Prenatal screening with cell-free DNA

Slides adapted in part from Nancy Rose, MD

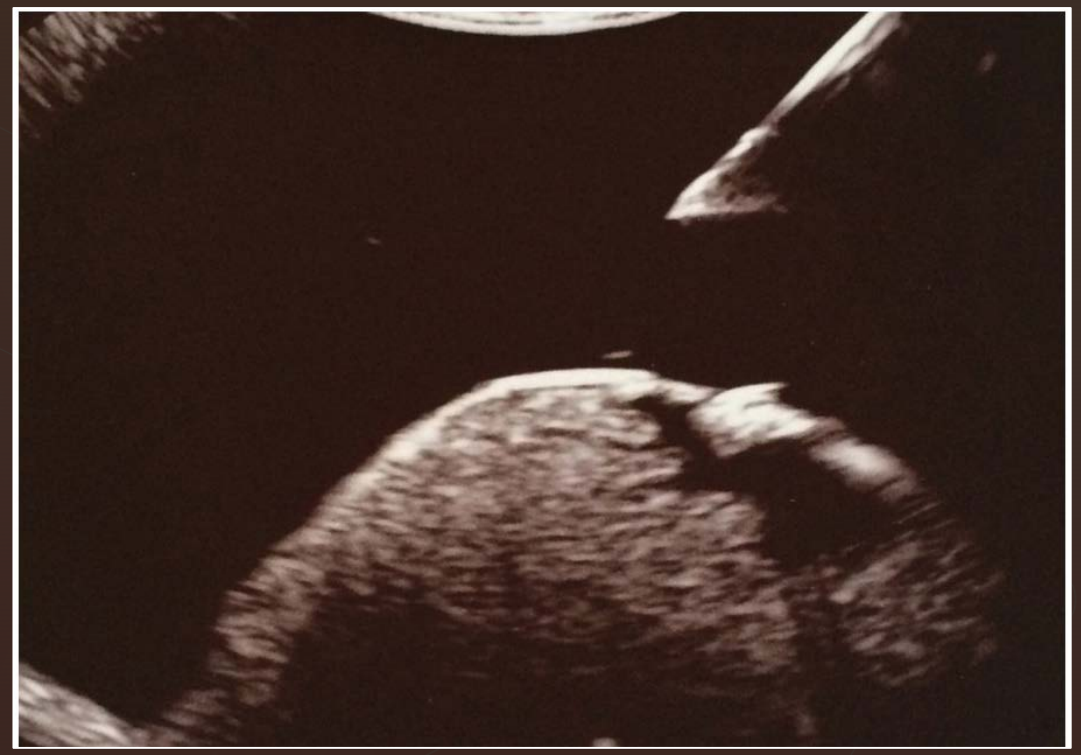
Disclosures & Objectives

- I have no conflicts of interest to disclose

- Describe the characteristics of cell-free DNA screening in comparison to other types of prenatal screening
- Understand the need for confirmatory testing and pregnancy implications
- Describe unexpected, particularly maternal, cell-free DNA test results and their clinical implications

Prenatal testing – A Brief History

- Amniocentesis fluid first cultured for karyotyping in 1966
- First amniotic-fluid diagnosis of T21 in 1968
- Motivated search for non-invasive routes of diagnosis
  - Maternal serum analytes
  - Ultrasound



Types of non-DNA based genetic screening

- Maternal age
- Ultrasound
- First trimester screen
  - Ultrasound nuchal translucency + maternal serum analytes – PAPP-A & hCG
  - 10-14 weeks gestation
- Quad screen
  - Maternal serum analytes – AFP, hCG, Inhibin A, uE3
  - 15-20/22 weeks gestation
- Integrated screening and stepwise sequential or contingent screening
  - First trimester US NT + PAPP-A
  - Second trimester quad screen analytes

Maternal age

Age at term*	Risk of T21	Risk of T21, 13, 18, & sex chromosome abnormalities
18 years	1: 1,556	1: 525
25 years	1: 1,340	1: 475
35 years	1: 353	1: 178
45 years	1: 35	1: 18

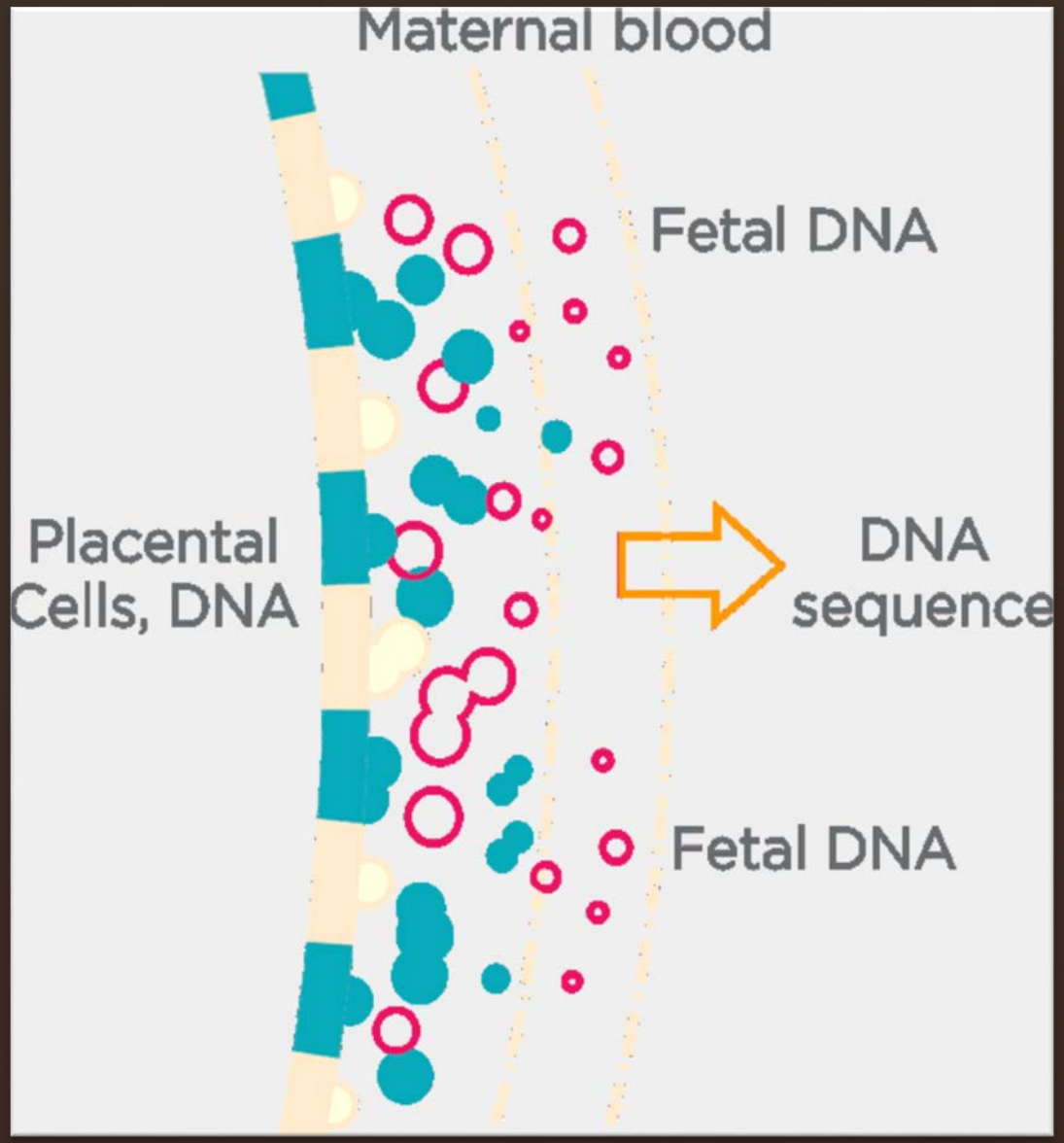
* Excludes those pregnancies ending in miscarriage or stillbirth due to chromosomal abnormalities

- Maternal age not an indication for offering/not offering screening
- 80% of T21 occurs in women <35yo

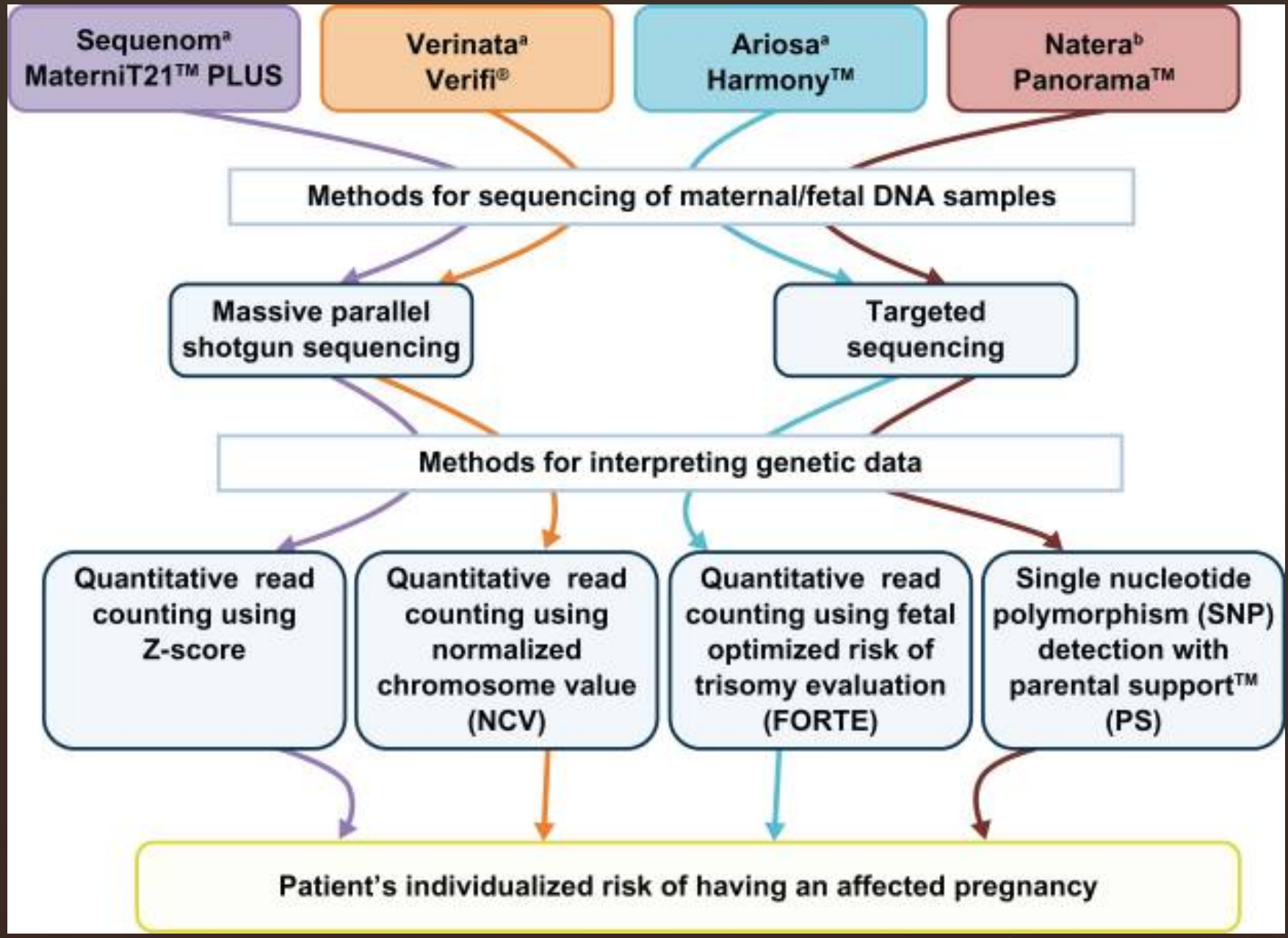
Cell-free DNA – A Brief History

- 1948: First identified Mandel and Metais
- 1966: Association of SLE and increased cfDNA levels.
- 1977: Higher levels in oncology patients
- 1997: Lo and colleagues: fetal CFDNA in maternal plasma.
  - Applied concept of tumor fragments to fetal development
- 2008: Association of cfDNA and aneuploidy
- 2011: Rapid commercial development with little transparency

Cell-free DNA



- Both maternal and “fetal”
  - Maternal: ~500bp
  - Fetal: ~200-300bp
- Fetal fraction derived from apoptosis of ***placental*** synciotrophoblasts
- Fetal fraction ~ 8-10% @10 weeks
  - Increases 0.1% per week to 20
  - Increases 0.6% per week >20
  - Undetectable 2h postpartum



Smith and Visootsak Int J Womens Health. 2013; 5: 125–131.

Side comments about fetal fraction

- It's not really fetal! It's placental!
  - 1-2% confined placental mosaicism (CPM)
  - Most commonly autosomal triploidy
  - Examples: triploidy rescue or monosomy rescue
- It changes with maternal habitus
  - Decreases 0.5% for every 10lb increase between 80 and 200 lbs
  - Dilutional effect?
  - Maternal adipocyte apoptosis?
  - 8-17% no call rate in BMI>35 vs 0.5-1% no call <35
- It changes with aneuploidy
  - Increased or decreased (↑T21, ↓T13/18/45X)
  - Overall a low fetal fraction raises risk for aneuploidy

What can we use cell-free DNA for?

AUTOSOMAL TRISOMIES

- Trisomy 21
- Trisomy 18
- Trisomy 13

SEX CHROMOSOME ANEUPLOIDIES

- 45,X
- 47,XXY
- 47,XYY
- 47,XXX

MICRODELETIONS

- DiGeorge (22q deletion)
- 1p36 deletion syndrome
- Angelman/Prader-Willi
- Cri-du-Chat syndrome
- Jacobsen syndrome
- Langer-Giedion syndrome

OTHER AUTOSOMAL TRISOMIES

- 9, 16, 22

What can we use cell-free DNA for?

- Sex determination for X-linked disorders
  - Hemophilia A
- Fetal RhD status
  - Allo-immunized Rh- mother, fetus at risk for hemolytic disease
- Paternally inherited AD gene
  - Neurofibromatosis, achondroplasia

- At this time, microdeletion/duplication testing via cell-free DNA is **not** recommended by ACOG/SMFM
- Other (relative) contraindications: twins, egg donor, transplant recipient

Screening Test	Gestational Age at Screening	Detection rate for T21	Screen Positive Rate	Analytes/measures obtained
First trimester screen	10w0d – 13w6d	82-87%	5%	NT PAPP-A hCG
Quad screen	15-22 (institution dependent)	81%	5%	hCG AFP uE3 DIA
Integrated	10w0d – 13w6d and 15-22	96%	5%	First trimester + Quad
Cell-free DNA	>9-10wk	99%	0.5%	Placentally-derived DNA in maternal circulation

Carlson and Vora Obstet Gynecol Clin North Am. 2017 Jun; 44(2): 245–256.

Positive predictive value matters, too!

- Cell-free detection rate is high and false negative rate is low
- HOWEVER – implications for a positive test depend upon the incidence in the tested population
  - PPV – likelihood that a positive test is a true positive
  - Initially limited to high risk population
  - First validated in high risk population

	Pooled Detection Rate	PPV(%), Age 25	PPV(%), Age 35	PPV (%), Age 45
Trisomy 21	99.2	51	79	98
Trisomy 18	96.3	15	39	90
Trisomy 13	91.7	7	21	N/A
Monosomy X	90.3	41	41	41

Perinatal Quality Foundation calculator used for PPVs

Carlson and Vora Obstet Gynecol Clin North Am. 2017 Jun; 44(2): 245–256.

Clinical pretest counseling – by the book

- Screening should be offered to every mother at 1st prenatal visit
- Cell free DNA may be offered to any mother
- Discuss pros/cons of each test
  - All tests are *pretty good*, better than age + US
  - Costs/insurance are important
  - Additional information from serum screen
- Include how to interpret (broadly) positive/negative results
- Cover possible unexpected results
- Given volume of information, videos, pamphlets, and infographics are being developed

What do I do with positive or no-call results?

- Positive test - requires confirmation before action
- No call test due to low FF in obese patient
  - Fetal fraction does not increase as much through pregnancy
  - >40% still have a no-call on subsequent test with BMI>35
  - Need additional testing (US, +/- amnio/CVS)
- No call or indeterminate for other reasons
  - High risk of aneuploidy (20-30%)
  - Offer genetic counseling and diagnostic testing
  - Repeat cell free DNA could be considered if very early in gestation (e.g., 11 weeks) but delays possible diagnosis

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

Society for Maternal-Fetal Medicine

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PRACTICE BULLETIN

CLINICAL MANAGEMENT GUIDELINES FOR OBSTETRICIAN–GYNECOLOGISTS

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(Replaces Practice Bulletin Number 77, January 2007)
(See also Practice Bulletin Number 162, *Prenatal Diagnostic Testing for Genetic Disorders*)

Screening for Fetal Aneuploidy

Prenatal genetic screening is designed to assess whether a patient is at increased risk of having a fetus affected by a genetic disorder. In contrast, prenatal genetic diagnostic testing is intended to determine, with as much certainty as possible, whether a specific genetic disorder or condition is present in the fetus. The purpose of prenatal screening for aneuploidy is to provide an assessment of the woman's risk of carrying a fetus with one of the more common fetal aneuploidies. This is in contrast to prenatal diagnostic testing for genetic disorders, in which the fetal chromosomes are evaluated for the presence or absence of abnormalities in chromosome number, deletions, and duplications, or the fetal DNA is evaluated for specific genetic disorders. The wide variety of screening test options, each offering varying levels of information and accuracy, has resulted in the need for complex counseling by the health care provider and complex decision making by the patient. No one screening test is superior to other screening tests in all test characteristics. Each test has relative advantages and disadvantages. It is important that obstetrician–gynecologists and other obstetric care providers be prepared to discuss not only the risk of aneuploidy but also the benefits, risks, and limitations of available screening tests. Screening for aneuploidy should be an informed patient choice, with an underlying foundation of shared decision making that fits the patient's clinical circumstances, values, interests, and goals.

Clinical Cases

- 37yo G2P0 at 11 weeks estimated gestation, first prenatal visit
- Normal BMI, no medical history, no history of aneuploidy or congenital defects
- Viability ultrasound:
  - Size consistent with dates
  - No obvious abnormalities (e.g. acrania)
- Would like genetic screening after discussion

Advanced maternal age: What options?

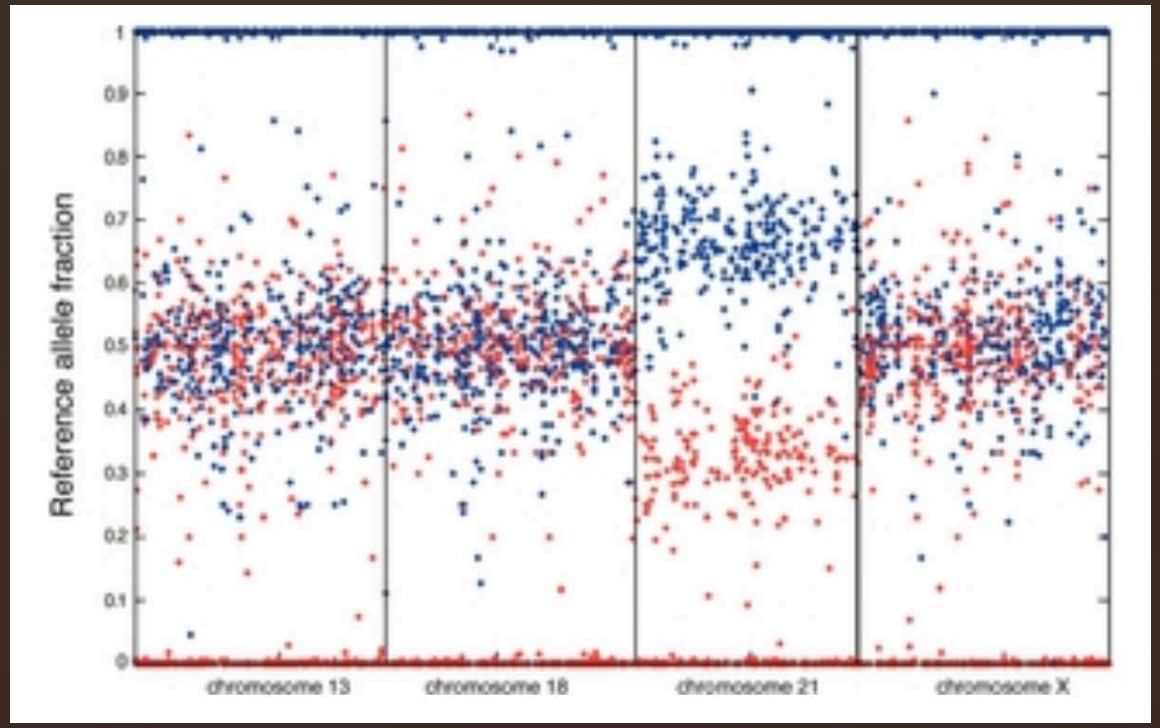
- Age dependent risks
  - 1:128 for T21
  - 1:104 for combined T21, T13, T18, SCA
- All options available
- Cell free DNA

Test

○ Harmony® ○ Materniti 21® ◉ Panorama® ○ Verifi®

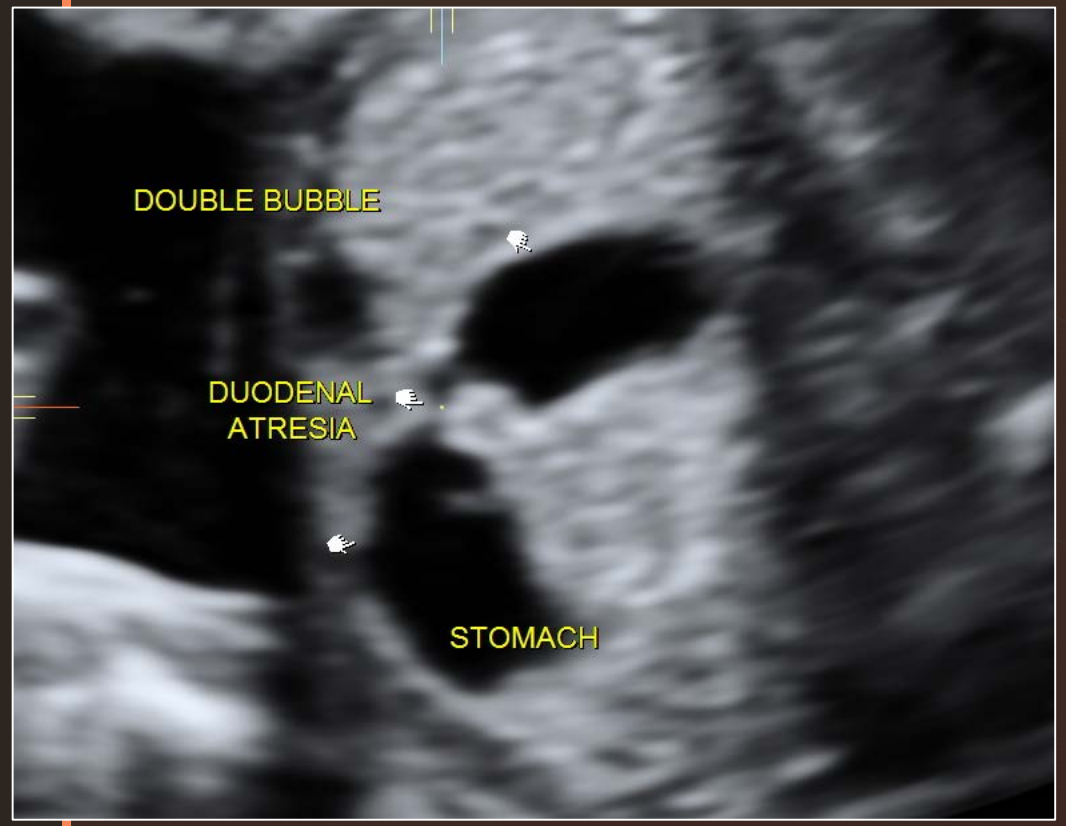
	Trisomy 21	Trisomy 18	Trisomy 13
Age-related risk	1:128	1:354	1:1116
Test Sensitivity	99	96.4	99
Test Specificity	99.9	99.9	99.9
PPV	89%	73%	47%

Test is high risk for T21



- PPV is 89%, counsel on likelihood that this represents a true positive
  - False +: Twin demise, screening variability, placental mosaicism, maternal malignancy
- Offer invasive diagnostic testing (CVS <15 weeks, amniocentesis >15-16 weeks)
  - ? CVS to verify cfDNA given 1-2% CPM

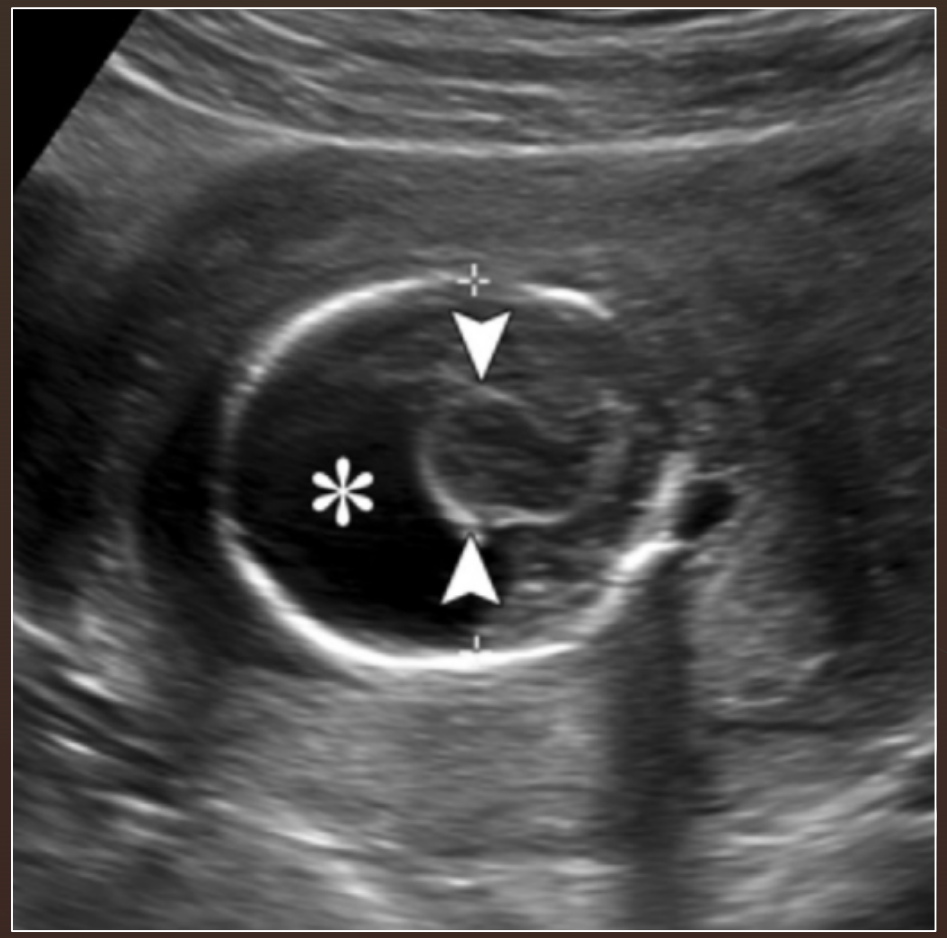
Anatomic survey (aka fetal anatomy scan)



- Double bubble = small bowel obstruction
  - Duodenal atresia
- 20% fetuses with T21 will have bowel obstruction
- Other common findings on ultrasound
  - Congenital heart defect (AVSD), ~50%
- Most abnormalities cannot be diagnosed on ultrasound

Clinical case #2

- 25 yo G3P2 at 18 weeks gestation by last menstrual period presenting for anatomy scan
- Declined genetic testing and ultrasound earlier in pregnancy
- Normal BMI, no history of recurrent miscarriage, aneuploidy, or congenital defects

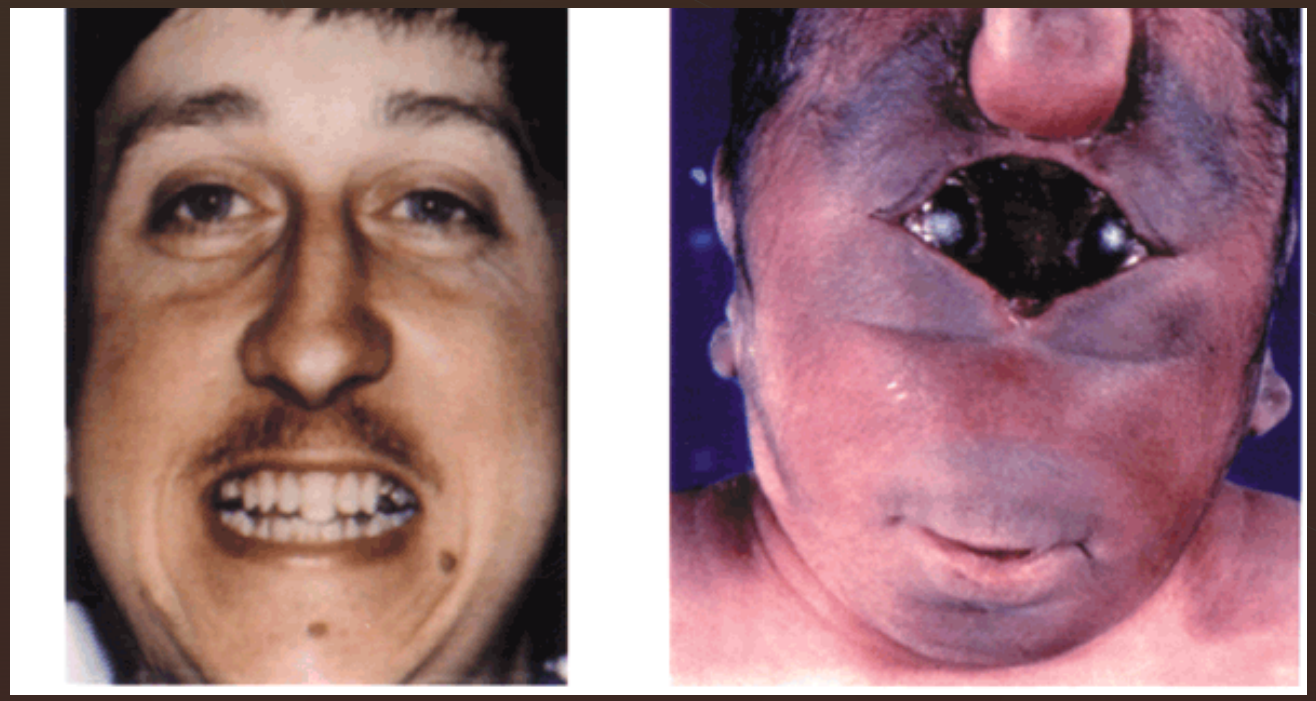


- Alobar holoprosencephaly
- 22-45% associated with T13 (Patau’s Syndrome)
- 10% have microdeletion/duplication
- 13 known HPE-associated genes, no strict genotype-phenotype correlation
- What to do next?

Ultrasound findings and cell-free DNA

- Standard text-book answer: do not use cell free to evaluate anomalies
- HOWEVER, when taking into account maternal preferences, some moms/families opt for cfDNA
  - Ultrasound findings very consistent with T13/18/21
  - Patient desires pregnancy to continue regardless of result
  - Desires not to perform invasive procedure for risk of pregnancy loss

- Side notes about microdeletion screening…
  - Genotype ≠ phenotype, and we can't see the baby
  - Differential penetration means microdel/dup could be mom and not baby

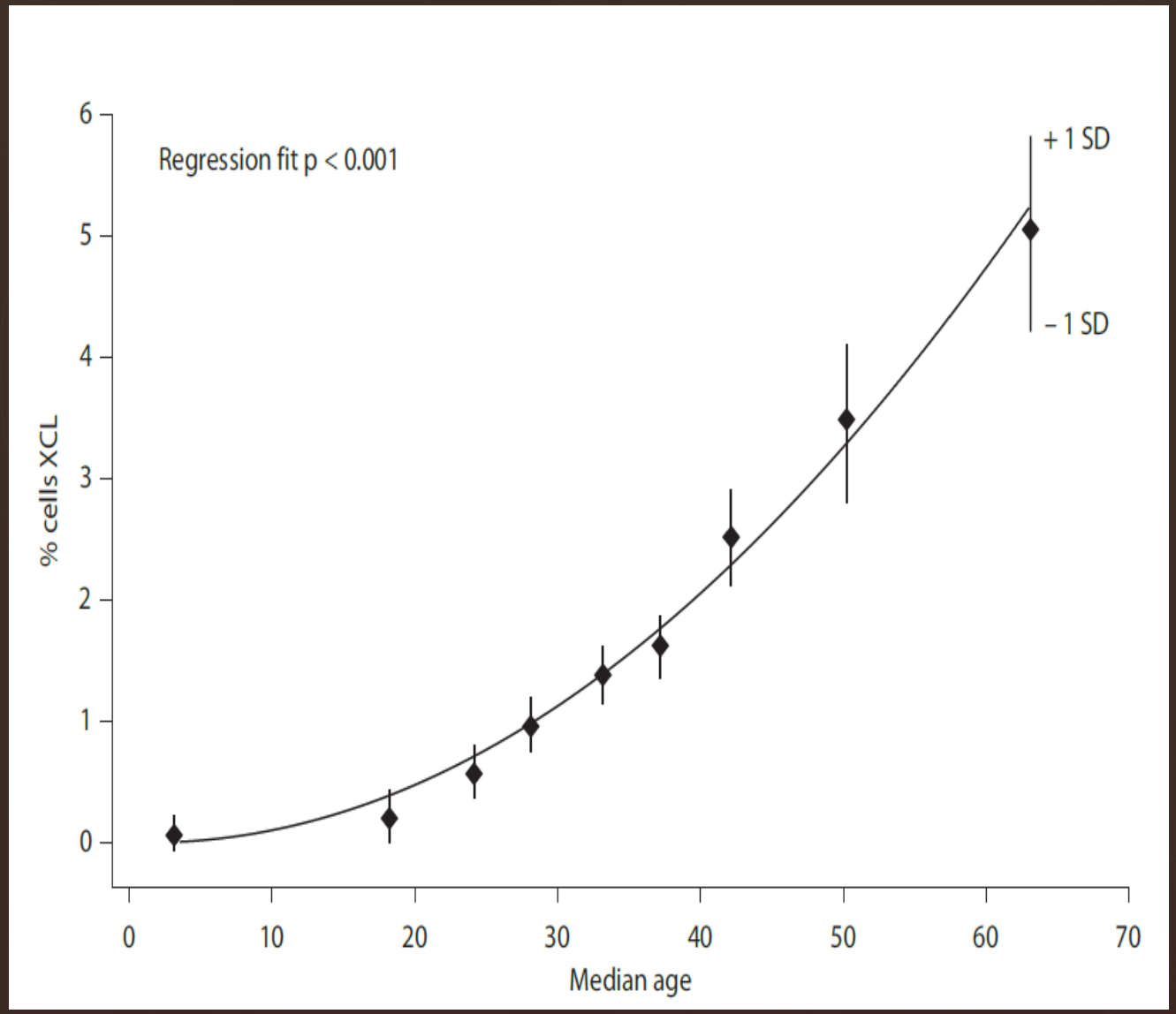


Same HPE microdeletion, very different penetrance/ phenotype

Unintended maternal consequences

- The test cannot distinguish a maternal from a fetal result!
- 45, X results can reflect older mothers, not fetuses
- The microdeletion syndrome you identify may be maternal
- Beware the patient with an organ transplant
- At least 26 cases of maternal cancer diagnosis

- A tale of two monosomy Xs…
  - 40 year old, first pregnancy
  - Nuchal translucency normal
  - CfDNA 45, X result
    - Sonogram: apparently normal female
    - Amnio: 46,XX
  - Maternal karyotype 45,X[5]/46,XX [45]
    - Interpretation: normal maternal aging



Russell et al. Cytogenet Genome Res. 116:181-185, 2007

- 35 Year old G1P0
  - cfDNA: 45, X
  - Fetal karyotype: 46, XX
  - Maternal karyotype:45 X (17), 46XX (35)
  - Phenotype:
    - Mild hearing loss, bone density issues, bicuspid aortic valve, short stature, normal intelligence
  - Clinical implications:
    - ECHO
    - Possible premature ovarian failure, with attendant increase in cardiovascular disease
    - Endocrine disorders

- Hip pain postpartum with abnormal cell-free DNA?
  - 37 Year old G2P1
    - cfDNA Screening Result: 47, XX+13
    - Anatomic survey: normal
    - Amniocentesis result: 46, XY
  - Normal male delivered, 3040 grams
  - Post partum hip pain:
    - Small cell carcinoma, vaginal origin
    - Cells with same karyotype as cell-free DNA result

Summary

- Cell-free DNA screening for fetal aneuploidy is highly sensitive
  - Must be partnered with ultrasound or AFP for anatomic/structural evaluation
- Positive predictive value falls with falling incidence
- Positive tests need confirmation!
- Range of applications is rapidly increasing
- Clinical implications are equally wide ranging and require counseling *pre-test*

Thank you!

- Thanks to Dr Toydemir, the fellows, medical directors, and lab staff who made my visit to ARUP educational and pleasant
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