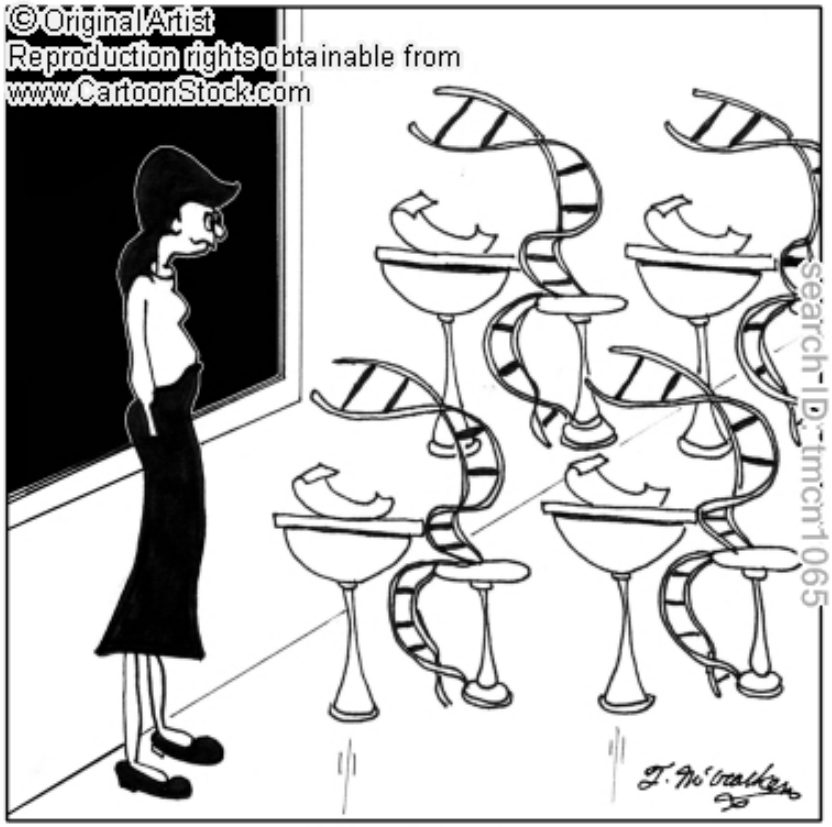
Updates in Genetic Screening

Pregnancy Care ECHO
December 18th

Amber Mathiesen, M.S., L.C.G.C

Outline

- Screening options:
  - **cfDNA testing**
  - **ACOG statement**
- Carrier testing:
  - **Ancestry based**
  - **Enhancements to fragile X and SMA**
  - **Expanded carrier testing**



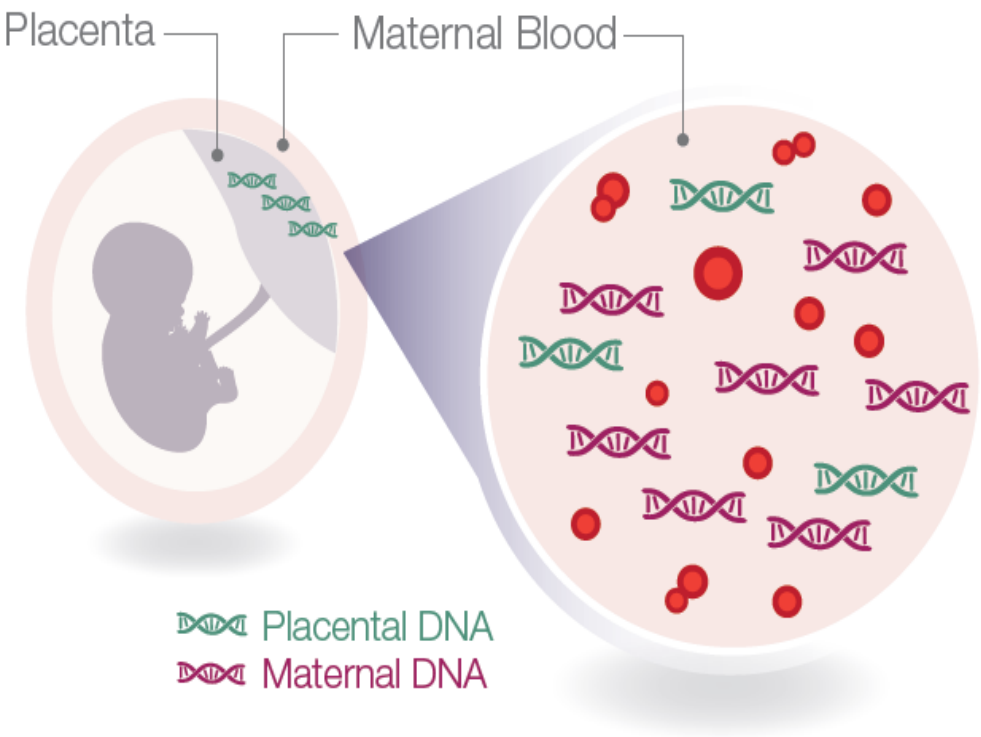
DNA Testing.

Advancements in Prenatal Screening

1960's	1980s	1988	1996	1997	2011-2012
Maternal Age	MSAFP	Triple Screen	Quad Screen	FTS NT/Serum	**cfDNA SNP**
Detection Rate					
27%	36%	60-74%	70-81%	80-95%	92->99%
Chromosome Abnormalities Screened					
All	T21	T21 T18	T21 T18	T21 T18 T13*	T21 T18 T13 SCA Triploidy* Microdeletions*

* some

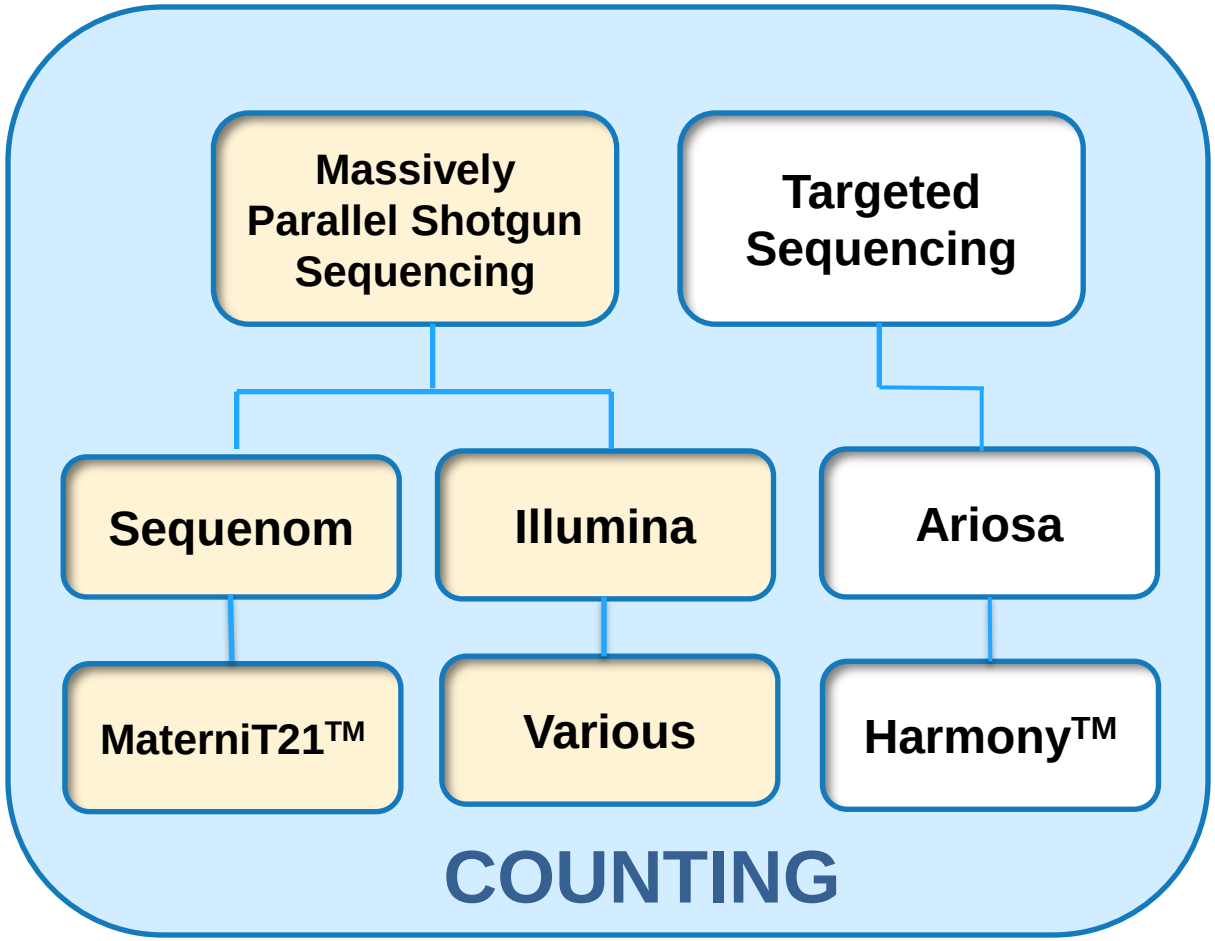
Cell-free DNA (cfDNA)

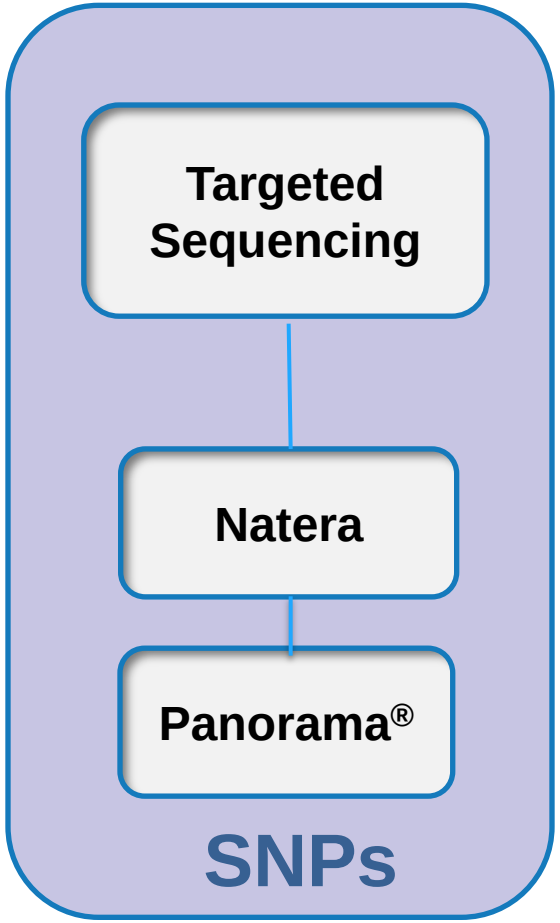


cfDNA comes from apoptotic cells derived from:

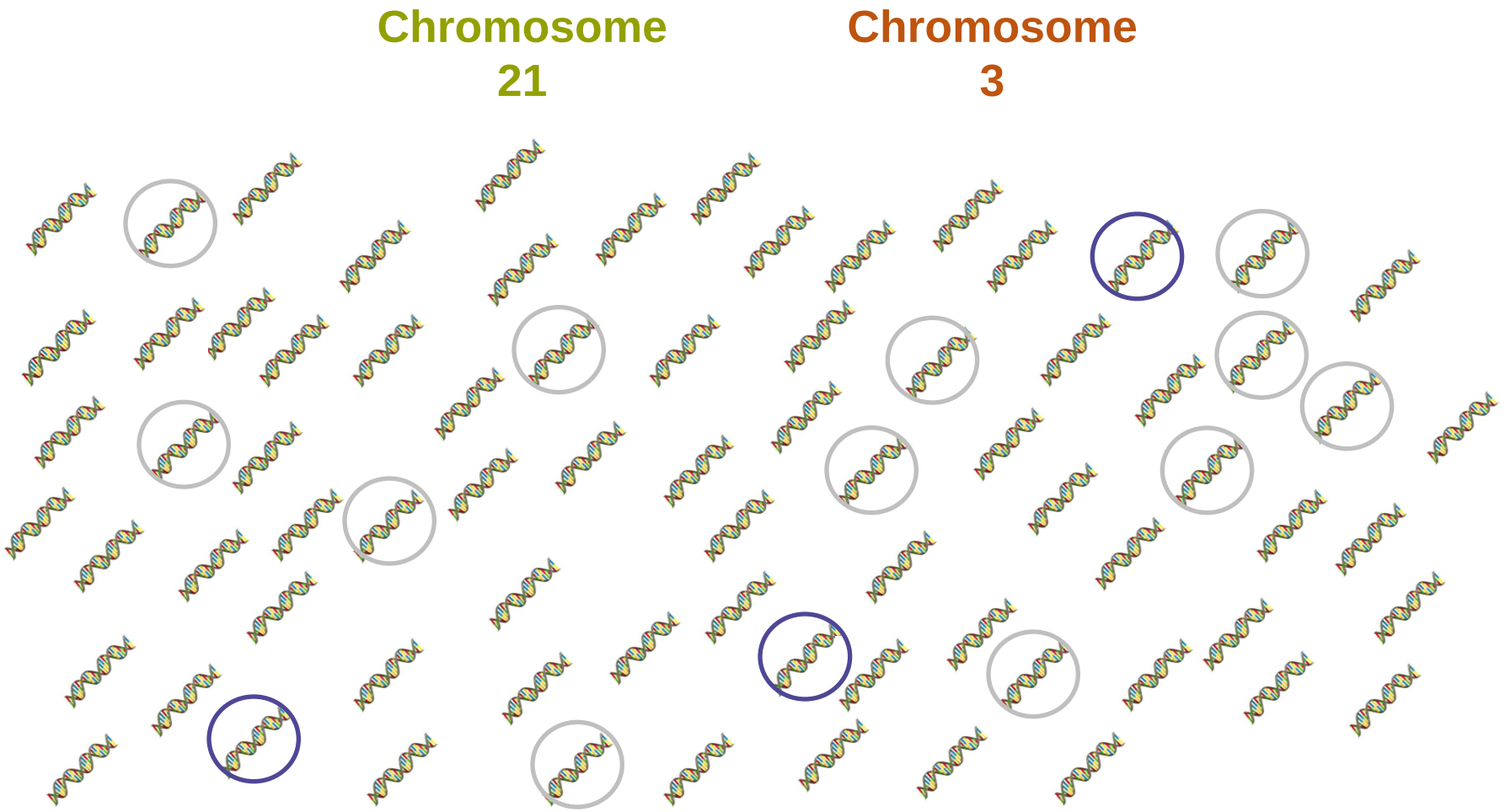
- Maternal Circulation
  - Adipocytes
  - White Blood Cells
- Fetal
  - Placental cells (trophoblasts) in the maternal circulation

Differentiating Methodologies



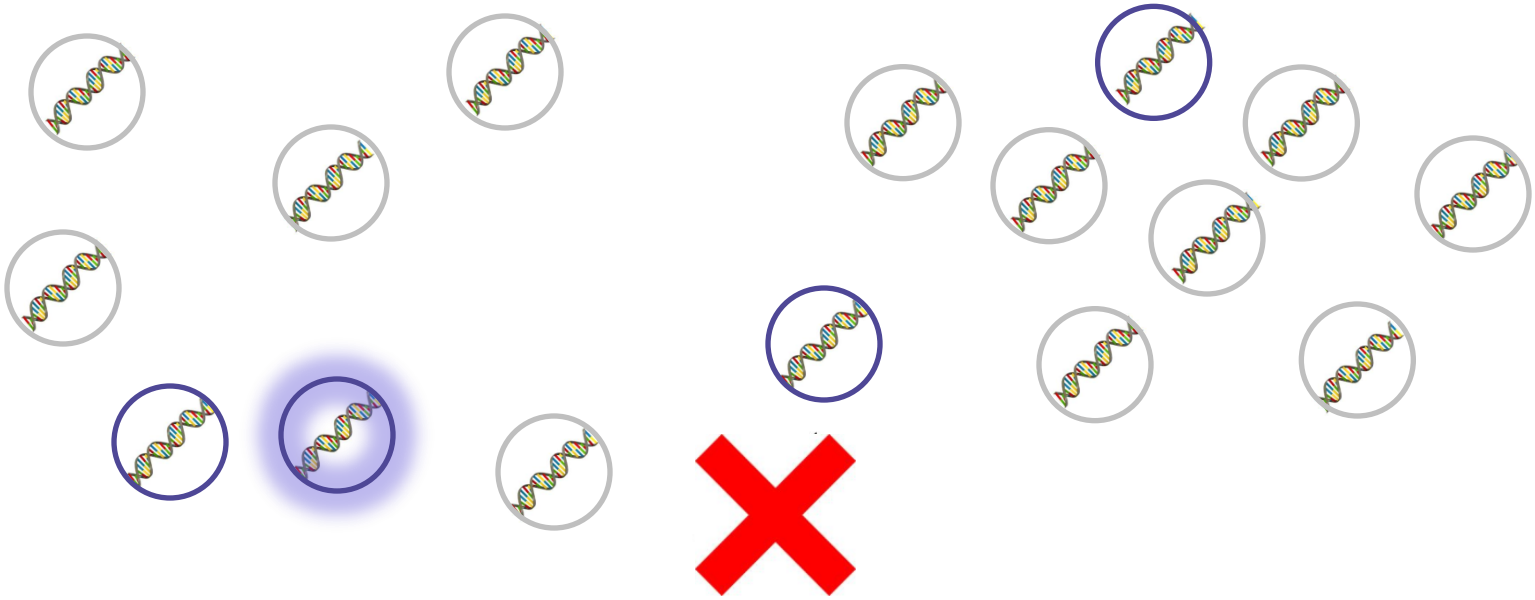


Counting

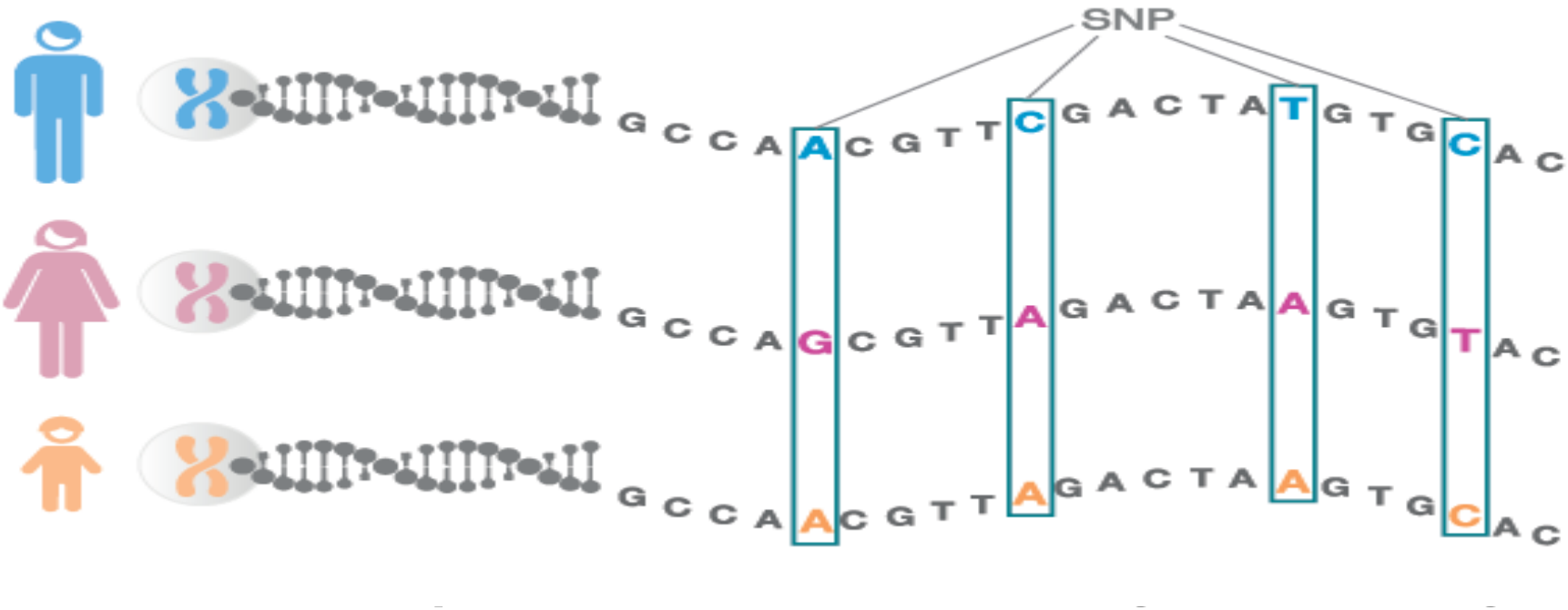


Counting

	Chromosome 21	Chromosome 3
Expected Amount:	20%	80%
Observed Amount:	25%	75%



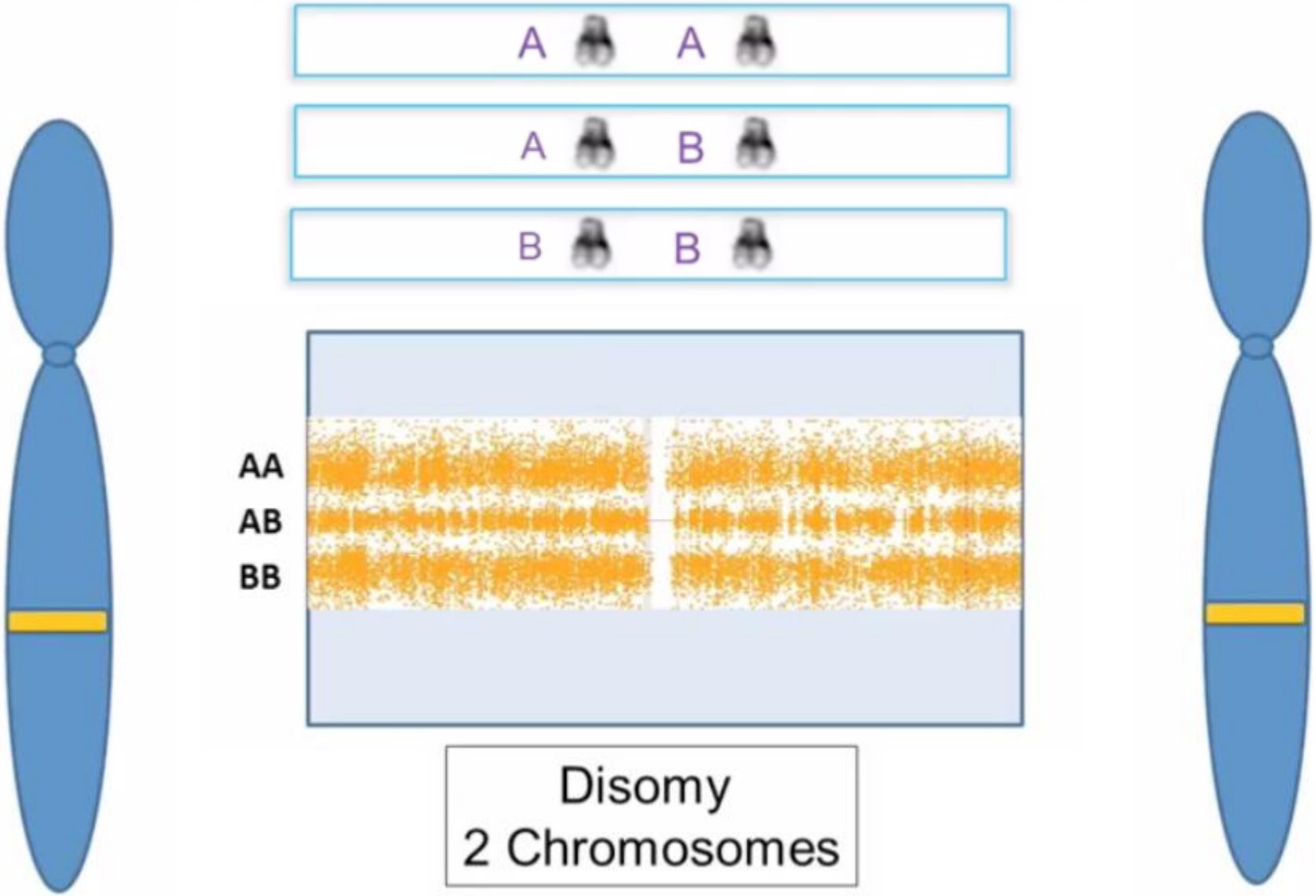
SNP = Single Nucleotide Polymorphism



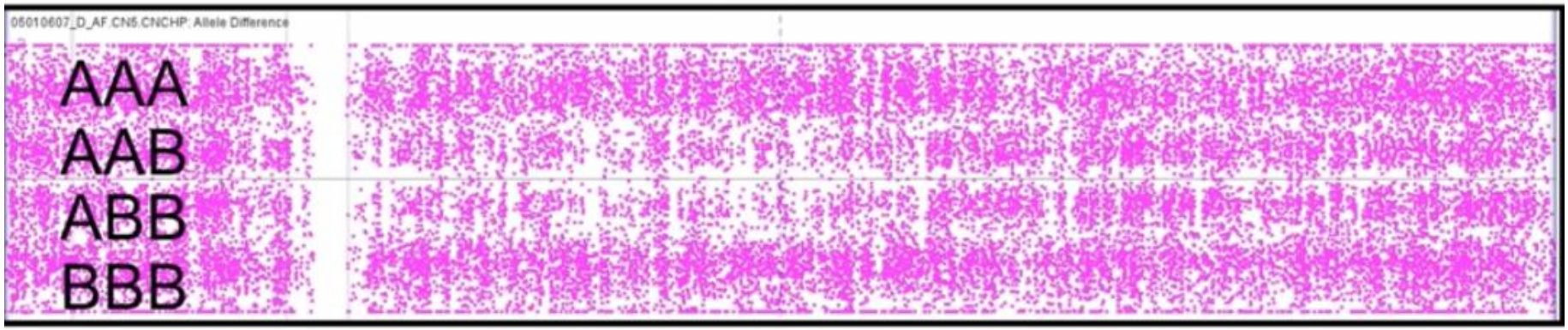
base pair (nucleotide) - A, T, C, or G – is changed.

- **These are normal genetic changes that occur in every person**

SNP



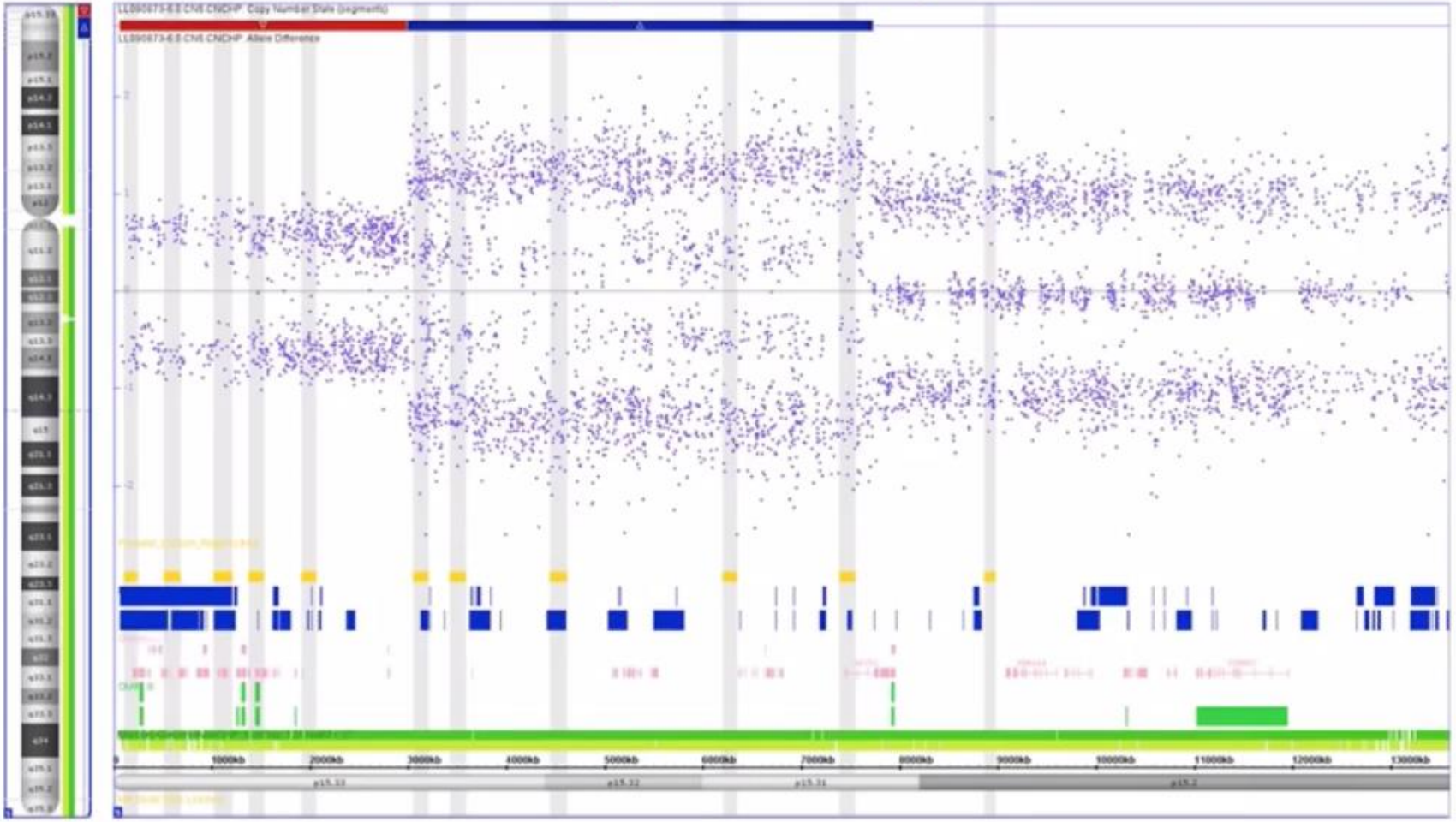
SNP



Trisomy
3 Chromosomes

SNP

Deletion Duplication Disomy



cfDNA - SNP

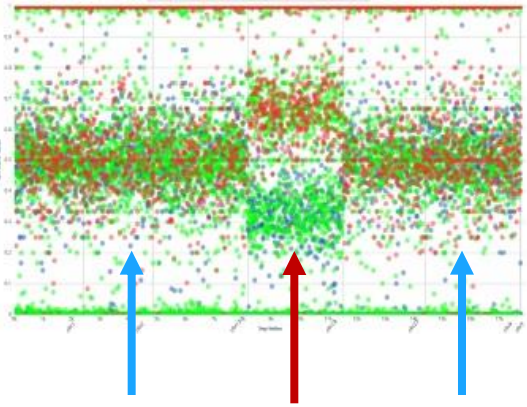
Maternal blood

Maternal + Fetal cfDNA

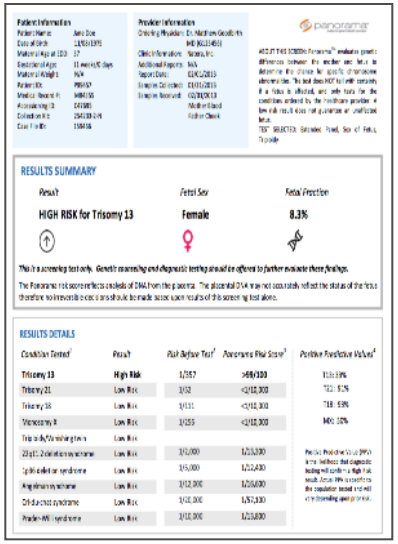
SNP Sequencing of Maternal + Fetal Genotype

Advanced Bioinformatics

Report



Disomy Trisomy Disomy



Reported Detection Rates

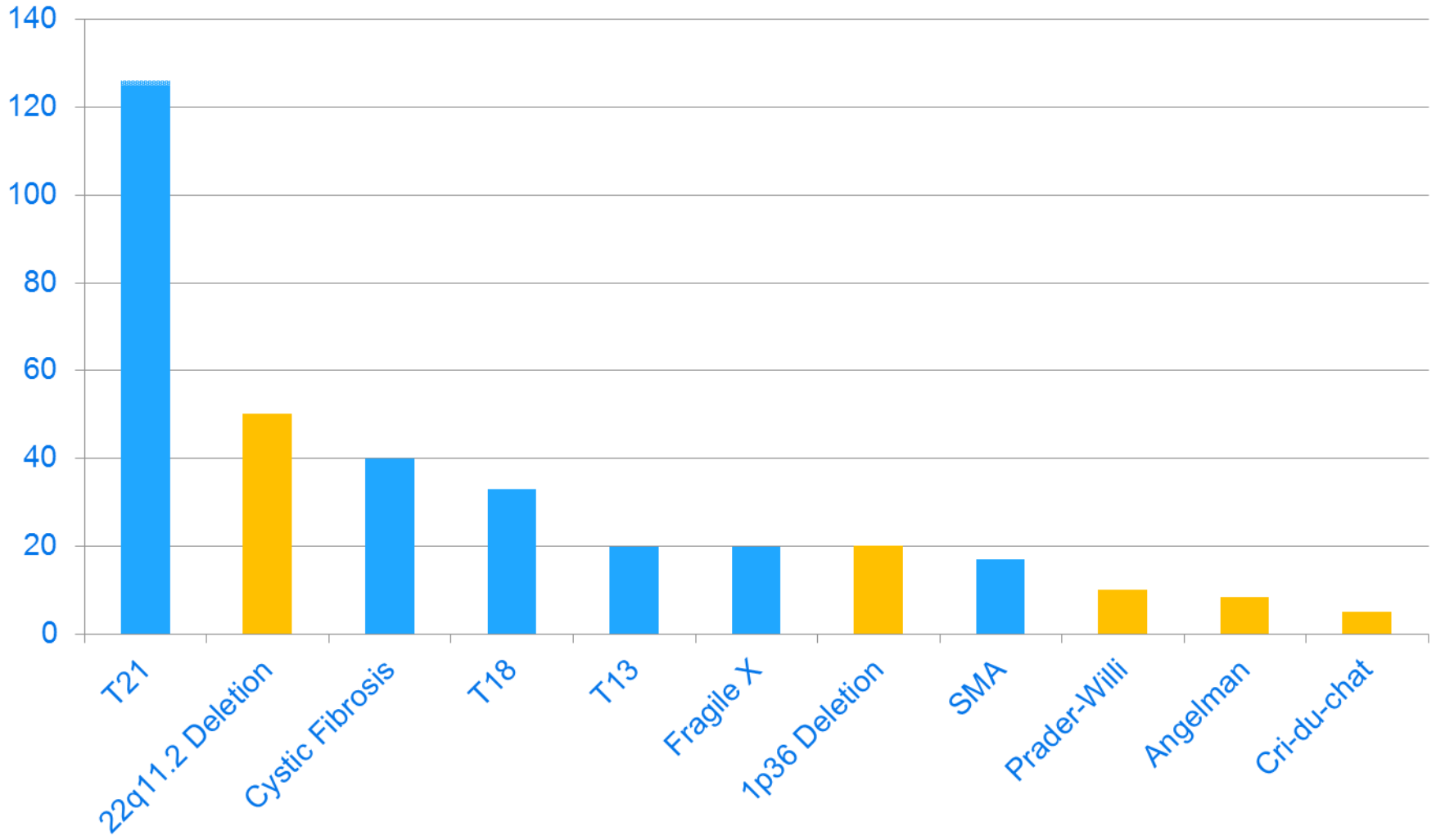
	Sequenom (MaterniT21)	Illumina (Verify)	Ariosa (Harmony)	Natera (Panorama)
Trisomy 21	99.1% 0.1%	>99.9% 0.2%	>99% <0.1%	>99% 0%
Trisomy 18	99.9% 0.4%	97.4% 0.4%	>98% <0.1%	96.4% <0.1%
Monosomy X	94.4% 0.6%	95% 1.0%	91.5% 0%	>99.9% 0%
Sex chromosome	96.2%	67-100%	99%	92.9% <0.1%
Female	99.1% 0.5%	97.6% 0.8%	99% 0%	>99.9% 0%
Male	99.4% 0.9%	99.9% 1.1%	100% 1%	>99.9% 0%
Triploidy	unable to detect	unable to detect	Unable to detect	>99%

Microdeletions

22q11 deletion (associated with DiGeorge syndrome)
15q11 deletion (associated with Prader-Willi / Angelman syndrome)
11q23 deletion (associated with Jacobsen syndrome)
8q24 deletion (associated with Langer-Giedion syndrome)
5p15 deletion (associated with Cri-du-chat syndrome)
4p16 deletion (associated with Wolf-Hirschhorn syndrome)
1p36 deletion syndrome

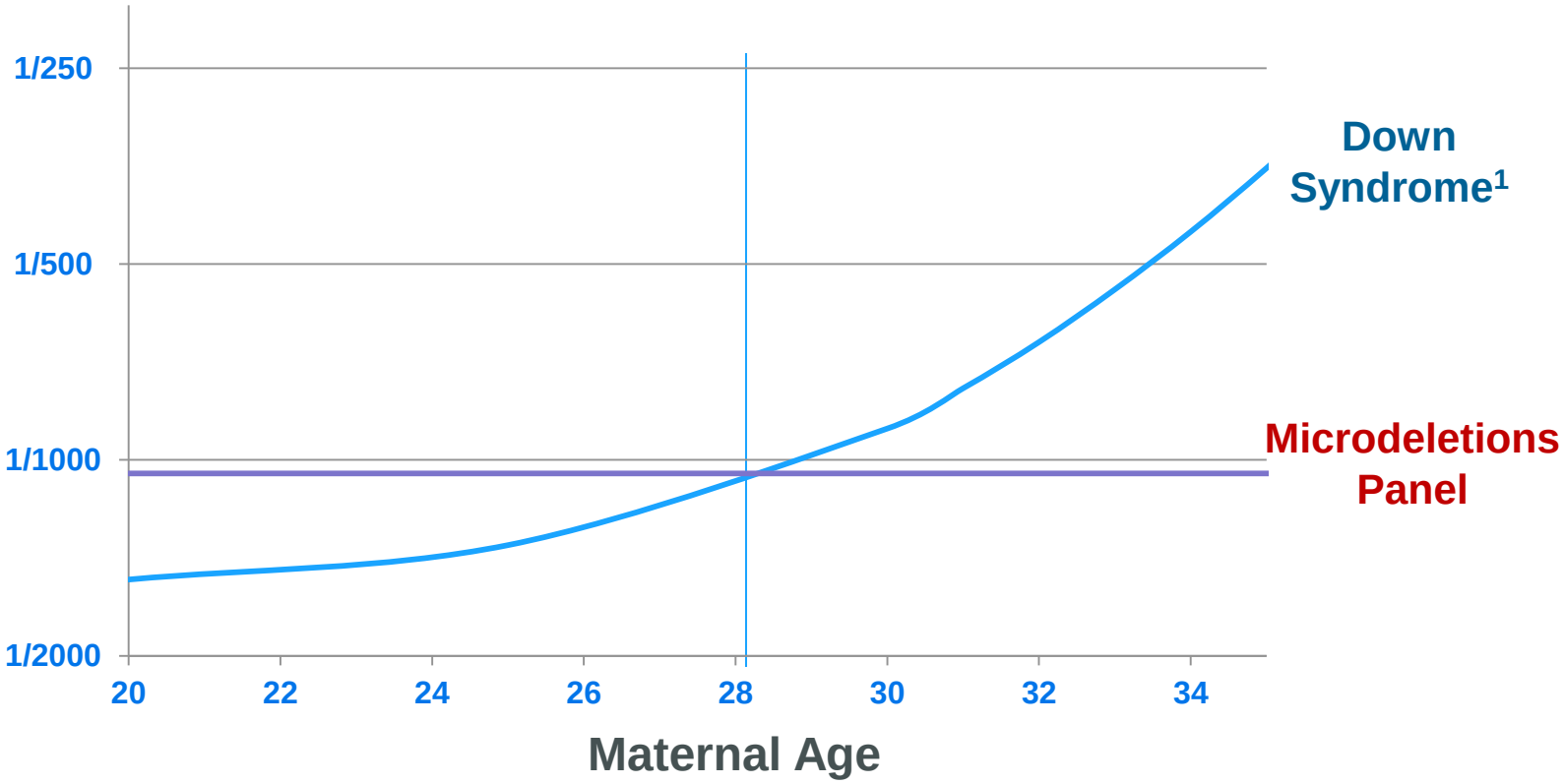
Microdeletions

Incidence out of 100,000 Live Births



Microdeletions

More Common Than Down Syndrome in Younger Women

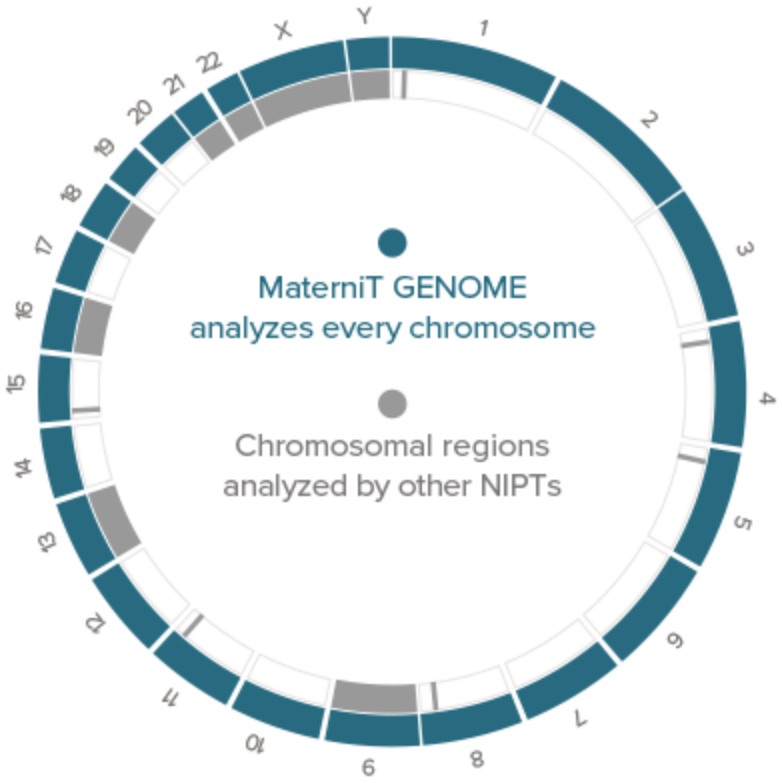


[1]Snijders, et al. Ultrasound Obstet Gynecol 1999;13:167–170.
[2]Combined prevalence using higher end of published ranges from Gross et al. Prenatal Diagnosis 2011; 39, 259-266; and www.genetests.org. Total prevalence may range from 1/1071 - 1/2206.

Sequenom's MaterniT Genome

Genome-wide deletions or duplications of 7 Mb and greater, and also detects select microdeletions below 7 Mb.

	Fetal karyotype test	MaterniT GENOME t
Analyzes every chromosome	Yes	Yes
Requires an invasive procedure	Yes	No
Detects large, unbalanced translocations	Yes	Yes
Detects marker chromosomes	Yes	Yes
Detects balanced translocations or inversions	Yes	No
Detects chromosome gains or losses as small as 7 mb	No	Yes
Detects select microdeletions	No	Yes
Detects Triploidy	Yes	No
Considered diagnostic	Yes	No

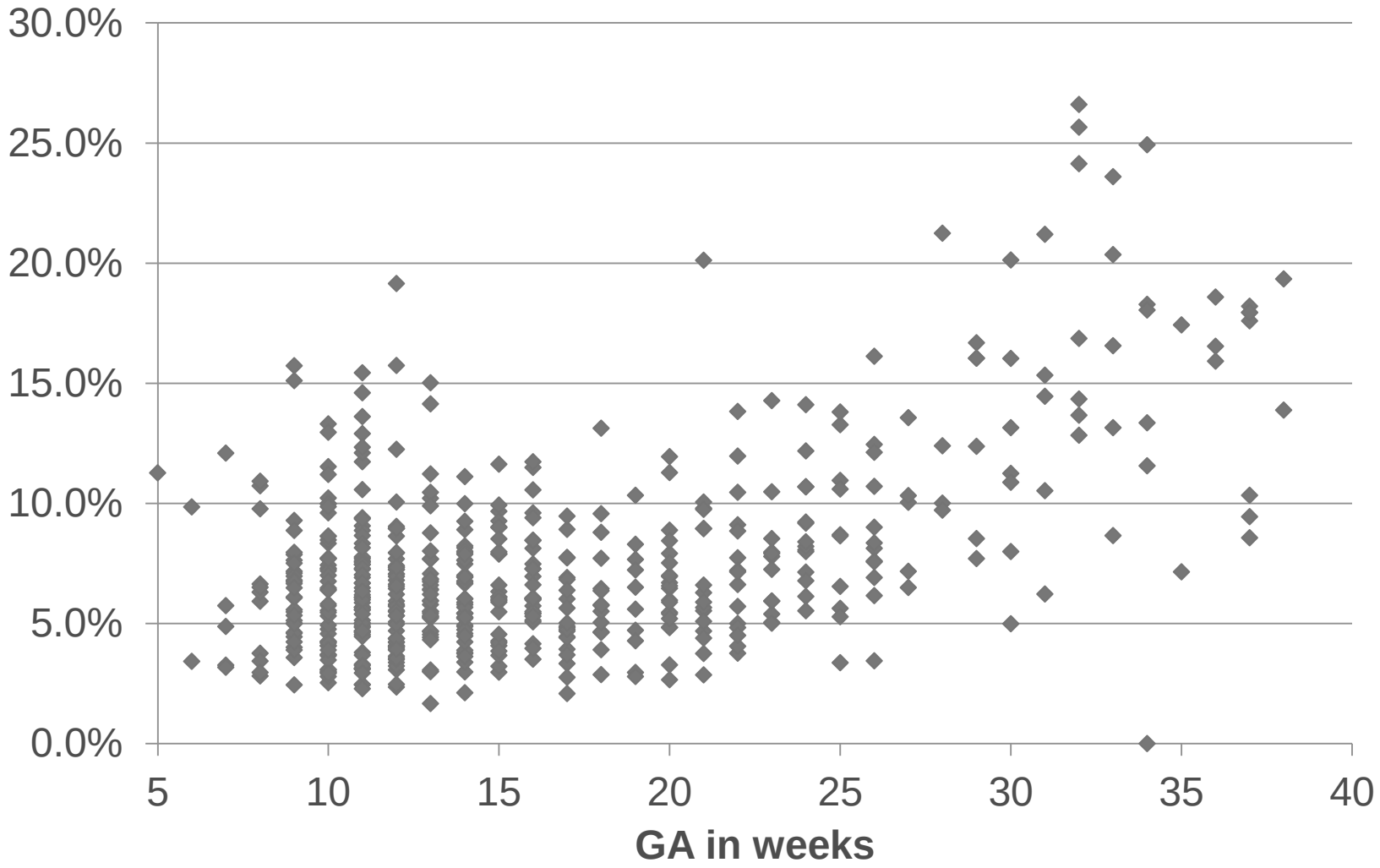


Sequenom's MaterniT Genome

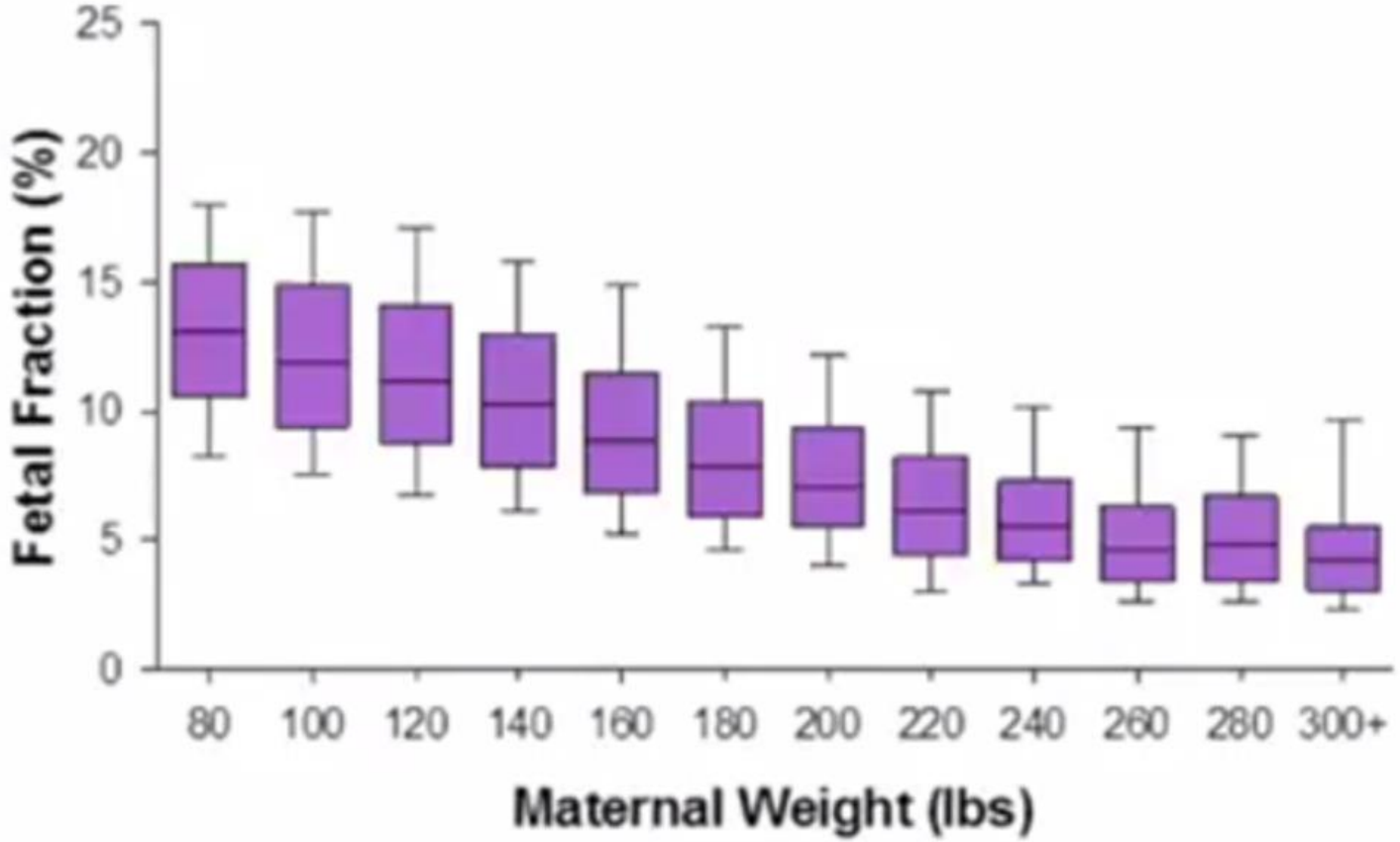
Known Causes of Discrepancies

Cause	Example	Reference
True fetal mosaicism	cfDNA: + or - Invasive: two cell lines	Canick et al., Prenatal Diagn 2013
Confined placenta mosaicism	cfDNA: - Invasive: 47,XY,+21	Wang et al., Prenatal Diagn, 2013
Twin pregnancy (vanishing twin)	cfDNA: + liveborn twin 46,XX Vanishing 47,XX, +21	Gromminger et al., JCM 2014
Maternal chromosome abnormality	47,XXX	Wang et al., Clin Chem 2014
Maternal somatic cell variation	cfDNA: + Maternal loss of X (45,X)	Wang et al., Clin Chem 2014
Maternal malignancy	cfDNA: +13 Invasive: 46,XY Cancer found in mom	Osborne et al., Prenat Diagn, 2013
Low fetal fraction	cfDNA: - Invasive: 47,XY, +21	Allen et al., ACMG meeting; abstract, 2013

Fetal Fraction Related to GA



Fetal Feraction Maternal Weight



Fetal fraction is inversely proportional to maternal weight:

↑ maternal DNA + ↓ fetal DNA

Fetal Fraction and Aneuploidy

- 17/31 samples with low fetal fraction (<3.4%) were aneuploid
- 119/1015 samples with above 3.4% fetal fraction were aneuploid

Translated to OR of 9.2X risk of aneupliody with low fetal fraction.

Pergament E, Cuckle H, Zimmermann B, Banjevic M, Sigurjonsson S, Ryan A, et al. Single-nucleotide polymorphism-based noninvasive prenatal screening in a high-risk and low-risk cohort. Obstet Gynecol 2014;124:210–8.

ACOG

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

ABSTRACT: Noninvasive prenatal screening that uses cell-free DNA from the plasma of pregnant women offers tremendous potential as a screening method for fetal aneuploidy. A number of laboratories have validated different techniques for the use of cell-free DNA as a screening test for fetal aneuploidy. All tests have a high sensitivity and specificity for trisomy 18 and trisomy 21, regardless of which molecular technique is used. Women whose results are not reported, indeterminate, or uninterpretable (a "no call" test result) from cell-free DNA screening should receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing because of an increased risk of aneuploidy. Patients should be counseled that cell-free DNA screening does not replace the precision obtained with diagnostic tests, such as chorionic villus sampling or amniocentesis and, therefore, is limited in its ability to identify all chromosome abnormalities. Cell-free DNA screening does not assess risk of fetal anomalies such as neural tube defects or ventral wall defects. Patients who are undergoing cell-free DNA screening should be offered maternal serum alpha-fetoprotein screening or ultrasound evaluation for risk assessment. The cell-free DNA screening test should not be considered in isolation from other clinical findings and test results. Management decisions, including termination of the pregnancy, should not be based on the results of the cell-free DNA screening alone. Patients should be counseled that a negative cell-free DNA test result does not ensure an unaffected pregnancy. Given the performance of conventional screening methods, the limitations of cell-free DNA screening performance, and the limited data on cost-effectiveness in the low-risk obstetric population, conventional screening methods remain the most appropriate choice for first-line screening for most women in the general obstetric population.

ACOG

- Offer to patients with high risk
  - Advanced maternal age;
  - Fetal ultrasound abnormality;
  - Personal or family history of Down syndrome or other chromosomal aneuploidy; and/or
  - Positive serum screening test
- Low risk population?
  - All women should receive information on all testing options
  - Any women can choose any method regardless of risk status
  - Conventional screening most appropriate for low risk given limitations (adverse pregnancy outcomes and PPV)

PPV/NPV

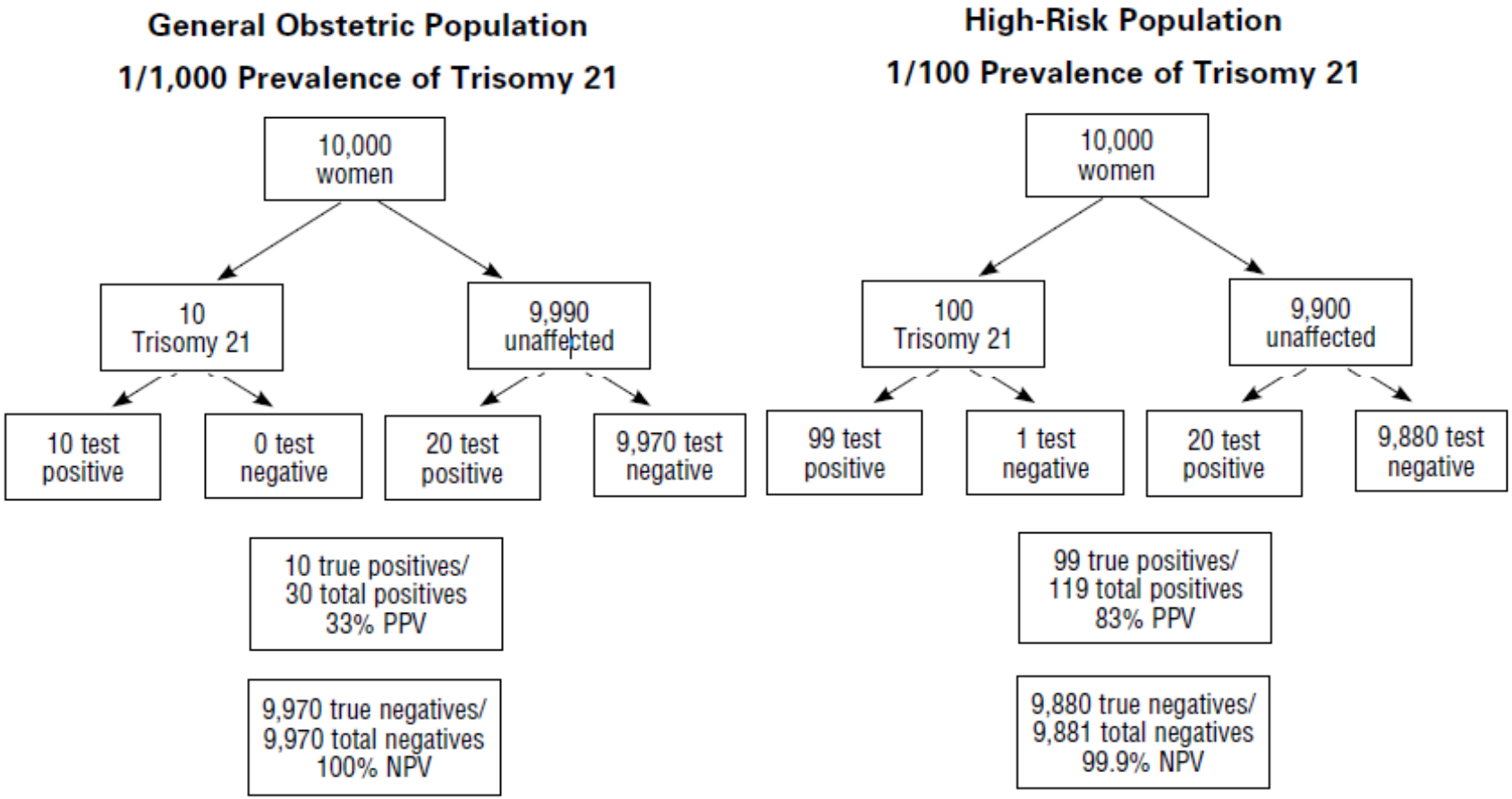


Fig.1. The importance of population prevalence on the predictive value for a screening test: an illustration with cell-free DNA.

Abbreviations: NPV, negative predictive value; PPV, positive predictive value

PPV

Test	DR	FPR	PPV (high risk 1/100)	PPV (low-risk 1/500)
FTS (NT, PAPPA, hCG	80%	3%	21%	5%
Quad	60%	3%	17%	4%
Sequential	93%	3%	24%	6%
cfDNA (all methods)	99.3%	0.1%	91%	67%

DR, FPR, for conventional screening: Benn *et al.* Prenat Diagn. 2013; 31:519-22
Based on meta-analysis of 19 validation studies (all methods)

ACOG

- Microdeletions
- Multiple pregnancies
- Recommend diagnostic testing for positive results
- Management decisions, including TOP, should not be made on cfDNA testing alone
- Should be offered MS-AFP
- If anomaly identified offer diagnostic testing
- Women with failed results should receive further counseling and offered ultrasound and diagnostic testing.

Carrier Testing

Current Guidelines – Ancestry

ACOG Guidelines

- African Americans,
- Southeast Asians,
- Mediterranean

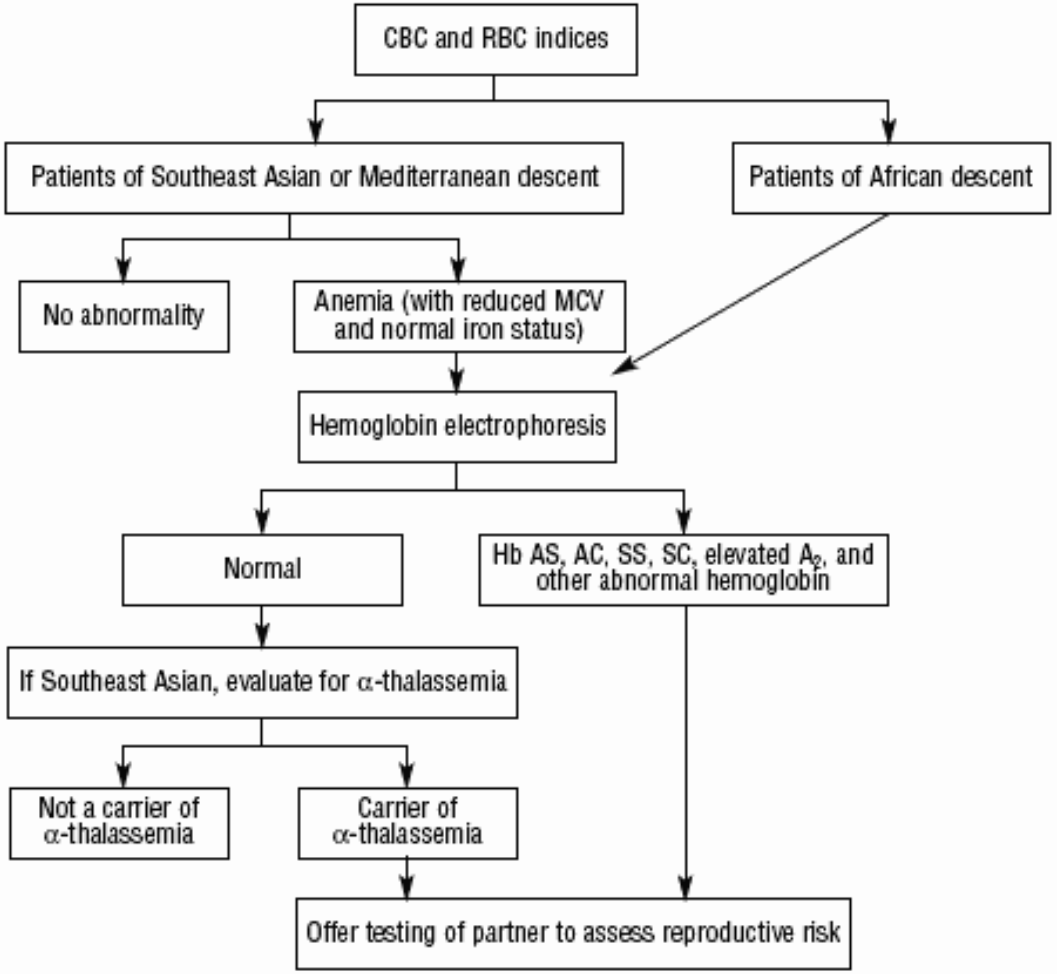


Figure 1. Specialized antepartum evaluation for hematologic assessment of patients of African, Southeast Asian, or Mediterranean descent. Patients of Southeast Asian or Mediterranean descent should undergo electrophoresis if their blood test results reveal anemia. Abbreviations: CBC, complete blood count; Hb, hemoglobin; MCV, mean corpuscular volume; RBC, red blood cell.

Current Guidelines – Ancestry

Gaucher Type I

*!Tay-Sachs

*Cystic Fibrosis

*Familial dysautonomia

*Canavan

Niemann-Pick A

Fanconi anemia C

Bloom syndrome

Mucolipidosis IV

Biochemical screening of hexosaminidase is the most sensitive screening in all populations.

*= For Ashkenazi Jewish ACOG recommended, ACMG recommends all

! = offer in those of French Canadian and Cajun ancestry

Current Guidelines – Panethnic

The American College of Obstetricians and Gynecologists
Women's Health Care Physicians

COMMITTEE OPINION

Number 486 • April 2011 *(Replaces No. 325, December 2005)*

Committee on Genetics

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.

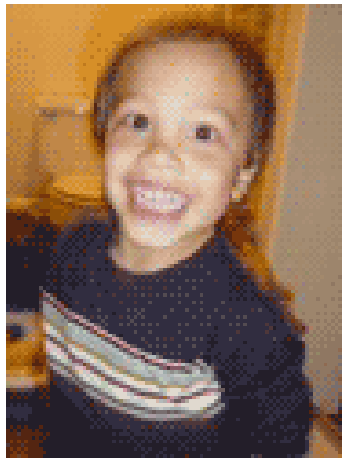
Update on Carrier Screening for Cystic Fibrosis

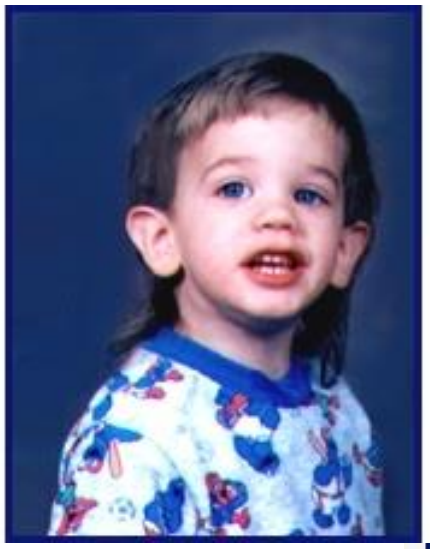
ABSTRACT: In 2001, the American College of Obstetricians and Gynecologists and the American College of Medical Genetics introduced guidelines for prenatal and preconception carrier screening for cystic fibrosis. The American College of Obstetricians and Gynecologists' Committee on Genetics has updated current guidelines for cystic fibrosis screening practices among obstetrician–gynecologists.

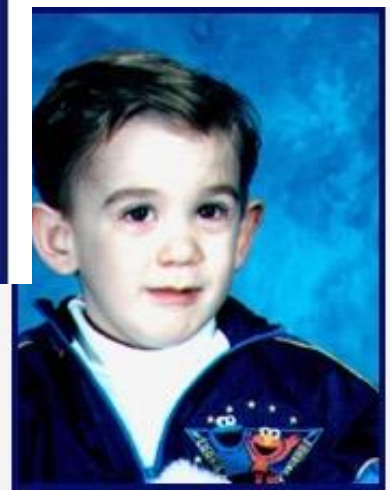
Every Patient

Current Guidelines – Targeted

- Fragile X
  - Pan-Ethnic carrier frequency: all women have ~1/250 or greater risk to be carriers
  - Severe disorder with no cure
- Offer testing:
  - Family history of fragile X
  - Family history of ID of unknown etiology
  - Family history of autism
  - Personal history of POI
  - Personal history of ataxia, esp male







CGG Repeat

- Trinucleotide repeat – CGG
  - **Expansion of CGG repeats within the gene occurs when inherited though the mother**

Fragile X Result	CGG Repeat Sizes
Normal	<45
Intermediate	45 – 54
Premutation (Carrier)	55 - 200
Full Mutation	>200

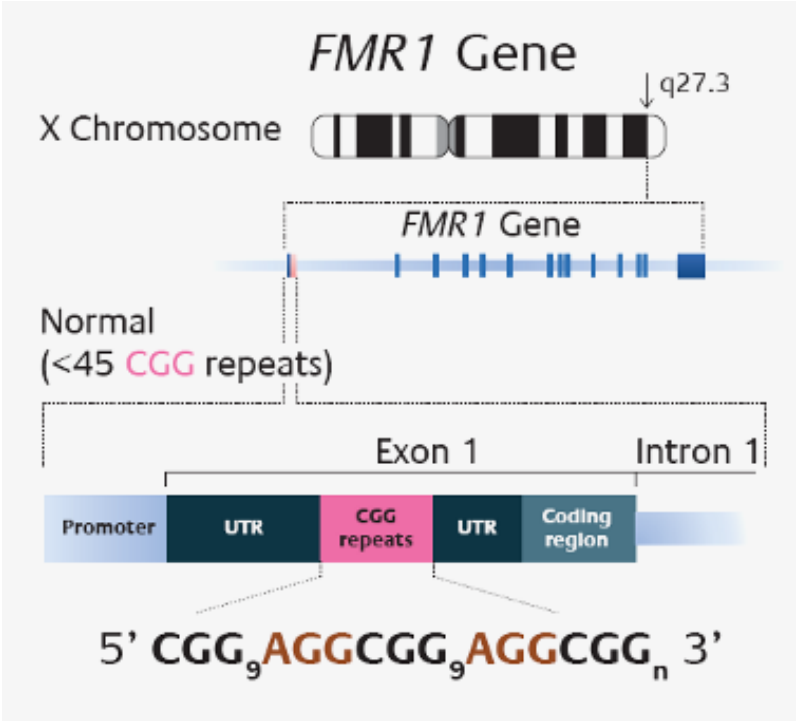
Anticipation

Number of Maternal Premutation CGG Repeats	Number which expanded to full mutations
55-59	1 (4%)
60-69	6 (5%)
70-79	28 (31%)
80-89	81 (58%)
90-99	89 (80%)
100-200	193 (98%)

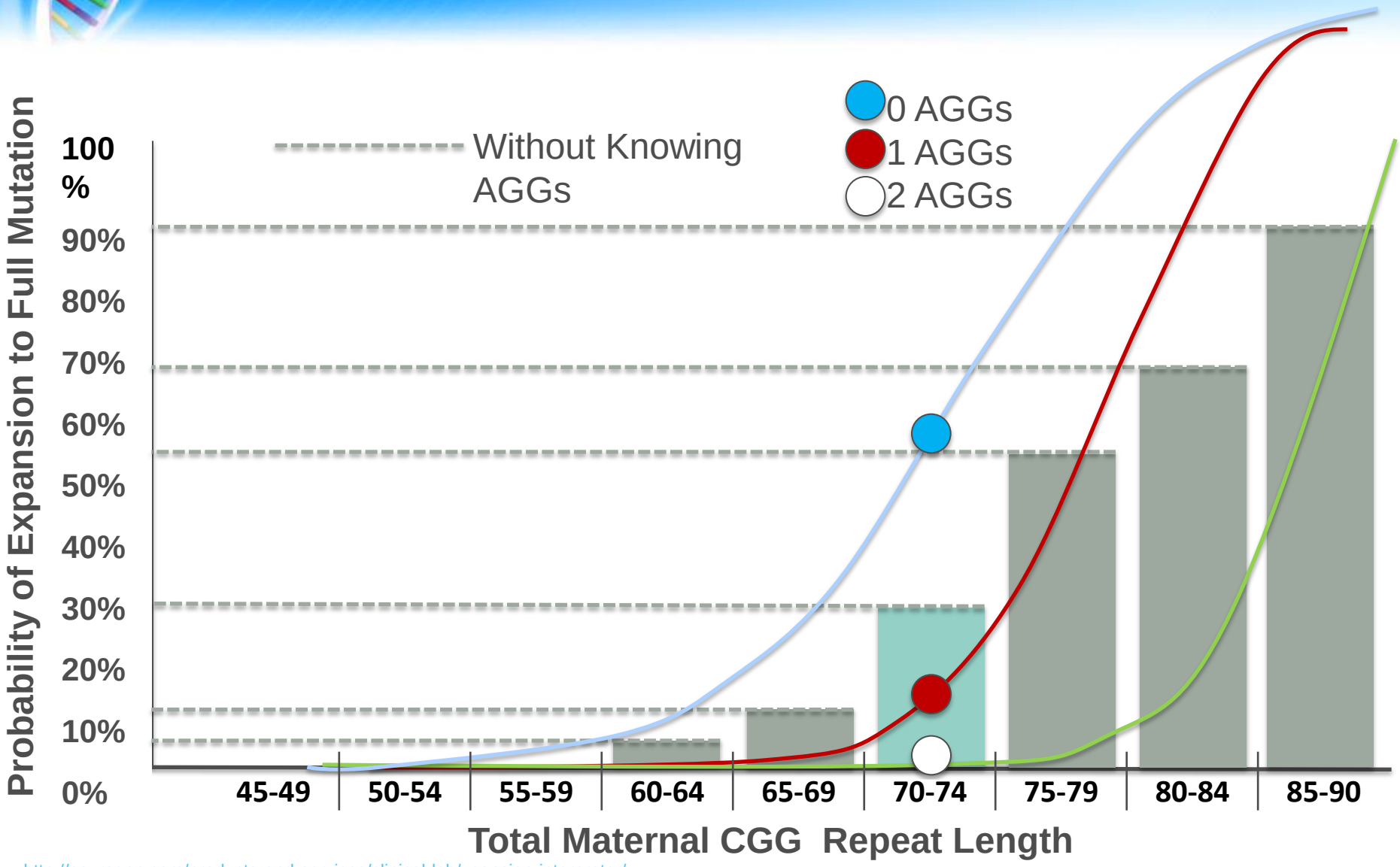
CGG repeat length is not the only factor accounting for instability

Stability of Allele

- AGG repeat
  - **In the normal population, CGG repeats interrupted by AGG at positions 10 and 20**
  - **Lead to stability to the repeat – think of it as an anchor**
  - **Some laboratories offer AGG interruption number**



Impact of AGGs



http://asuragen.com/products-and-services/clinical-lab/xpansion-interpreter/

Nolin et al., American Journal of Medical Genetics A. 2013;161(4):771-8.

Coppinger et al., Platform Presentation, American College of Medical Genetics Annual Clinical Genetics Meeting. 2013

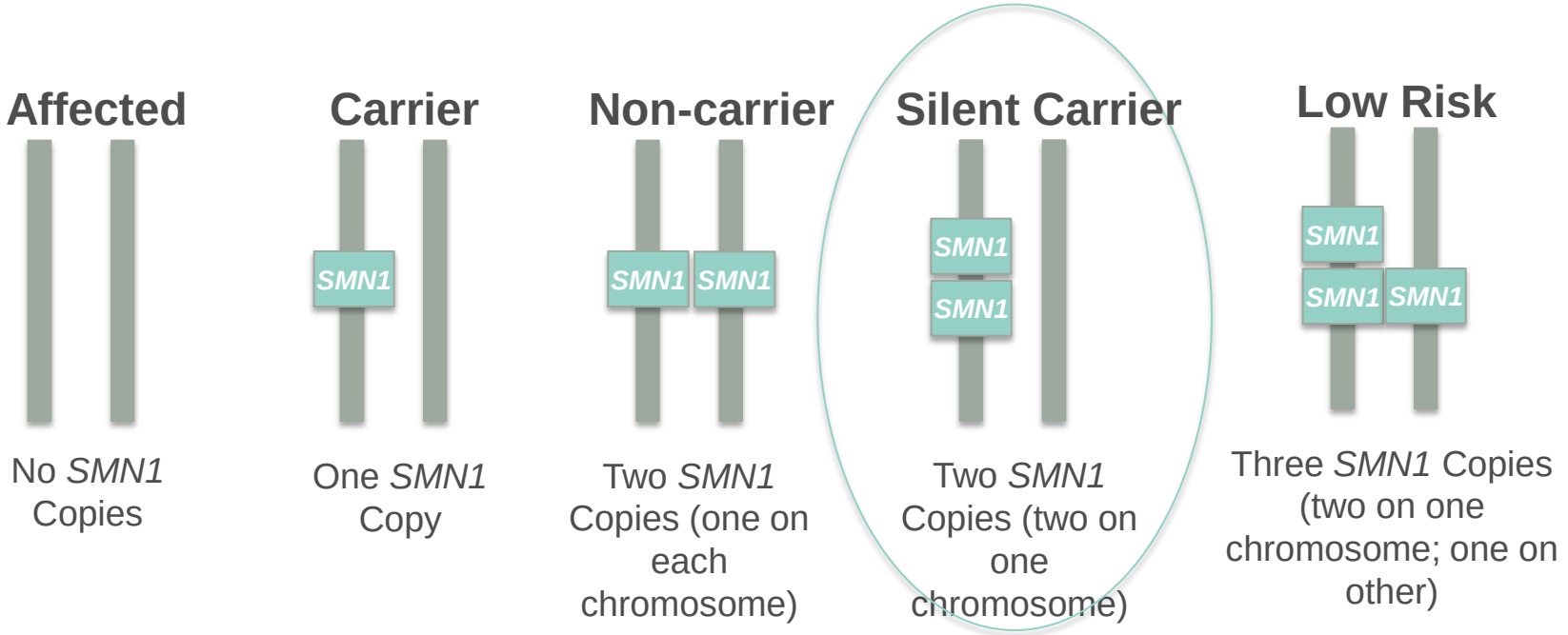
Current Guidelines – Targeted

- Spinal Muscular Dystrophy (SMA)
  - Severe Autosomal recessive Neuromuscular disease
  - Pan-ethnic carrier frequency: ~ 1:50
  - 1/6000 – 1/10,000
  - Standard SMA carrier screening determines SMN1 exon7 copy number

- Guidelines
  - ACOG – only with family history
  - ACMG – offer regardless of ancestry or family history

Gene Copy Number

- Routine carrier screening looks at *SMN1* gene copy number
- 2 copies of the *SMN1* gene could mean a non-carrier OR a silent carrier (2+0)

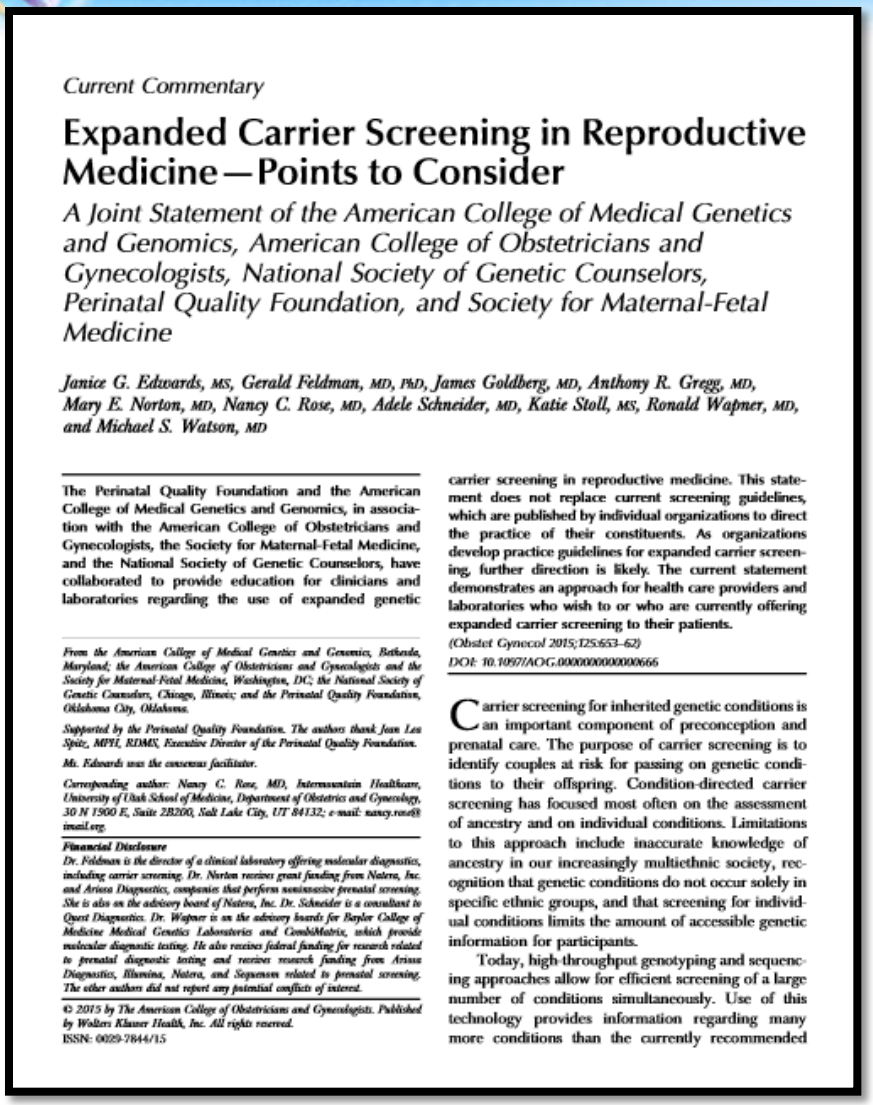


SNP Analysis for Silent Carriers

- SNP analysis
  - SNP in intron 7 of SMN1 (g.27134t>G) associated with “Silent Carriers”
  - If patient carries two SMN1 copies and the SNP is present, there is an increased liklihod of being a silent carrier
    - Ashkenazi Jewish or Asian – likely carrier
    - Caucasian, African American, Hispanic – risk increased
  - Routine screening would miss ~4% of carriers

1. Hendrickson BC et al. Differences in SMN1 allele frequencies among ethnic groups within North America. J Med Genet. 2009;46:641-644.
2. Luo M et al. An Ashkenazi Jewish SMN1 haplotype specific to duplication alleles improves pan-ethnic carrier screening for spinal muscular atrophy. Genet Med. 2014 Feb;16(2):149-56.

Expanded Carrier Testing



Current Commentary

Expanded Carrier Screening in Reproductive Medicine—Points to Consider

A Joint Statement of the American College of Medical Genetics and Genomics, American College of Obstetricians and Gynecologists, National Society of Genetic Counselors, Perinatal Quality Foundation, and Society for Maternal-Fetal Medicine

Janice G. Edwards, MS, Gerald Feldman, MD, PhD, James Goldberg, MD, Anthony R. Gregg, MD, Mary E. Norton, MD, Nancy C. Rose, MD, Adele Schneider, MD, Katie Stoll, MS, Ronald Wapner, MD, and Michael S. Watson, MD

The Perinatal Quality Foundation and the American College of Medical Genetics and Genomics, in association with the American College of Obstetricians and Gynecologists, the Society for Maternal-Fetal Medicine, and the National Society of Genetic Counselors, have collaborated to provide education for clinicians and laboratories regarding the use of expanded genetic carrier screening in reproductive medicine. This statement does not replace current screening guidelines, which are published by individual organizations to direct the practice of their constituents. As organizations develop practice guidelines for expanded carrier screening, further direction is likely. The current statement demonstrates an approach for health care providers and laboratories who wish to or who are currently offering expanded carrier screening to their patients.

(Obstet Gynecol 2015;125:653–62)
DOI: 10.1097/AOG.0000000000000666

From the American College of Medical Genetics and Genomics, Bethesda, Maryland; the American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine, Washington, DC; the National Society of Genetic Counselors, Chicago, Illinois; and the Perinatal Quality Foundation, Oklahoma City, Oklahoma.

Supported by the Perinatal Quality Foundation. The authors thank Jean Lee Spitz, MPH, RDMS, Executive Director of the Perinatal Quality Foundation.

Ms. Edwards was the consensus facilitator.

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Financial Disclosure

Dr. Feldman is the director of a clinical laboratory offering molecular diagnostics, including carrier screening. Dr. Norton receives grant funding from Natera, Inc. and Ariosa Diagnostics, companies that perform noninvasive prenatal screening. She is also on the advisory board of Natera, Inc. Dr. Schneider is a consultant to Quest Diagnostics. Dr. Wapner is on the advisory boards for Baylor College of Medicine Medical Genetics Laboratories and CombiMatrix, which provide molecular diagnostic testing. He also receives federal funding for research related to prenatal diagnostic testing and receives research funding from Ariosa Diagnostics, Illumina, Natera, and Sequenom related to prenatal screening. The other authors did not report any potential conflicts of interest.

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Carrier screening for inherited genetic conditions is an important component of preconception and prenatal care. The purpose of carrier screening is to identify couples at risk for passing on genetic conditions to their offspring. Condition-directed carrier screening has focused most often on the assessment of ancestry and on individual conditions. Limitations to this approach include inaccurate knowledge of ancestry in our increasingly multiethnic society, recognition that genetic conditions do not occur solely in specific ethnic groups, and that screening for individual conditions limits the amount of accessible genetic information for participants.

Today, high-throughput genotyping and sequencing approaches allow for efficient screening of a large number of conditions simultaneously. Use of this technology provides information regarding many more conditions than the currently recommended

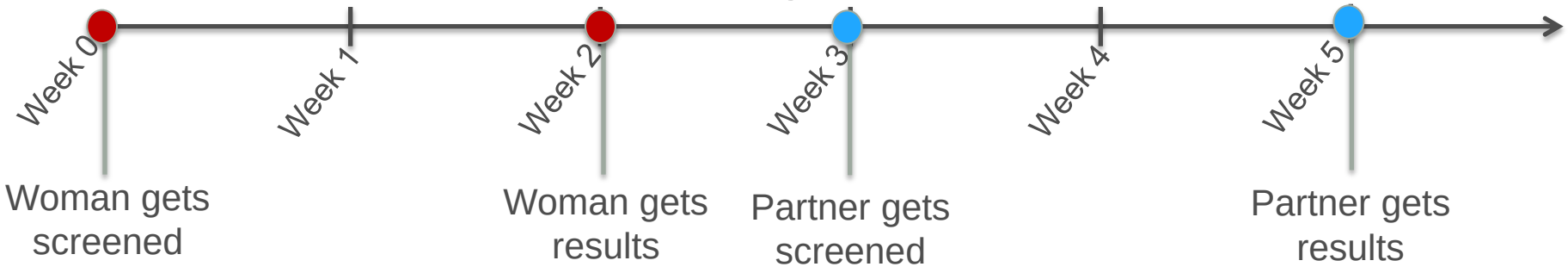
Conditions included on a carrier screening panel should encompass one or more of the following criteria:

- Cognitive disability
- Need for surgical or medical intervention
- Effect on quality of life
- Prenatal diagnosis may result in prenatal intervention to improve perinatal outcomes, delivery management, or prenatal education of parents to prepare for special needs after birth

Concurrent Carrier Screening

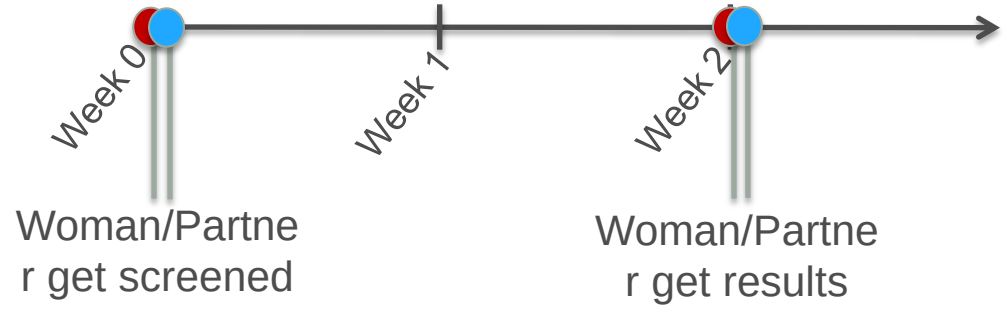
Speeds Up Identification of Potential Risk to the Fetus

Timeline of Individual Screening



Total turnaround time can be 5+ weeks…With two positives, is there still time for CVS or amnio? And what about the emotional stress of waiting?

Timeline of Concurrent Screening



Done in about 2 weeks = more time for CVS or amnio AND reduced emotional burden

Considerations

- Hex A and CBC/hemoglobin electrophoresis still most appropriate.
- Recommend pre and post test counseling
  - **Varying degrees of severity and inheritance**
  - **Risk reduction not elimination**
  - **Patient could be found with two mutations**
  - **Paternal information is needed for accurate risk**
  - **Sequencing possible but not routinely recommended**
  - **Offer follow-up if applicable**

If You Would Like to Refer a Patient or Have a Question …

We'd be happy to help!
Call Perinatal Genetics at 581-7825