Non-invasive prenatal screening for aneuploidies and microdeletions

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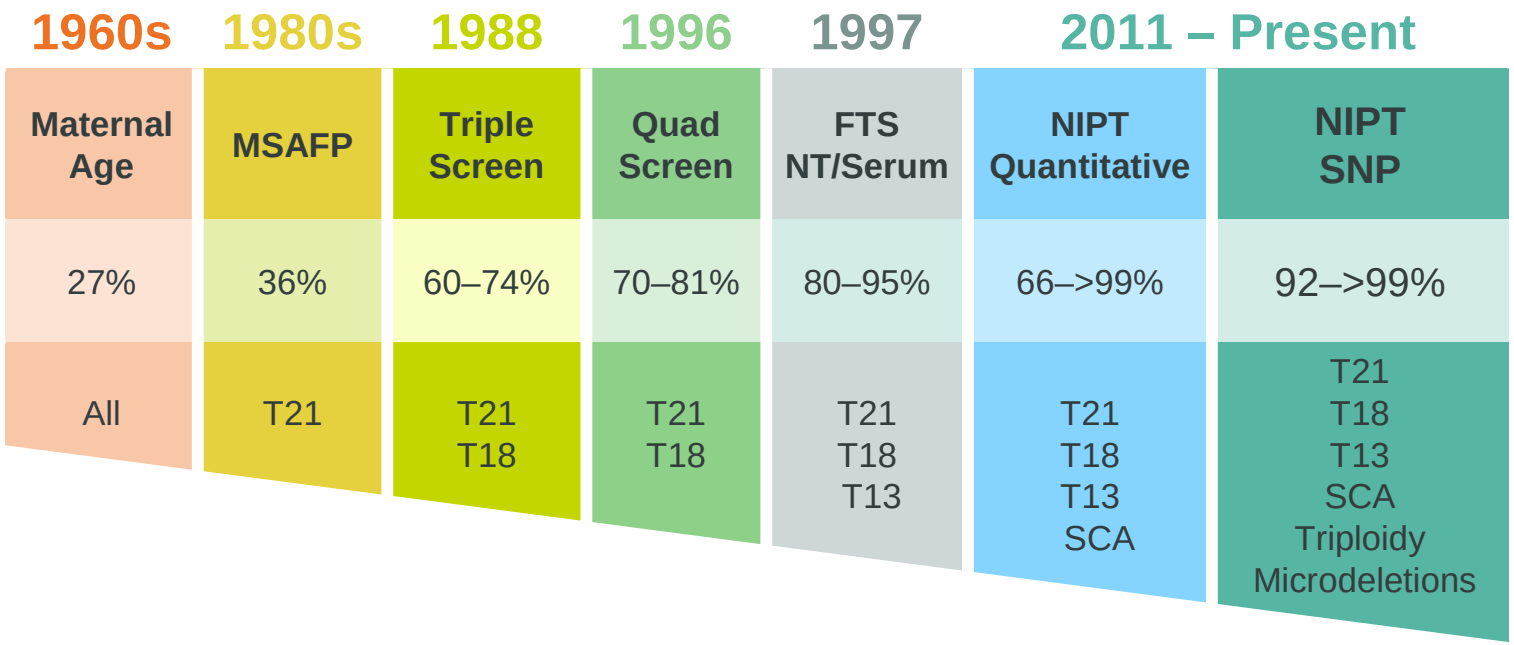
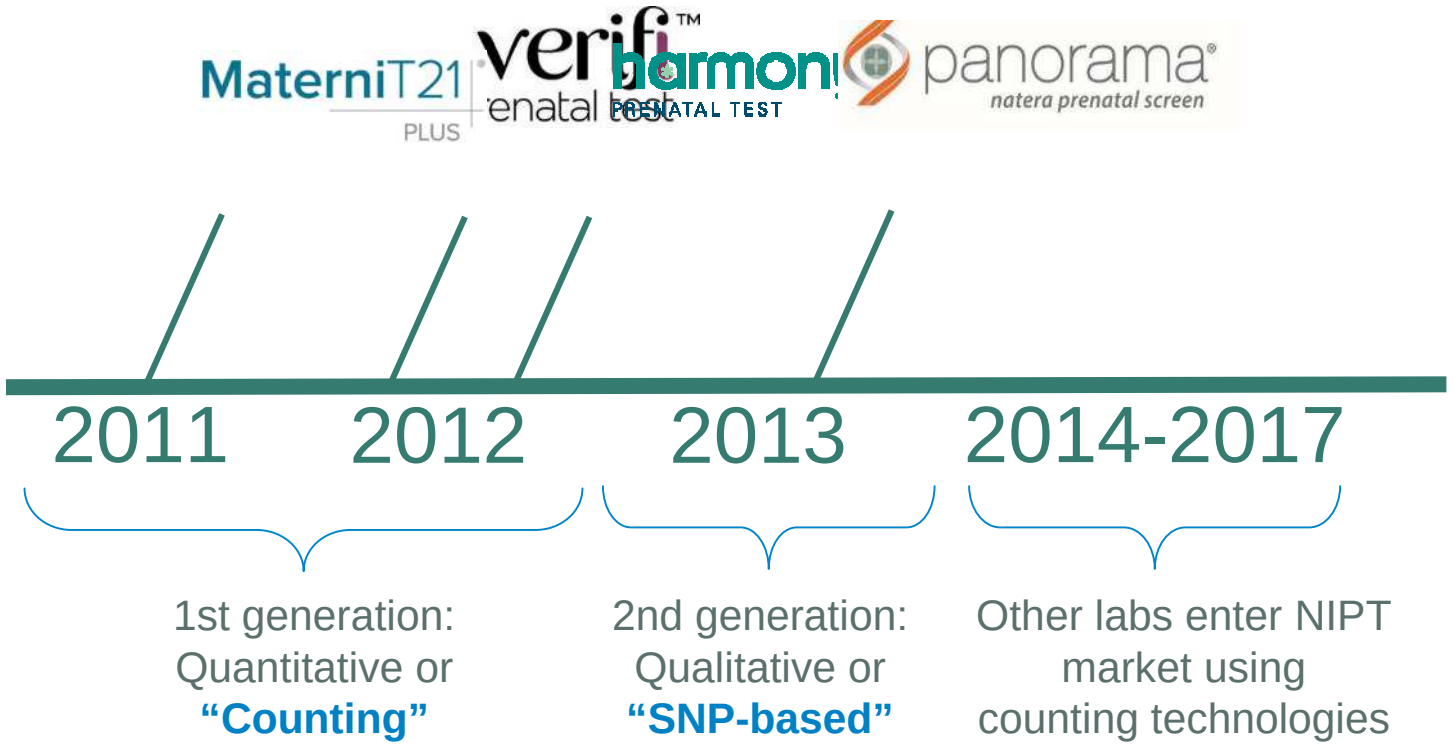
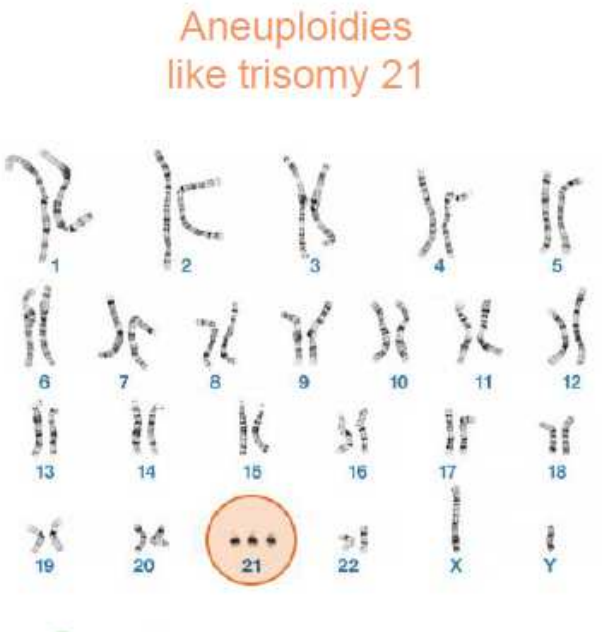
Prenatal Screening



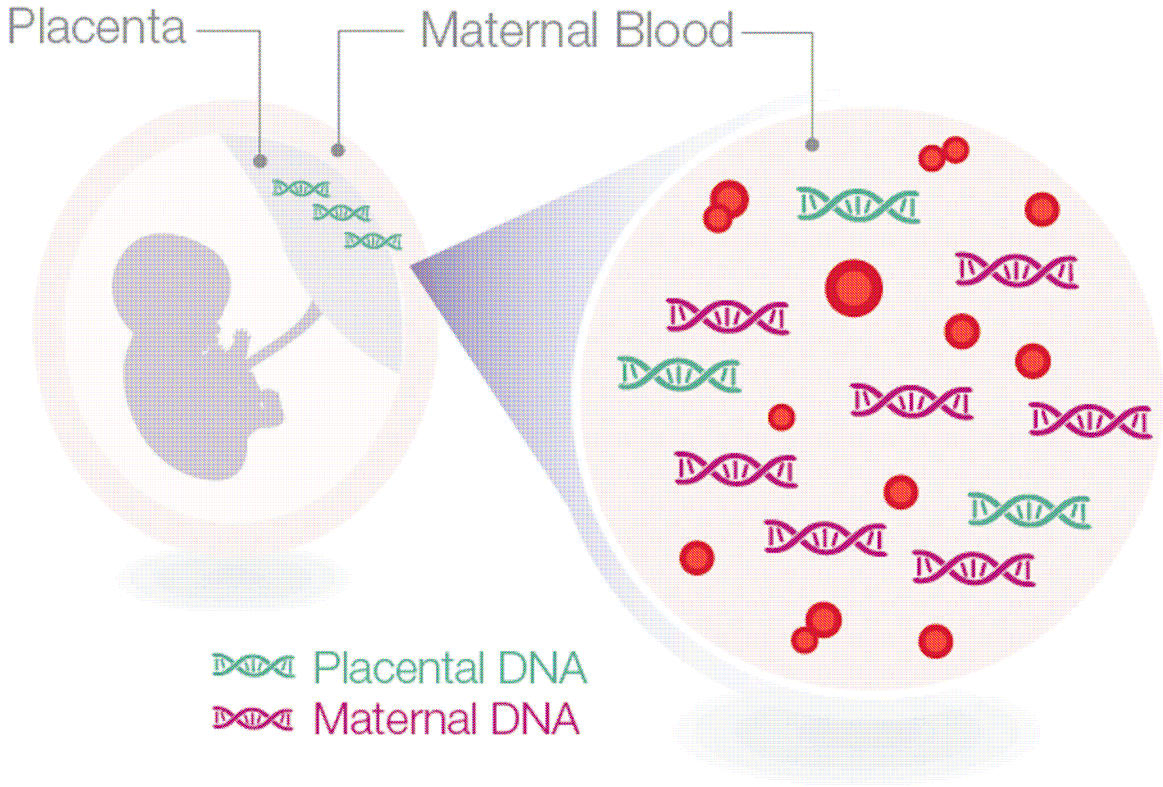
Evolution of NIPT



Evolution of NIPT



Cell-free DNA (cfDNA)



Conditions screened for by NIPT

- Trisomy 21, 18, 13
- Monosomy X
- 22q11.2 deletion syndrome
- Additional microdeletions
- Fetal sex
- Sex chromosome trisomies
- Triploidy
- Vanished twin

Comparing Screening Options

	Maternal Serum Screening[1–6]	SNP-based NIPT[7–8]
Gestational age	~11–22 wks	9+ wks
Nuchal Translucency	FTS	No
Open Neural Tube Defects	AFP	No
T21 Positive Predictive Value	3.4%	91%
False positive rate	5%	<1%

[1]Nicolaides K H et al. Ultrasound Obstet Gynecol. 2005; 25(3)221-6.
[2]Wapner R et al. N Engl J Med. 2003; 349 (15); 1405-13.
[3]Malone FD et al. N Eng J Med. 2005; 353(19): 2001-11.
[4]PerkinElmer Labs / NTD 2013, http://ntdlabs.com/maternal-marker-testing/.
[5]Quest Diagnostics 2014, www.questdiagnostics.com
[6]Norton M et al. NEJM. 2015 Apr 23;372(17):1589-97
[7]Pergament et al. Obstet Gynecol. 2014 Aug;124(2 Pt 1):210-8
[8]Dar P et al. Am J Obstet Gynecol 2014 Nov;211(5):527

Trisomy 21 Positive Predictive Value

Maternal Serum Screening

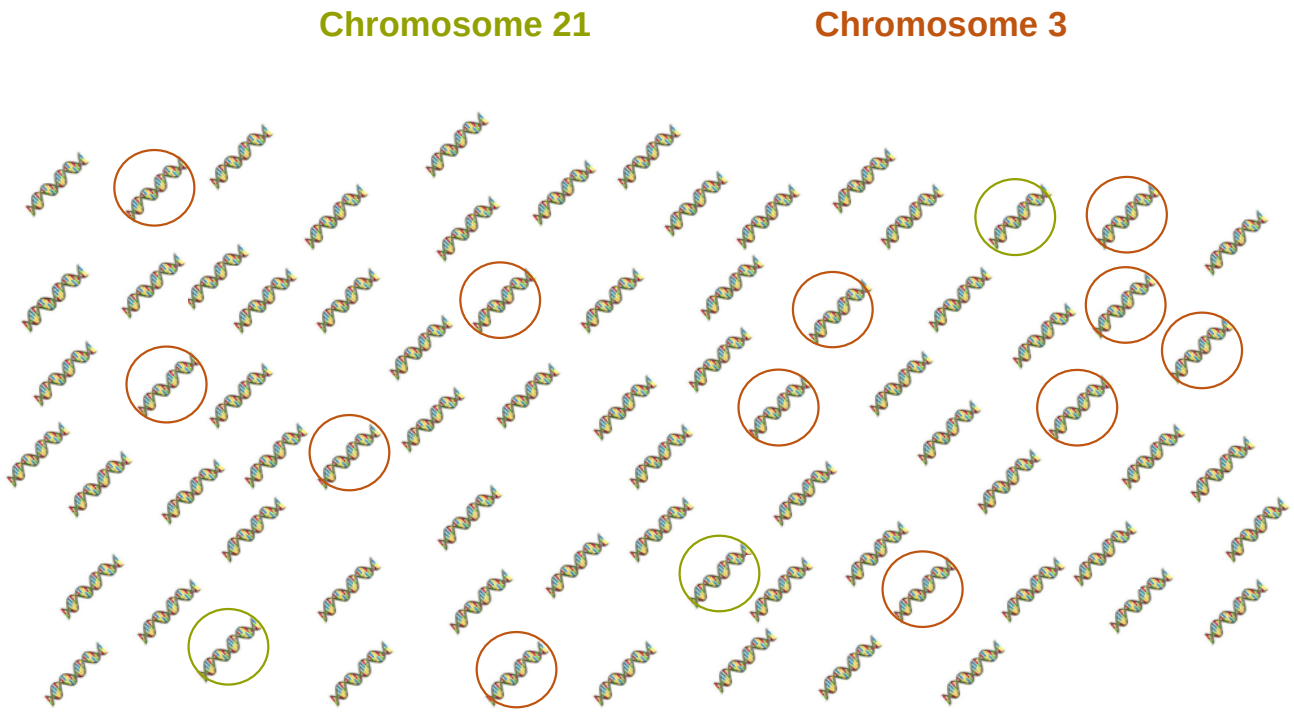
265 women will undergo invasive testing to discover **9** true positives.[1]

1Norton et al, N Engl J Med 2015.
2Dar et al. Am J Obstet Gynecol 2014 Nov;211(5):527

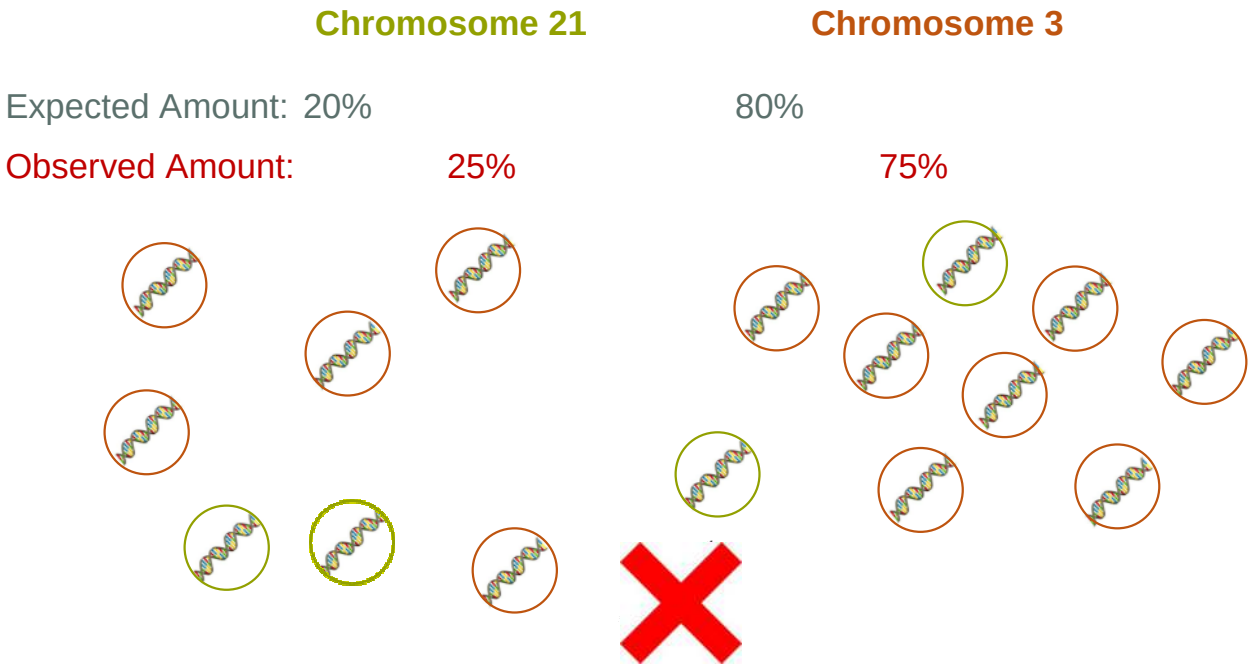
NIPT Methodologies

- Counting (quantitative)
  - Massively Parallel Shotgun Sequencing
  - Targeted Sequencing
- SNP (qualitative)
  - Targeted Sequencing

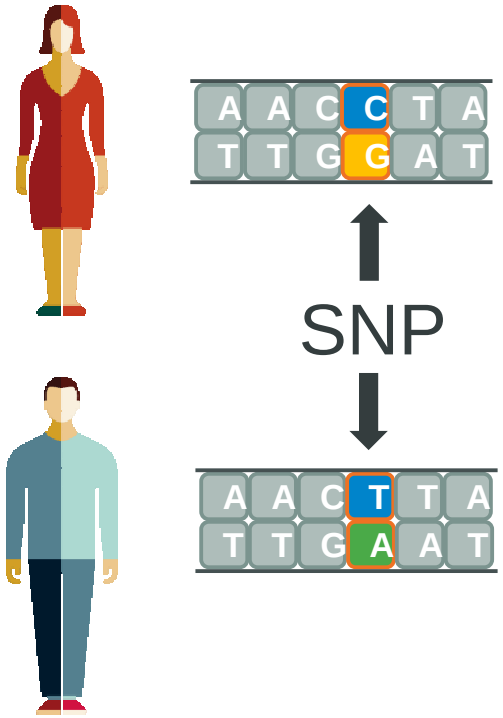
Counting



Counting

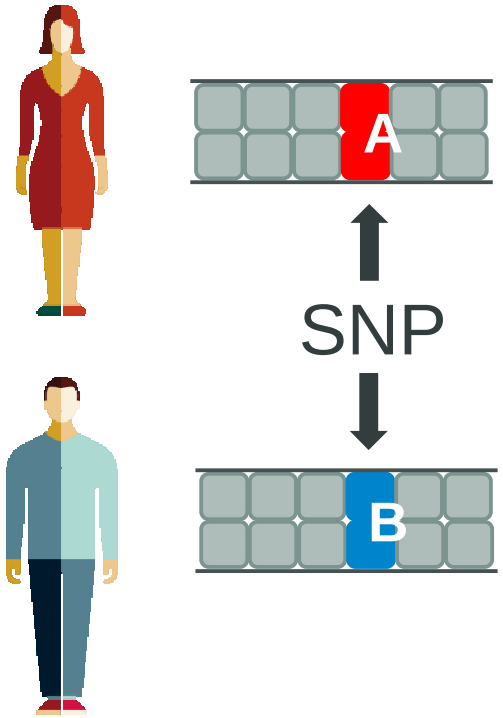


SNP = Single Nucleotide Polymorphism



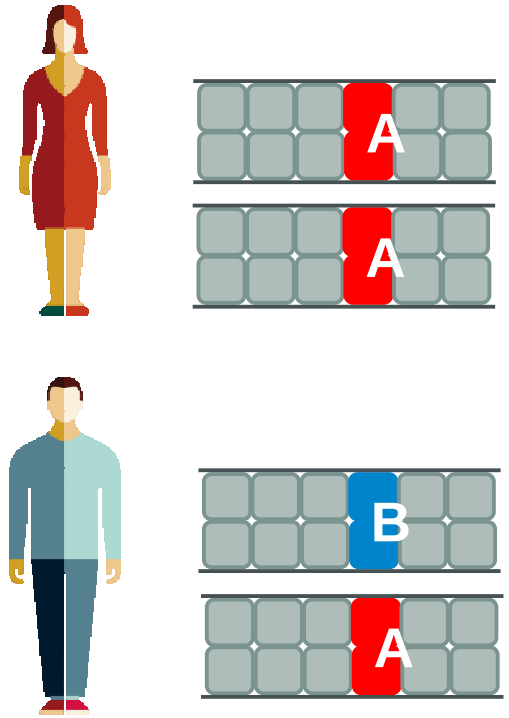
- A DNA sequence variation occurring when a single base pair is changed
- Normal genetic changes that occur in every person

SNP = Single Nucleotide Polymorphism



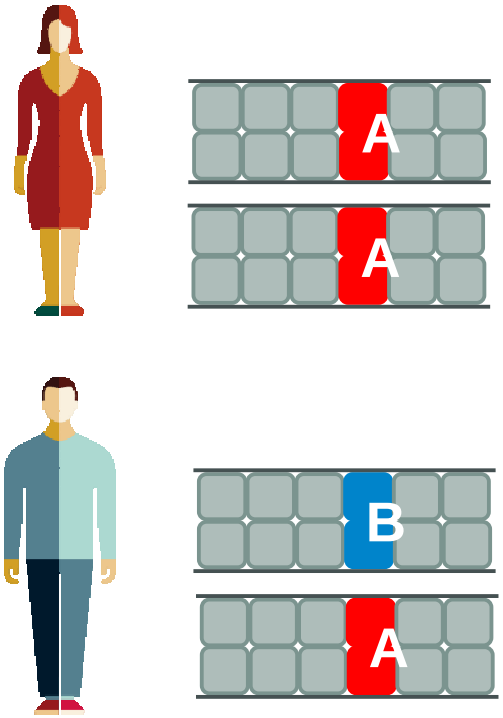
- All analyzed SNPs are assumed to be biallelic
- For simplicity, we designate this as **A** and **B**

SNP = Single Nucleotide Polymorphism

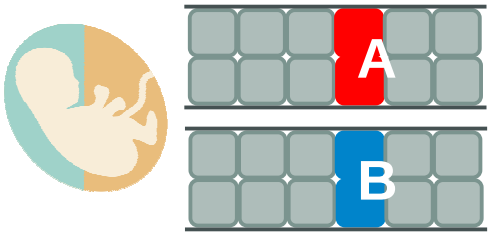


- Since chromosomes come in pairs, we would inherit two SNPs at the same location, and could be **A/A**, **A/B**, or **B/B** for each SNP

SNP = Single Nucleotide Polymorphism

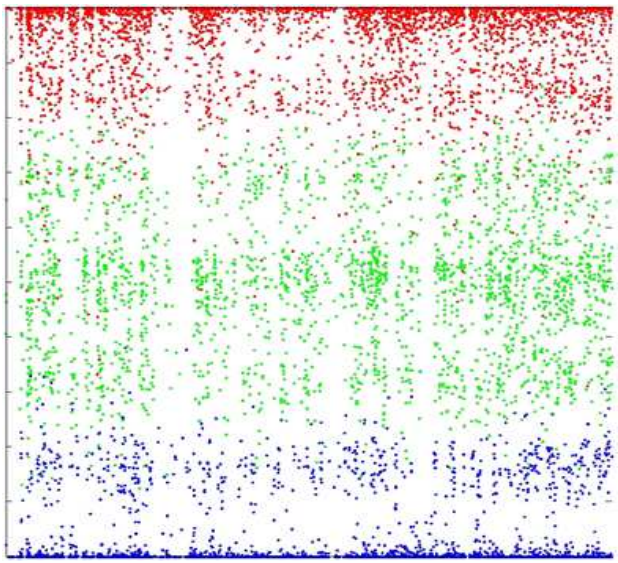


- Baby inherits one SNP from mom and one from dad. In this example baby inherits **A** from mom and **B** from dad. Baby is **A/B** for this SNP.



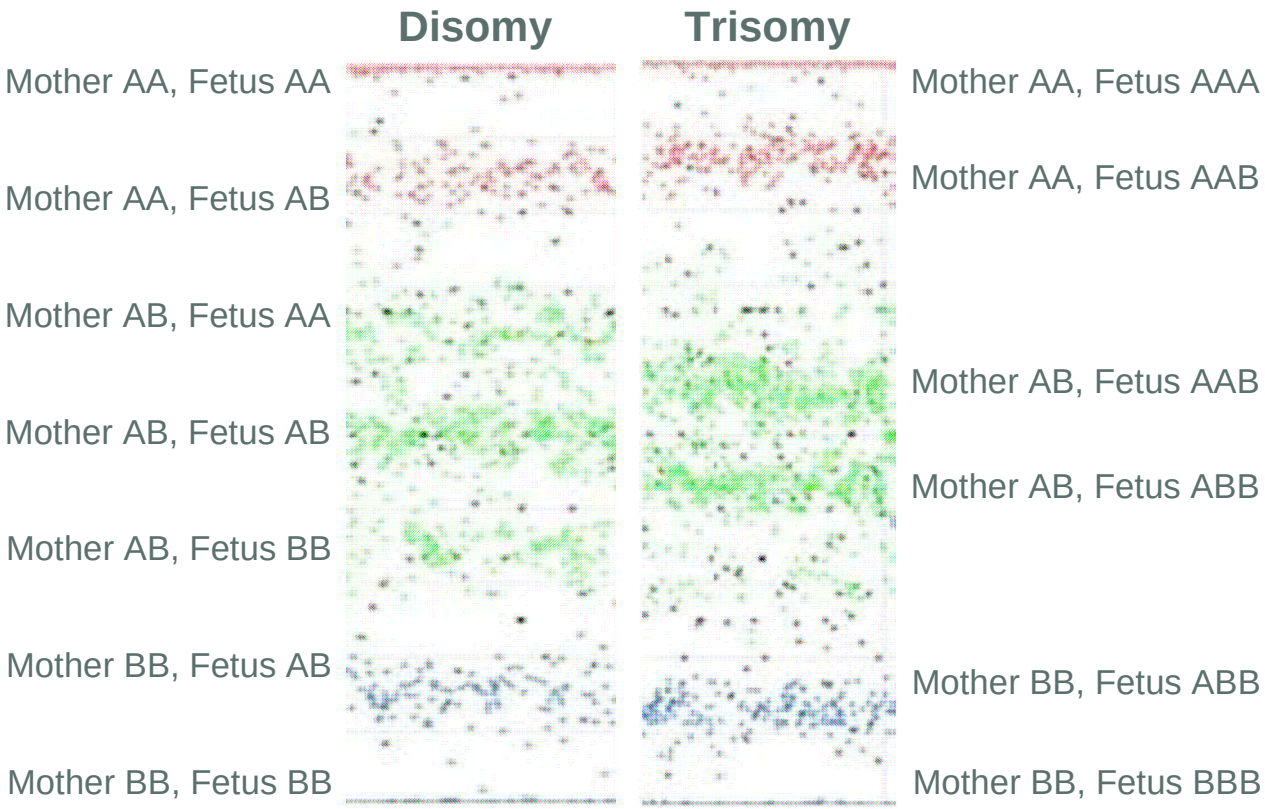
SNP-based NIPT analyzes >13,000 SNPs

- Analysis is based on allele ratios at SNPs (genotypes)
- Plasma allele ratios reflect mixture of maternal and fetal genotypes
- Fetal genotypes reflect ploidy state



Each dot is one SNP and is the **sum** of both the maternal and fetal contribution

Breaking down a SNP profile



DNA “fingerprints”

- SNP-based NIPT is the only NIPT on the market that distinguishes between maternal and fetal DNA.
- SNP-based NIPT can identify different DNA “fingerprints” in a maternal blood sample, such as those from a vanishing twin.

Clinical Advantages of SNP-based NIPT

Uniquely differentiates between maternal and fetal DNA

- Fetal fraction
- Maternal contribution
- Vanishing twins
- Fetal sex accuracy
- Triploidy

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Fetal Fraction (FF) Matters

"…the measurement of fetal cfDNA is a basic quality metric required to ensure reliable interpretation of test results."[1]

$$\frac{\text{Fetal DNA}}{\text{Fetal DNA} + \text{Maternal DNA}}$$

- Average fetal fraction is 10–12%*
- Varies by gestational age, maternal weight, placental and pregnancy factors
- SNP-based NIPT cutoff: 2.8%

[1]Takoudes et al., *Ultrasound Obstet Gynecol* 2015; 45: 112–116
*at time of NIPT

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Maternal X Mosaicism

Without differentiating maternal and fetal DNA, counting methods may incorrectly report a fetal sex chromosome abnormality.

Clinical	NIPT findings	Total
NIPT follow-up	Abnormal NIPT for SCA, n	187
	Normal maternal karyotype, n	171
	Abnormal maternal karyotype, n	16
	Maternal mosaicism rate	8.56%

Russell LM, et al. X chromosome loss and ageing. Cytogenet Genome ,116:181-185.

Clinical Advantages of SNP-based NIPT

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Case Example: Vanishing twin

- 35 year-old G2P1
- History of “vanishing twin” at 8 weeks gestation
- SNP-based NIPT at 10 weeks: “Results consistent with possible triploid or vanishing twin”
- Counting NIPT at 13 weeks: “Normal male fetus”
- Ultrasound at 17 weeks: female fetus
- Delivery: **normal female, 46 XX chromosomes**

Su B et al. Discordant NIPT and ultrasound results from vanishing twin pregnancy . Contemporary Ob/GYN April 2015.

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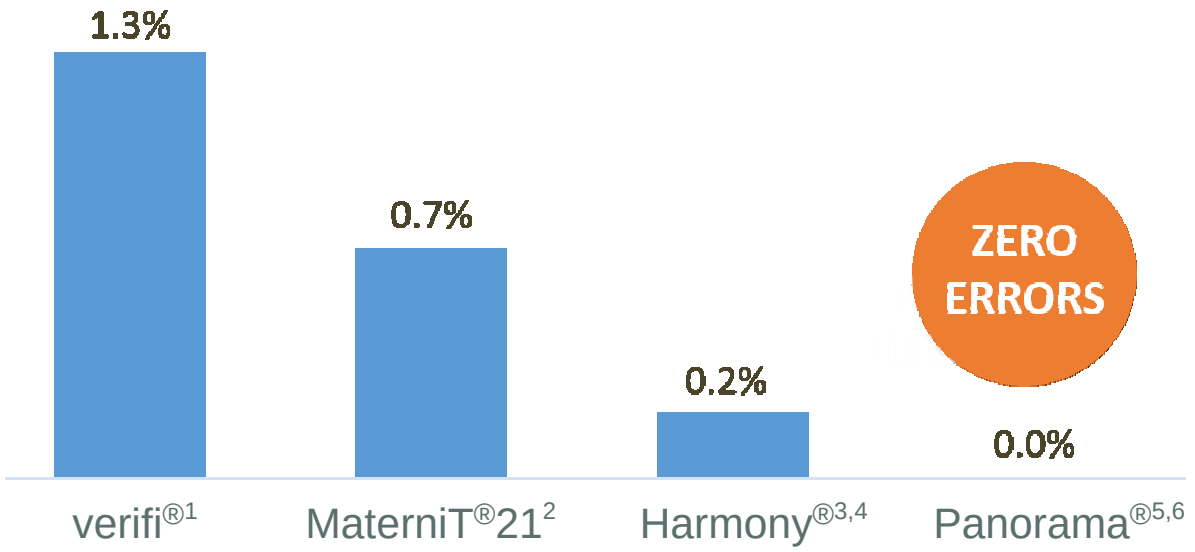
- Triploidy

Error Rate in Sex Determination

In validation studies, as many as 1 in 75 cases can have gender discrepancy when using counting methodologies.



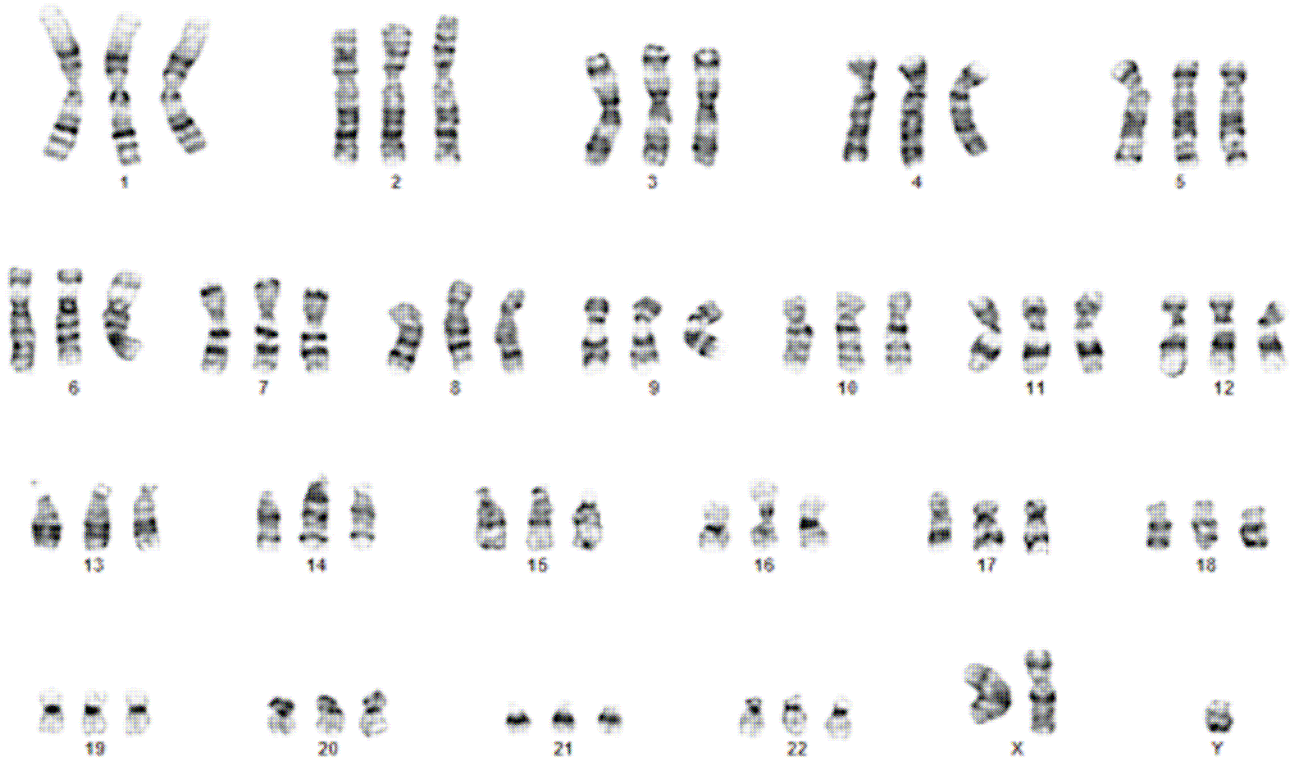
1.Verinata white paper. Analytical validation of the Verifi prenatal tes: enhanced test performance for detecting trisomies 21, 18 and 13 and the option for classification of sex chromosome status. 2012
2.Mazloom et. al. 2013. Prenat Diagn.
3.Nicolaides et. al. 2013. Fetal Diagn Ther.
4.Hooks et al. 2014. Prenat. Diagn.
5.Pergament et al. 2014. Obstetrics & Gyn.
6.Nicolaides et. al. 2013. Prenat Diagn.

Clinical Advantages of SNP-based NIPT

Uniquely differentiates between maternal and fetal DNA

- Fetal fraction
- Maternal contribution
- Vanishing twins
- Fetal sex accuracy
- Triploidy

Triploidy



Only SNP-based NIPT Can Detect Triploidy

- 1 in 1000 pregnancies at 10 weeks[1]
- Risk for partial molar pregnancy
- Provides risk assessment for couples with prior pregnancy with triploidy
- Counting method cannot recognize extra copy of all chromosomes

1Snijders, et al. Fetal Diagn Ther 1995; 10:357-9.
2Berkowitz, RS and Goldstein, DP, Cancer 1995; 76: 2079–2085.
3Soper, J. Obstet Gynecol 2006; 108:176–87
4Chromosome Abnormalities and Genetic Counseling, Gardner and Sutherland, 2004.

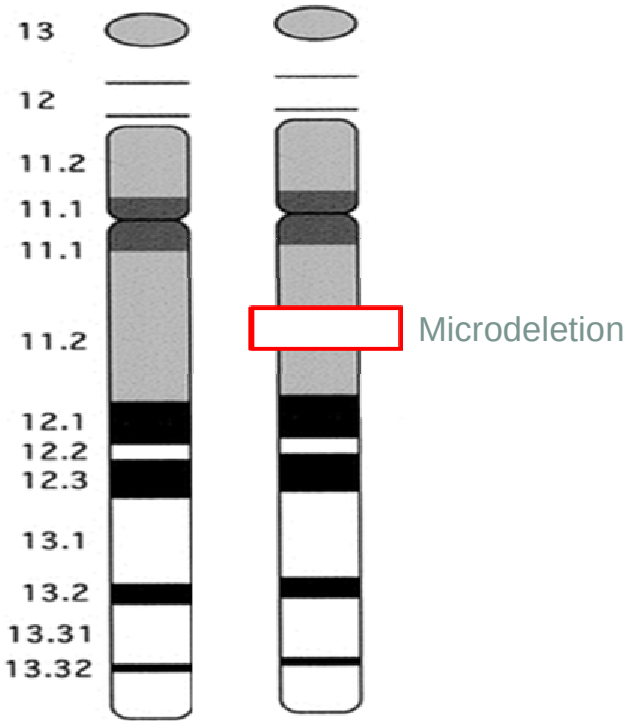
Clinical Advantages of SNP-based NIPT

Uniquely differentiates between maternal and fetal DNA

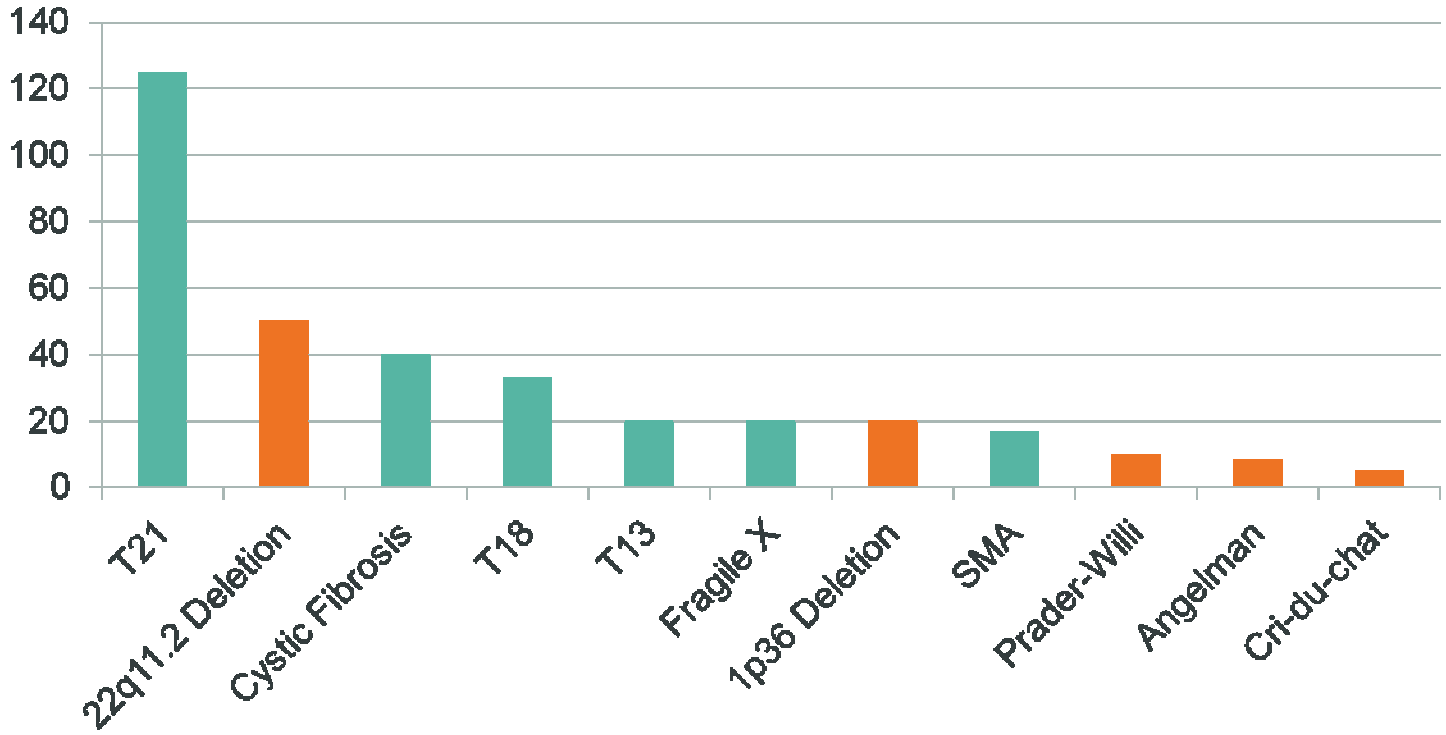
- Fetal fraction
- Maternal contribution
- Vanishing twins
- Fetal sex accuracy
- Triploidy
- Microdeletions

Screening for microdeletions

- Microdeletions vary in size
- Karyotype can usually only visually detect >7–10 MB
- Clinical outcome depends on size & genes involved

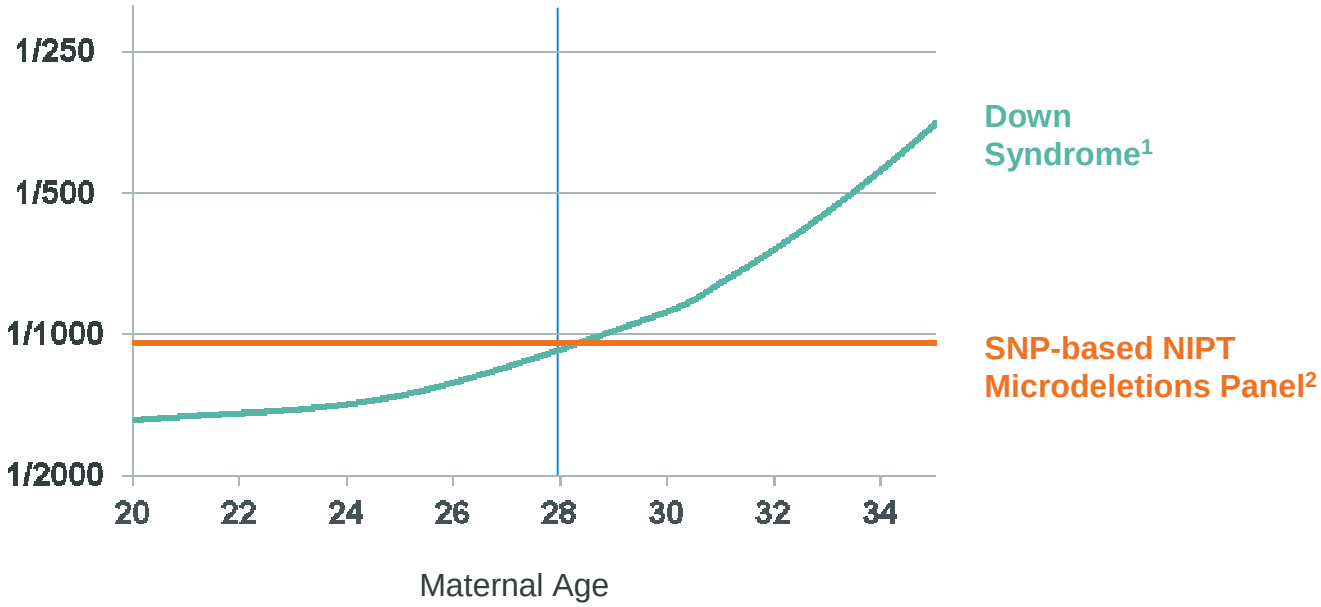


Incidence out of 100,000 Live Births



Rethink screening

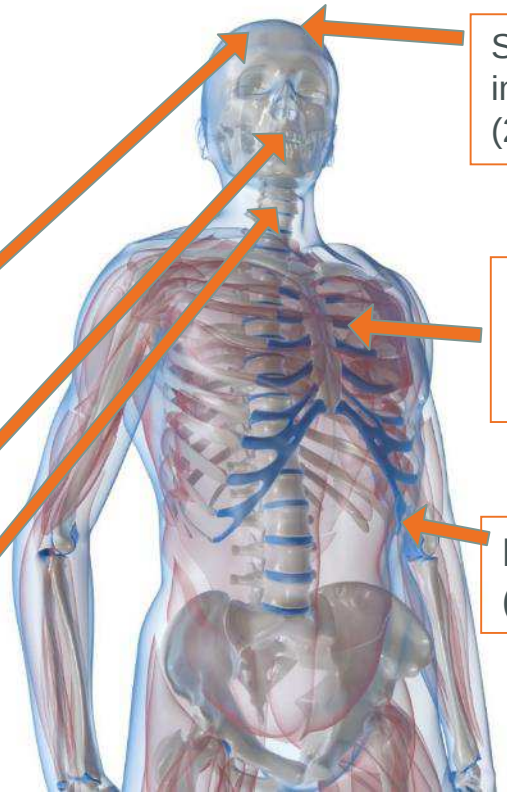
Microdeletions are more common than Down syndrome in younger women



1Snijders, et al. Ultrasound Obstet Gynecol 1999;13:167–170.
2Combined 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.

22q11.2 Deletion Syndrome

"…occurs in approximately 1 in every 2,000 to 4,000 live births, although this is likely a gross underestimate of its prevalence. It is thought to be almost as common as Down syndrome."[1]



- Schizophrenia in adulthood (25%)
- Congenital heart defects (75%)
- Hypocalcemia (77%)
- Developmental delay and learning disabilities (70-90%)
- Palatal abnormalities (70%)
- Immune deficiencies (75%)

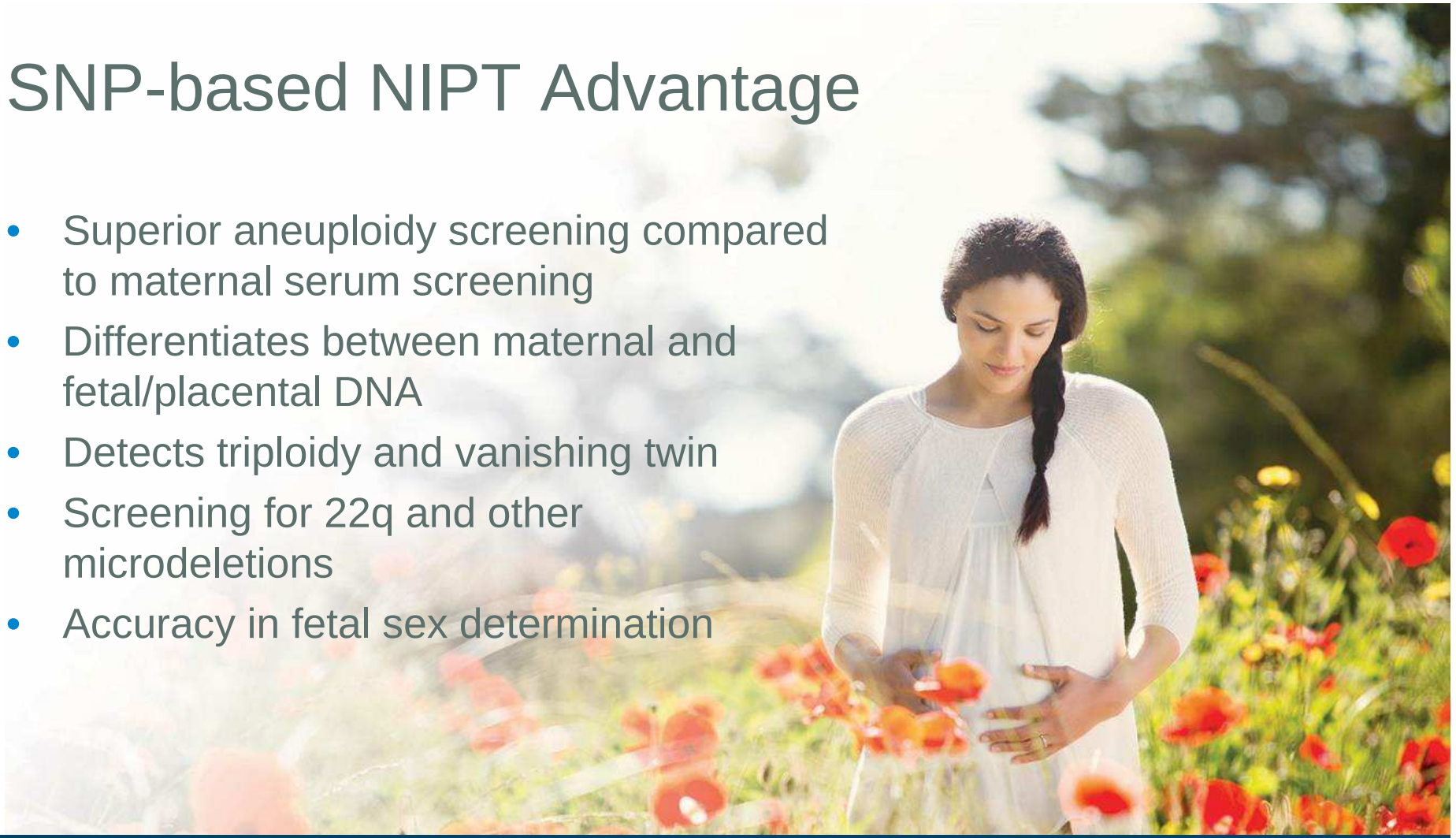
[1]International 22q11.2 Foundation – Handbook and www.22q.org
[2]www.genereviews.org
[3]Wapner R et al. NEJM 2012; 367:(23) 2175-2184.
[4]Grati F et al. Prenat Diag 2015. In press.

Early Intervention Matters

- Deliver baby at tertiary care center
- Delay in administering live vaccines
- Monitor calcium levels
- Check palate for clefting and velopharyngeal insufficiency

SNP-based NIPT Advantage

- Superior aneuploidy screening compared to maternal serum screening
- Differentiates between maternal and fetal/placental DNA
- Detects triploidy and vanishing twin
- Screening for 22q and other microdeletions
- Accuracy in fetal sex determination




Thank you.