

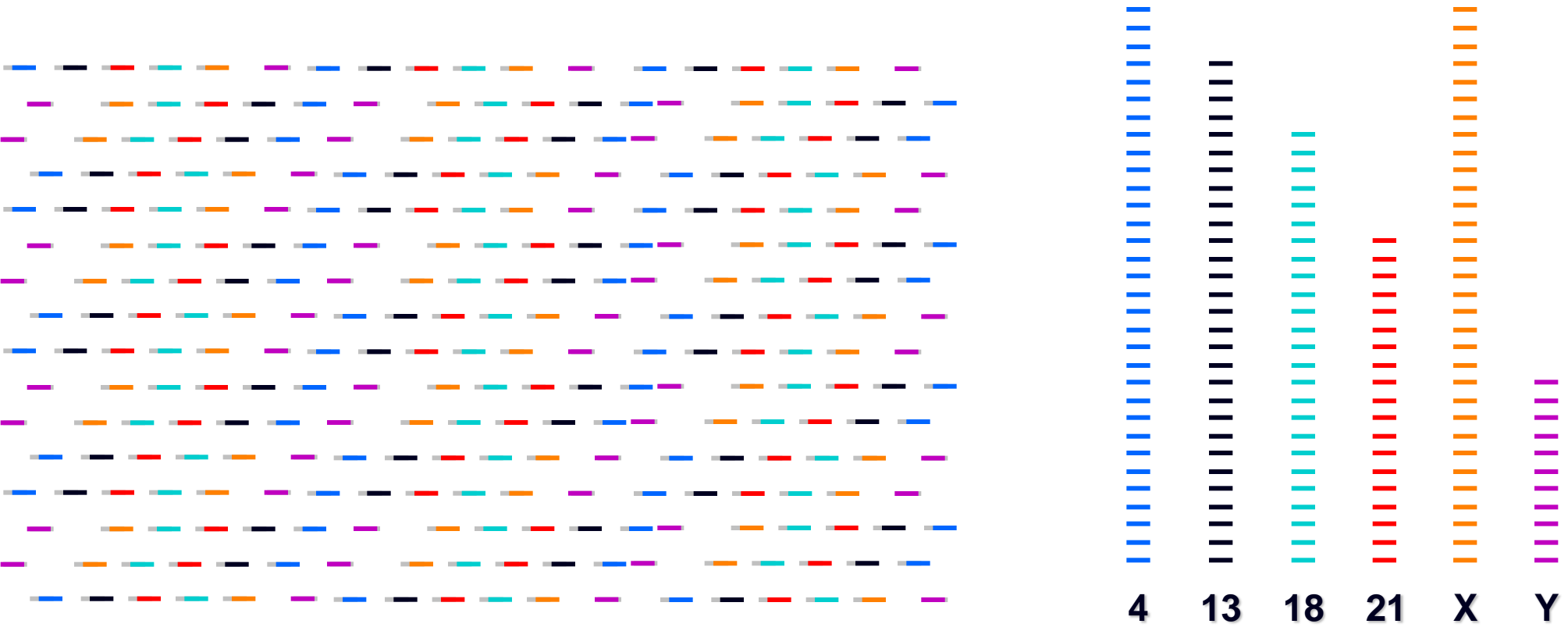


Screening for Down syndrome

cell-free DNA analysis in maternal blood



Cell-free DNA in maternal blood

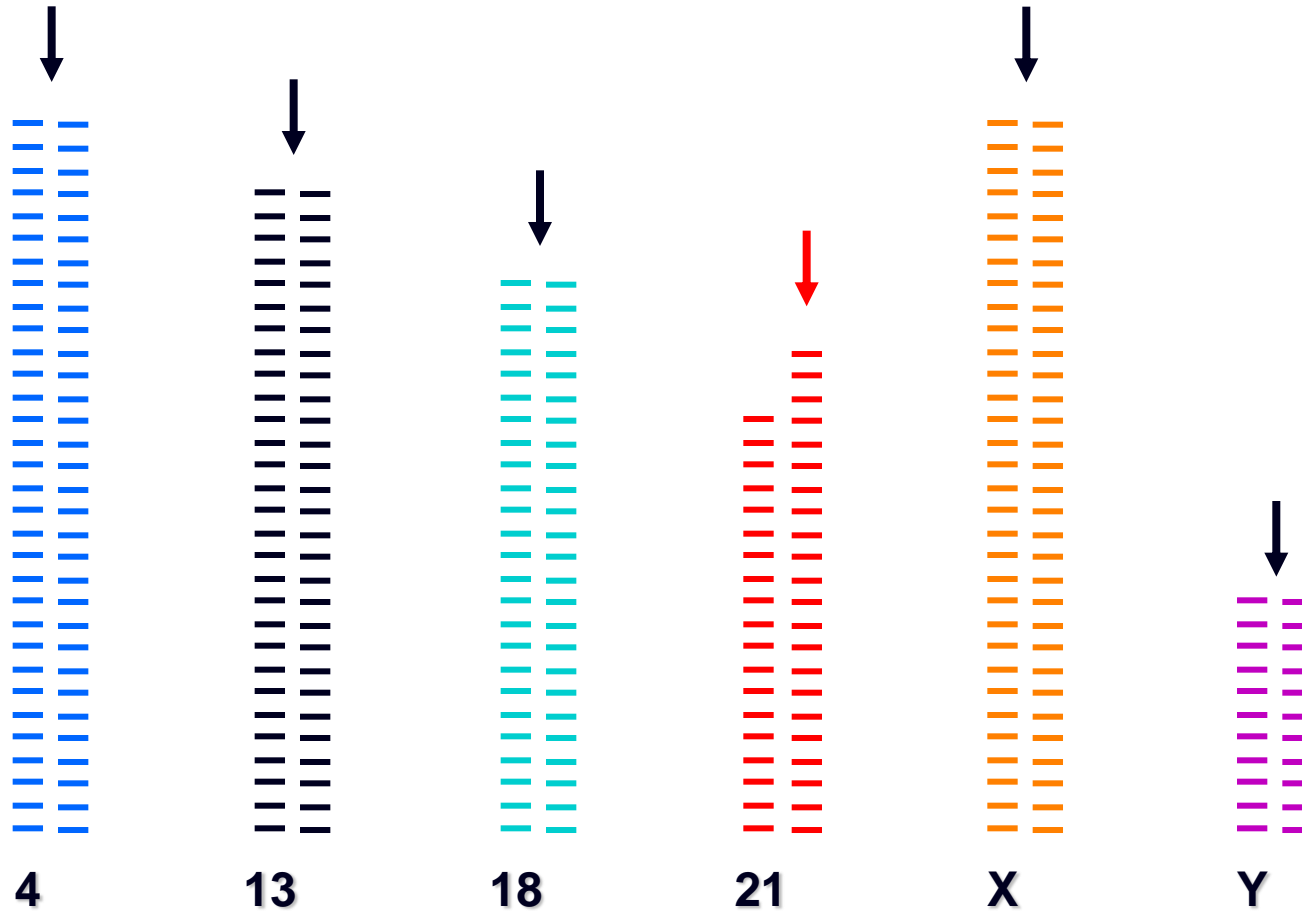


There are many millions of cf DNA fragments in maternal plasma

The cf DNA fragments are sequenced, their chromosome origin is established and the quantity of each chromosome is determined



Cell-free DNA in maternal blood

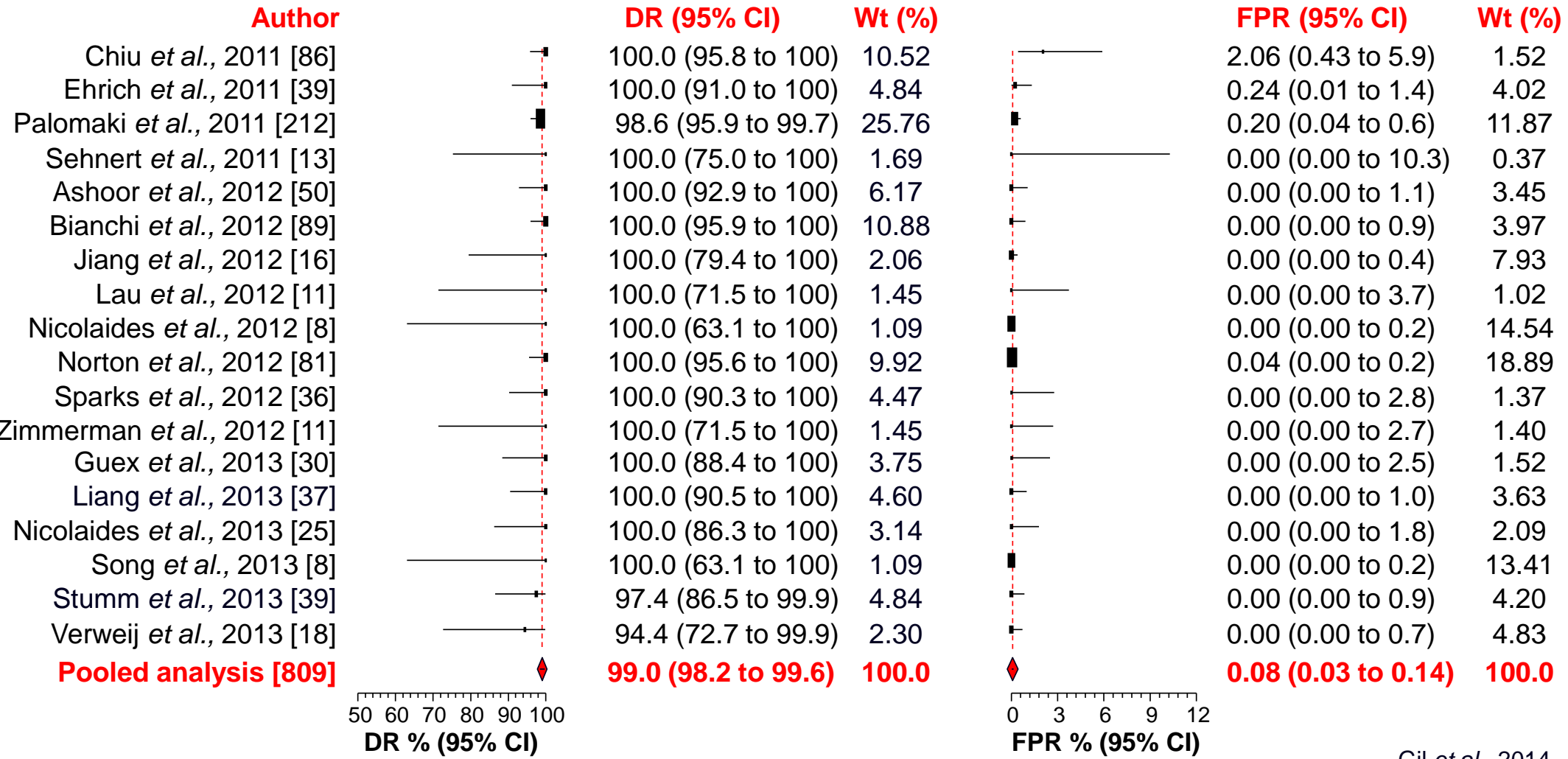


In **trisomy 21** cfDNA fragments from chromosome 21 are increased

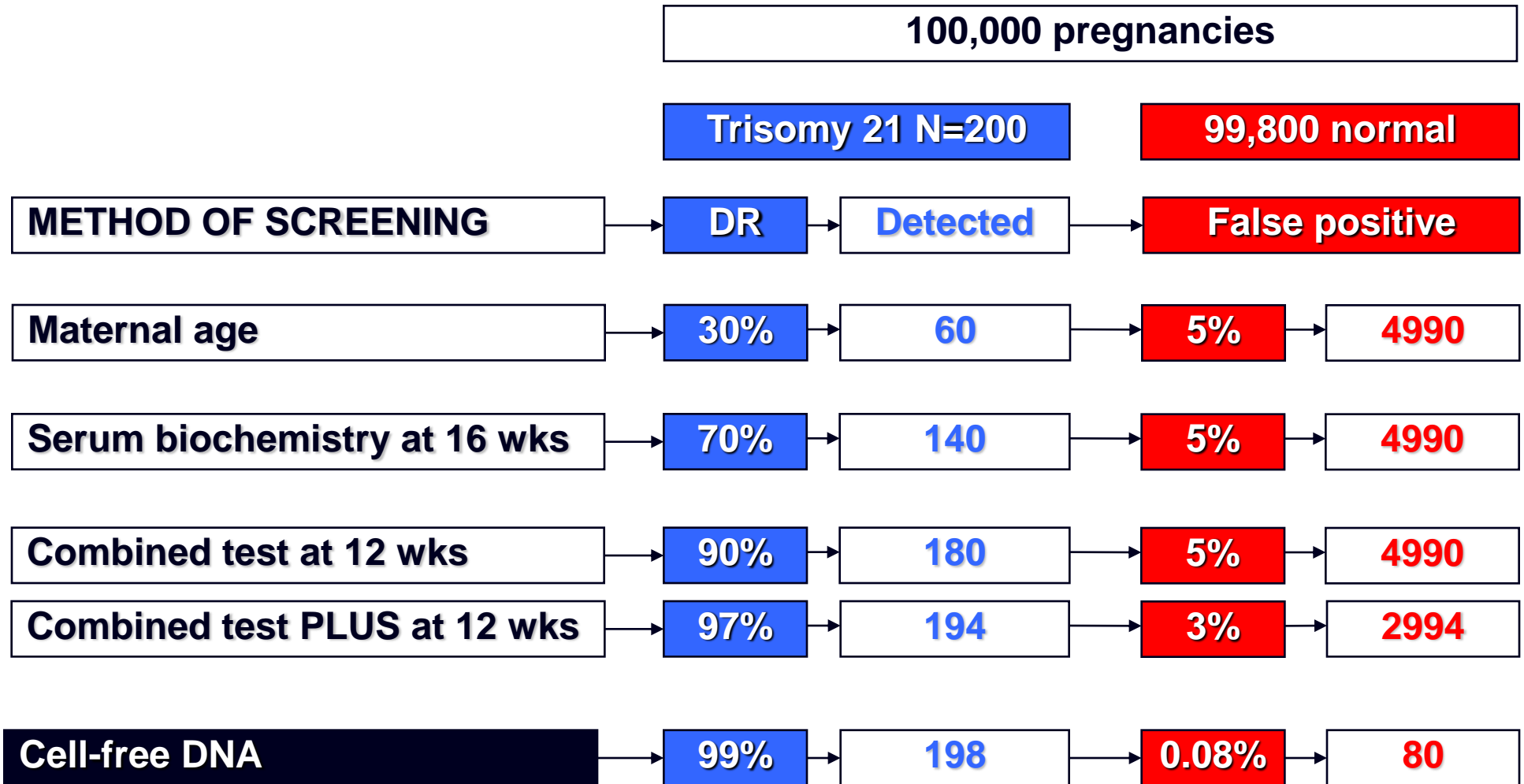


Cell free DNA test

Trisomy 21



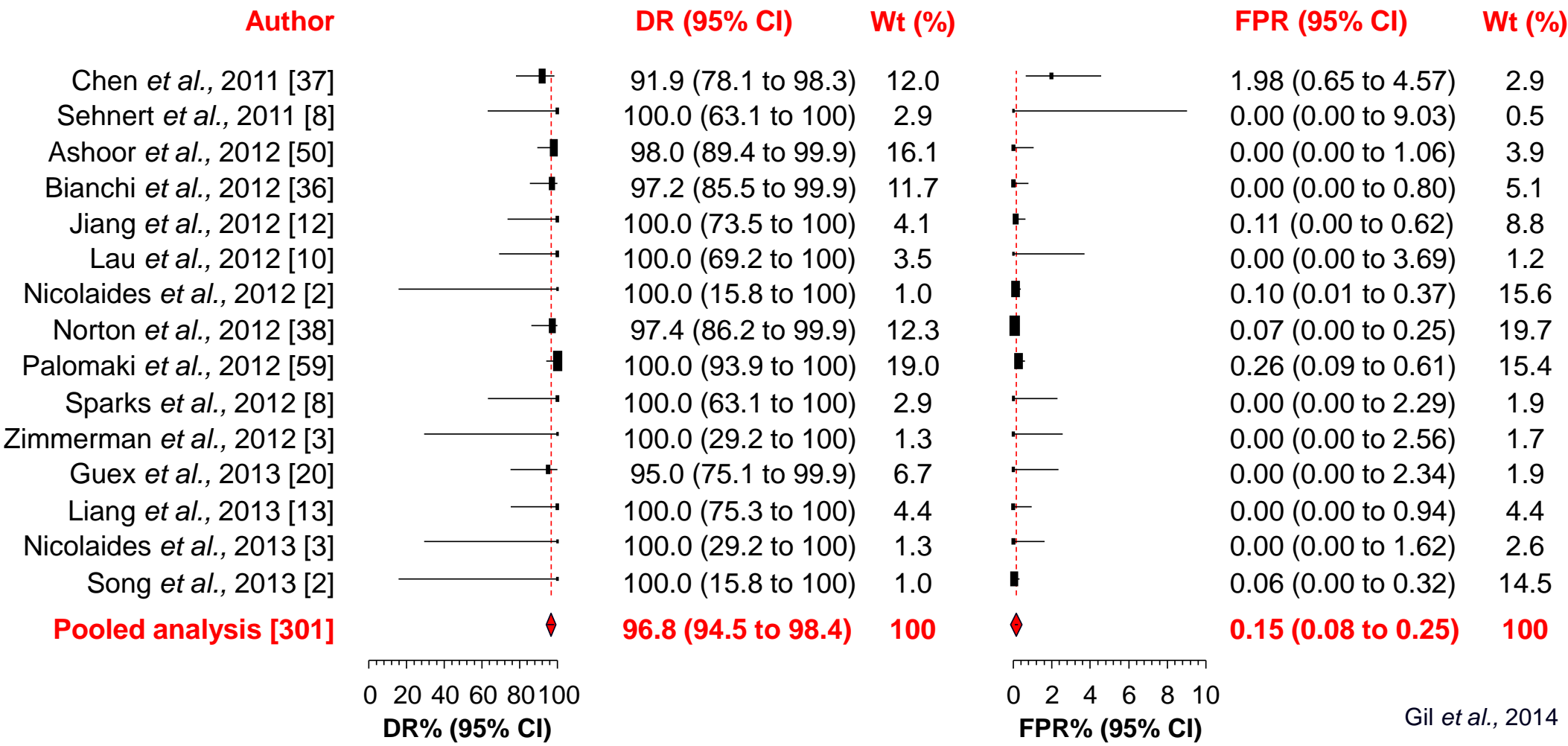
Screening for trisomy 21 1960-2014





Cell free DNA test

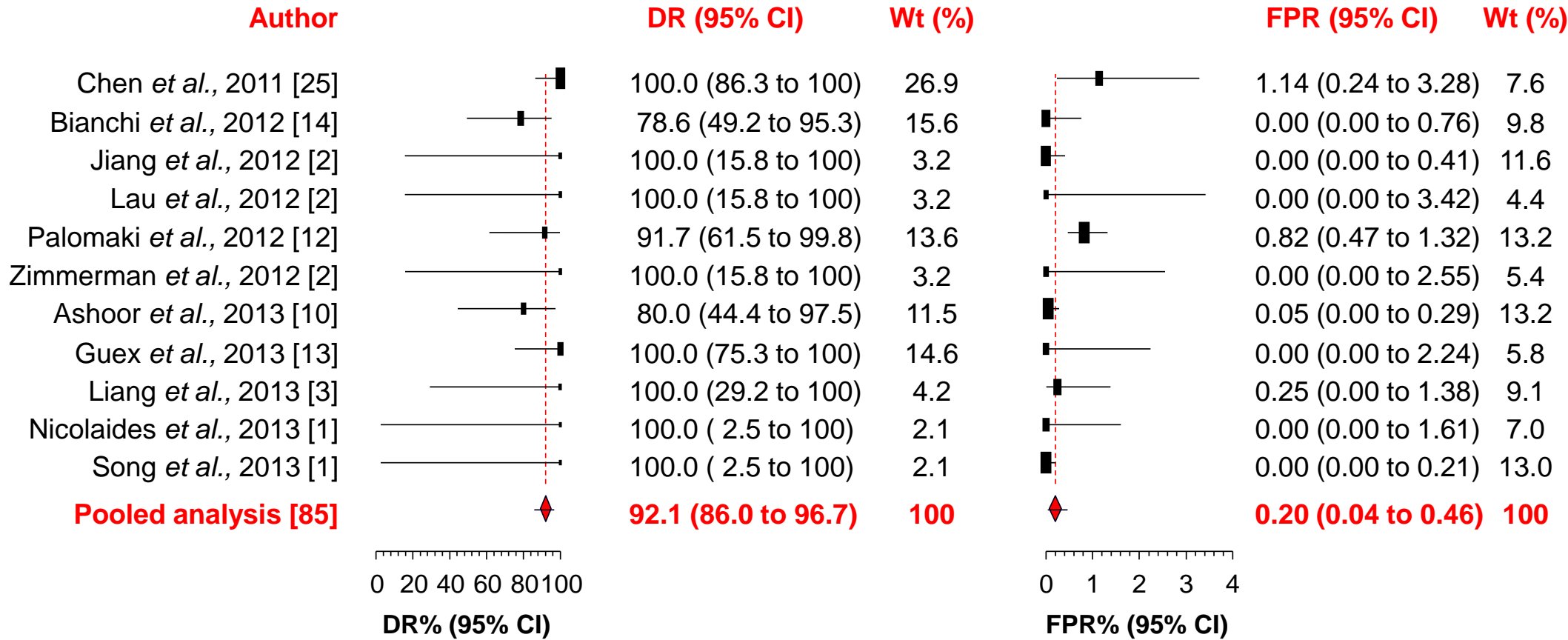
Trisomy 18





Cell free DNA test

Trisomy 13





Meta-analysis: clinical implementation / validation studies

				DR/FPR	1/ (100-DR)/ (100-FPR)
		DR	FPR	LR +ve	(LR -ve)
Trisomy 21	n=809	99.0%	0.08%	1238	1000
Trisomy 18	n=301	96.8%	0.15%	645	31
Trisomy 13	n= 85	92.1%	0.20%	461	13

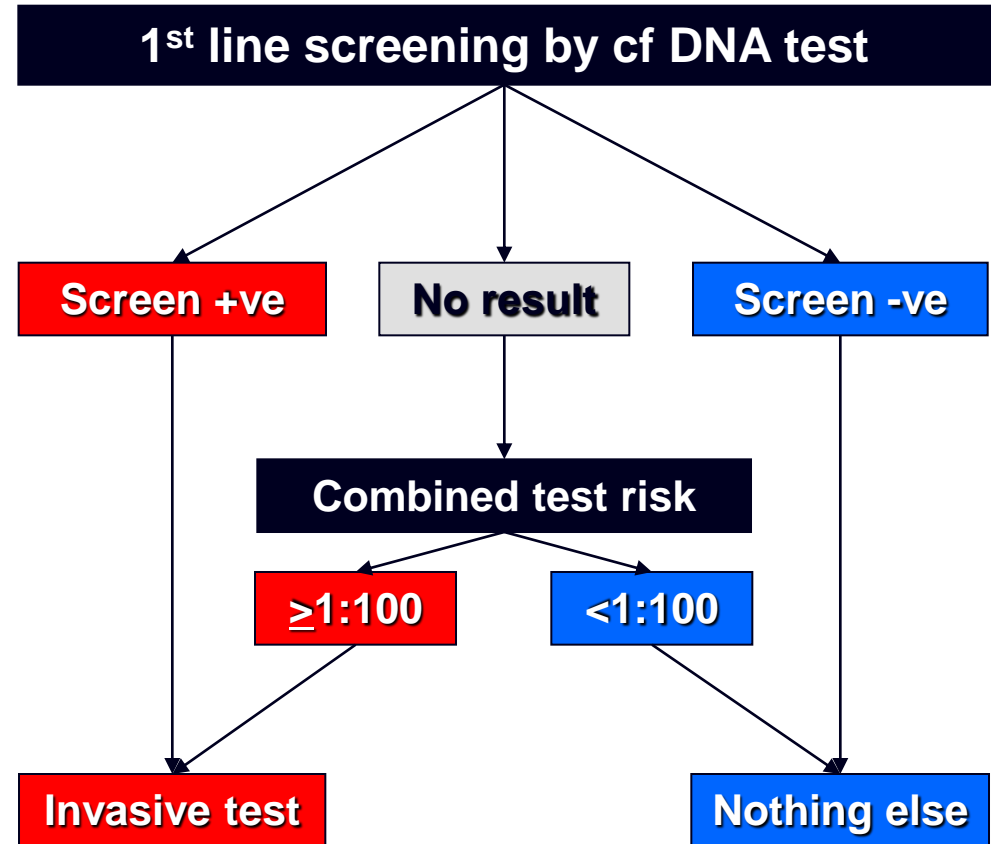
$$1 / (7.9 / 99.8) = 1 / 0.079 = 13$$

Chiu *et al.*, 2011; Chen *et al.*, 2011; Ehrich *et al.*, 2011; Sehnert *et al.*, 2011; Palomaki *et al.*, 2011; Ashoor *et al.*, 2012; Bianchi *et al.*, 2012; Jiang *et al.*, 2012; Lau *et al.*, 2012; Nicolaides *et al.*, 2012; Norton *et al.*, 2012; Palomaki *et al.*, 2012; Sparks *et al.*, 2012; Zimmerman *et al.*, 2012; Ashoor *et al.*, 2013; Guex *et al.*, 2013; Liang *et al.*, 2013; Nicolaides *et al.*, 2013; Song *et al.*, 2013; Stumm *et al.*, 2013; Verweij *et al.*, 2013



Model 1 Universal screening

Detection T21	98-99%
Detection T18/13	95%
Invasive testing	1%



10 weeks' gestation

Scan for viability, number of fetuses and gestational age

Blood sampling for cfDNA testing

12 weeks' gestation

Combined test for trisomies, fetal defects and pregnancy complications

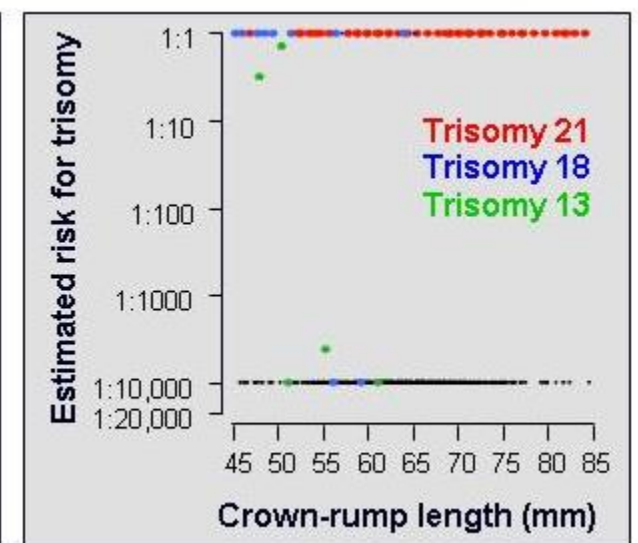
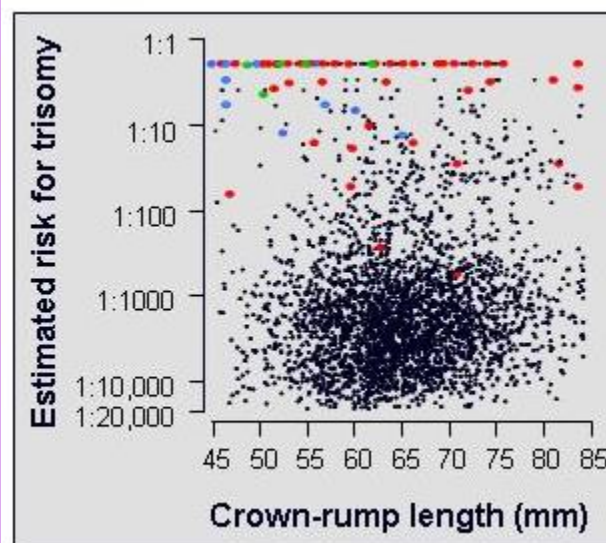
Lessons learnt

Interval to results:

Median 9 days
98% by 14 days

No results:

First draw 4%
Second draw 2%



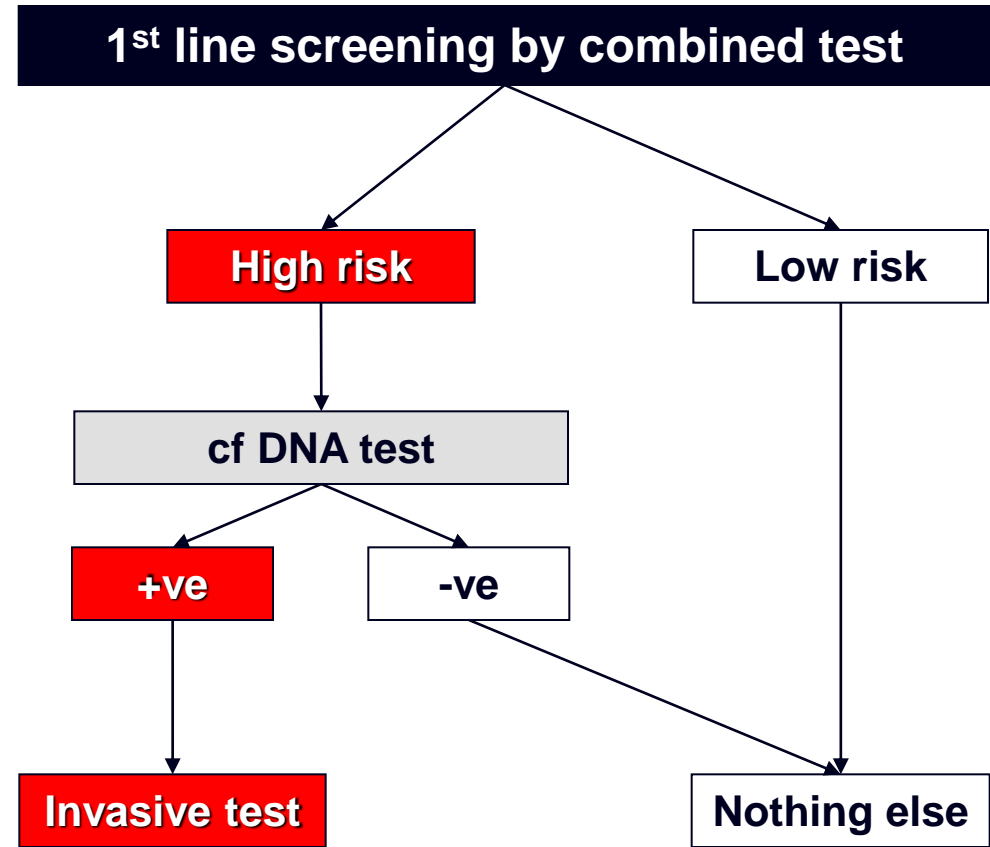


Cell free DNA test

Clinical implementation

Model 2 cf DNA test contingent on results of combined test

Detection T21	86%
Detection T18/13	89%
Invasive testing	0.4%

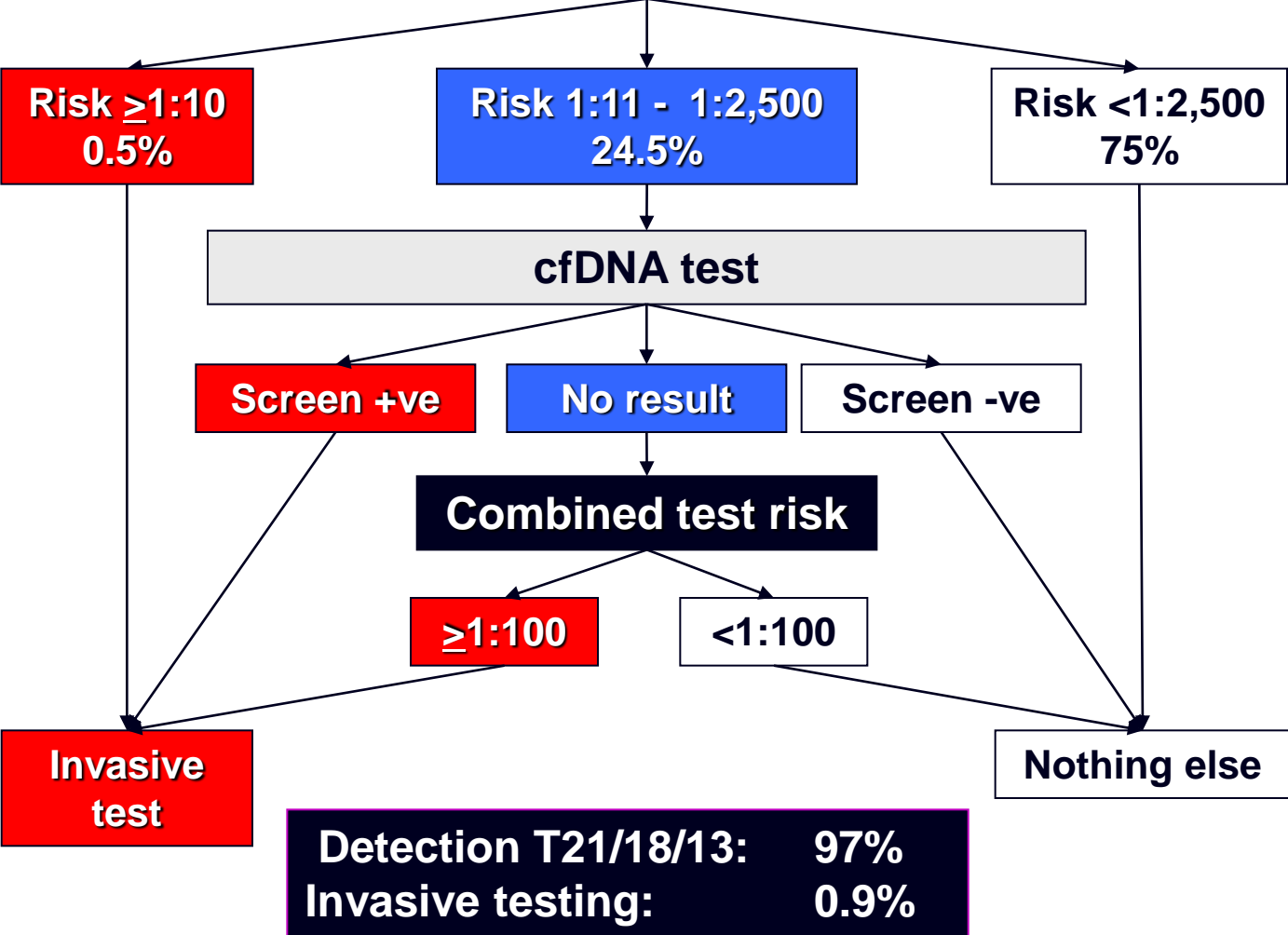




Cell free DNA test

Clinical implementation

First - line screening by the combined test



Combined test	25%
+ PLGF, AFP	17%
+ PLGF, AFP, DVPI	9%

Detection T21/18/13: 97%
 Invasive testing: 0.9%

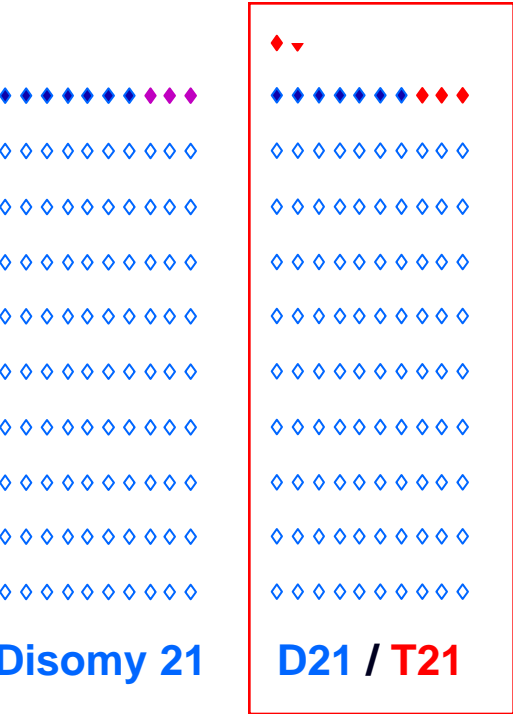
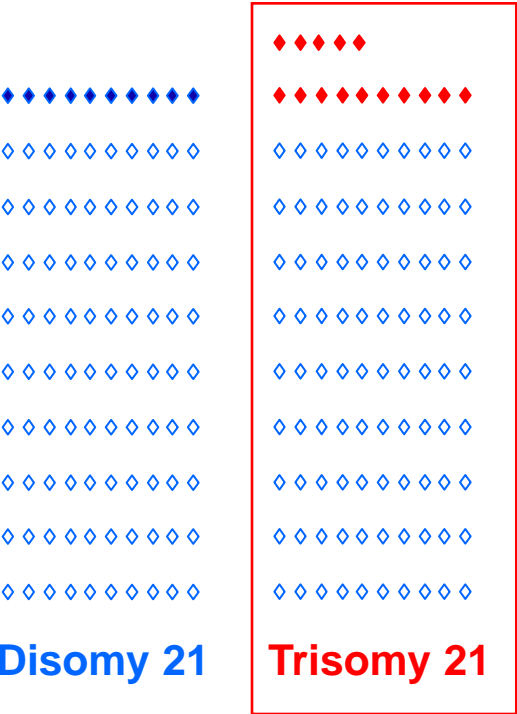
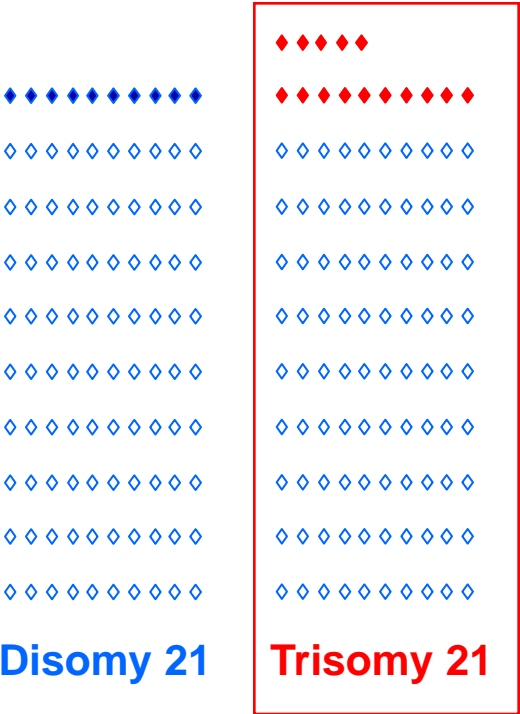


Cell free DNA test in twins

Singletons ff 10%

MC twins ff 10%

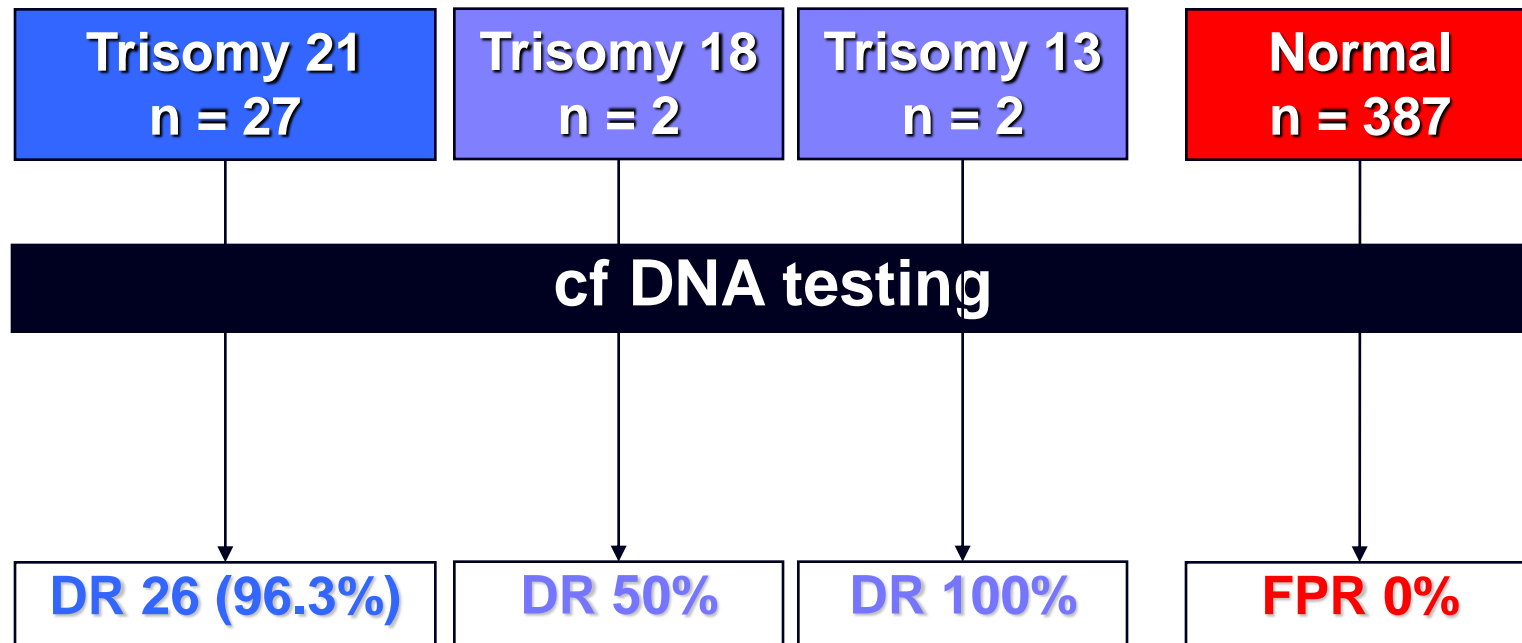
DC twins ff 10%





Cell free DNA test in twins

Clinical validation studies



Trisomy 21: Canick *et al.*, 2012 (n=7); Gil *et al.*, 2013 (n=10); Lau *et al.*, 2013 (n=1); Huang *et al.*, 2014 (n=9)

Trisomy 18: Huang *et al.*, 2014 (n=2)

Trisomy 13: Canick *et al.*, 2012 (n=1); Gil *et al.*, 2013 (n=1)



The Fetal Medicine
Foundation



Cell free DNA test

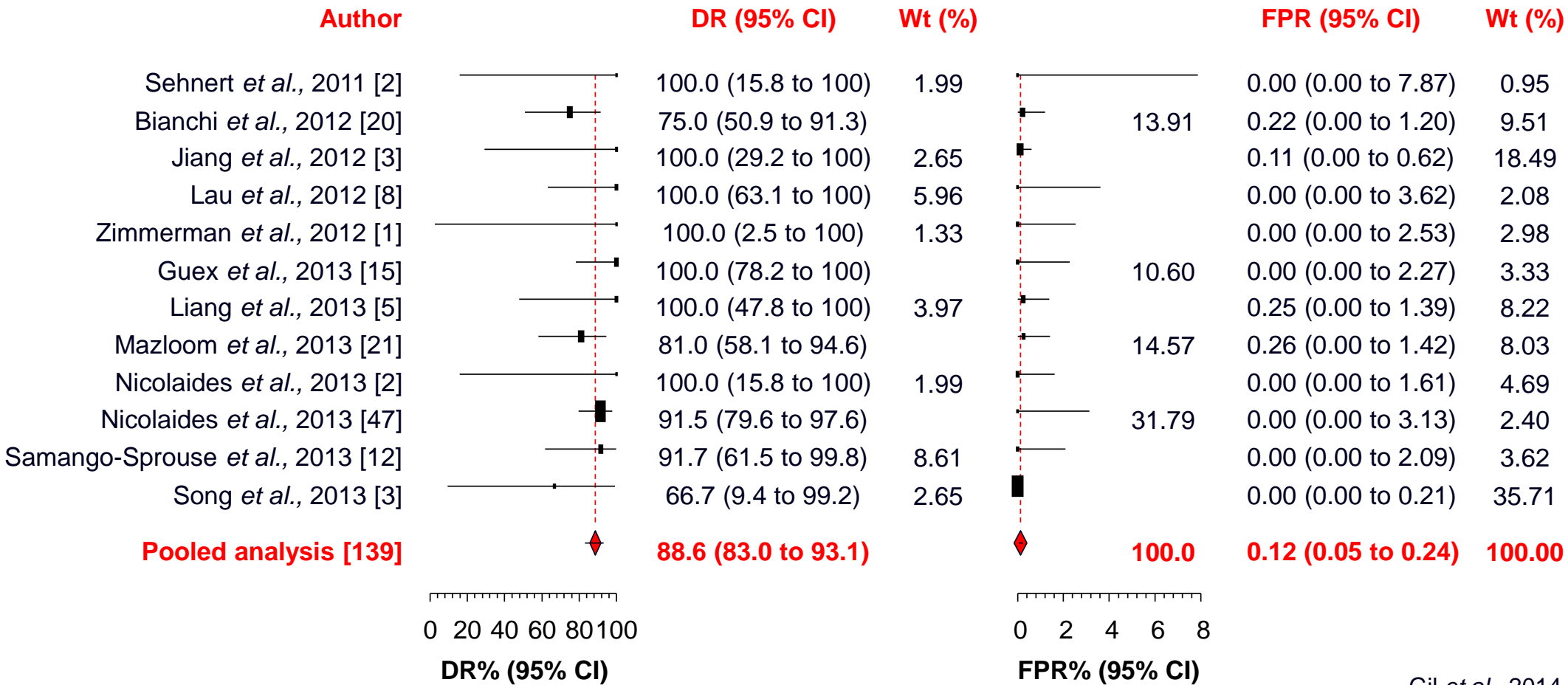
Clinical implementation

Chromosomal abnormalities beyond trisomies 21, 18 and 13



Cell free DNA test

Monosomy X





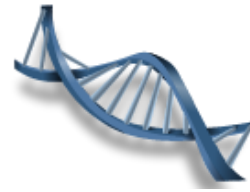
Cell free DNA test

47,XXX, 47,XYY, 47,XXY

Bianchi <i>et al.</i> , 2012 (Verinata)	No call rate 9.5% (T21 1.4%)	DR 8/9	FPR 0%
Mazloom <i>et al.</i> , 2013 (Sequenom)	No call rate 5.1% (T21 1.0%)	DR 8/8	FPR 0%
Samango-Sprouse <i>et al.</i> , 2013 (Natera)	Failure rate 7.0% (T21 5.4%)	DR 3/3	FPR 0%
Jiang <i>et al.</i> , 2012 (BGI)	Failure rate 0.0% (T21 0.0%)	DR 3/3	FPR 0%
Liang <i>et al.</i> , 2013 (Berry Genomics)	Failure rate 2.8% (T21 2.8%)	DR 3/3	FPR 0%
Nicolaidis <i>et al.</i> , 2013 (Ariosa)	Failure rate 2.8% (T21 2.8%)	DR 9/9	FPR .9%

- **50% of sex chromosome aneuploidies are mosaics**
- **High incidence of maternal mosaicism**

Performance of screening	High?
No result rate	High



- **Most cases are mild without intellectual disability**

- **47,XXX:** **1:1000 female births**
Only 10% are diagnosed clinically
IQ 90 (55-110), normal IQ 100 (70-130)
Epilepsy 10-15% (good control by drugs)
Most are fertile, premature menopause a few

- **47,XXY:** **1:500 male births**
Normal phenotype until puberty; small testes, gynecomastia
IQ 10-15% decrease with each extra X
Most are infertile

- **47,XYY:** **1:1000 male births**
IQ Normal, behavioural problems
Most are fertile

Screening by cf DNA for abnormalities beyond those for 21, 18, 13, X and Y

SEQUENOM[®] MaterniT21 PLUS

• Trisomies 21, 18, 13 and sex chromosome aneuploidies

• Trisomy 22 (common cause of miscarriage)	lethal	→ DR >99%
• Trisomy 16 (commonest cause of miscarriage)	lethal	

Microdeletions:

	1:x births	
• DiGeorge syndrome (22q11.2 deletion) [3 Mb]	2,000	} DR 60-85%
• 1p36 deletion syndrome [3-5 Mb]	5,000	
• Prader-Willi (pat15q11-13 del) [5-6 Mb]	15,000	
• Angelman syndrome (mat15q11-13 del) [5-6 Mb]	15,000	
• Cri-du-chat syndrome (5p deletion) [9-11Mb]	50,000	→ DR 85-90%

FPR 1%

Screening by cf DNA for abnormalities beyond those for 21, 18, 13, X and Y



Panorama™

Microdeletions:

	1:x births	
• DiGeorge syndrome (22q11.2 deletion)	2,000	→ DR 97.8% (45/46) FPR 0.8% (3/392)
• 1p36 deletion syndrome	5,000	} DR 96.9% (63/65) FPR 0.7% (3/404)
• Prader-Willi (pat15q11-13 deletion)	15,000	
• Angelman syndrome (mat15q11-13 deletion)	15,000	
• Cri-du-chat syndrome (5p deletion)	50,000	
• Wolf-Hirschhorn (4p16.3 deletion)	50,000	
• Phelan-McDermid (22q13.3 deletion)	<100,000	
• Miller-Dieker syndrome (17p13.2 deletion)	<100,000	

Total FPT 1.5%
No result 6.0%



Clinical implementation

Trisomy 21: DR 99% FPR 0.08 (Combined DR 90%) 5%

Trisomy 18: DR 97% FPR 0.15 (Combined DR 95%)

Trisomy 13: DR 92% FPR 0.20 (Combined DR 95%)
FPR 0.43

Monosomy X: DR 89% FPR 0.12 (Combined DR 86%)

Other sex: ?DR 97% FPR 0.90 (Combined DR 8%)
FPR 1.45 No result: 5-10%

Microdeletions: ?DR60-97% FPR 1.50 No result: 6%
FPR 3%

Thank you