SNP-based Non-invasive Prenatal Testing (NIPT)

Savina Adamo, PhD

"Pre-implantation Genetic Diagnosis (PGD) e Non Invasive Prenatal Testing (NIPT): Nuove frontiere in diagnosi prenatale"
Padova, 18-19 Gennaio 2016

Agenda

- SNP-NIPT concept and analysis
- Cromosomes and counting with MPSS
- Clinical advantages of SNP-NIPT

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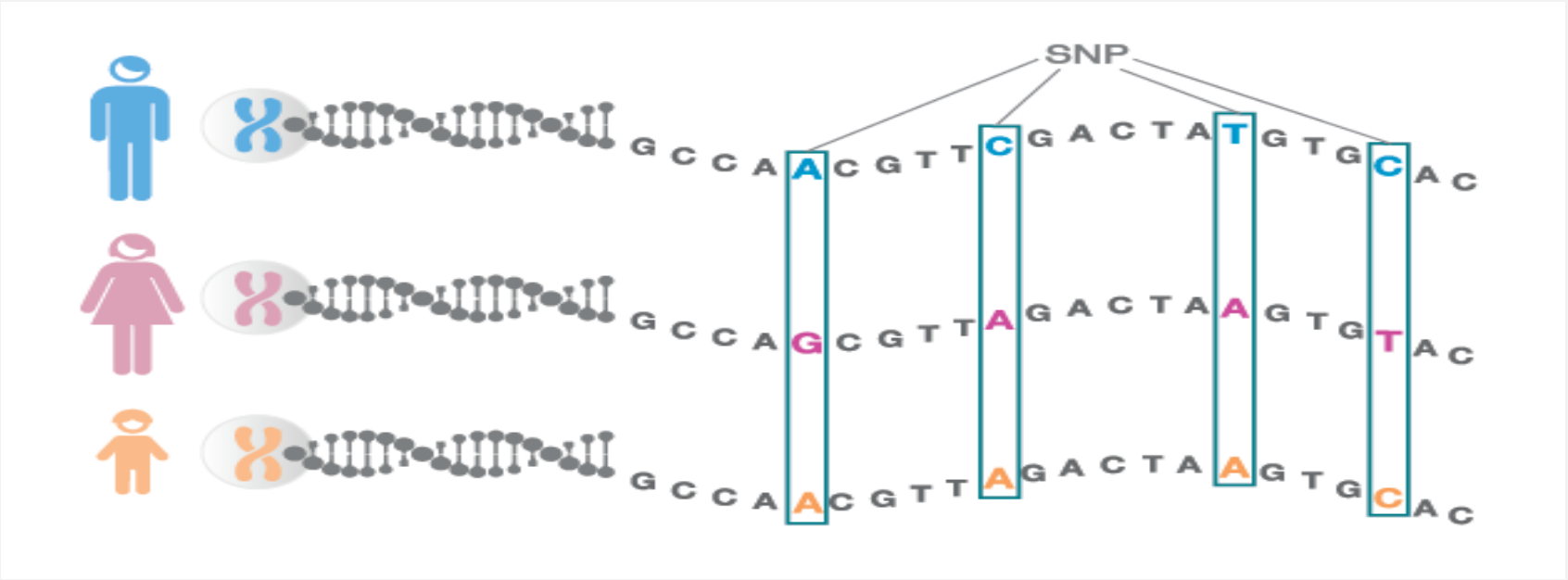
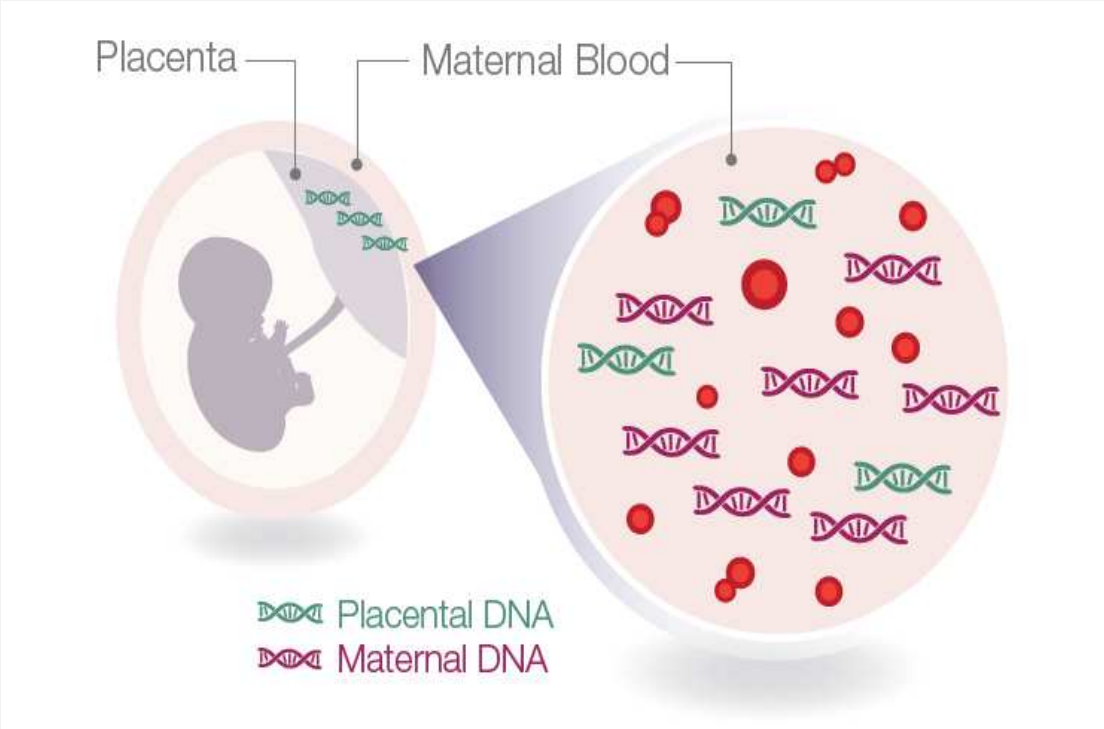
SNP = Single Nucleotide Polymorphism



- A DNA sequence variation occurring when a single base pair (nucleotide) - A, T, C, or G – is changed.
- These are **normal** genetic changes that occur in every person

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

How a SNP-NIPT works

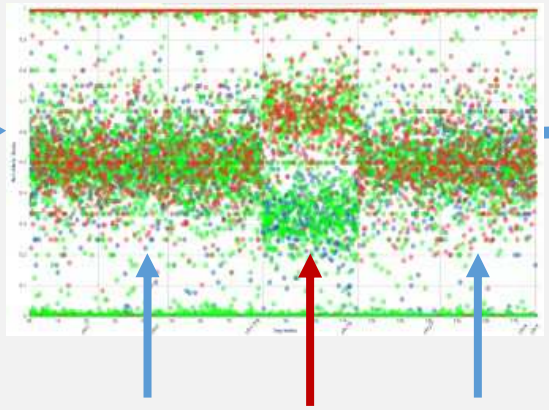
Maternal blood

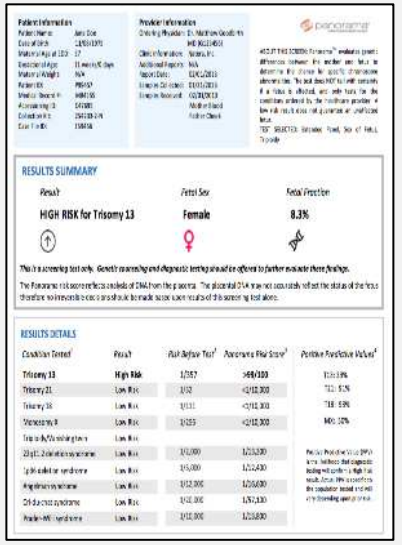
Maternal + Fetal cfDNA

SNP Sequencing of Maternal + Fetal Genotype

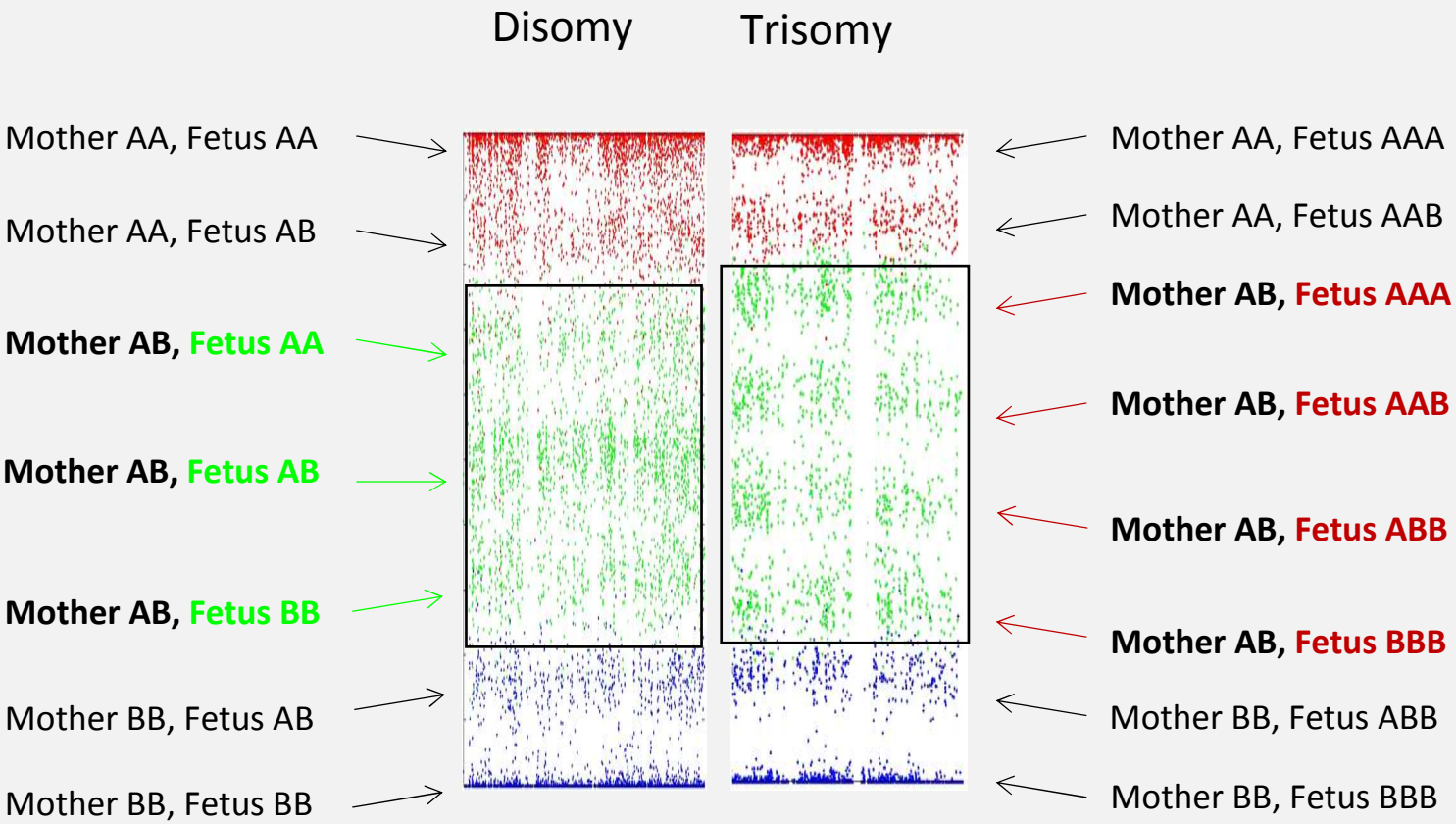
Advanced Bioinformatics

Report
(Including fetal fraction for all results and PPV for positive results)





Breaking down a SNP profile



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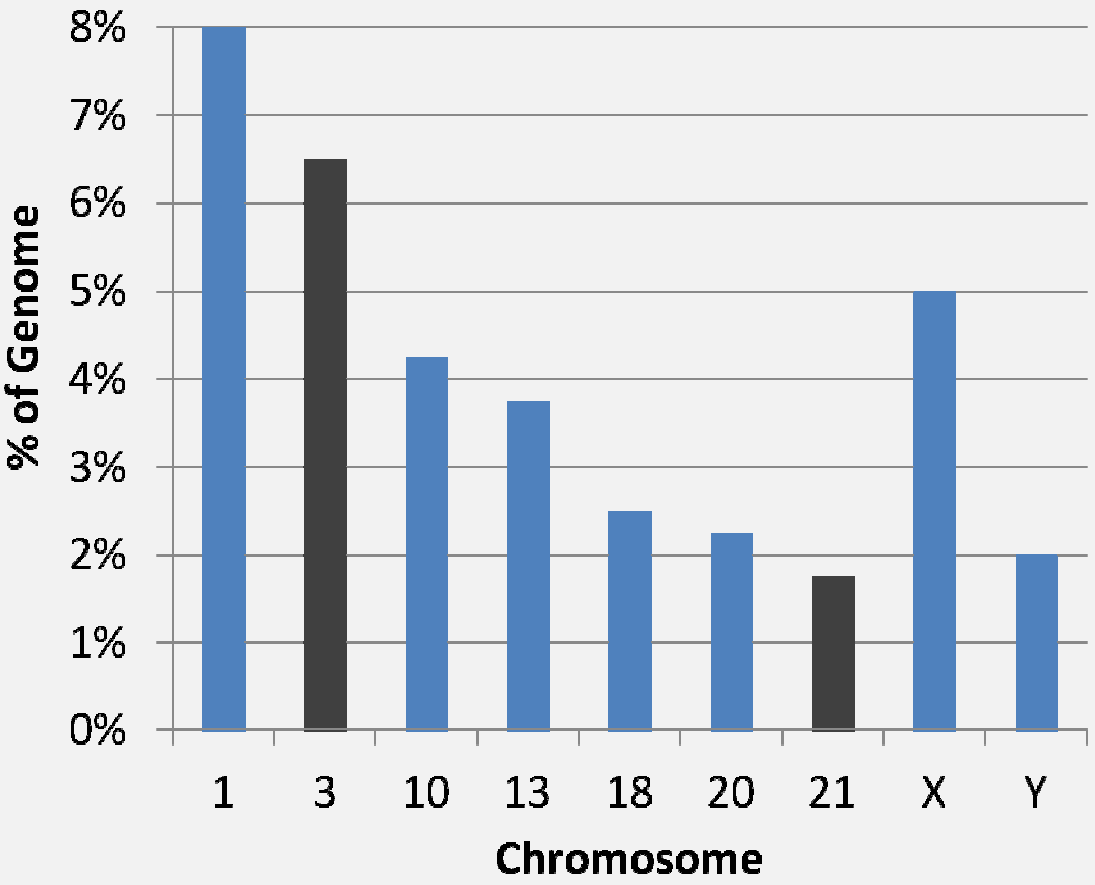
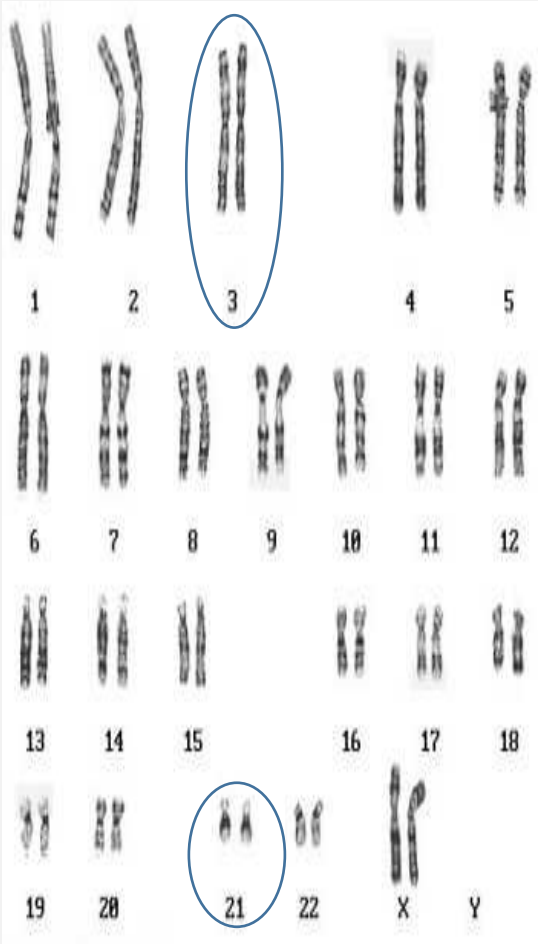
- Cromosomes and counting
- Clinical advantages of SNP-NIPT

Relative Size of Chromosomes



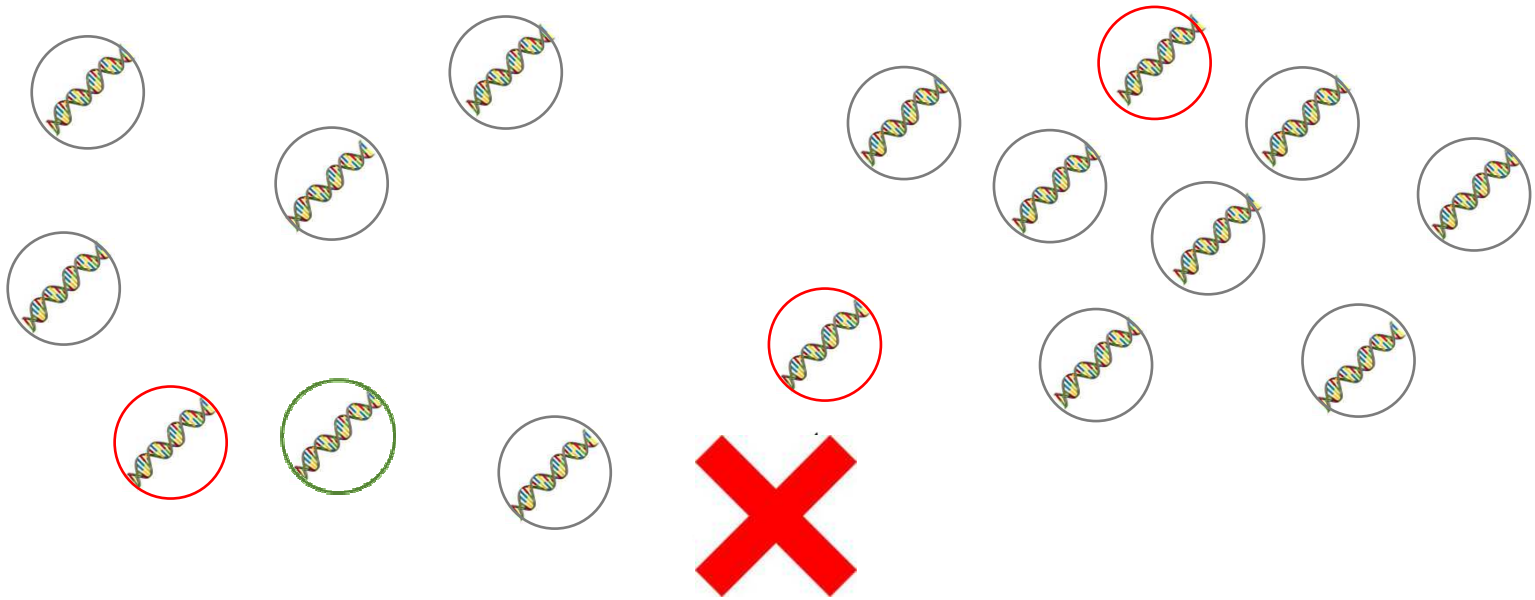


Counting

Size Ratio 1:4 ~

Counting

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



Can you distinguish between mother and child?

Only SNP-based NIPT allows differentiation of maternal and fetal DNA.

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- Clinical advantages of SNP-NIPT

Clinical Advantages of SNP

SNP-NIPT uniquely differentiates between maternal and fetal DNA.

Fewer false positives:

- Maternal contribution
- Vanishing twins
- Fetal sex accuracy

SNP-based NIPT Detects Maternal Contribution[1,2]

- SNP-based NIPT analyzes maternal DNA contribution to decrease false positives related to maternal mosaicism and copy number variants

Table 2. Contribution of an abnormal ChrX maternal karyotype in a prospective study of 187 discordant SCAs.

Clinical	NIPT findings	NIPT ChrX gain	NIPT ChrX loss	Total
NIPT follow-up	Abnormal NIPT for SCA, n	63	124	187
	Normal maternal karyotype, n	57	114	171
	Altered maternal karyotype, n	6	10	16
	Maternal mosaicism rate	9.52%	8.06%	8.56%

16/187 = 8.56%

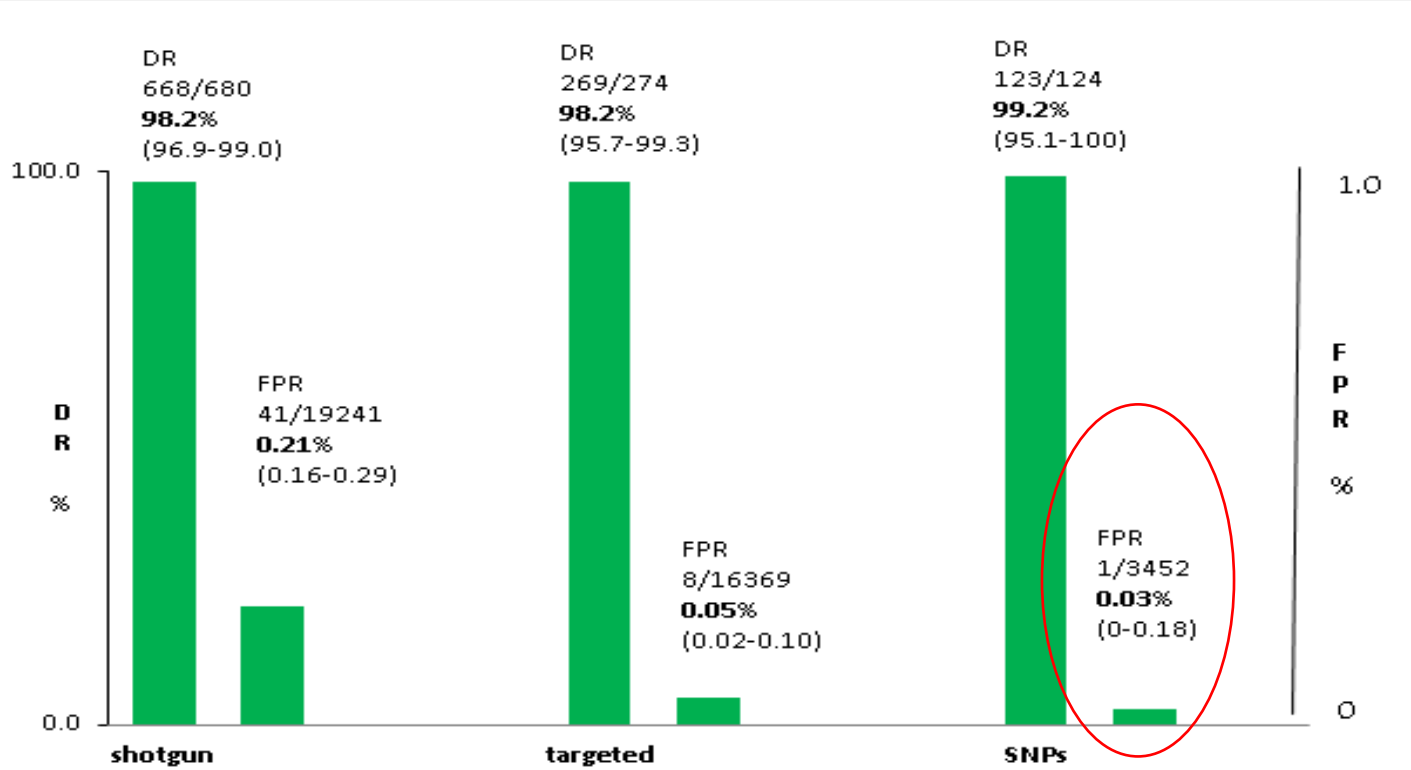
By counting method, 8.56% of results positive for sex chromosome aneuploidies were FP due to maternal mosaicism.

[1]**Wang Y, et al. Clinical Chemistry 2014; v. 60, p.251-259**.
[2]Snyder M, et al. N Engl J Med . Epub ahead of print April 1, 2015. DOI:10.1056/NEJMoa1408408

SCAs: sex chromosome aneuploidies

SNP-based NIPT has Lowest FPR for Autosomes 21, 18, 13[1]

Other NIPT methods report up to 7x higher FPR[1]



[1]Benn P., J Clinical Medicine 2014; 3, 537-565. Non-Invasive Prenatal Testing Using Cell Free DNA in Maternal Plasma: Recent Developments and Future Prospects

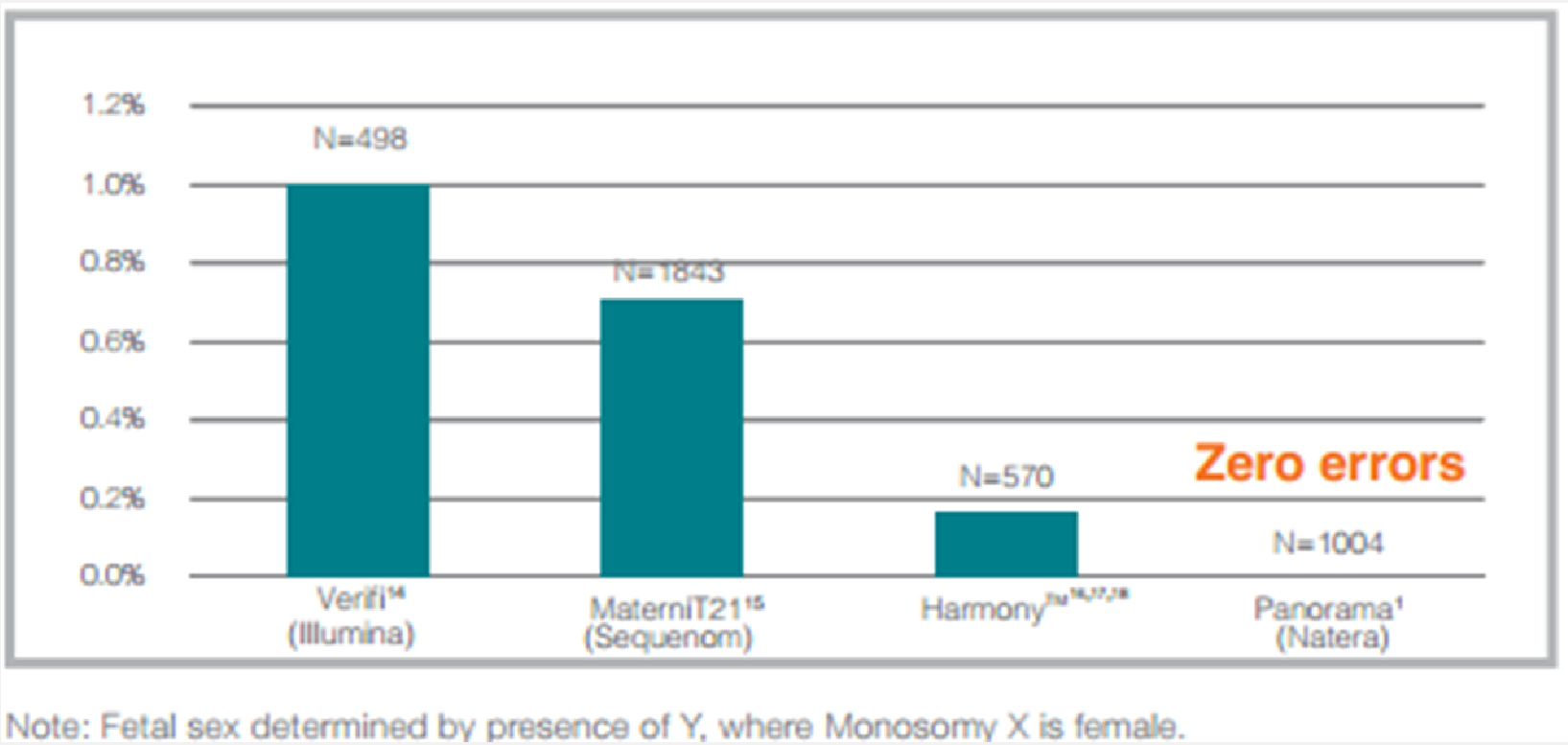
SNP-based NIPT Calls Vanishing Twin

- Identifies vanishing twin, which contributes an additional SNP haplotype
  - 0.2% of commercial cases[1]
  - Seen up to 8 weeks post-demise
  - Case study reported ~50% of cffDNA was from vanished twin 6+ weeks after demise noted[2]
- Leads to false positives, incorrect gender calls by counting method
  - >15% of discordant commercial results in counting methodology involved vanishing twin[3]
  - 1/3 trisomy 21 false positives attributed to vanishing twin[4]

[1]Curnow et al. Am J Obstet Gynecol. 2015 Jan;212(1):79.
[2]Gromminger et al. J Clin Med 2014: 3;679-692.
[3]Futch, et al. Prenat Diagn 2013 Jun;33(6):569-74
[4]Porreco RP et al. Am J Obstet Gynecol 2014:211:365.e1-12

Error Rate – Sex Determination

As many as 1/100 cases can have gender discrepancy when using counting methodologies.



Note: Fetal sex determined by presence of Y, where Monosomy X is female.

1. Dar P et al. Clinical experience and follow-up with large scale single-nucleotide polymorphism-based non-invasive prenatal aneuploidy testing. Am J Obstet Gynecol 2014; 211(5):527e1-527 e17. 14. Illumina internal data (www.verifitest.com). 15. Mazloom A et al. Noninvasive prenatal detection of sex chromosomal aneuploidies by sequencing circulating cell-free DNA from maternal plasma. Prenat Diagn, 2013; 33(6):581-7. 16. Nicolaides KH et al. Assessment of fetal sex aneuploidy using directed cell-free DNA analysis. Fetal Diagn Ther, Epub 2013 Dec 11. 17. Nicolaides KH et al. Validation of targeted sequencing of single-nucleotide polymorphisms for non-invasive prenatal detection of aneuploidy of chromosomes 13, 18, 21, X and Y. Prenat Diagn, 2013; 33(6):575-9. 18. Palomaki GE et al. DNA sequencing of maternal plasma reliably identified trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. Genet Med2012; 3:296-305.

Clinical Advantages of SNP

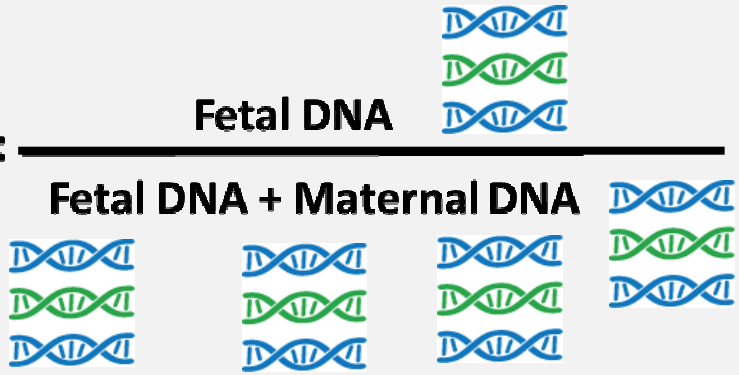
SNP-NIPT uniquely differentiates between maternal and fetal DNA.

Fewer false negatives:

- Fetal fraction
- Triploidy

Fetal Fraction Matters

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



- Average fetal fraction between 10 and 22 weeks gestation is 10-12%.
- Varies by gestational age, maternal weight, placental and pregnancy factors
- Lower cut off for analysis by SNP-NIPT :2.8%

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

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

Ultrasound Obstet Gynecol 2014
Published online in Wiley Online Library (wileyonlinelibrary.com).

Letter to the Editor

Fetal Fraction Matters

Performance of non-invasive prenatal testing when fetal cell-free DNA is absent

Numerous studies have validated the accuracy of non-invasive prenatal testing (NIPT) using fetal cell-free DNA (cfDNA) to assess the risk of fetal aneuploidies early in pregnancy[1], and we have used this technology in our practice since 2012 in both low- and high-risk women[2].

We are aware that several factors influence the fraction of fetal cfDNA present in maternal blood. Such factors include gestational age and maternal weight[3], as well as methods of sample collection and shipping conditions that may lead to maternal cell hemolysis. Some commercial laboratories assert that the accuracy of cfDNA testing is influenced by the amount of fetal cfDNA relative to that of maternal cfDNA. In these laboratories that report fetal fraction, the performance claims for NIPT are based on testing that requires a minimal amount of fetal cfDNA to be present. We are also aware that some commercial laboratory providers assert that measurement of fetal cfDNA is unnecessary and that reliable results can be provided without prior knowledge of the amount of fetal cfDNA analyte in the sample.

In order to assess the reliability of NIPT, blood samples from two 44-year-old non-pregnant women were drawn and submitted to five American commercial laboratories

This example raises concerns about the need for quality standards in NIPT. We feel that the measurement of fetal cfDNA is a basic quality metric required to ensure reliable interpretation of test results. With karyotyping or fluorescence *in-situ* hybridization analysis, it is standard to require a minimum number of fetal cell colonies to be counted before reporting a result. It seems reasonable that for NIPT, an analogous control measure should be applied. While the promise of accurate performance with NIPT has been acknowledged widely in publications and realized in many clinical experiences, we urge professional medical and laboratory societies to set and enforce appropriate quality-control guidelines for NIPT that are consistent with standard laboratory practice as in other commercially available tests.

Disclosure

Ariosa Diagnostics Inc. provided financial support for test costs.

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DOI: 10.1002/uog.14715

Table 1 Non-invasive prenatal test (NIPT) results for two non-pregnant women from five commercial laboratories

	Patient 1		*Patient 2*	
Laboratory	*Test result available*	*Details*	*Test result available*	*Details*
Lab A	No	Insufficient fetal cfDNA for accurate NIPT evaluation	No	Insufficient fetal cfDNA for accurate NIPT evaluation
Lab B	No	Unable to report due to low fetal fraction (fetal fraction reported as 0.6%)	No	Unable to report due to low fetal fraction (fetal fraction reported as 0.6%)
Lab C	Yes	Negative, consistent with female fetus (fetal fraction 4.3% reported on request)	Yes	Negative, consistent with female fetus (fetal fraction 3.9% reported on request)
Lab D	Yes	No aneuploidy detected, two sex chromosomes (XX)	Yes	No aneuploidy detected, two sex chromosomes (XX)
Lab E	Yes	No aneuploidy detected, two sex chromosomes (XX)	Yes	No aneuploidy detected, two sex chromosomes (XX)

cfDNA, cell-free DNA.

Only SNP-based NIPT Can Detect Triploidy

- Although most miscarry, incidence is 1/1000 at 10 weeks[1]
- Paternal triploidy carries risk for partial molar pregnancy
  - Up to 5% risk for gestational trophoblastic disease with partial molar pregnancy[2,3]
  - Risk for malignant tumors
- Maternal triploidy can be recurrent in future pregnancies[4]
- Provides risk assessment for couples with prior pregnancy with triploidy

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

SNP-based Clinical Trial Data[1,2]:

(mosaics included)

	Sensitivity	Specificity
Trisomy 21	83/83 **>99%** (CI: 95.6-100%)	1,108/1,108 **>99%** (CI: 99.7-100%)
Trisomy 18	27/28 **96.4%** (CI:81.7-99.9%)	1,164/1,165 **>99%** (CI: 99.5-100%)
Trisomy 13	13/13 **>99%** (CI: 75.3-100%)	1,180/1,180 **>99%** (CI: 99.7-100%)
Triploidy	8/8* **>99%** (CI: 47.8-100%)	272/272 **>99%** (CI: 92.0-100%)
Monosomy X	13/14 **92.9%** (CI: 66.1-99.8%)	1,179/1,180 **>99%** (CI: 99.5-100%)
Presence of Y	533/533 **>99%** (CI: 99.3-100%)	469/469 **>99%** (CI: 99.2-100%)

*5/5 paternal triploidy cases correctly called; 3/3 maternal triploidy correctly called based on extremely low fetal fraction

[1] Pergament E, et al. Obstet Gynecol. 2014 Aug;124(2 Pt 1):210-8

[2]Nicolaides et al.. *Prenatal Diagnosis*. 2013;33:1-5

Clinical Outcomes Study[1]

	Combined (4 indications)	Per Indication (T21/18/13/45X)
Number of samples	17,885[a]	
Aneuploidy detected (%)	2.0%	
Aneuploid calls with karyotype	222 (62%)	154 / 29 / 21 / 18
False Positives	38	14 / 2[b] / 13[c] / 9
Positive Predictive Values (PPV)	83%	91/93/38/50

PPV for Trisomies 21 and 18 is >90%.

[a]Two partner laboratories accounting for 38% of cases did not participate in follow-up efforts; all cases from these partners were excluded from outcome calculations [b]Includes one confined placental mosaicism (CPM) case. [c]Includes two CPM cases

[1] Dar P et al. Am J Obstet Gynecol 2014 Nov;211(5):527

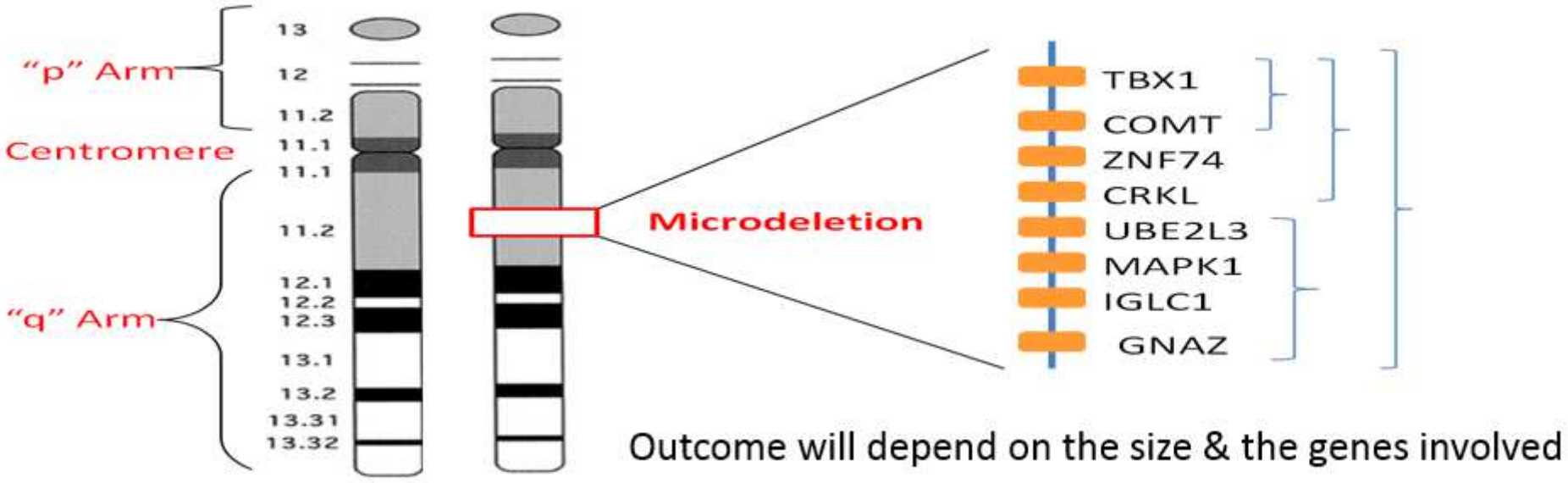
Clinical Advantages of SNP

SNP-NIPT is used also for microdeletions detection

Higher detection rate
of microdeletions

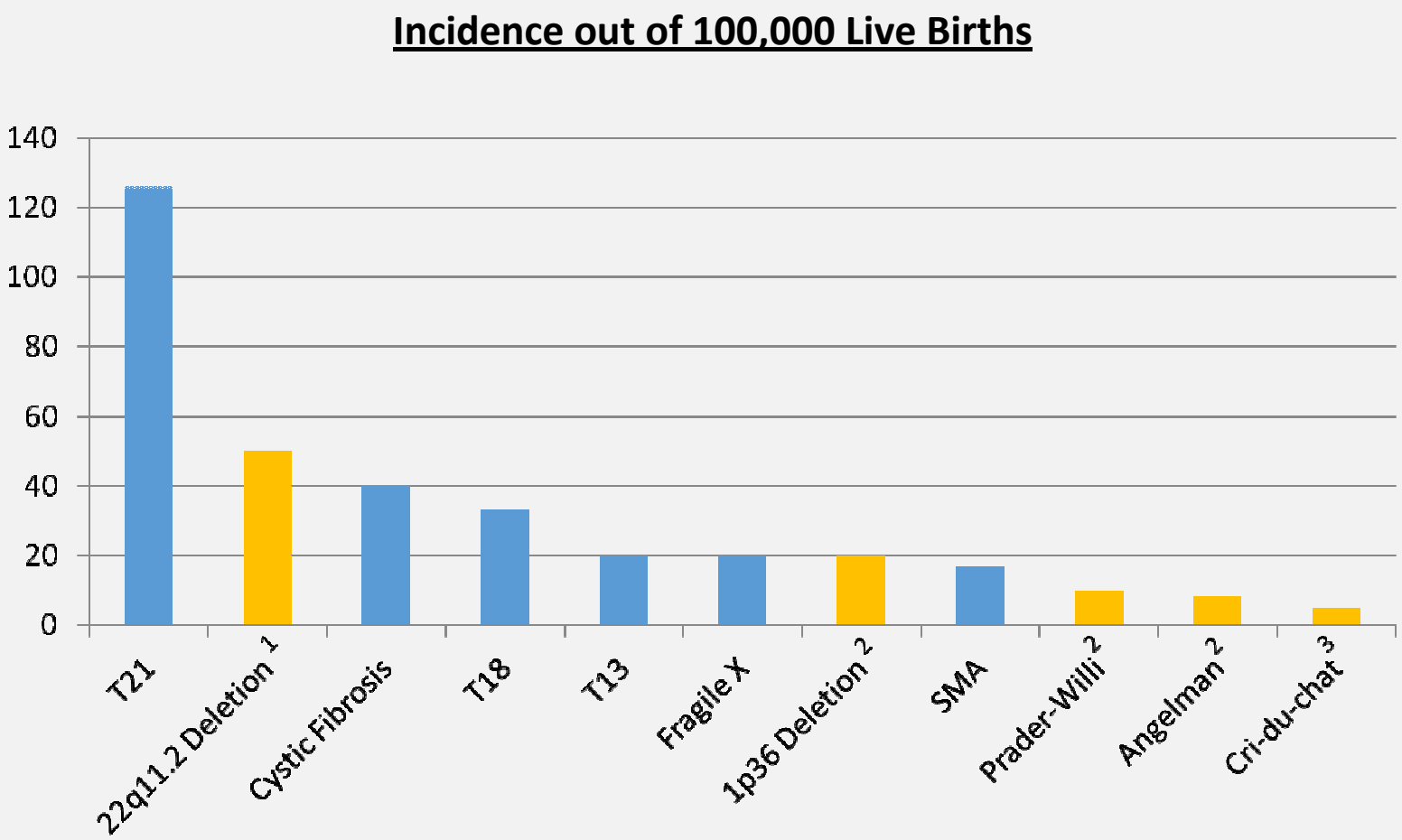
What is a Microdeletion?

- 1MB (megabase) = 1 million base pairs
- Microdeletions are 100kb to several MB
- Karyotype can usually only visually detect ≥7-10 MB



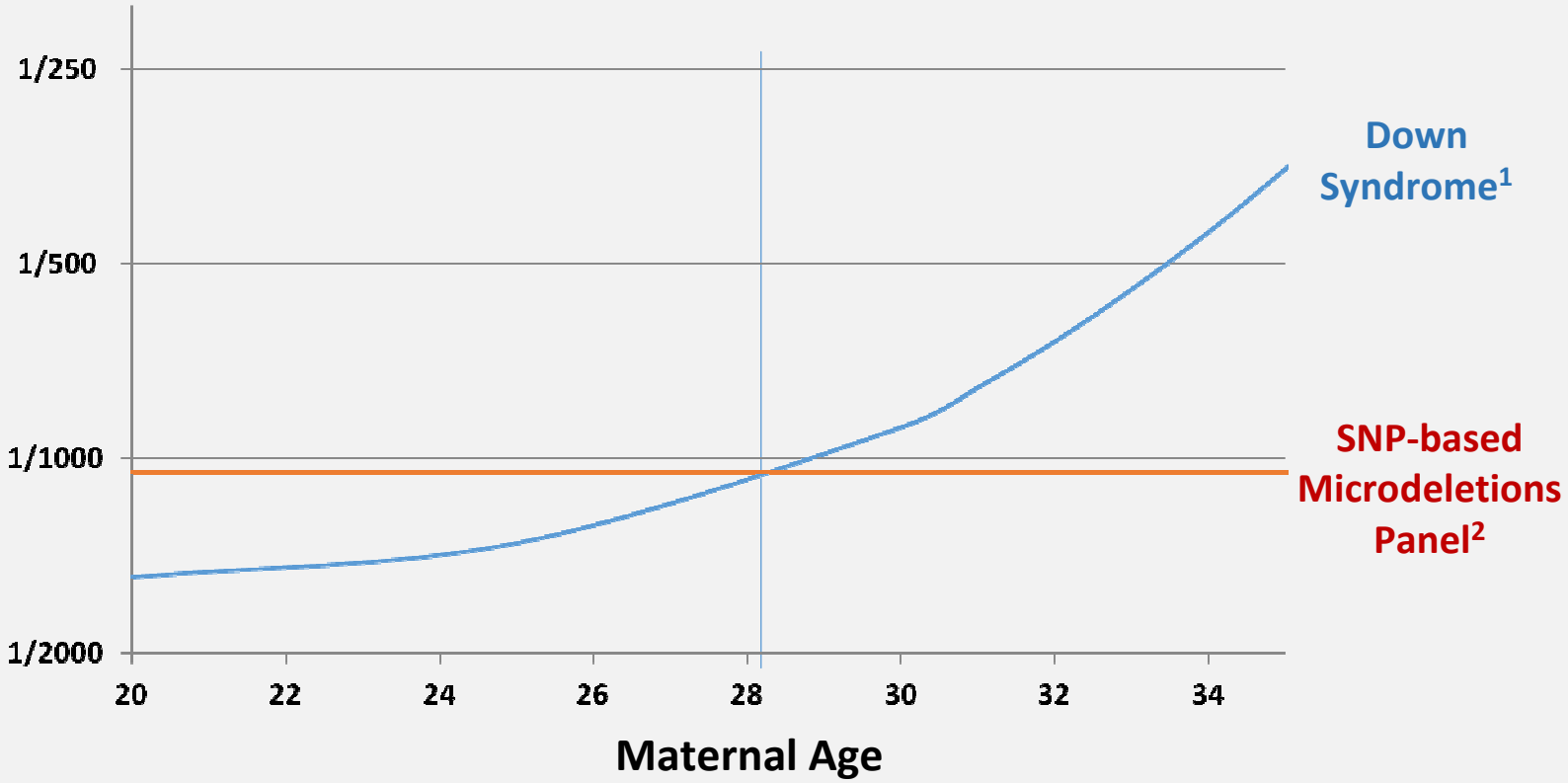
Outcome will depend on the size & the genes involved

High Incidence Conditions



[1]Nussbaum et al. 2007. Thompson and Thompson Genetics in Medicine (7th edn). Oxford Saunders: Philadelphia
[2]http://www.genetests.org.
[3]http://ncbi.nlm.nih.gov

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.

22q11.2 Deletion Syndrome[1,2]

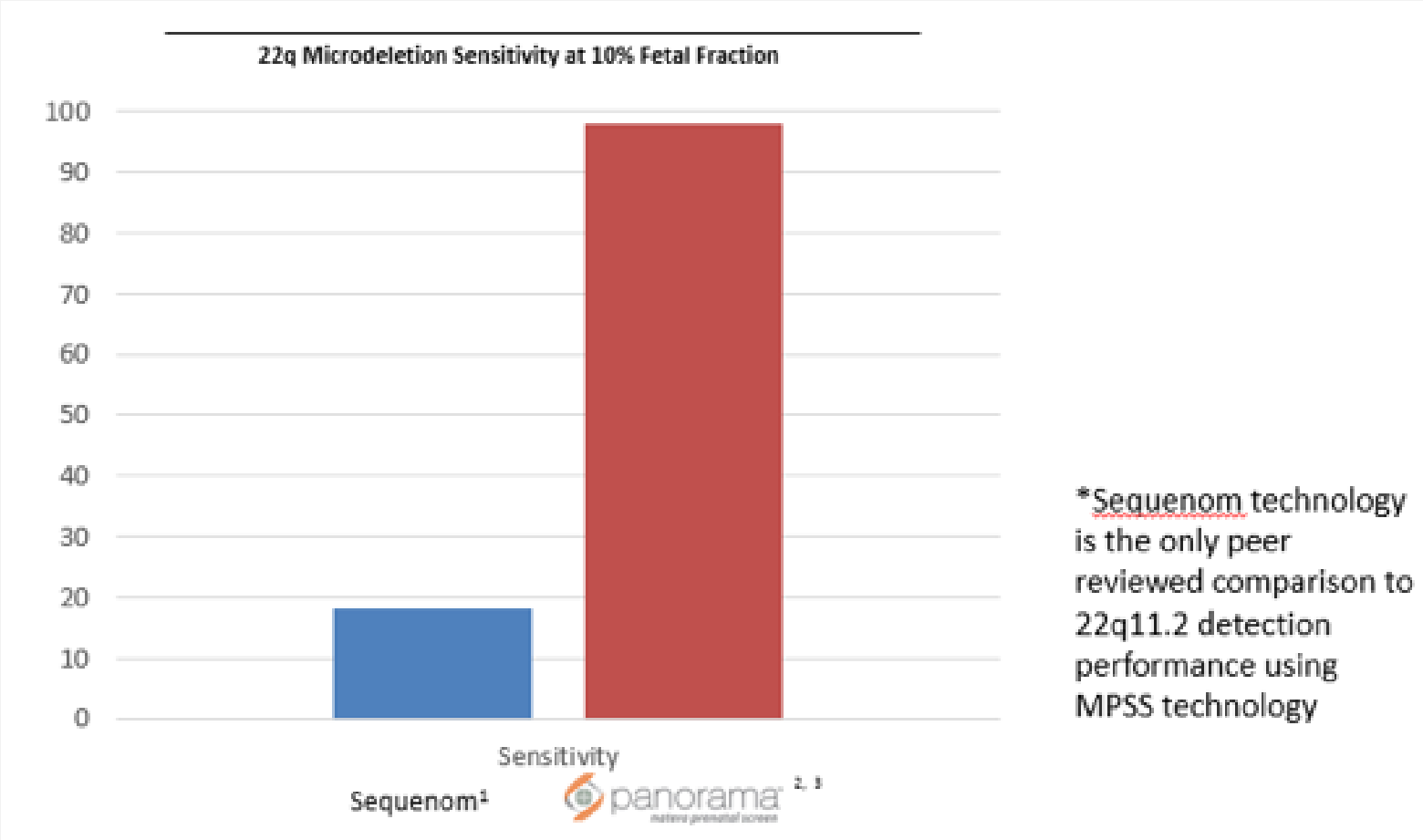
- Population incidence ~1 in 2000, though may be as high as 1 in 1000 [3,4]
- Several other names: DiGeorge, Velo-Cardio-Facial Syndrome (VCFS)
- Often unrecognized at birth
- Common features:
  - Congenital heart defect (75%)
  - Immune deficiencies (75%)
  - Palatal abnormalities (70%)
  - Schizophrenia in young adulthood (25%)
  - Hypocalcemia (77%)
  - Developmental delay and learning disabilities (70-90%)

[1]International 22q11.2 Foundation – Handbook
[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.

22q: Early Intervention Matters

- Prepare to deliver baby at tertiary care center
- Delay in administering live vaccines
- Monitor calcium levels to reduce or eliminate adverse outcomes secondary to hypocalcemic seizures
- Check palate for clefting

Importance of Testing for Microdeletions Using SNPs



[1]Zhao, et al., Detection of Fetal Subchromosomal Abnormalities; Figure 3C; *Clinical Chemistry.* April, 2015

[2]Wapner, et al, Expanding the Scope of Noninvasive Prenatal Testing; *American Journal of Obstetrics and Gynecology*, November 30, 2014; Table 3. Sensitivity based on deletions of approximately 2.9Mb

[3]Hall, M., Panorama non-invasive prenatal screening for microdeletion syndrome, Natera Inc. 2013

SNP-based Microdeletion Validation Data[1]

(partial calls and no calls counted as low-risk)

[1]Wapner R et al. Am J Obstet Gynecol 2014; 212(3):332.

- 469 samples tested with 110 confirmed positives

	Sensitivity*		Specificity*	
22q11.2 deletion	45/47	95.7% (CI: 85.5-99.5%)	419/422	99.3% (CI: 97.9-99.9%)
Prader-Willi	15/16	93.8% (CI: 69.8-99.8%)	453/453	100% (CI: 99.2-100%)
Angelman	21/22	95.5% (CI: 77.2-99.9%)	447/447	100% (CI: 99.2-100%)
1p36 deletion	1/1	100% (2.5-100%)	468/468	100% (CI: 99.2-100%)
Cri-du-chat	24/24	100% (CI: 85.8-100%)	444/445	99.8% (CI: 98.8-99.9%)

469 tot: 352 unaffected pregnancies, 6 affected pregnancy plasmas, 111 plasmART samples derived from: 8 affected child and their unaffected mother +unaffected child

Hindawi Publishing Corporation
Case Reports in Obstetrics and Gynecology
Volume 2015, Article ID 813104, 3 pages
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Hindawi

Case Report

The First Case Report in Italy of Di George Syndrome Detected by Noninvasive Prenatal Testing

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12 weeks gestational age; high risk (38 y)

The SNP-NIPT based Difference

Summary:

- Utilizes a SNP technology
- Differentiates between maternal and fetal/placental DNA
- Assesses risks for vanishing twin and triploidy
- Provides a specific risk score for each microdeletion syndrome

Thank you!

Questions?

Appendix

Maternal Serum Screening (MSS) vs NIPT

	MSS[1-6]	NIPT (Panorama®)[7-8]
	Analytes from fetus and placenta	cfDNA
Nuchal Translucency	YES	NO
Gestational age	~11-22 wks	9+ wks
Open Neural Tube Defects	YES	NO
Conditions	21, 18, +/-13	21,13,18,X,Y Triploidy Microdeletions*
T21 Sensitivity	81-90+%	>99%
T21 Positive Predictive Value (PPV)	3.4%	91%
False positive rate	5%	<1%

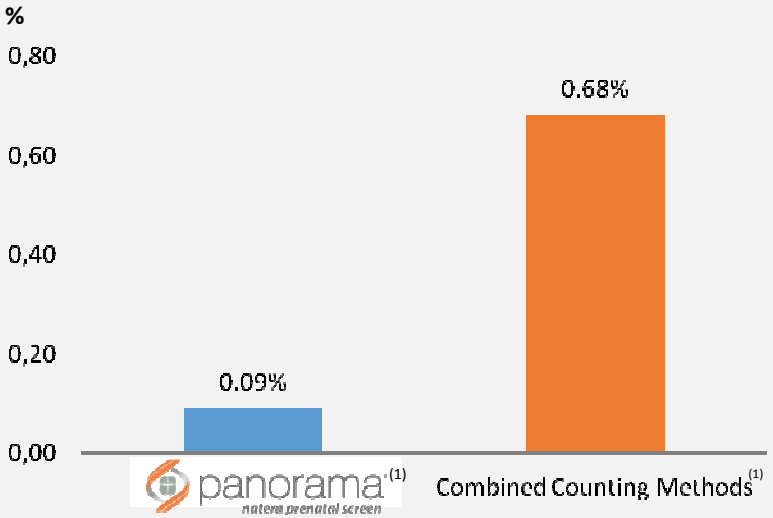
*some

[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/testcenter/testguide.action?dc=TS_Integrated_Screen
[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

Demonstrated Clinical Superiority

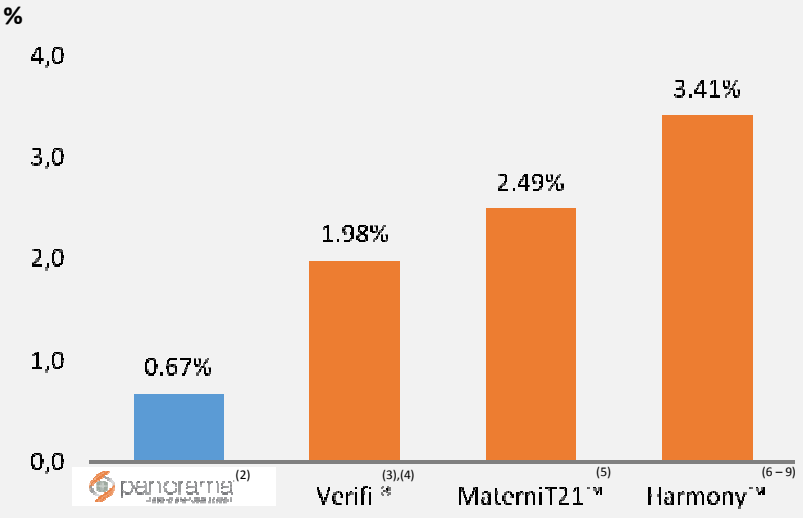
Fewer False Positives

False Positive Rate by Autosomes



Fewer False Negatives

False Negative Rate (T21, T18, T13, MX combined)



"… Combined specificity for the three autosomal trisomies was 99.91% (1,103/1,104 total negative samples, CI: 99.5-100%); the overall specificity of the combined quantitative methods was 99.32% (4,084/4,112, CI: 99.02-99.55%). This is a statistically significant difference (p=0.0085)." [1]

PPV	T21	T18	T13	MX
	90.9%	93.1%	38.1%	50.0%

[2]

1. Pergament et al. Obstet Gynecol 2014
2. Dar et al. Am J Obstet Gynecol 2014
3. Futch et al. Prenat Diagn 2013
4. Bianchi et al. NEJM 2014
5. Porreco et al. Am J Obstet Gynecol 2014
6. Verweij et al. Prenat Diagn 2013
7. Gil et al. Ultrasound Obstet Gynecol 2013
8. Jackson et al. Prenat Diagn 2013
9. Norton et al. NEJM 2015

Targeted Sequencing: Greater Depth, Superior Results

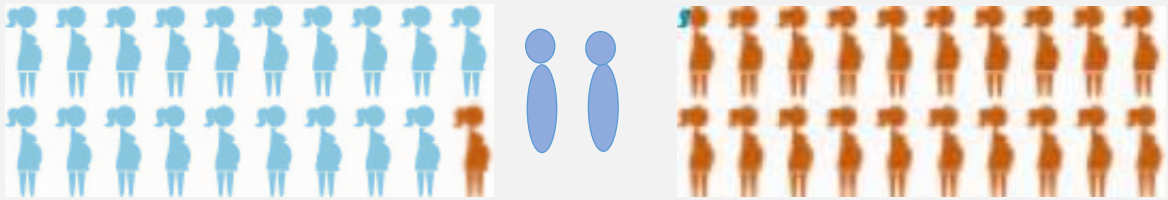
- Targeted only on regions of interest
- More sequence reads per chromosome than MPSS
  - 11X depth on Chr 21 with Low Depth Protocol compared to VeriSeq, 30X depth on High Depth Protocol
  - 20-100x more reads in microdeletion regions

				NextSeq V2 (targeted)	
	% genome	ILMN (Verifi)	ILMN (VeriSeq)	Natera (LDOR)	Natera (HDOR)
Sample Plex			48	64	24
Total Reads		22,800,000	~6,000,000	4,500,000	12,000,000
Chr 21	1.50%	342,000	90,000	1,032,556	2,753,510
Chr 18	2.50%	570,000	150,000	1,189,026	3,170,736
Chr 13	3.70%	843,600	222,000	1,184,826	3,159,536
Chr X, Y	6.80%	1,550,400	408,000	1,281,082	3,416,219
22q	0.09%	20,520	5,400	585,938	585,938
1p36	0.32%	72,960	19,200	1,004,464	1,004,464
5p-	0.64%	145,920	38,400	1,004,464	1,004,464
15q11-13	0.19%	43,320	11,400	1,004,464	1,004,464

Increasing sequencing depth increases statistical precision of aneuploidy calls and is correlated to clinical performance

Perché fare un test non-invasivo su DNA fetale (NIPT)?

- Il test NIPT fornisce un tasso di falso positivo (FPR) più basso ed un più elevato Valore Predittivo Positivo (PPV)
  - Con i vecchi metodi di screening biochimico (o bitest nel primo trimestre), per ogni 20 risultati ad alto rischio o rischio positivo, solo 1 paziente risulterà affetto dalla malattia (vero positivo), mentre con Panorama i veri positivi saranno in media 18*



- Il test NIPT presenta un livello di sensitività più elevato
  - ~99% per l'NIPT vs 80-95% per il bitest
- Il test NIPT può effettuare lo screening per più sindromi
  - Microdelezioni, Aneuploidie dei Cromosomi sessuali e Triploidia

* For Trisomy 18 and 21

What is PPV?

- PPV = Positive Predictive Value

$$\frac{TP}{TP+FP}$$

- How likely is it that the pregnancy is truly affected given a high risk or positive result
- Factors that affect PPV
  - Sensitivity/Detection rate
  - Specificity/False positives
  - Incidence of condition