Overview of SNP-based Method for NIPT

April 2014

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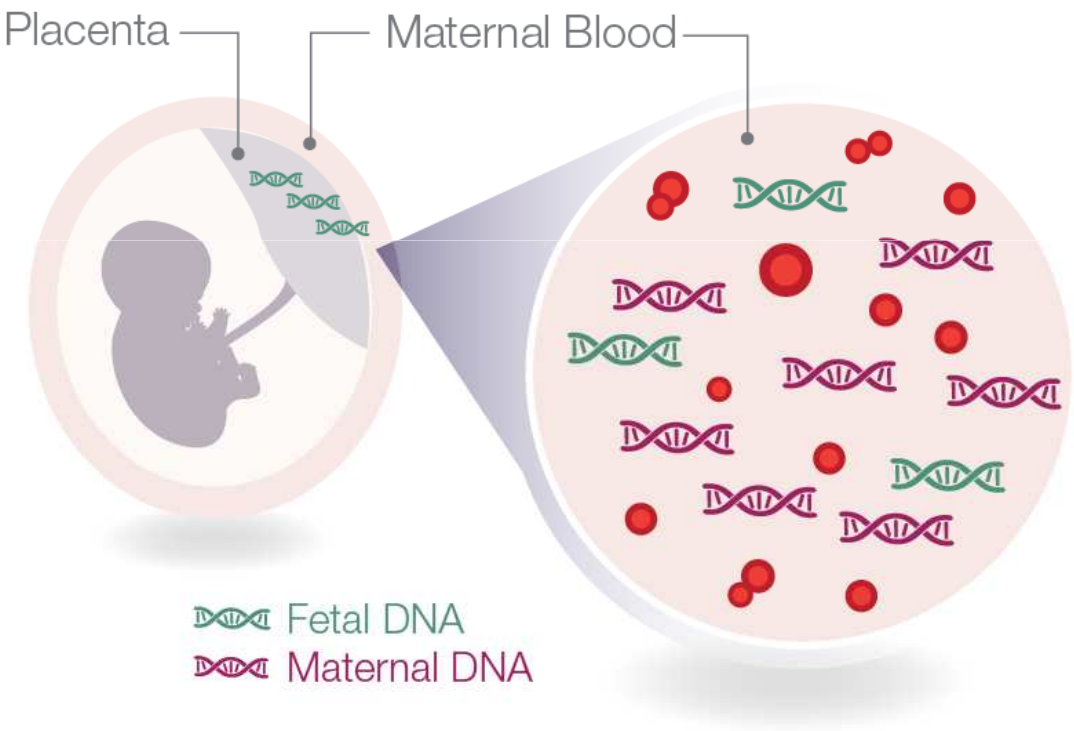
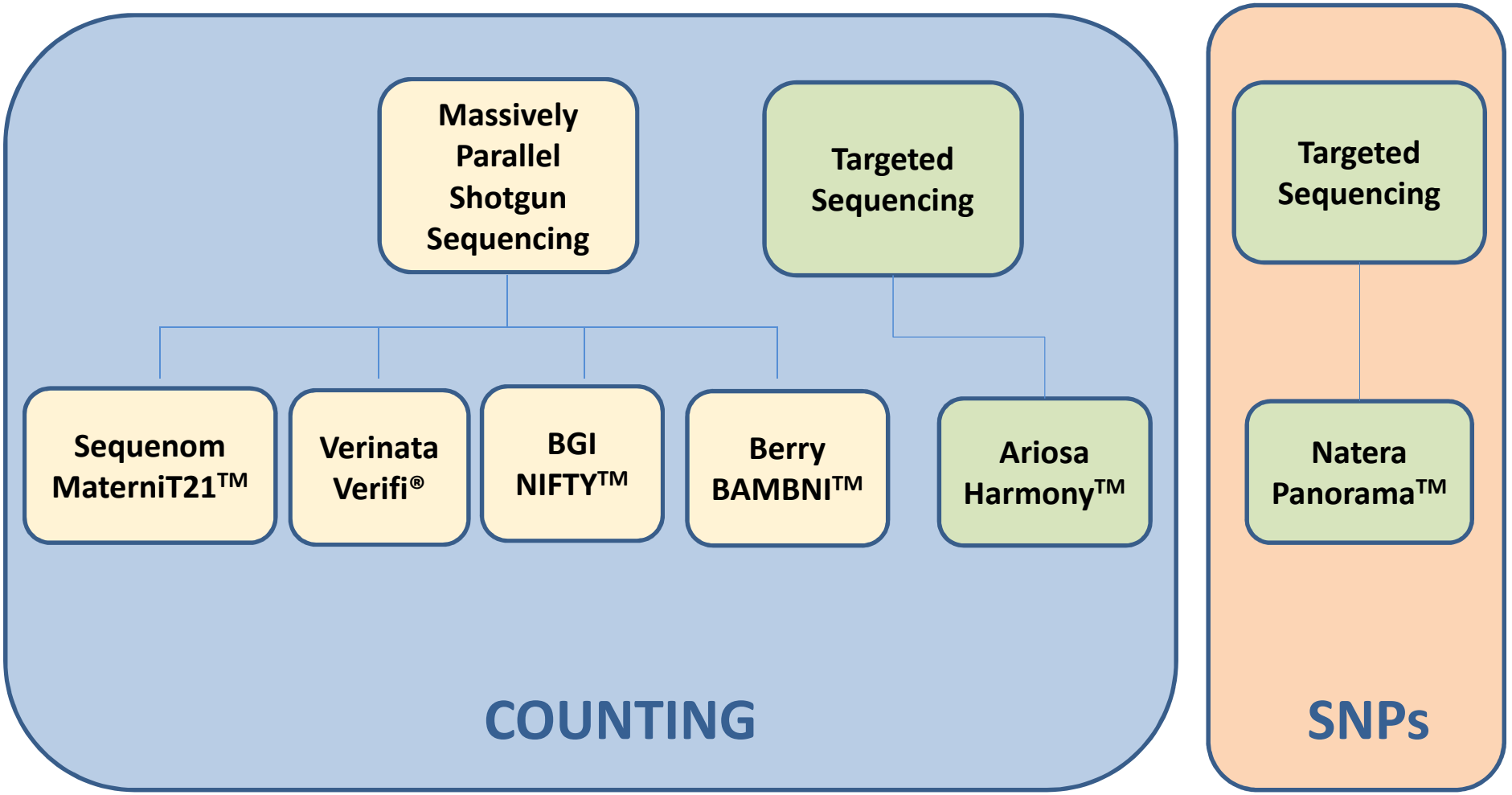
Cell-free DNA (cfDNA)



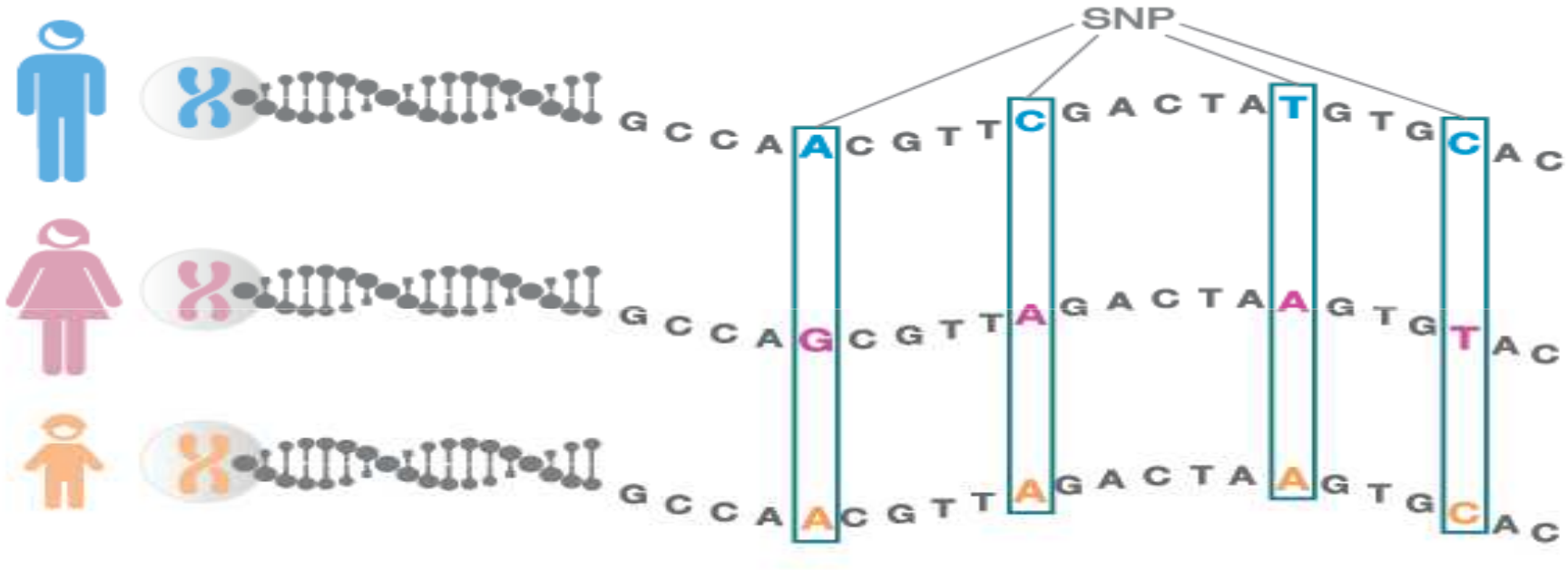
cfDNA comes from apoptotic cells derived from:

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

Differentiating NIPT Methodologies



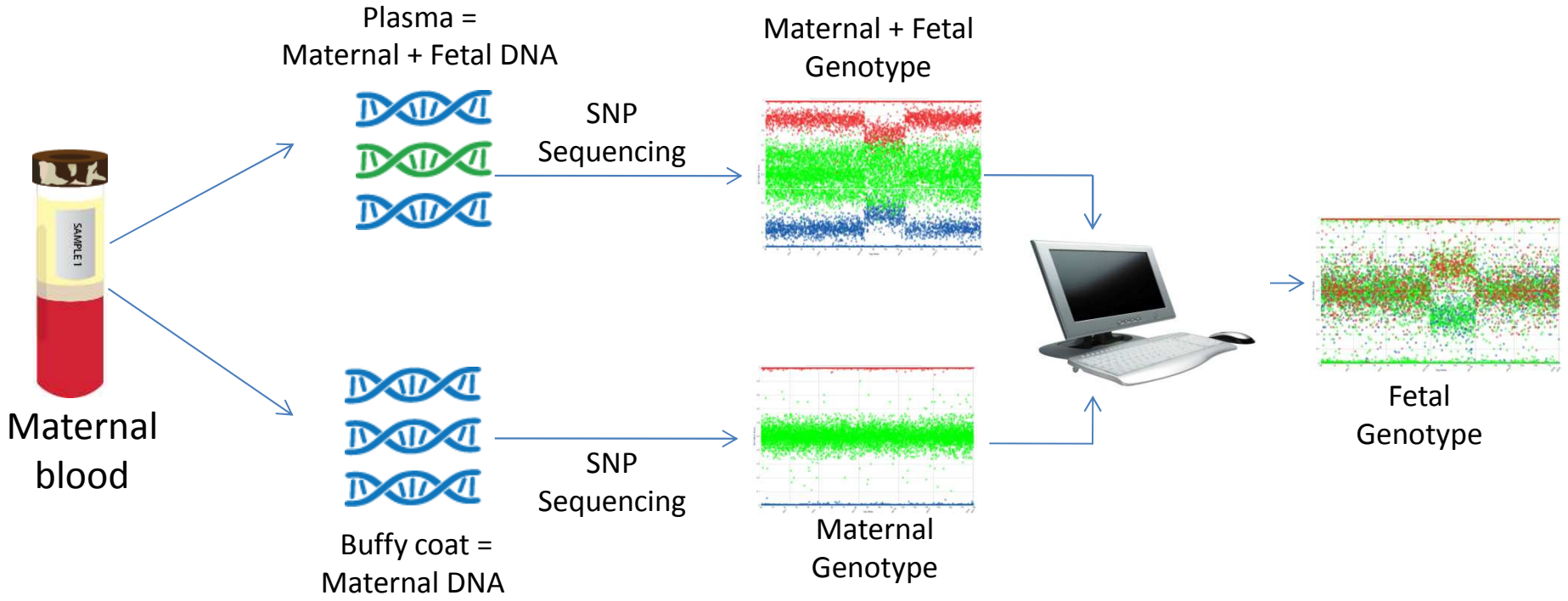
SNP = Single Nucleotide Polymorphism



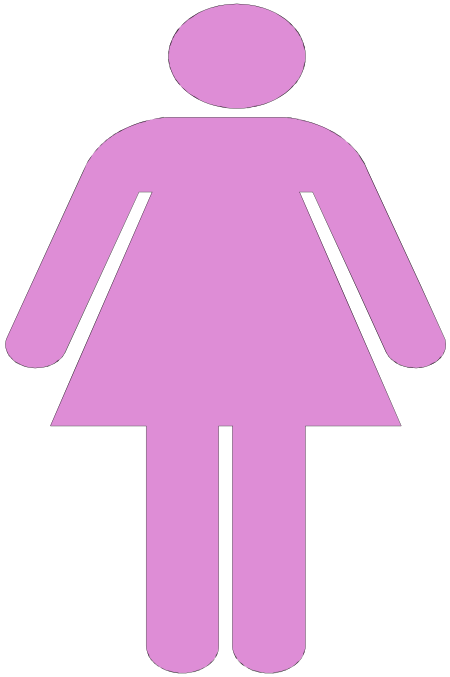
- Polymorphisms occur when a single base pair (nucleotide) is changed: A, T, C, or G
- These are **normal** genetic changes that occur in every person and mark where people differ from one another

Sequencing the Buffy Coat to Get Maternal SNP Genotype

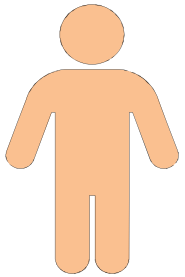
Panorama simultaneously targets 19,488 SNPs



Panorama Differentiates



Panorama differentiates between maternal and fetal DNA in the plasma



Published Data

- 166 Samples – Zimmermann *et al*. (redraw 12.6%) Older Methodology, never used clinically — 11,000 SNP
- **242 Samples – Nicolaides *et al.* (Prospective blinded study, redraw 5.4%)** — **19,488 SNP**
- **201 Samples – Samango-Sprouse *et al.* (Sex Chromosome case control study)** — **19,488 SNP**
- **56 Samples – Nicolaides *et al.* (Triploidy case control study)** — **19,488 SNP**
- **1051 Samples – Pergament *et al*. *(submitted)* (Prospective blinded study, redraw 5.9% > 10 wks GA)** — **19,488 SNP**

Panorama Validation: Nicolaides *et al*.

Study population

- 242 singleton pregnancies
- Undergoing CVS at 11-13 weeks
- High risk pregnancies
- Blood drawn for NIPT prior to CVS
- Natera was blinded to CVS results

Results

- 229 reported NIPT results
- 13 cases were “no-results” due to failed quality metrics (5.4%)
- All calls made were correct

	Correct Calls
Trisomy 21	25/25
Trisomy 18	3/3
Trisomy 13	1/1
Monosomy X	2/2
XY	117/117
XX	109/109**
Triploidy	1/1

Prenatal Diagnosis, 2013.

Validation of targeted sequencing of single-nucleotide polymorphism for non-invasive prenatal detection of aneuploidy of chromosomes 13, 18, 21, X, and Y

Samango-Sprouse *et al.*, Prenatal Diagnosis 2013

Study population

- 201 singleton pregnancies
- Confirmation by invasive test, cord blood, POC testing, or child karyotype

Results

- 187 samples passed quality metrics (no result on one 45,X)

Condition	Correct Calls
45,X*	11/12
47,XXY	2/2
47,XYY	1/1
Fetal Sex (XX/XY)	172/172

*One 45,X was called XX

Prenatal Diagnosis, 2013.
SNP-based non-invasive prenatal testing detects sex chromosome aneuploidies with high accuracy

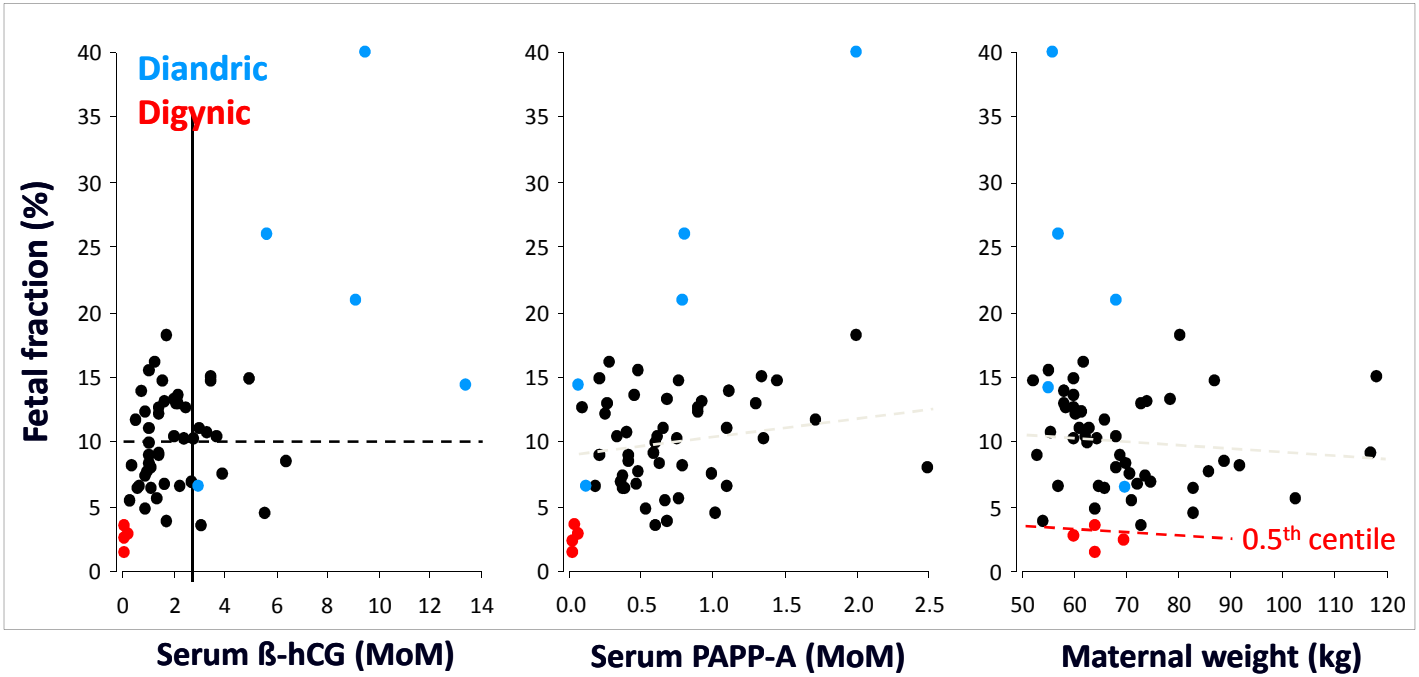
Triploidy Detection by SNP: Nicolaides *et al.*

Nicolaides KH, *et al.*
Prenatal Detection of Fetal Triploidy from Cell-Free DNA Testing in Maternal Blood.
Fetal Diagn Ther(2013)
DOI: 10.1159/000355655

Retrospective study
Stored maternal plasma (4 mL) at 11-14 w

Triploidy, Diandric	4	Correct	4
Triploidy, Digynic	4	'Low FF'	3
		Failed	1
Normal	48	Correct	44
		Failed	4

The Fetal Medicine Foundation



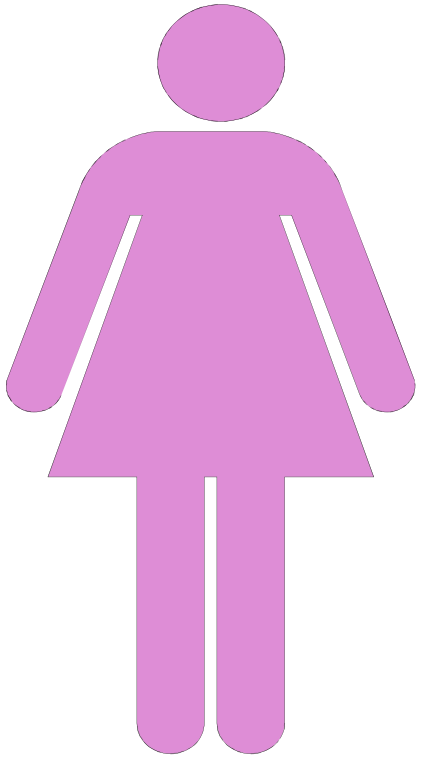
Expanded Validation in High and Low Risk Cohort

- 1051 singleton pregnancies
- 966 samples received a result
- No-result rate:
  - 5.9% (53/900) ≥10 weeks
  - 10.7% (6/56) at 9 weeks
  - 27.7% (26/94) at 8 weeks

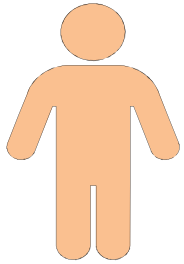
	Sensitivity	Specificity
Trisomy 21	**58/58 (100%)** CI:[93.8% - 100%]	**905/905 (100%)** CI:[99.6% - 100%]
Trisomy 18	**24/25 (96%)** CI:[79.7% -99.9%]	**938/939 (99.9%)** CI:[99.4% - 99.98%]
Trisomy 13	**12/12 (100%)** CI:[73.5% - 100%]	**953/953 (100%)** CI:[99.6% - 100%]
Monosomy X	**9/10 (90%)** CI:[55.5% - 99.8%]	**953/954 (99.9%)** CI:[99.4% - 99.98%]
Presence of Y (gender)	**418/418 (100%)** CI:[99.1% - 100%]	**358/358 (100%)** CI:[99.0% - 100%]

Manuscript submitted; Lead authors E. Pergament, H. Cuckle

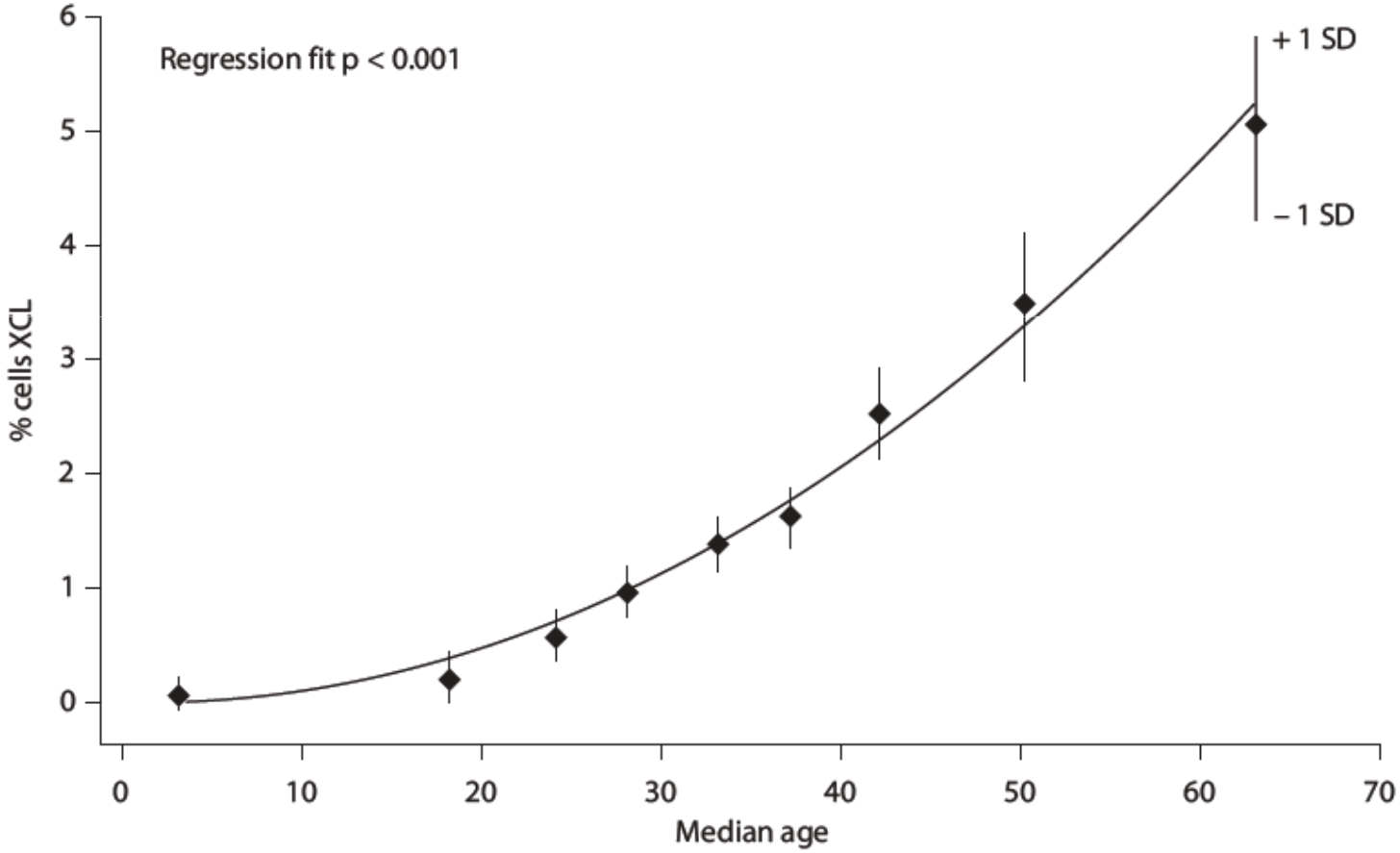
Differentiating between maternal and fetal DNA



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



Panorama Detects Maternal Contamination from Mosaic X Chromosomes



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

Panorama Detects Vanishing Twin

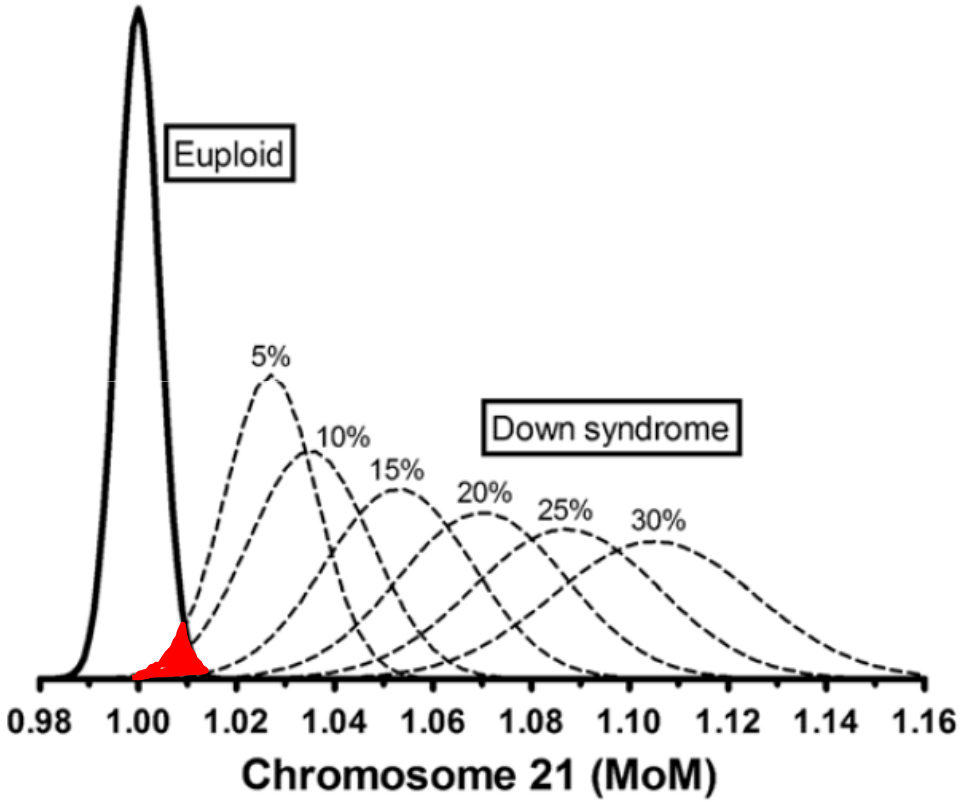
- Vanishing twin contributes additional SNP haplotype
  - 0.2% of commercial cases (Natera internal data)
  - Could be up to 0.6% (Landy, et al. Am J Obstet Gynecol 1986 July; 155(1):14-9)
  - Seen up to 8 weeks post-demise
- More false positives by counting method
  - >15% of discordant commercial results in counting methodology involved vanishing twin
    (Futch, et al Prenat Diagn 2013 Jun;33(6):569-74)

Panorama Detects Triploidy

- Although most miscarry, incidence is 1/1000 at 10 weeks[1].
- Paternal triploidy carry higher risks for partial mole which can be associated with choriocarcinoma and pose risks for the mother[2].
- Maternal triploidy can be recurrent in future pregnancies[3].

1. Snijders, *et al.* Fetal Diagn Ther 1995; 10:357-9.
2. Seckl MJ *et al.*.Lancet 2000 Jul 1;356(9223):36-9.
3. Chromosome Abnormalities and Genetic Counseling, Gardner and Sutherland, 2004.

Counting Methods Suffer at Low Fetal Fraction



When using the counting methodology, as fetal fraction decreases, there is less distinction between the euploid and aneuploid distributions

Canick, et al. Prenat Diagn 2013, 33, 1–8

SNP-based Method for Screening Microdeletions

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Panorama Microdeletion Panel

- 22q11.2 Deletion/DiGeorge

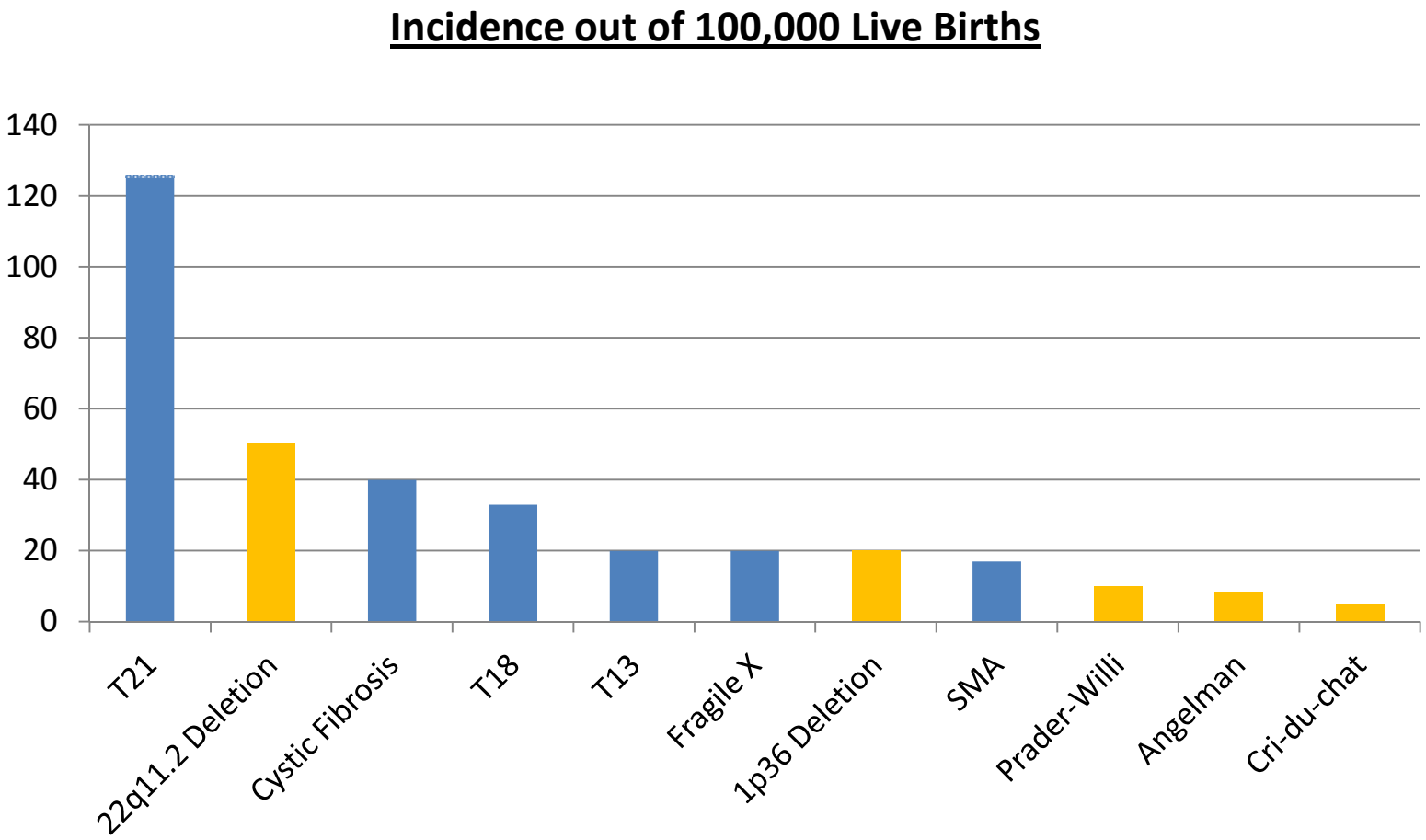
Cardiac indications on ultrasound but many missed

- 1p36 Deletion
- Angelman
- Prader-Willi
- Cri-du-chat

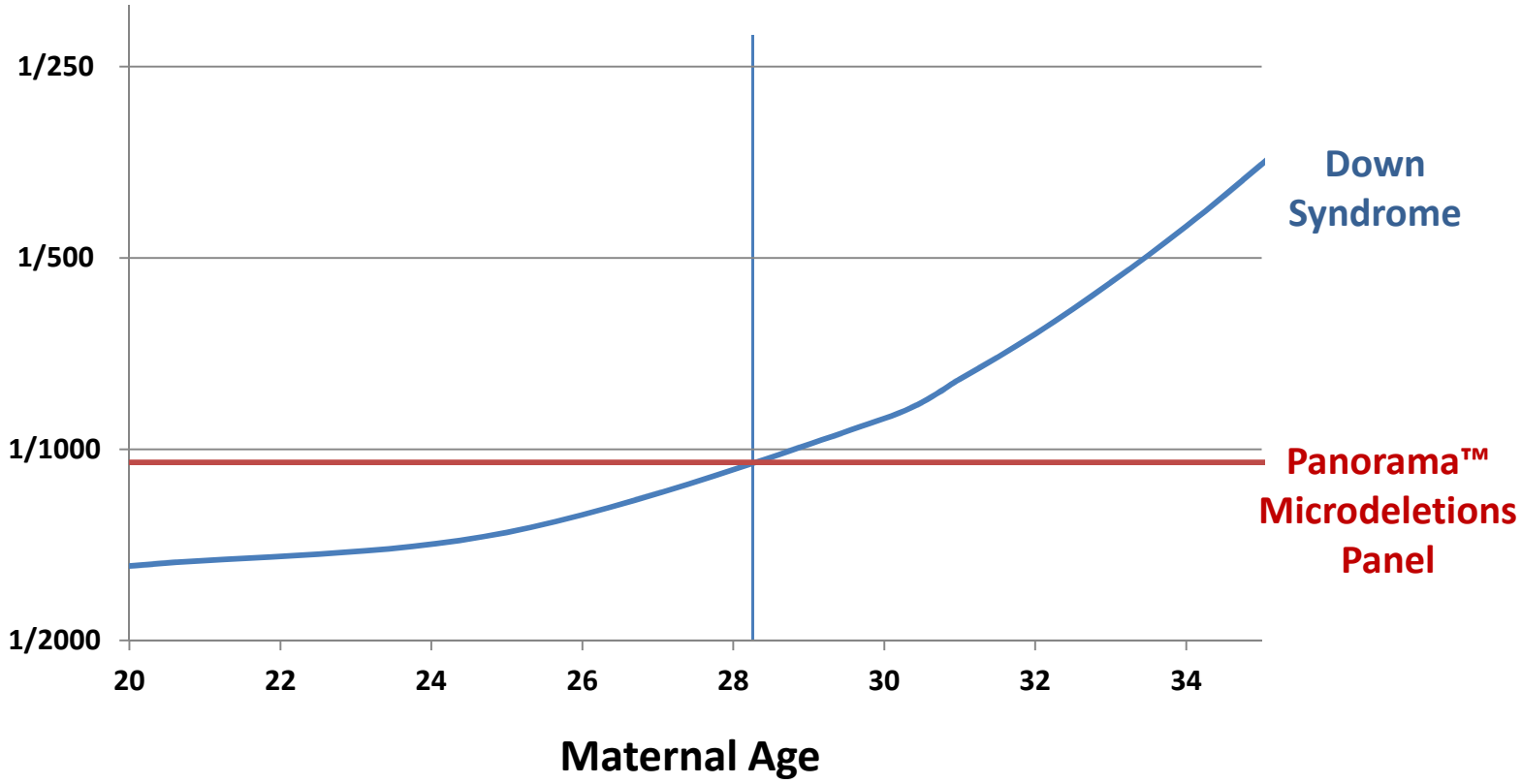
Vast majority missed on ultrasound

Combined prevalence approximately 1/1,000

High Incidence Conditions



More Common Than Down Syndrome in Younger Women



Down Syndrome prevalence from Snijders, et al. *Ultrasound Obstet Gynecol 1999;13:167–170.*
Total prevalence shown for 6 microdeletions 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 1/1049 - 1/2113.

Microdeletion Validation Data

- 469 samples tested
  - 6 positive pregnancy plasmas
  - 104 positive PlasmART samples

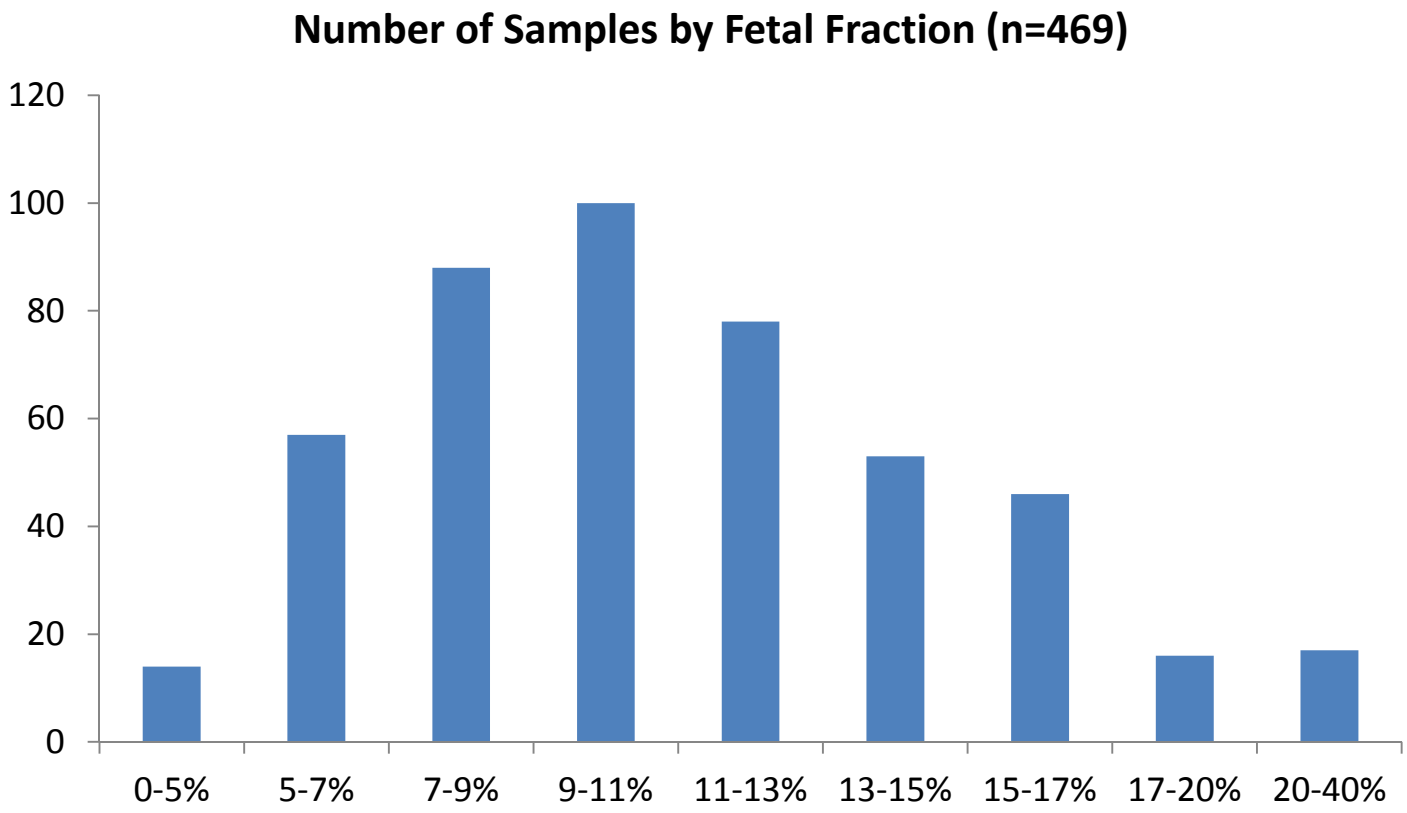
	Sensitivity*		Specificity*	
22q11.2 Deletion	45/47	95.7% (CI: 85.5-99.5%)	419/422	99.3% (CI: 97.9-99.9%)
Prader-Willi	15/16	93.8% (CI: 69.8-99.8%)	453/453	100% (CI: 99.2-100%)
Angelman	21/22	95.5% (CI: 77.2-99.9%)	447/447	100% (CI: 99.2-100%)
1p36 Deletion	1/1	100% (2.5-100%)	468/468	100% (CI: 99.2-100%)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

*Sensitivity and specificity calculated considering as negatives the samples that did not return a call.

Validation Sample Cohort

- 469 pregnancy plasmas total
  - 352 confirmed unaffected
  - 6 affected pregnancy plasmas
    - 3 22q11.2 deletion
    - 2 Cri-du-Chat
    - 1 1p36
  - 111 plasmART samples derived from 6 affected children and their unaffected mothers
    - 2 22q11.2 deletion
    - 1 Cri-du-Chat
    - 1 Angelman
    - 1 Prader-Willi
    - 1 unaffected

Validation Across Fetal Fractions



Odds of Being Affected Given a Positive Result (OAPR)

- Results are reported as a risk score using OAPR
- OAPR takes into account
  - Clinical incidence of the syndrome
  - % of cases caused by deletion of the entire covered region
- Using a risk score allows accurate counseling about the actual risks to the pregnancy

Risk Scores on Panorama Test Reports

SYNDROME	PRIOR RISK (all women)	RISK SCORE IF POSITIVE*	RISK SCORE IF NEGATIVE*
22q11.2 Deletion	1 in 2,000	1/19	1/13,330
Prader-Willi	1 in 10,000	1/22	1/13,882
Angelman	1 in 12,000	1/26	1/16,658
1p36 Deletion	1 in 5,000	1/6	1/12,494
Cri-du-chat	1 in 20,000	1/19	1/57,110

*Risk scores for fetal fractions ≥ 6%

Microdeletion Disorders are Severe

Syndrome	Incidence	Sensitivity [1]	Specificity [1]	Location (Size of Region) # SNPs	Lifespan	Mental Effects	Heart Defects	Other features
22q11.2 Deletion/ DiGeorge	1 in 2,000 [2]	95.7% (45/47) (85.5-99.5%) [5]	>99% (419/422) (97.9-99.9%) [5]	22q11.2 (2.9 MB) 672 SNPs	Reduced	Mild to moderate intellectual disorder & schizophrenia	Yes	Palate and feeding Immune problems, low calcium, seizures
Prader-Willi	1 in 10,000 [3]	93.8% (15/16) (69.8-99.8) [5]	>99% (453/453) (99.2-100%) [5]	15q11-q13 Paternal (5.9 MB) 1,152 SNPs	Reduced	Mild to severe intellectual disorder & behavioral problems	No	Hypotonia in babies, insatiable appetite
Angelman	1 in 12,000 [3]	95.5% (21/22) (77.2-99.9%) [5]	>99% (447/447) (99.2-100%) [5]	15q11-q13 Maternal (5.9 MB) 1,152 SNPs	Normal	Severe intellectual disorder	No	"Happy" affect, ataxia, microcephaly, no speech, seizures
Cri-du-chat	1 in 20,000 [4]	>99% (24/24) (85.8-100%) [5]	>99% (444/445) (98.8-99.9%) [5]	5p15.2 (20 MB) 1,152 SNPs	Infancy to adult	Moderate to severe intellectual disorder & behavioral problems	No	Cat like cry, growth problems, wide set eyes
1p36 Deletion	1 in 5,000 [3]	>99% (1/1) (2.5-100%) [5]	>99% (468/468) (99.2-100%) [5]	1p36 (10 MB) 1,152 SNPs	Normal in most	Severe intellectual disorder & behavioral problems	Yes	Limited/no language, hearing loss, abnormal ears, seizures, 2:1 M:F

Total incidence: approximately 1 in 1,000

[1] Performance specifications reflect presence or absence of the entire region

[2] Nussbaum et al 2007. Thompson and Thompson Genetics in Medicine (7th edn). Oxford Saunders: Philadelphia

[3] http://www.genetests.org.

[4] http://ncbi.nlm.nih.gov/entrez/disponim.cgi?id=123450

[5] 95% confidence interval

The Panorama prenatal test was developed by Natera, Inc., a laboratory certified under the Clinical Laboratory Improvement Amendments (CLIA). This test has not been cleared or approved by the U.S. Food and Drug Administration (FDA).

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