# Non-Invasive Prenatal Screening (NIPS) in the detection of microdeletions

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# Disclosures

Institutional research support for the SMART study: Natera Inc, San Carlos, CA (ended 2021)

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# Prenatal genetic screening: A (very) brief review

# Prenatal Genetic Screening

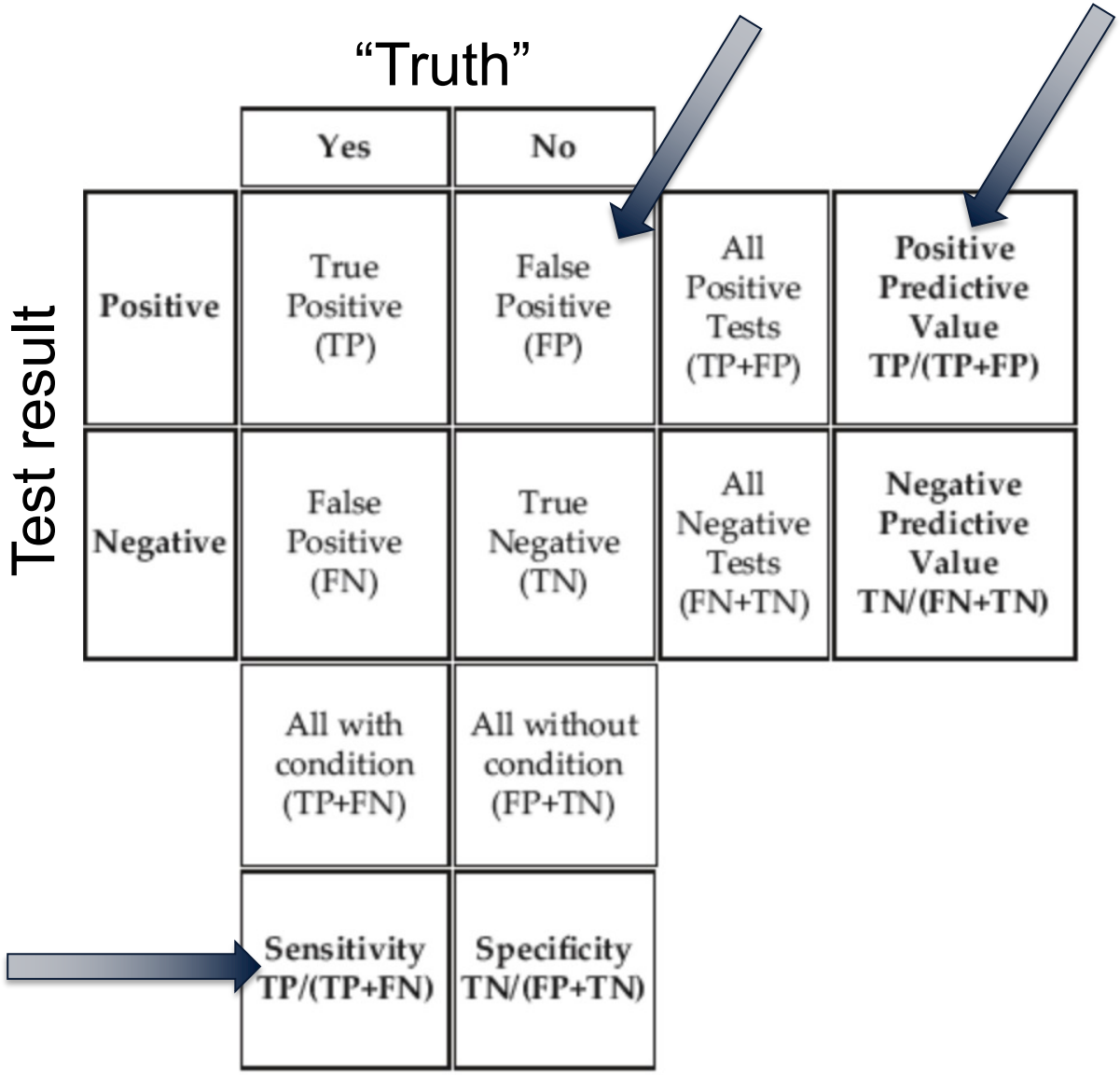
| | 1960's | 1984 | 1988 | 1996 | 1998-2006 | 2011 |
|---|---|---|---|---|---|---|
| | Maternal Age | MS-AFP | Triple Screen | Quad Screen | FTS: NT+serum | NIPS with cfDNA |
| Detection rate | 27% | 36% | 60-74% | 70-81% | 80-95% | 99% |
| Gestational age | N/A | 15 wks+ | 15 wks+ | 15 wks+ | 10-11wks+ | 9+ |
| Screened abnormality | T21 | T21, T13, T18 | T21, T13, T18 | T21, T13, T18 | T21, T13, T18 | T21, T13, T18 + |
| False positive T21 | 25% | NA | 5% | 5% | 5% | 0.1% |
| PPV for T21 | | | | | 3% | 95% |

# Screening performance

- ***False-positive:*** what is the chance that someone has a wrong high-risk screening results in the entire cohort?

- ***Sensitivity:*** How many of the affected patients will be detected?

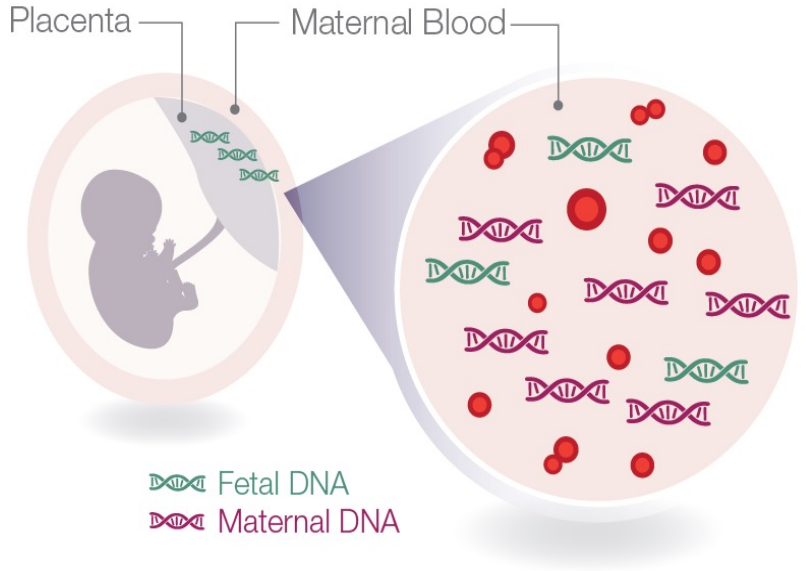
- ***Positive predictive value (PPV):*** From those that received positive results, what is the chance of it being a true positive?



# NIPS (Non-Invasive Prenatal Screening)

- Increasingly used as a primary method to screen pregnancies for the common whole-chromosome fetal aneuploidies due to high sensitivity and extremely low false positive (FP) rate

- NIPS uses “fetal” cfDNA in maternal serum that primarily arises from apoptosis of placental trophoblasts



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| Screened abnormality | T21 | T21, T13, T18 | T21, T13, T18 | T21, T13, T18 | T21, T13, T18 | T21, T13, T18 + |
| Screen positive | | | | | **534/10,000** | **42/10,000** |
| False positive T21 | 25% | NA | 5% | 5% | **500/10,000** | **2/10,000** |
| True positives | | | | | **40/10,000** | **40/10,000** |
| True negatives | | | | | **6/10,000** | **<1/10,000** |
| PPV for T21 | | | | | **3%** | **95%** |

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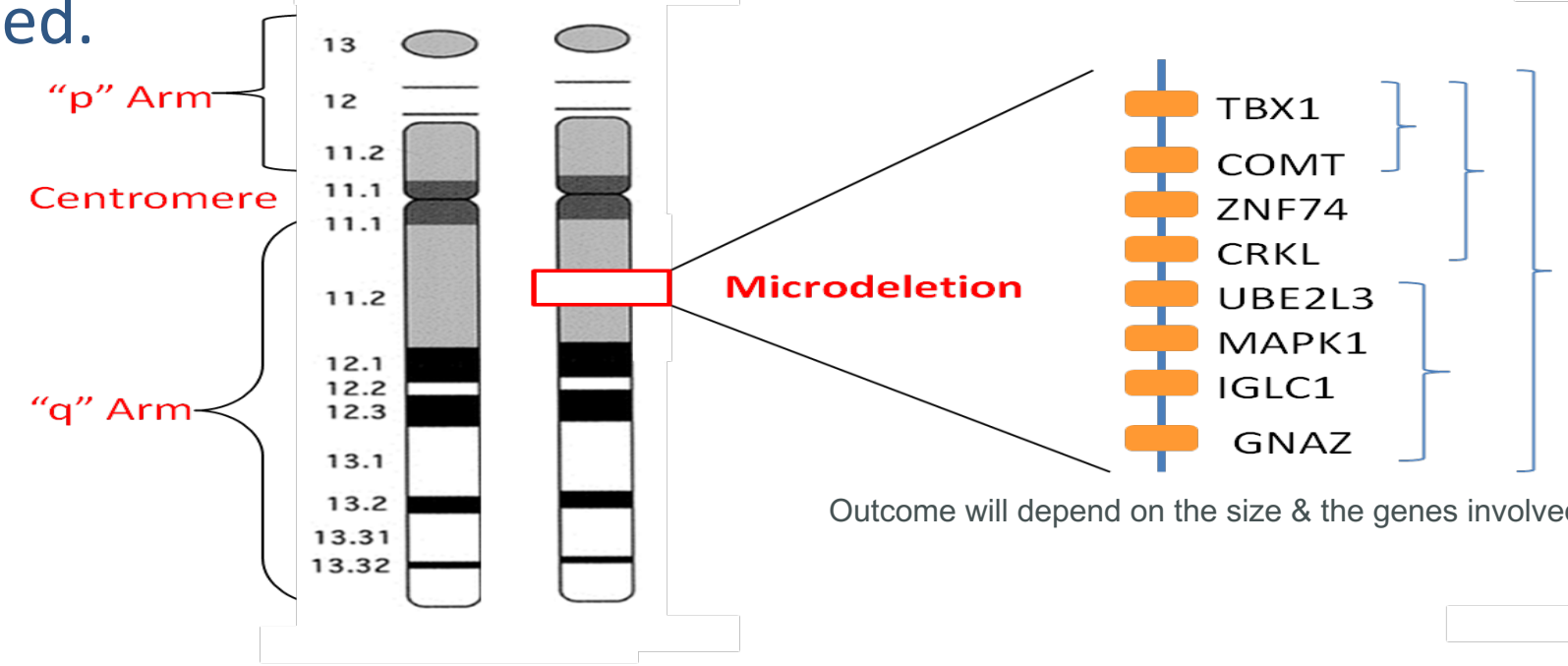
# Microdeletion syndromes

# What is a Microdeletion (or duplication)?

- Chromosomal deletions that are too small to be detected by light microscopy using conventional cytogenetic methods
- Karyotype can usually only visually detect ≥7-10 MB
- Size ranges 100kb to several MB. The larger the deletion, more genes are included.




Outcome will depend on the size & the genes involved

The NEW ENGLAND JOURNAL of MEDICINE

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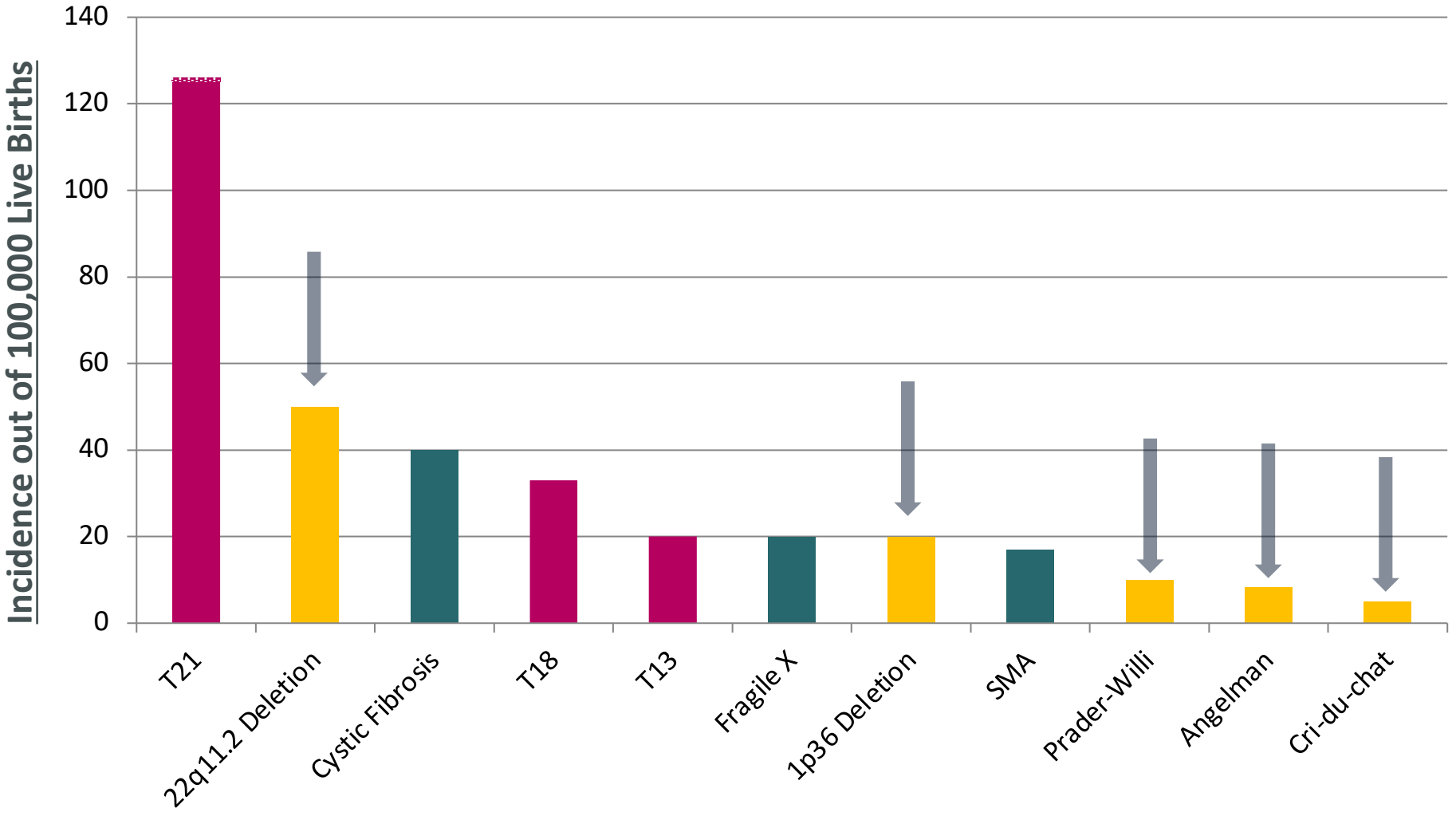
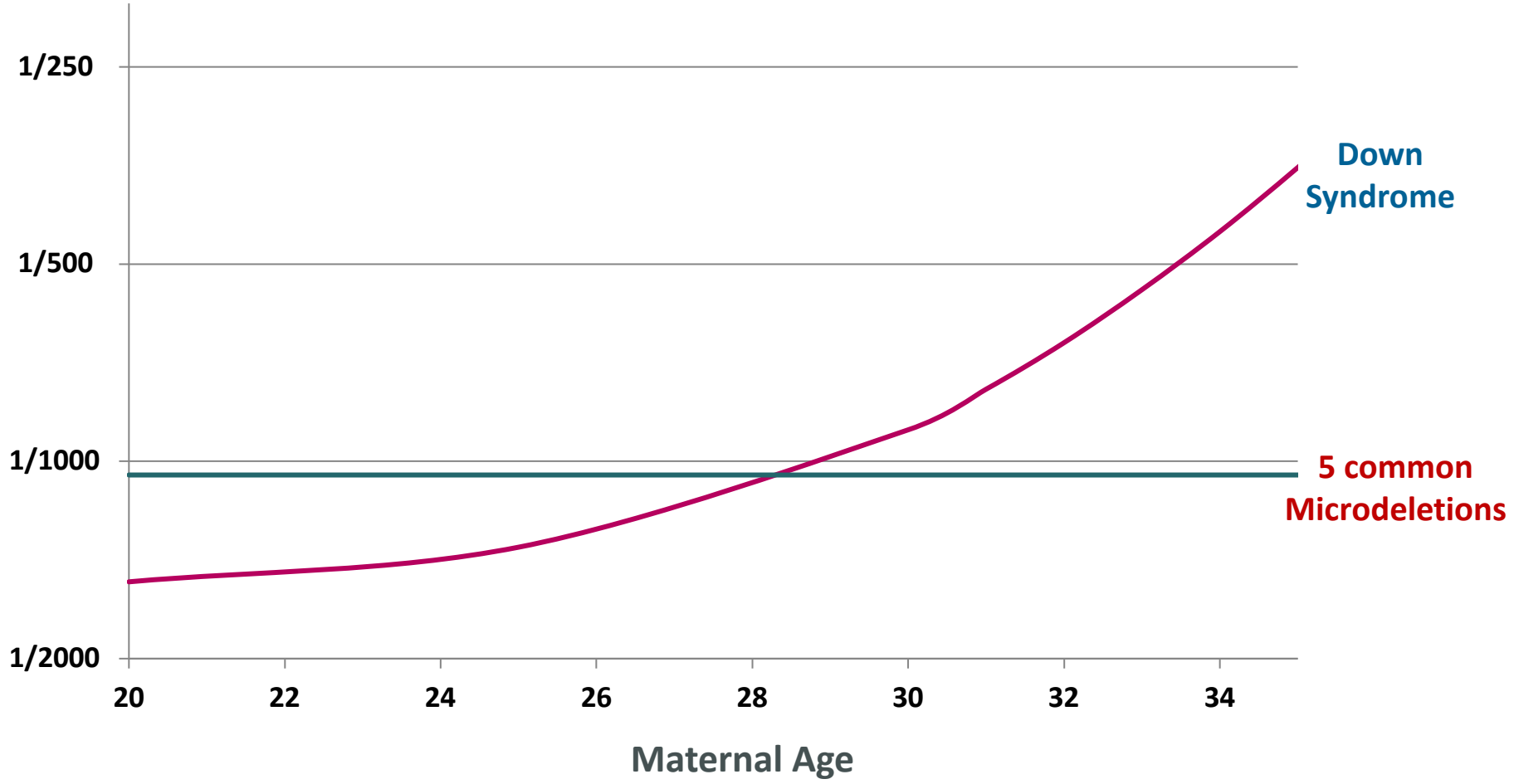
# Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis

Ronald J. Wapner, M.D., Christa Lese Martin, Ph.D., Brynn Levy, M.Sc.(Med.), Ph.D., Blake C. Ballif, Ph.D., Christine M. Eng, M.D., Julia M. Zachary, Melissa Savage, M.S., Lawrence D. Platt, M.D., Daniel Saltzman, M.D., William A. Grobman, M.D., M.B.A., Susan Klugman, M.D., Thomas Scholl, Ph.D., Joe Leigh Simpson, M.D., Kimberly McCall, B.S., Vimla S. Aggarwal, M.B., B.S., Brian Bunke, B.S., Odelia Nahum, M.Sc., Ankita Patel, Ph.D., Allen N. Lamb, Ph.D., Elizabeth A. Thom, Ph.D., Arthur L. Beaudet, M.D., David H. Ledbetter, Ph.D., Lisa G. Shaffer, Ph.D., and Laird Jackson, M.D.

# High Incidence Conditions



# 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.

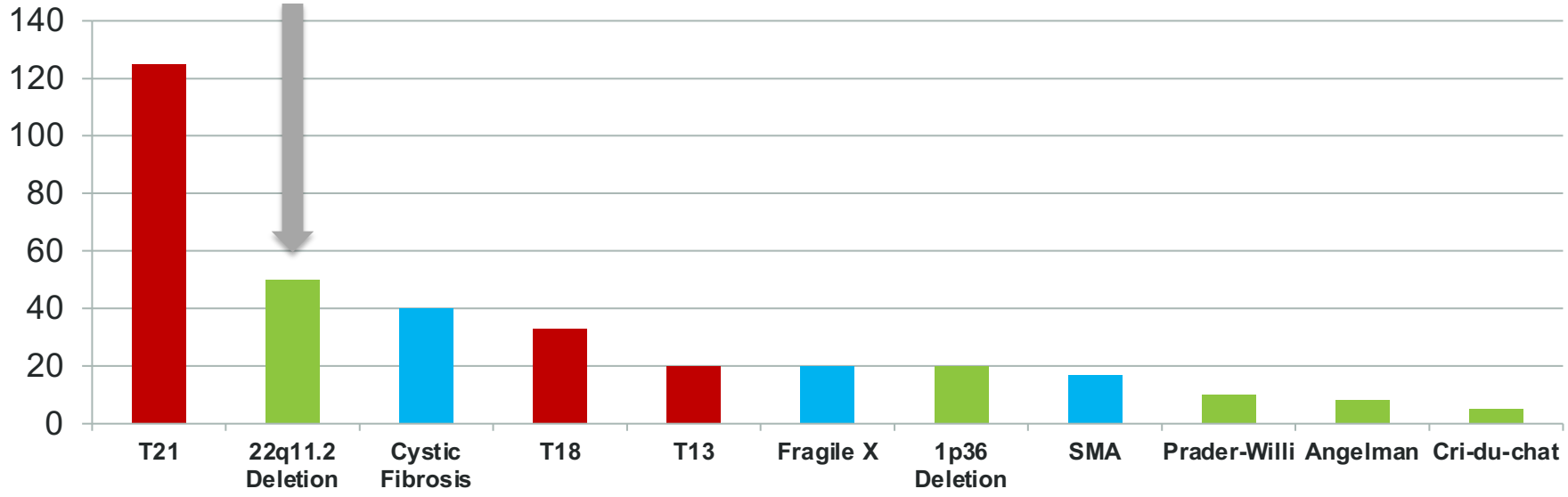
# Common microdeletions

| Microdeletion Syndrome | Additional names | Common Genomic defect | Prevalence | Clinical manifestations |
|---|---|---|---|---|
| **22q11.2 deletion syndrome** | Di-George; Velo-Cardio-Facial | 22q11.2 **3Mb del** | 1:2,000 | Cardiac and other anomalies, ***intellectual disability*****,** immune deficiency, hypocalemia, schizophrenia |
| **Prader-Willi syndrome** | | 15q11.2 **5Mb del**, Maternal UPD | 1:10,000 | ***intellectual disability***, short stature, genital hypoplasia, obesity, psychiatric disorders |
| **Angelman syndrome** | | 15q11.2-q13 **4Mb del**; UBE3A mutation; Paternal UPD | 1:12,000 | ***Severe intellectual disability***, seizures, problems with balance and walking. |
| **1p36 deletion syndrome** | 1p36 monosomy | 1p36 del **(1.5->10Mb)** | 1:5,000 | ***intellectual disability***, seizures, hearing loss, birth defects |
| **Cri-Du - Chat syndrome** | 5p- | 5p del **(5 to 40 Mb)** | 1:20,000 | ***Severe intellectual disability***, cardiac anomalies, scoliosis and short stature |

# 22q11.2 deletion syndrome

- 22q11.2DS (DiGeorge or Velo-Cardio-Facial syndrome) is the most common microdeletion in humans and a leading cause of congenital heart defects and neurodevelopmental delay

- Affects approximately 1 : 3-6,000 live births



# Clinical features of 22q11.2DS

| Feature | prevalence |
|---|---|
| Typical facial features | 100% |
| Congenital heart defects | 65% |
| Palate abnormalities | 70% |
| Gastrointestinal anomalies | 30% |
| Renal anomalies | 20% |
| Immunodeficiency | 75% |
| Neonatal hypocalcemia | 50% |
| Developmental delay | 90% |
| Psychiatric disorders | 60% |

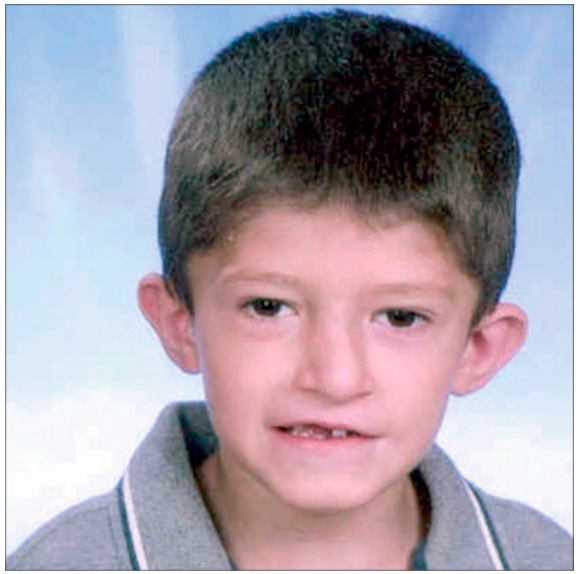
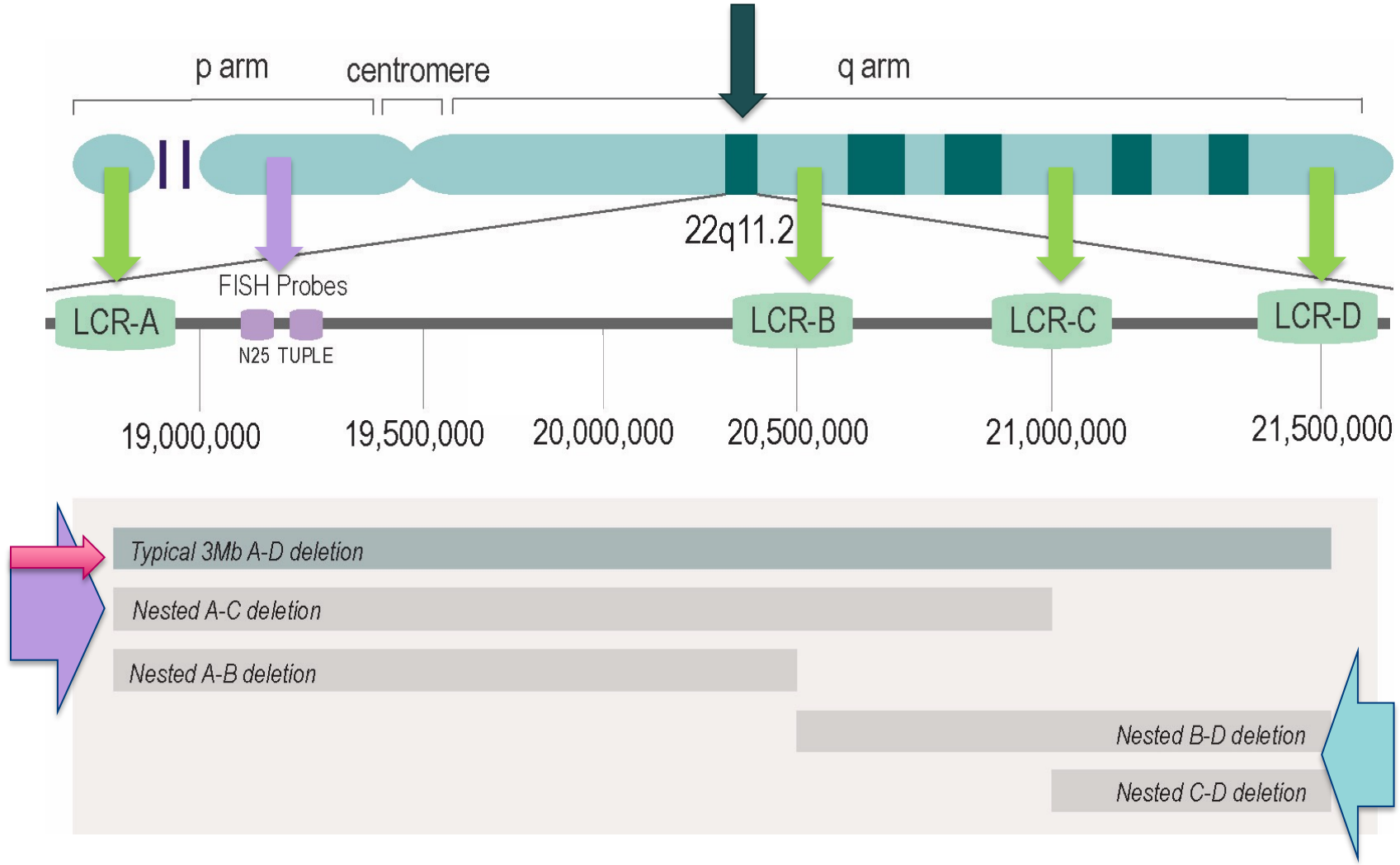


Image from: Lisa J Kobrynski, Kathleen E Sullivan. Velocardiofacial syndrome, DiGeorge syndrome: the chromosome 22q11.2 deletion syndromes, The Lancet; 370,9596, 2007,1443-1452

# 22q11.2DS - early intervention matters

- Plan delivery of a fetus with cardiac anomaly at a center capable of caring for complex cardiac anomalies
- Monitor calcium levels after birth to prevent long term sequalae that is is associated with hypocalcemia
- Delay administering live vaccines due to thymus hypoplasia associated immunodeficiency

# Chromosome 22



# Prader-Willi Syndrome (PWS)

- Affects 1:12,000-30,000 live births
- Clinical features:
  - Mild to moderate intellectual disability
  - Delayed motor development
  - Speech and behavioral problems
  - Distinct facial features
  - Food craving and obesity
  - Hypogonadism
  - Short stature

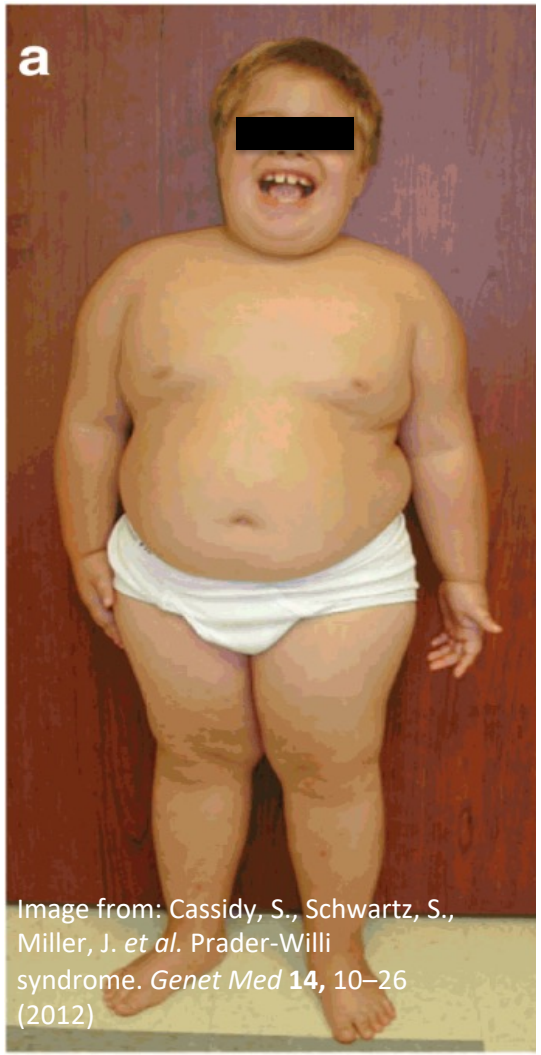



Image from: Cassidy, S., Schwartz, S., Miller, J. *et al.* Prader-Willi syndrome. *Genet Med* **14,** 10–26 (2012)

# Genetics of PWS

- The 15q11.2-q13 region includes mostly maternally imprinted genes that are controlled by the imprinting center (IC).

- Two main genetic mechanisms cause PWS:
  - In 75%. the 15q11.2-q13 region from the fathers ch #15 is deleted
  - In 25% 2 maternal ch #15 are transferred to the child (Maternal Uniparental Disomy or UPD)

- Deletion of the maternal chromosome in the same region will result in a different syndrome - Angelman syndrome

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# Prenatal screening for Microdeletion syndromes

# Why screen for microdeletions?
# Current criteria for prenatal genetic screening

- Must have a significant clinical impact and/or proven immediate postnatal intervention
- The disease is prevalent
- Test performance is reasonable
- Cost is reasonable

# Why screen for microdeletions?

- A common cause for intellectual disability and developmental delay
- Leading cause of genetic disorders in younger women and in aggregate are more prevalent than the common trisomies
- Can be easily missed in routine prenatal care or after birth
- Prenatal detection has the potential to improve short- and long-term infant and childhood outcomes in some syndromes

# NIPS for microdeletions and duplications

- cfDNA screening introduced the potential to target any region of the genome – i.e. the opportunity to extend routine screening beyond the detection of aneuploidies
- Introduced in 2014 and isoffered by several commercial companies
- Companies claim high detection rate and decent PPV but data is based on small validation studies or retrospective cohorts
- Concern: Data on actual disease prevalence and real-world test performance using genetic confirmation in a large cohort was lacking

Original Research ajog.org

OBSTETRICS

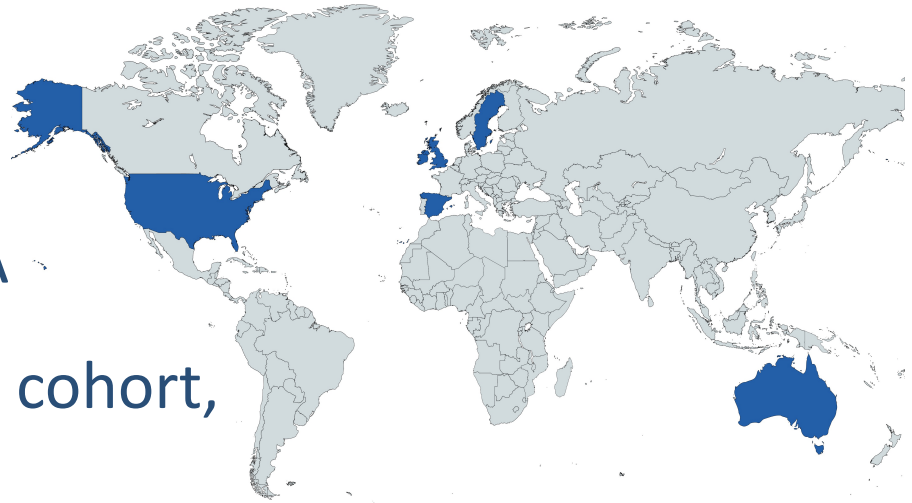
# Cell-free DNA screening for prenatal detection of 22q11.2 deletion syndrome

Pe'er Dar, MD; Bo Jacobsson, MD, PhD; Rebecca Clifton, PhD; Melissa Egbert, MS; Fergal Malone, MD; Ronald J. Wapner, MD; Ashley S. Roman, MD; Asma Khalil, MD; Revital Faro, MD; Rajeevi Madankumar, MD; Lance Edwards, MD; Noel Strong, MD; Sina Haeri, MD; Robert Silver, MD; Nidhi Vohra, MD; Jon Hyett, MD; Zachary Demko, PhD; Kimberly Martin, MD; Matthew Rabinowitz, PhD; Karen Flood, MD; Ylva Carlsson, MD, PhD; Georgios Doulaveris, MD; Sean Daly, MD; Maria Hallingström, PhD; Cora MacPherson, PhD; Charlly Kao, PhD; Hakon Hakonarson, MD, PhD; Mary E. Norton, MD

SMART
SNP-based Microdeletion and Aneuploidy Registry Trial

## Primary Objective of the SMART study

To assess the performance of SNP-based cfDNA screening for 22q11.2DS in a large, prospective cohort, using genetic confirmation in all pregnancies



# SMART Methods unique aspects

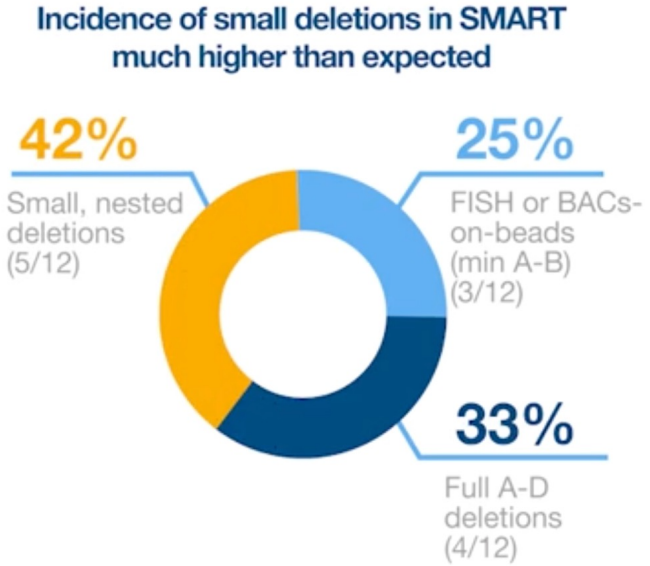
- Prospectively obtained DNA samples for genetic confirmation by Chromosomal Microarray from over 18,000 fetuses and newborns.
- Included very small deletions (>500Kb) in the analysis
- Data was re-analyzed with an updated cfDNA algorithm using for machine learning technologies to optimize the identification of of those very small deletions

# SMART Results

**Enrolled n=20,887**

**Final cohort for analysis**
(cfDNA results and genetic confirmation available)
**n=18,290 (87.5%)**

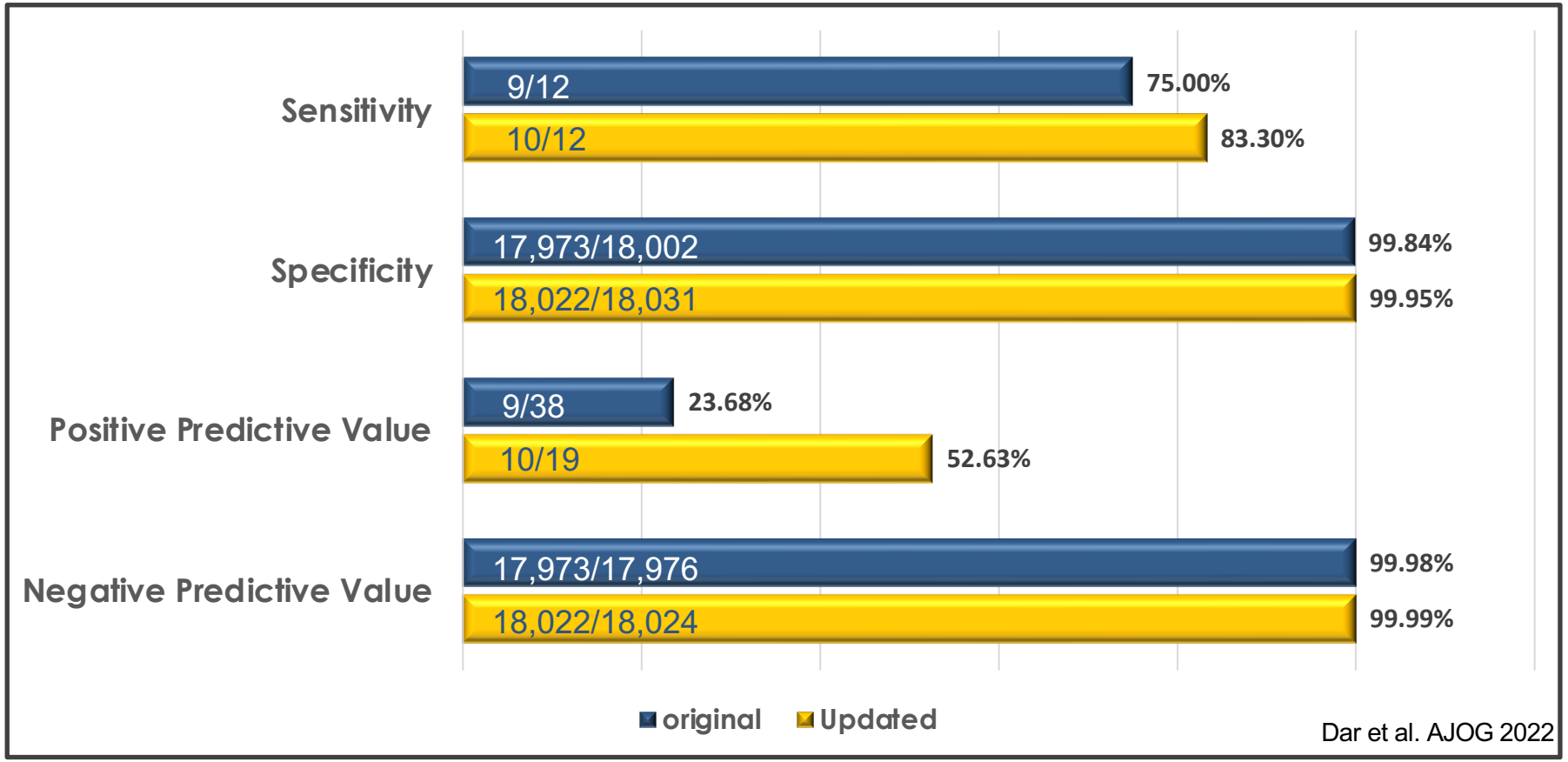
**12 confirmed 22q11.2 deletions**




**Prevalence 1:1524**

Dar et al. AJOG 2022

# 22q11.2 deletion screening performance




Dar et al. AJOG 2022

# Additional microdeletions (n=10,971)

- CMA confirmed 5 PWS cases (1:2,194), and one case of PWS/AS, and one Cri-Du-Chat case
- 6/7 microdeletion cases, were detected by cfDNA (sensitivity of 85.7%). The Cri-Du-Chat case was missed
- cfDNA was reported as high-risk in 14 cases (0.13%), 6 true and 8 false positives. The PPV for PWS was 62.5% (5/8).

Table 1. Characteristics of Deletions and Loss-of-Heterozygosity Cases Identified by Microarray

| Diagnosis | Size of Deletion or Region with LOH | GA at cfDNA (weeks) | NT (mm) | SGA | Prenatal Anomalies Detected | Postnatal Malformation and Short-term Outcome | Fetal or newborn Genetic Testing | cfDNA Screening Result |
|---|---|---|---|---|---|---|---|---|
| *Confirmed Syndrome by Microarray* | | | | | | | | |
| Cri-du-Chat | 2Mb | 10 | 1.6 | No | No | No | No | Low-Risk |
| PWS | 4.8Mb | 18 | 1.5 | Yes | No | Left club foot | No | High-Risk |
| PWS | LOH 13.7Mb | 12 | 1.6 | No | No | Micrognathia, hypotonia, respiratory failure | Newborn | High-Risk |
| PWS | 4.8Mb | 13 | 2.3 | No | No | No | No | High-Risk |
| PWS | LOH 7.8Mb | 13 | 1.7 | No | No | No | No | High-Risk |
| PWS | LOH 11.9Mb | 32 | N/A | No | 32 weeks Pierre Robin Sequence, CL/P, Heterotaxy | No | No | High-Risk |
| PWS/AS[£] | LOH 11.3Mb | 11 | 1.9 | No | No | No | No | High-Risk |

**Table 1.** *LOH – Loss of heterozygosity within the critical region and including disease-associated genes was considered diagnostic and suggestive of uniparental disomy; £ CMA inconclusive without maternal sample available to differentiate between PWS and AS; GA- gestational age; NT-Nuchal translucency; CL/P – cleft lip and palate; SGA- small for gestational age

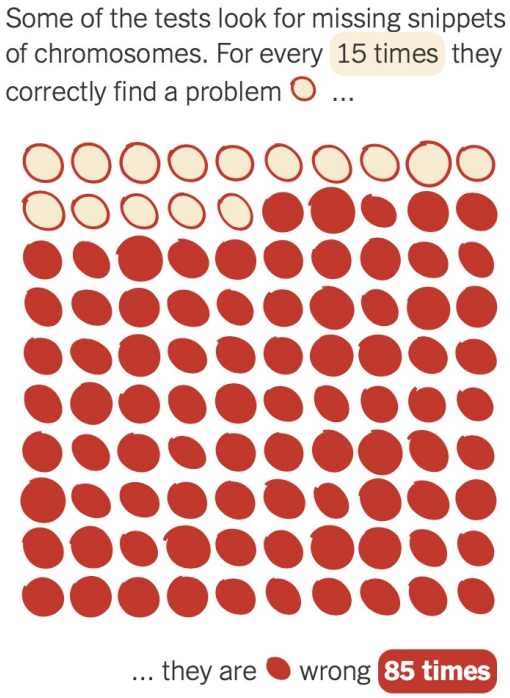
# Summary of SMART study findings

- 22q11.2DS prevalence in our cohort was 1:1,524
- cfDNA screening detected 83% of 22q11 microdeletions that are >500kb with a false positive rate of <0.1% and PPV of 52.6%
- Using the updated algorithm, NIPS detected all PWS cases with a low false-positive rate  but screening performance for the other microdeletions could not be determined

The New York Times

TheUpshot

*When They Warn of Rare Disorders, These Prenatal Tests Are Usually Wrong*




FDA U.S. FOOD & DRUG ADMINISTRATION

# Genetic Non-Invasive Prenatal Screening Tests May Have False Results: FDA Safety Communication

The New York Times

Chance positive results are wrong

**DiGeorge syndrome**
Affects 1 in 4,000 births
Can cause heart defects and delayed language acquisition. (May appear on lab reports as "22q.")

81% wrong

**1p36 deletion**
1 in 5,000 births
Can cause seizures, low muscle tone and intellectual disability.

84% wrong

**Cri-du-chat syndrome**
1 in 15,000 births
Can cause difficulty walking and delayed speech development.

80% wrong

**Wolf-Hirschhorn syndrome**
1 in 20,000 births
Can cause seizures, growth delays and intellectual disability.

86% wrong

**Prader-Willi and Angelman syndromes**
1 in 20,000 births
Can cause seizures and an inability to control food consumption.

93% wrong

TABLE 4

**Estimated positive predictive value and negative predictive value**

| Disorder | Incidence (1:n) | Frequency of deletion evaluated | Positive predictive value,[a] % | Negative predictive value,[b] % |
|---|---|---|---|---|
| 22q11.2 del | 2000 | 0.87 | 5.3 | >99.99 |
| Prader-Willi | 10,000 | 0.28 | 4.6 | >99.99 |
| Angelman | 12,000 | 0.28 | 3.8 | >99.99 |
| 1p36 del | 5000 | 0.60 | 17.0 | >99.99 |
| Cri-du-chat | 20,000 | 0.65 | 5.3 | >99.99 |

[a] Calculated by multiplying population incidence, the frequency of the deletion evaluated, and the positive likelihood ratio (detection rate/false-positive rate); [b] Calculated by multiplying population incidence, the frequency of the deletion evaluated, and the negative likelihood ratio ([1-detection rate]/[1-false-positive rate]).

*Wapner. Noninvasive screening for fetal microdeletion syndromes. Am J Obstet Gynecol 2015.*

# How to interpret NIPS results

- While low-risk results are expected in most cases and are reassuring, false-negative results, although rare, can still occur.

- NIPS is a screening test and not a diagnostic test and false positives are possible and even common for microdeletions.

- High-risk results should be confirmed with diagnostic testing and decisions should not be made solely on NIPS results.

# Screening for microdeletions is complicated

- The rarer the deletion, it is more difficult to assess test performance. A lower PPV is expected.
- Deletions can be of different sizes or in different locations within the syndrome-related region.
- Some deletions may not include the syndrome critical region or genes
- The clinical implication of very small deletions is less clear
- Syndromes that are associated with imprinted genes require further testing

# Should we challenge the current paradigm of prenatal genetic testing?

- The current prenatal screening model follows a paradigm that is based on the  prenatal screening model for T21, i.e. disease must be prevalent
- New technologies, such as whole-exome sequencing, allow screening of the entire genome including for rare single-gene disorders
- If the real question asked by parents is: “Is my child healthy?” Should the paradigm change from adding disorders, approved by professional societies in a salami method to an all-inclusive assessment of the fetal genome with parents’ autonomy to make their own decisions?

# Conclusions

- Screening for microdeletion syndromes is clinically reasonable as they are associated with severe sequelae
- Detection of microdeletions by cfDNA is complicated, but we have now data that at least for 22q11.2DS it is accurate with clinically reasonable PPV
- Before expanding NIPS to additional microdeletions or to all-inclusive screening, professional societies should reassess the goals of modern prenatal genetic screening.
- NIPS has false negatives and false positives and patients need to be aware that it is a screening and not a diagnostic test

# SMART investigators

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# Questions?

# High-Risk 22q11 Results Example

## FINAL RESULTS SUMMARY

| *Result* | *Fetal Sex* | *Fetal Fraction* |
|---|---|---|
| **HIGH RISK for 22q11.2 deletion syndrome** | **Male** | **8.3%** |

***This is a screening test only. Genetic counseling and diagnostic testing with a microarray should be offered to further evaluate these findings.***

The Panorama risk score reflects analysis of DNA from the placenta. The placental DNA may not accurately reflect the status of the fetus; therefore, no irreversible decisions should be made based upon results of this screening test alone.

## RESULTS DETAILS

| *Condition tested*[1] | *Result* | *Risk Before Test*[2] | *Panorama Risk Score*[3] |
|---|---|---|---|
| Trisomy 21 | Low Risk | 1/152 | <1/10,000 |
| Trisomy 18 | Low Risk | 1/111 | <1/10,000 |
| Trisomy 13 | Low Risk | 1/357 | <1/10,000 |
| Monosomy X | Low Risk | 1/256 | <1/10,000 |
| Triploidy/Vanishing twin | Low Risk | | |
| **22q11.2 deletion syndrome** | **High Risk** | 1/2,000[4] | **1/19** |

1. Excludes cases with evidence of fetal and/or placental mosaicism. 2. Based on maternal age, gestational age, and/or general population, as applicable. References available upon request. 3. Based on a priori risk and