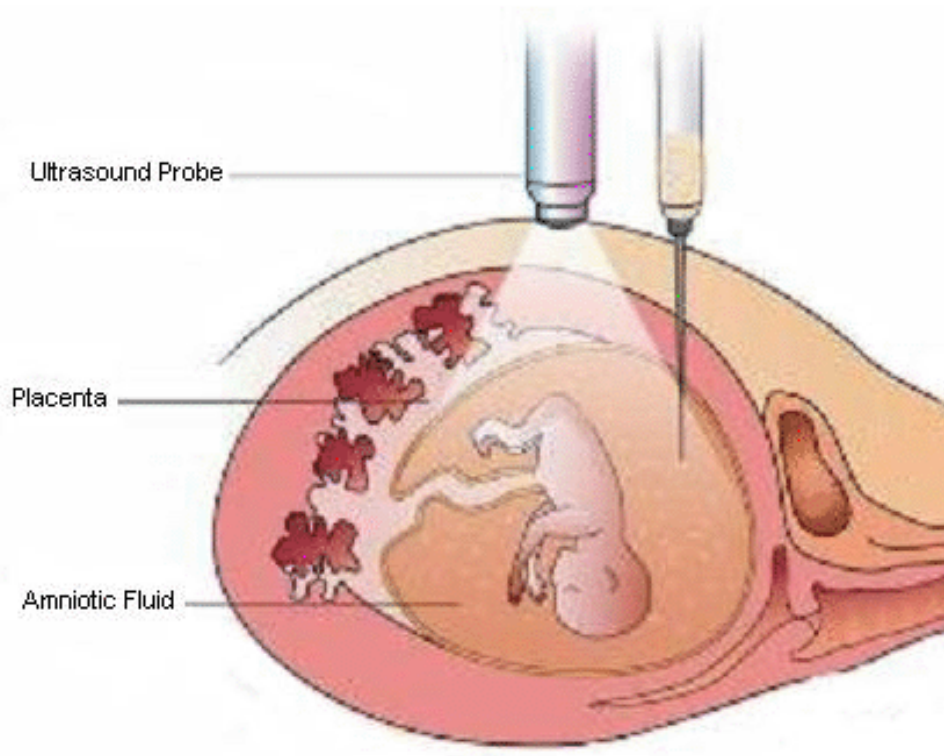
Setting cutoff in this way assumes that women weigh risk of SAB vs possibility of Down syndrome birth equally



But recent studies indicate this is NOT the case, and that women vary widely in this!

*Kuppermann et al, Prenat Diagn. **1999** Aug;19(8):711-6.*

Amniocentesis



- Sample of amniotic fluid (20cc) removed by insertion of needle through maternal abdomen into uterus
- At 15-20 wks, volume of AF is ~150-250 cc
- Fluid for AFP determination as well as culturing for karyotype and/or DNA

Amniocentesis: Risks

- Miscarriage risk somewhat difficult to measure (background miscarriages, esp. in high risk patients)

- 1/200 standard quoted loss rate historically, but not based on reliable data, origins obscure

*Tabor et al, Lancet. **1986** Jun 7;1(8493):1287-93*

Amniocentesis: Risks

Caughey et al, 2006

- Compared loss rates of CVS (n=9900) and amnio (n=31,000) at UCSF over 20 years
- Loss rate for both decreased, now both ~1/350

➢ *Loss rate generally agreed to be lower than previously quoted, ~1/300-400*

*Caughey et al, Obstet Gynecol. **2006** Sep;108:612-6.*

Amniocentesis Loss Rate

51,557 singletons at 15-22 wks, 1990-2006

Group	Amnio	No amnio	
Total #	11,746	39,811	
Fetal loss<24 w	0.97%	0.84%	p=0.33

Attributable fetal loss: 0.13% = 1/769

Odibo et al, Obstet Gynecol. 2008 Mar;111(3):589-95

Chorionic villus sampling

- Performed between 10-13 weeks
- Villi removed from developing placenta
  - Transcervical or transabdominal
- Indications same as for amniocentesis, except that AFP testing of amniotic fluid cannot be performed
- Primary advantage is timing

Chorionic Villus Sampling: Risks

- Miscarriage risk from studies in 1980's was 0.6-0.8% higher than with amnio (NS)
- Risk often quoted as 0.5-1%
- Risks decrease with provider experience
- Recent data indicates risk similar to amniocentesis in experienced hands
- 1/300-400

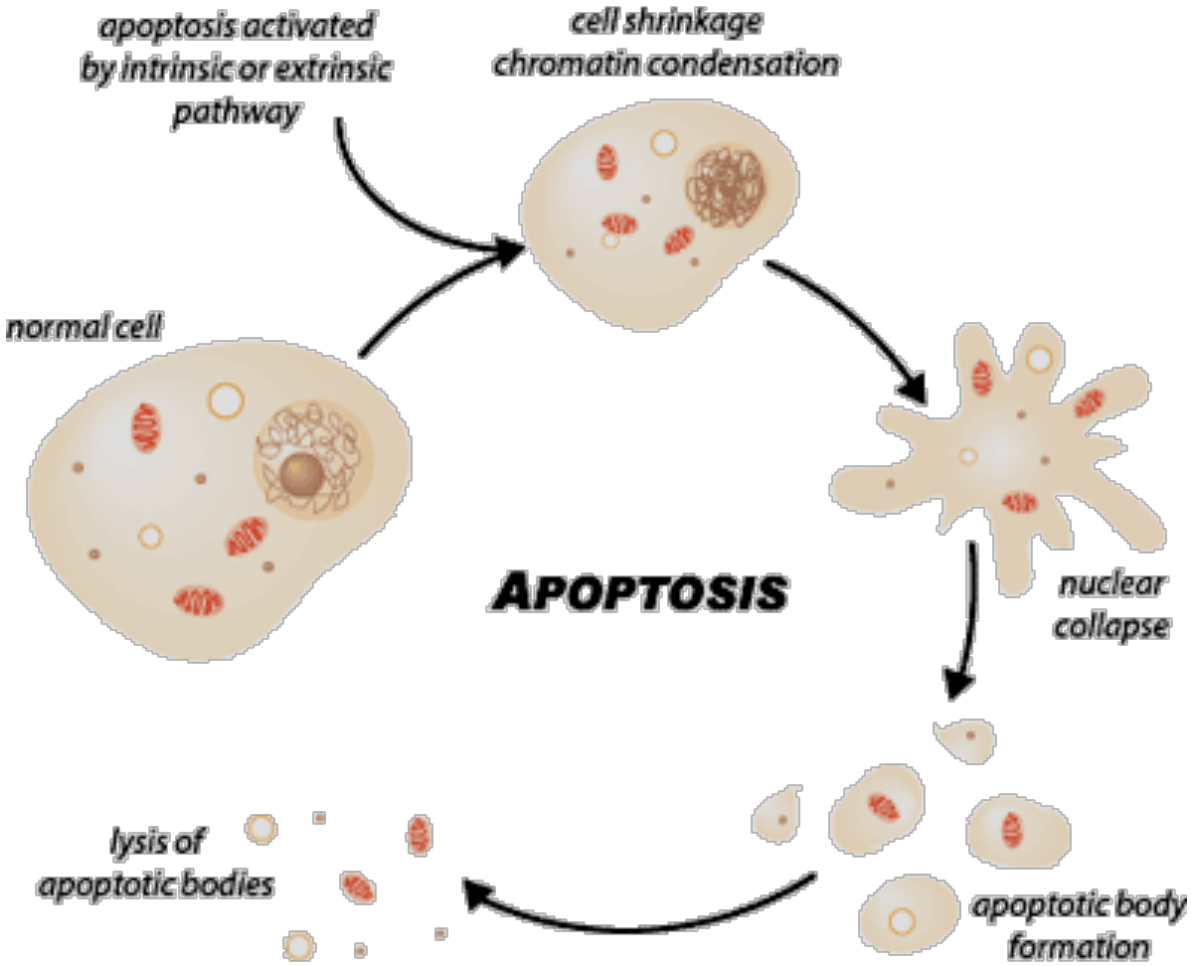
*Caughey et al, Obstet Gynecol. **2006** Sep;108:612-6; Canadian Collaborative CVS-Amniocentesis Clinical Trial Group.Lancet 1989;1:1–6; Lancet 1991;337:1491–9; Prenat Diagn 1991;11:529–37; N Engl J Med 1989;320:610–617*

Cell free fetal DNA

- In 1997, Lo et al reported on presence of fetal DNA in serum of pregnant women
- Subsequently confirmed by other investigators
- Big advance allowing cfDNA testing has been development of next generation sequencing

Lancet. 1997 Aug 16;350(9076):485-7

Cell free DNA results from apoptosis



Fetal DNA in Maternal Plasma: Characteristics

- cffDNA represents ~10% of total DNA in maternal plasma (Lo 1998, Chiu 2011)
- Much higher percentage than intact fetal cells
- cffDNA made up of short (<200 bp) DNA fragments (*Chan 2004*)
- Reliably detected after 7 wks gestation (*Birch 2005*)
- Higher concentrations late in gestation
- Short half life (16 min), undetectable by 2 hrs postpartum (*Lo 1999*)

Am J Hum Genet. 1998;62:768-75; BMJ 2011;342:1-9; Clin Chem. 2004 Jan;50(1): 88-92; Clin Chem. 2005 Feb;51(2):312-20; Am J Hum Genet. 1999 Jan;64(1):218-24

cffDNA: Clinical Challenges

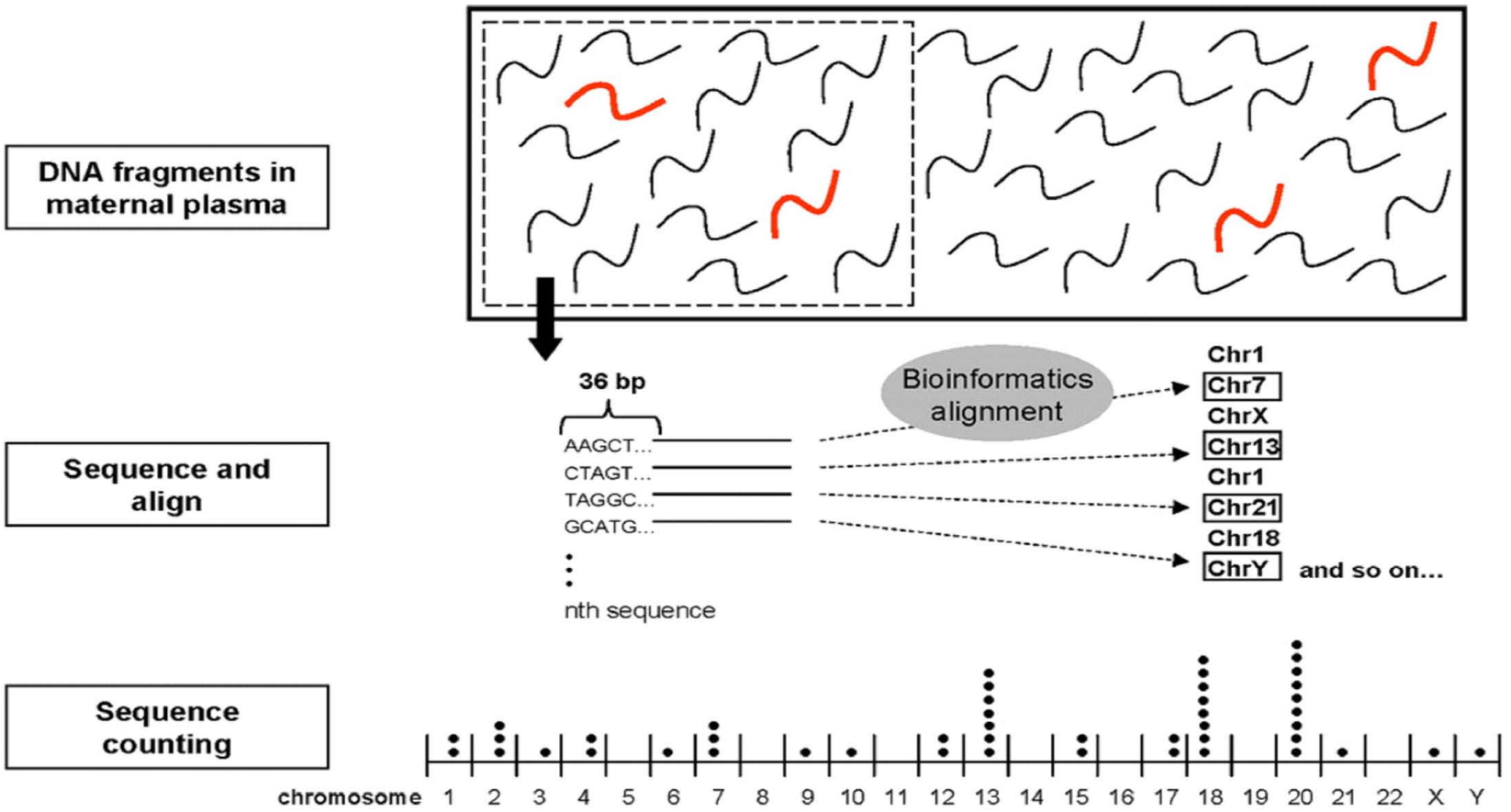
False negatives/failed results:

- Failure to extract adequate material
- Individual variation in amount of cffDNA
- Placental mosaicism

False positives:

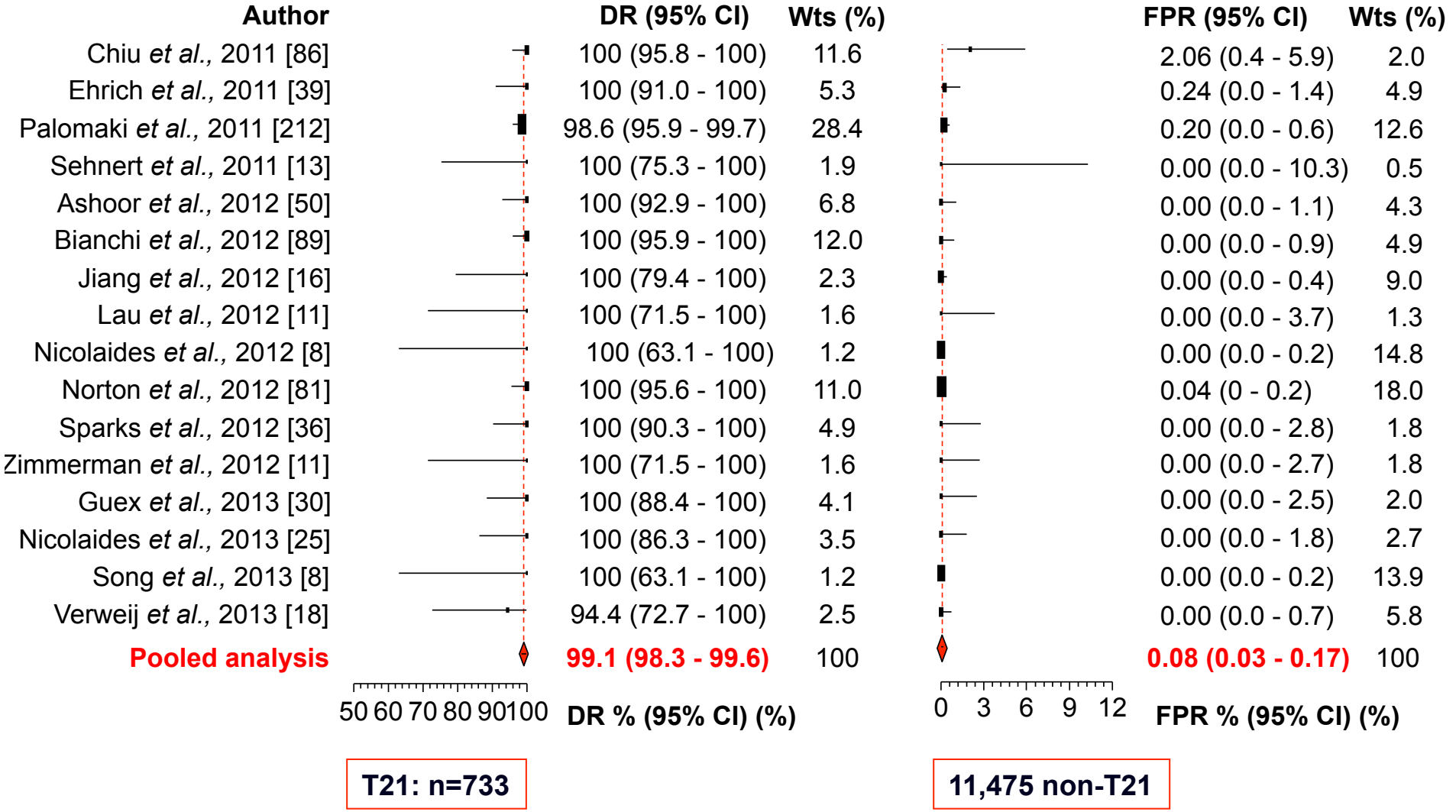
- Contamination
- Unrecognized or vanishing twin
- Placental mosaicism
- Low level maternal mosaicism

Analysis of fetal DNA



Zhong, X, Holzgreve, W, Glob. libr. women's med 2009

Trisomy 21 performance cfDNA testing: meta-analysis



Chromosomal Microarray (CMA) for Prenatal Diagnosis

The NEW ENGLAND JOURNAL of MEDICINE

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Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis

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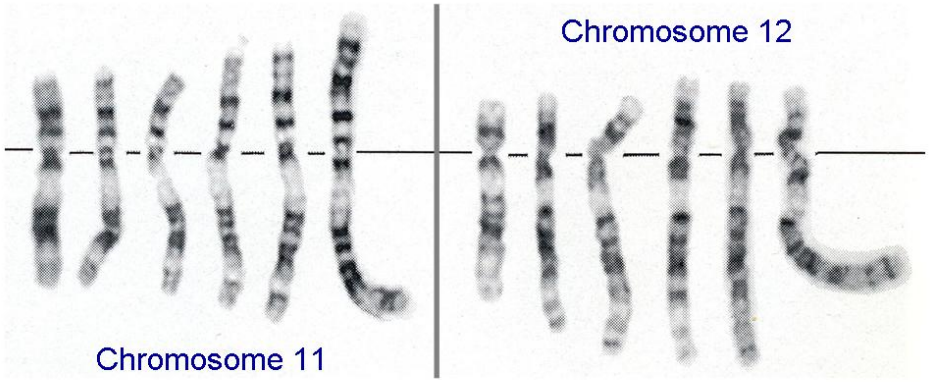
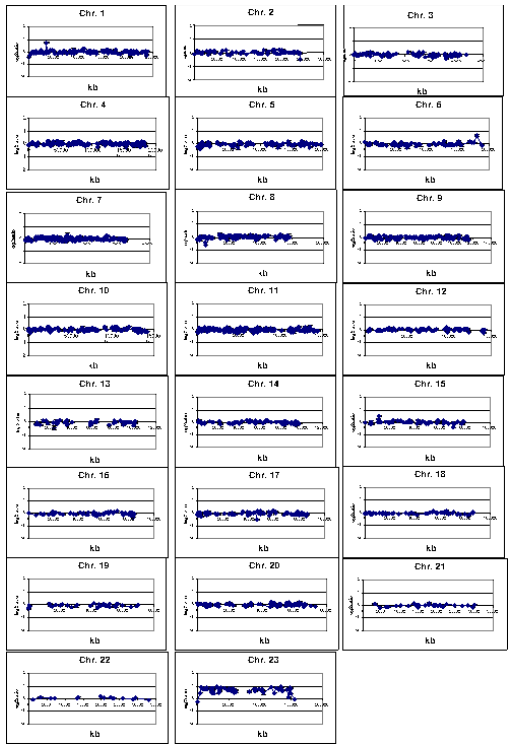
Karyotype

Chromosomal Microarray





Resolution:

>7-10 Million Base Pairs

(7-10 Mb)

Resolution:

< 0.5 Million Base Pairs

(< 500 kb)

Diagnostic Yield in Cases with Normal Karyotype

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

Prenatal Genetic Testing

- Prenatal genetic testing has undergone tremendous advances in recent years
- Current screening and diagnostic strategies continue to evolve and improve
- Cell free DNA and chromosomal microarrays have both been tremendous additions to the field of prenatal diagnosis
- Further genomic advances will continue to expand prenatal testing options

Question:

- It is recommended that all women aged 35 years and older undergo amniocentesis, true or false?

Question:

- It is recommended that all women aged 35 years and older undergo amniocentesis, true or false?

- Answer: false. Prenatal screening and diagnostic testing choice should be driven primarily by patient preferences.