Counseling patients in times of NIPT

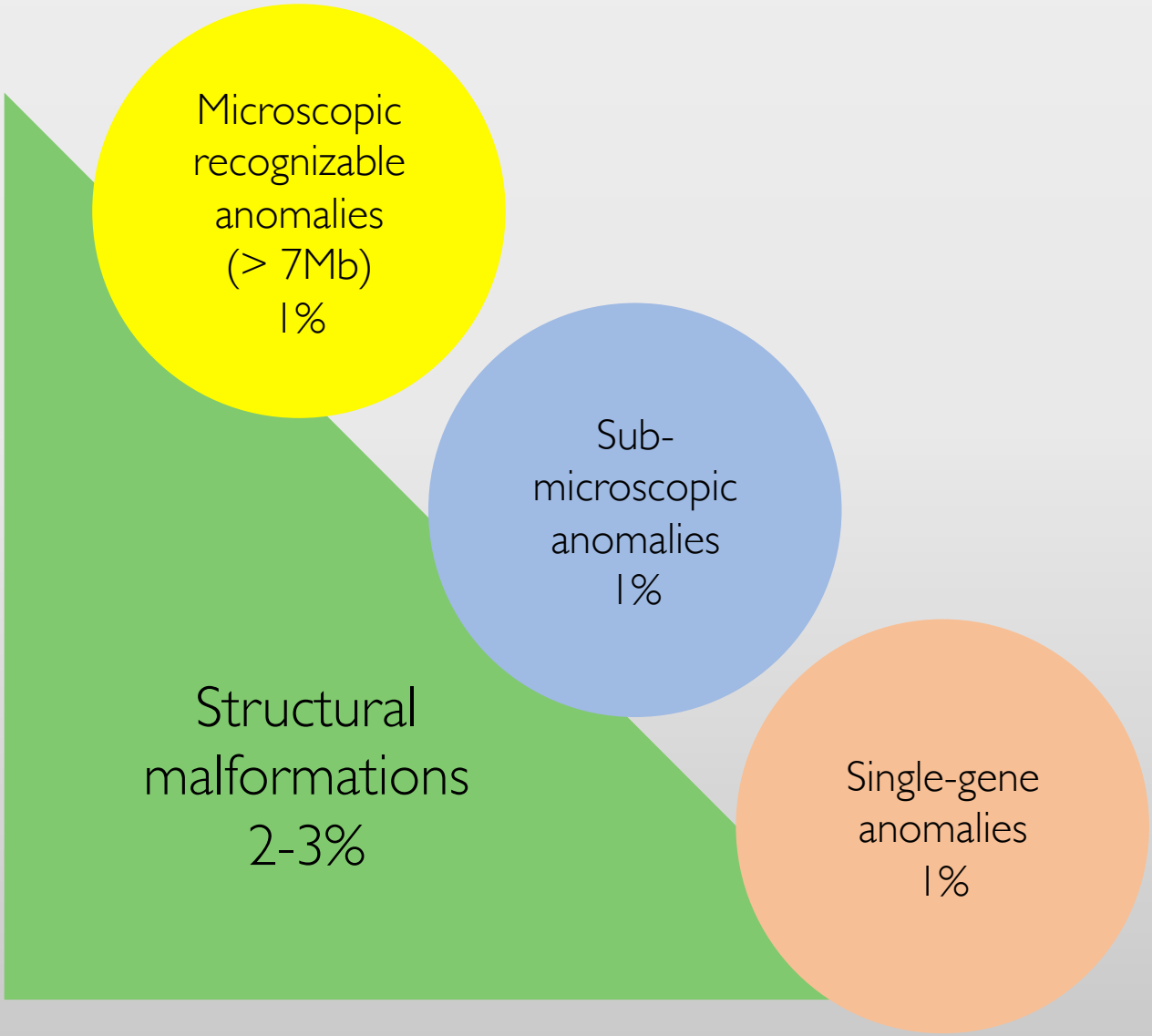
Peter Kozlowski
praenatal.de

Thermo Fisher International Workshop on Prenatal Screening
Berlin June 1-2, 2018

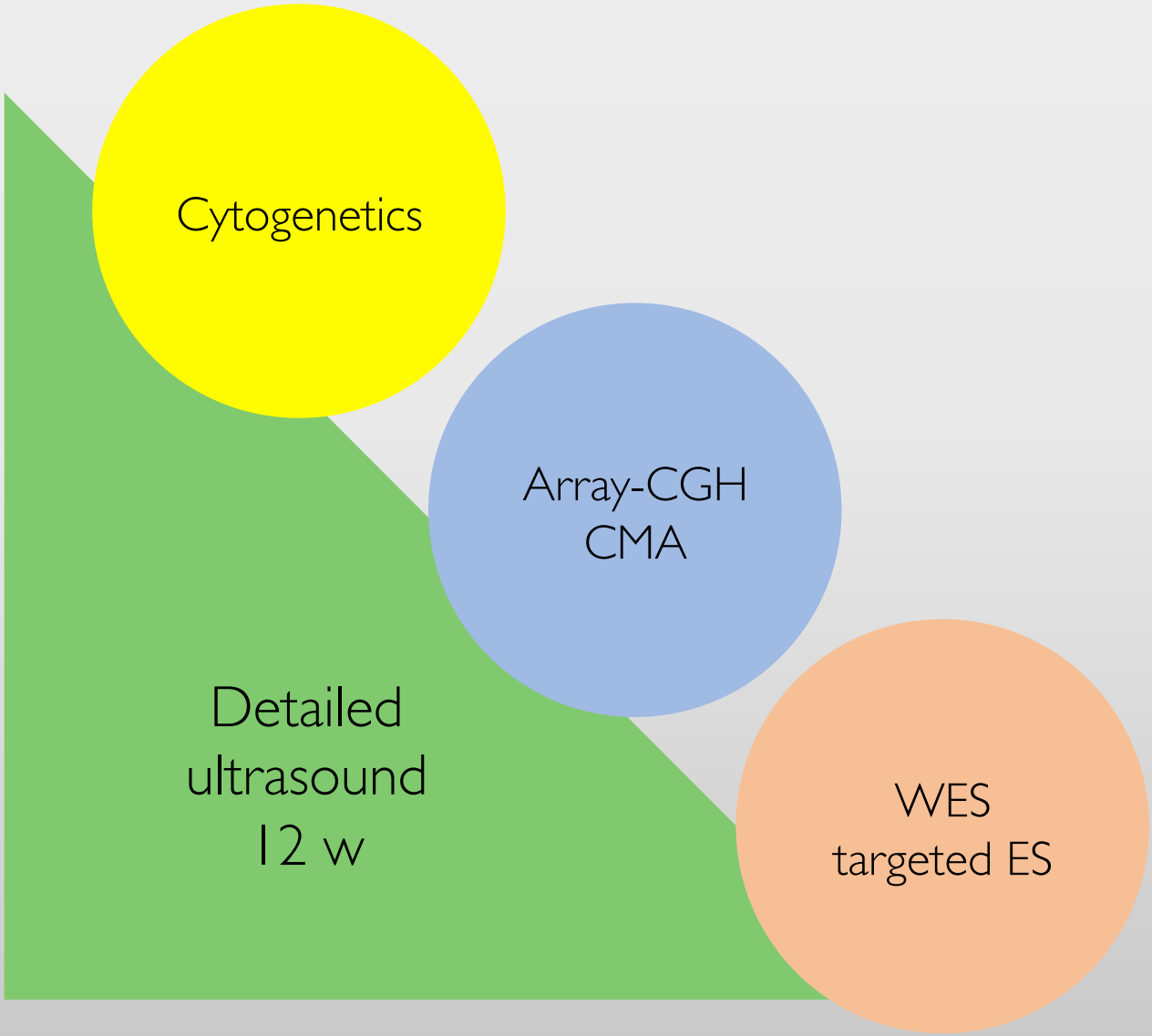
cfDNA

- Introduction of cfDNA screening for trisomies and gonosomal aneuploidies ⟶ reducing the spectrum of anomalies
- What do pregnant women expect from prenatal screening:
  - screening auf trisomie 21 *or*
  - screening for causes of severe disease?
- Age dependent anomalies
- Sensitivity, specificity and positive predictive value
- Ethical and medicolegal considerations
- Costs

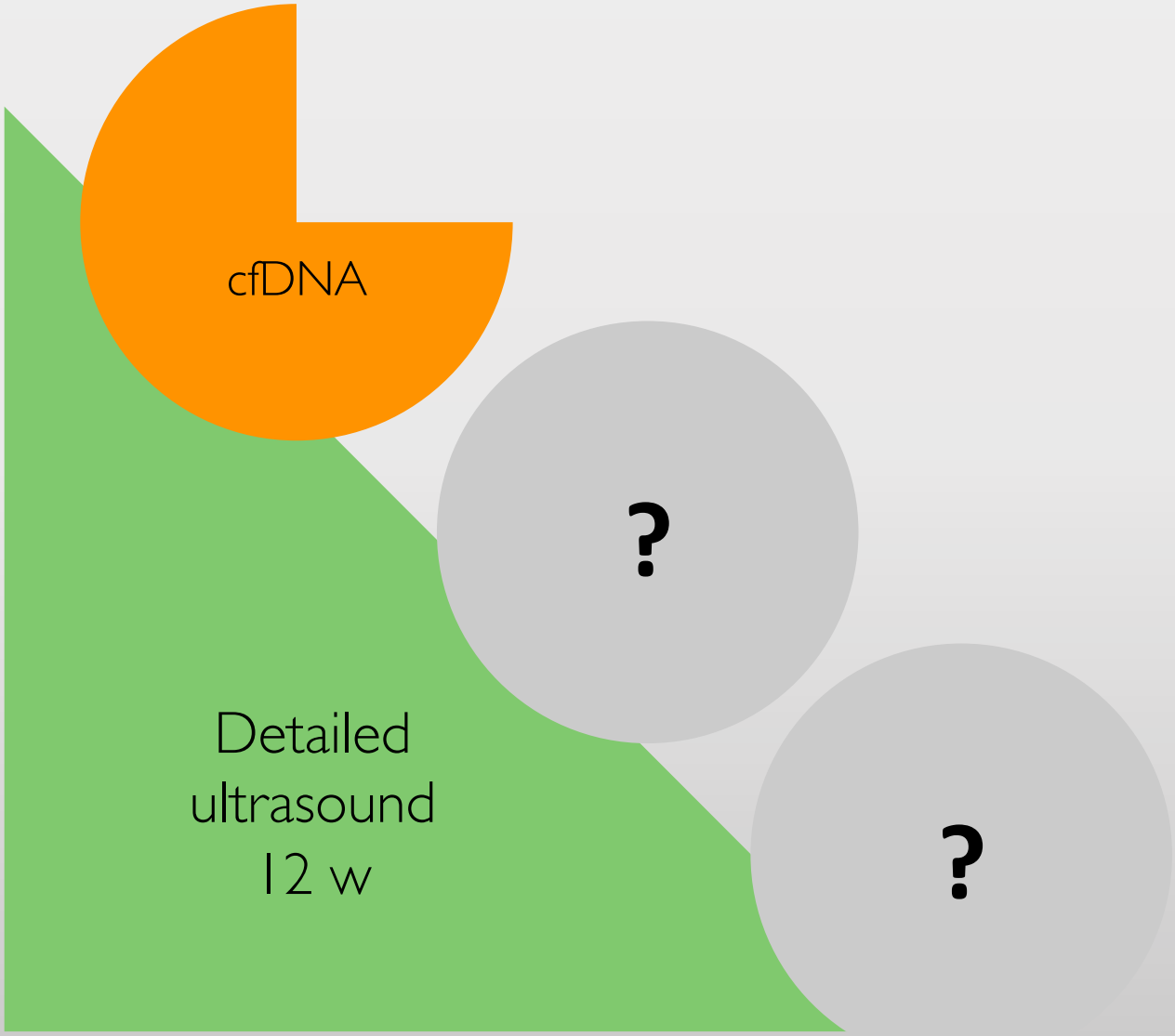
Fetal anomalies 11-13 weeks



Fetal anomalies 11-13 weeks



Fetal anomalies 11-13 weeks



Central role of US at 11-13 w

► **Table 2** Categories of the detectability of important anomalies at 11^{+0} – 13^{+6} weeks.

(almost) always able to be detected	potentially able to be detected	rarely or never able to be detected
anencephaly/exencephaly	hand and foot abnormalities	microcephaly
holoprosencephaly	diaphragmatic hernia	anomaly of the corpus callosum
omphalocele	lethal skeletal dysplasia	ventriculomegaly
gastroschisis	severe heart defects	tumors
body stalk anomaly	spina bifida aperta	ovarian cysts
megacystis	facial clefts	pulmonary lesions
		gastrointestinal obstructions

Ultrasound Obstet Gynecol 2017; **50**: 302–314
Published online 27 July 2017 in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.17484

Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis

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KEYWORDS: cell-free fetal DNA; fetal aneuploidy; monosomy X; non-invasive prenatal testing; sex chromosome aneuploidy; trisomy 13; trisomy 18; trisomy 21; Turner syndrome

- Metaanalysis of 35 studies
- Jan 2011 until Dec 2016
- Independent of method

Aneuploidy	Triue positive %	False positive %
Trisomy 21	99,7	0,04
Trisomy 18	97,9	0,04
Trisomy 13	99,0	0,.04
Monosomy X	95,8	0,14
SCA	100,0	0,04

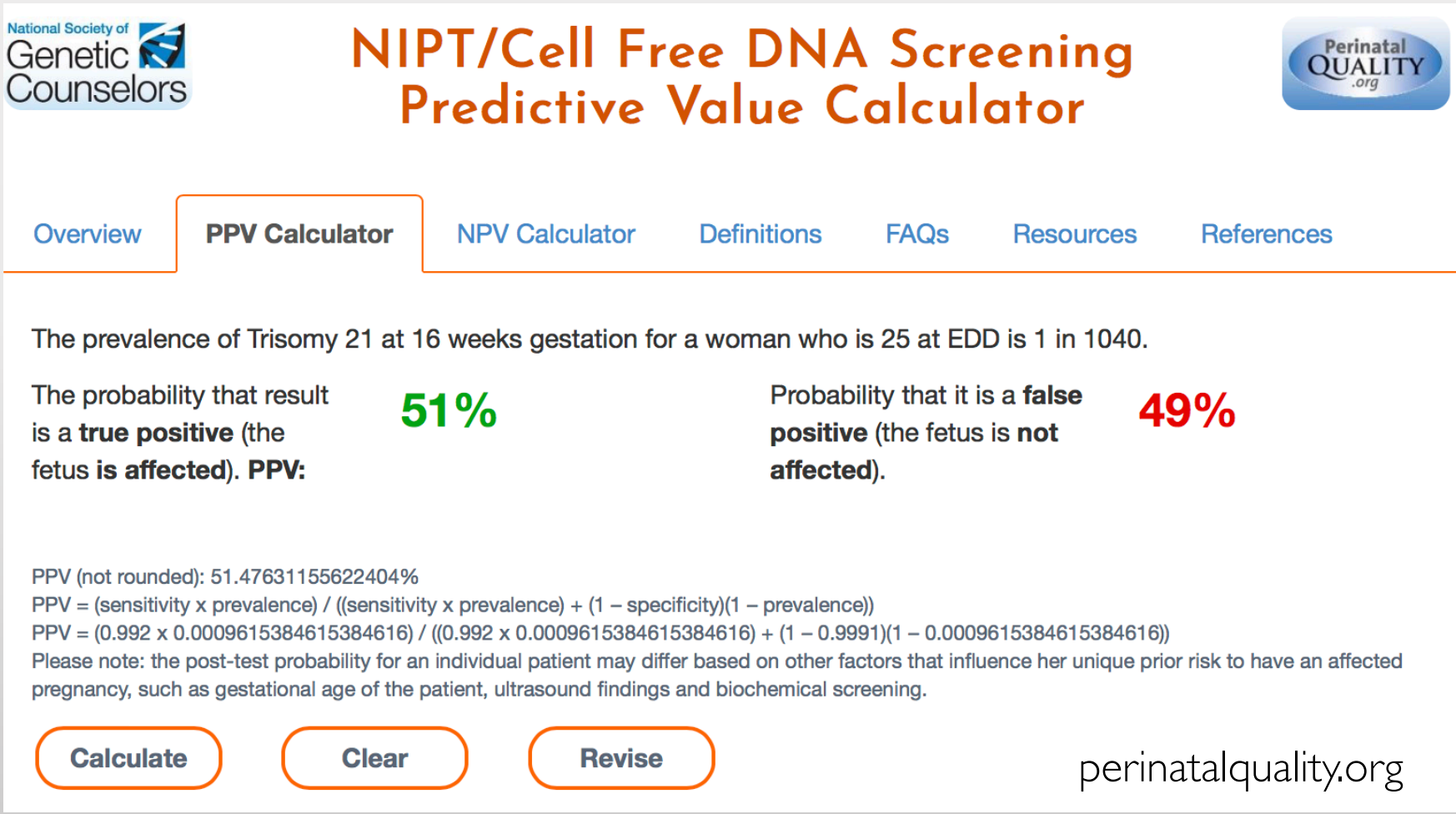
Gil 2017 UOG

cfDNA in „general population“

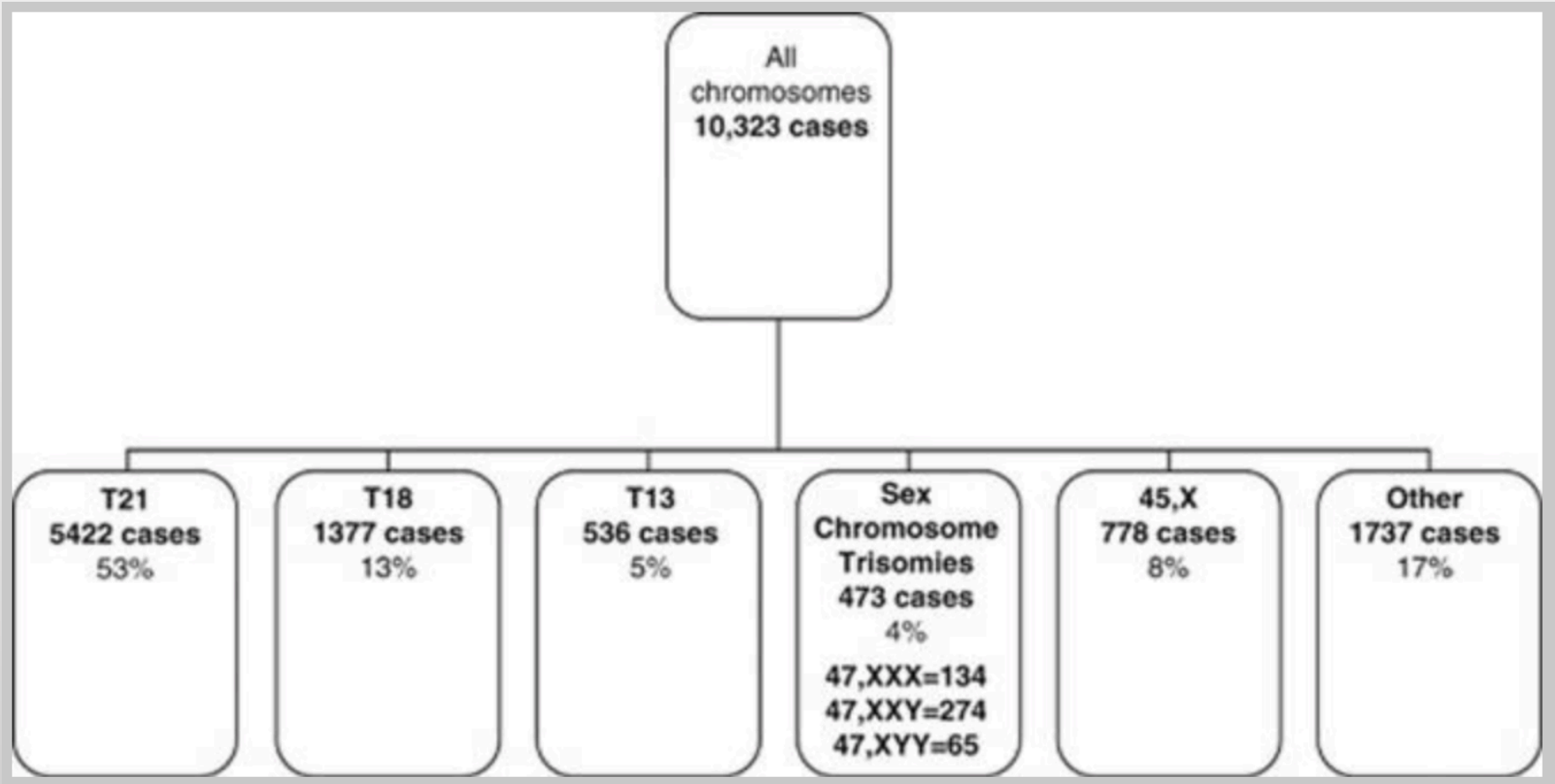
	Trisomy 21	Trisomy 18	Trisomy 13
Prevalence	1:230	1:1.000	1:2.000
Sensitivity	95,9 %	86,5 %	77,5 %
Spezifity	99,9 %	99,8 %	99,9 %
PPV	81,6 %	36,6 %	48,8 %
NPV	99,98 %	99,98 %	99,98 %

Taylor-Phillips 2016 BMJ Open

Positive predictive value

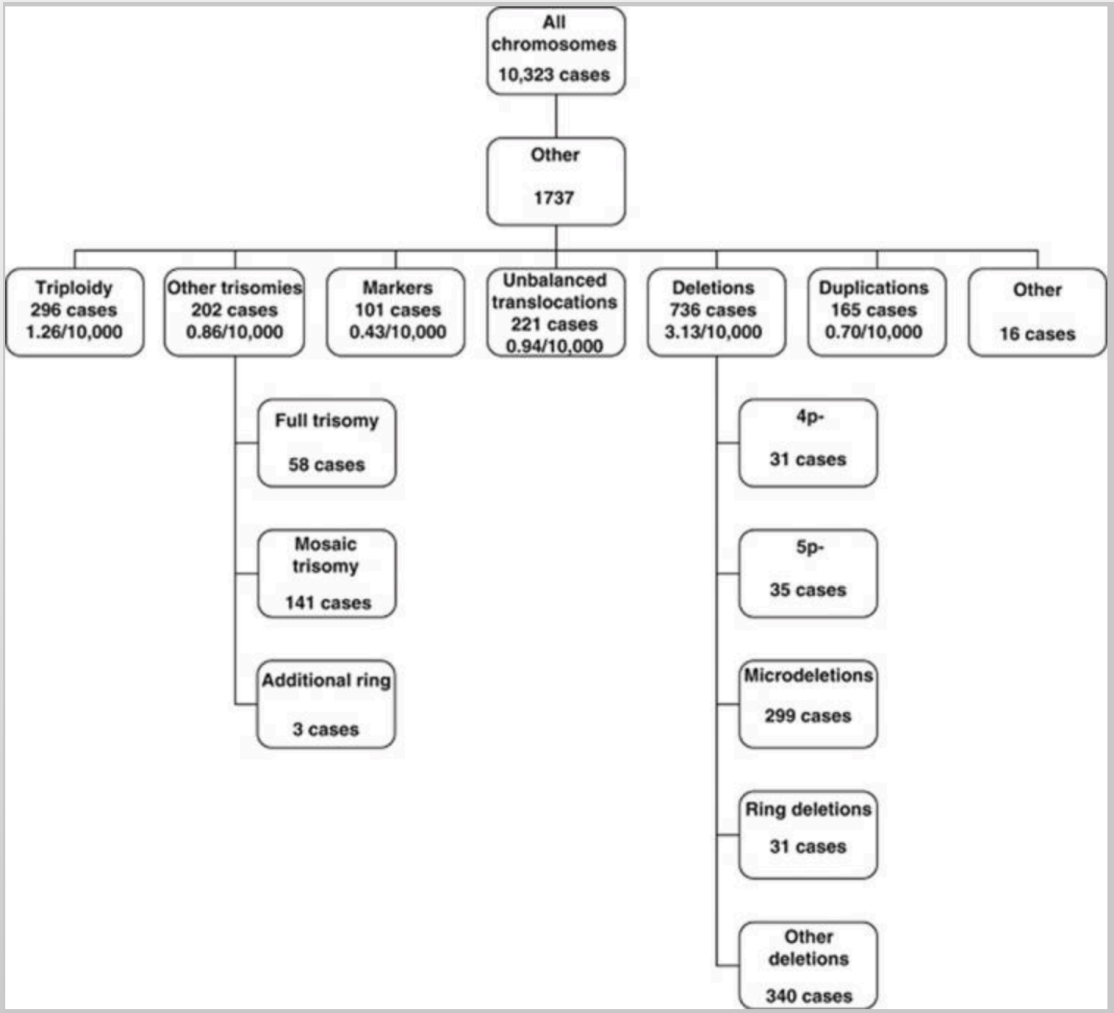


Chromosomal abnormalities



Wellesley 2012 EJHG

Chromosomal abnormalities



Wellesley 2012 EJHG

Arch Gynecol Obstet (2016) 294:219–224
DOI 10.1007/s00404-016-4077-y

REVIEW

Discordance between ultrasound and cell free DNA screening for monosomy X

Karl Oliver Kagan[1] · Markus Hoopmann[1] · Sylke Singer[2,3] · Karin Schaeferhoff[2,3] · Andreas Dufke[2,3] · Ulrike A. Mau-Holzmann[2,3]

FNR 5,90 %
FPR 0,53 %

Study	Monosomy X		Euploid	
	N	Detection rate *n* (%)	*N*	False positive rate *n* (%)
Bianchi [19][a]	16	15 (93.8)	417	1 (0.24)
Guex [15]	15	15 (100)	261	0 (0)
Mazloom [16][b]	26	25 (96.2)	1814	18 (0.99)
Samango-Sprouse [17][c]	12	11 (91.7)	174	0 (0)
Hooks [13][d]	27	26 (96.3)	380	2 (0.53)
Nicolaides [12][e]	47	43 (91.5)	116	0 (0)
Pergament [18][f]	10	9 (90.0)	954	1 (0.10)
Total	153	144 (94.1)	4116	22 (0.53)

[a] 16 cases with no DNA, 37 cases with censored complex karyotype and 49 cases with unclassified examinations were excluded

Kagan 2016

Cave: „Lost to follow up“ cases more often in SCA

Rare Aneuploidies and SCA

- Prevalence of rare trisomies 0,3-0,8%
  PPV 8% Benn 2016, Pescia 2017
- plazental mosaiks, in 13% present in fetus
  Malvestiti 2015
- Prevalence of SCA 0,8-1,0% (2/3 45,X)
  Bianchi 2015
- PPV for 45,X and normal US 53% (suspicious 99%)
  Grati 2017
- Reasonable to screen for SCA?

Spectrum of genetic anomalies

n=3.182 Cytogenetic exams
n=1.037 plus Microarray

220 (7%) Chromosomal Anomalies
57% Tris 21,18,13 und SCA
22% Mosaics, unbalanced translokations
21% pathologic microarrays

Shani 2016 AJOG

Ultrasound Obstet Gynecol 2014; **43**: 265–271
Published online in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.13270

Potential diagnostic consequences of applying non-invasive prenatal testing: population-based study from a country with existing first-trimester screening

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193.638 ETS in DK 2008-2010

1.122 Aneuploidies (prevalence 0,6%)

23,4% not recognizable by cfDNA

1,6% prevalence of aneuploidies if

- PAPP-A < 0,2 MoM
- ß-HCG < 0,2 MoM or > 5 MoM
- maternal age > 45
- NT > 95. percentile

Petersen 2014 UOG

Conclusions *A significant proportion of karyotypic abnormalities will be missed by targeted NIPT. Women of advanced maternal age, or with increased fetal NT or abnormal biochemistry, have a higher risk of having a fetus affected by an atypical abnormal karyotype and need to be counseled accordingly when considering NIPT.*

Prevalence of atypical anomalies

Ultrasound Obstet Gynecol 2018; **51**: 487–492
Published online in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.18979

Prenatal diagnostic testing and atypical chromosome abnormalities following combined first-trimester screening: implications for contingent models of non-invasive prenatal testing

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KEYWORDS: aneuploidy; combined first-trimester screening; NIPT; non-invasive prenatal testing; PAPP-A; serum screening

2014-2015
n=103.319 comb. FTS
>1:300 reported as „high risk"

Probability of atypical abnormalities 4,6% in cFTS risk >1: 10
1,4% in >1:300

90% Detection of atypical abnormalities if CMA performed in
cFTS risk >1:100, ß-HCG a/o PAPP-A >0,2 MoM
a/o US structural abnormality

Individual serum markers should be considered independently in a decision pathway

Chromosomal anomalies & cFTS

► **Table 7** Rate of chromosomal anomalies depending on first-trimester screening finding and NT measurement (publications with partial inclusion of chromosomal microarrays).

author	criterion	n	karyotype and CMA pathol. (%)	percentage of all pathol. karyotypes and CMAs (%)	trisomies 13, 18, 21 and SCAs (%)	other aneuploidies	abnormal CMAs (%)	percentage of all pathol. CMAs (%)
Maya 2017 [93]	NT ≤ 2.9 mm	462	8 (1.7)	21.1	2 (25)	2 (25)	4 (50)	40
	NT ≥ 3 mm	308	30 (9.7)	78.9	20 (66.6)	4 (13.3)	6 (20)	60
	NT ≥ 3.5 mm	138	19 (13.8)	50.0	13 (68.4)	3 (15.8)	3 (15.7)	30
Vogel 2017 [80]	comb. first-trimester screening risk > 1:300	575	51 (8.9)	100	28 (54.9)	8 (28.6)	13 (25.4)	100[1]
	comb. first-trimester screening risk > 1:100	274	35 (12.8)	68.0	23 (65.7)	5 (14.3)	5 (14.2)	38.4
	comb. first-trimester screening risk > 1:50	139	23 (16.5)	45.1	20 (86.9)	2 (8.7)	0 (0)	0

CMA: chromosomal microarray, SCA: sex chromosome anomaly. Special features of the studies: Maya: isolated NT, no anomalies. Only pathological CNVs; Vogel: isolated NT ≤ 3.5 mm, no anomalies. Additional CMA findings 6 "susceptibility mutations", 2 "likely pathogenic".

[1] No data regarding the population with first-trimester screening risk < 1:300.

Kozlowski et al 2018

CMA in routine population

Ultrasound Obstet Gynecol 2018; **51**: 445–452
Published online in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.17533

Frequency of submicroscopic chromosomal aberrations in pregnancies without increased risk for structural chromosomal aberrations: systematic review and meta-analysis

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KEYWORDS: array; background risk; CNV; prenatal diagnosis; review; submicroscopic chromosomal aberrations

Metaanalysis n=10.614
Diagnostic „routine“procedures (AMA/ANX)

Srebniak 2018 UOG

CMA in routine population

Table 3 Estimation, by age category, of risks in live births of chromosomal microscopic aberrations according to Hook *et al.*[5] and of submicroscopic aberrations according to data gathered in this review

MA (years)	*Risk for Down syndrome (Hook et al.[5], minimal prevalence)*	*Risk for clinically relevant microscopic chromosomal aberrations (Hook et al.[5])*	*Risk for pathogenic submicroscopic aberrations associated with syndromic early-onset disorders (this review)*	*Risk for all chromosomal aberrations (both microscopic and submicroscopic)*
20	1:2000	1:555	1:270	1:179
30	1:1111	1:384	1:270	1:159
35	1:400	1:178	1:270	1:108
40	1:117	1:63	1:270	1:51
45	1:35	1:19	1:270	1:17

Conclusion *This systematic review shows that a significant proportion of fetuses in a general pregnant population carry a submicroscopic pathogenic CNV. Based on these figures, all women should be informed on their individual risk for all pathogenic chromosomal aberrations and not only for common trisomies.*

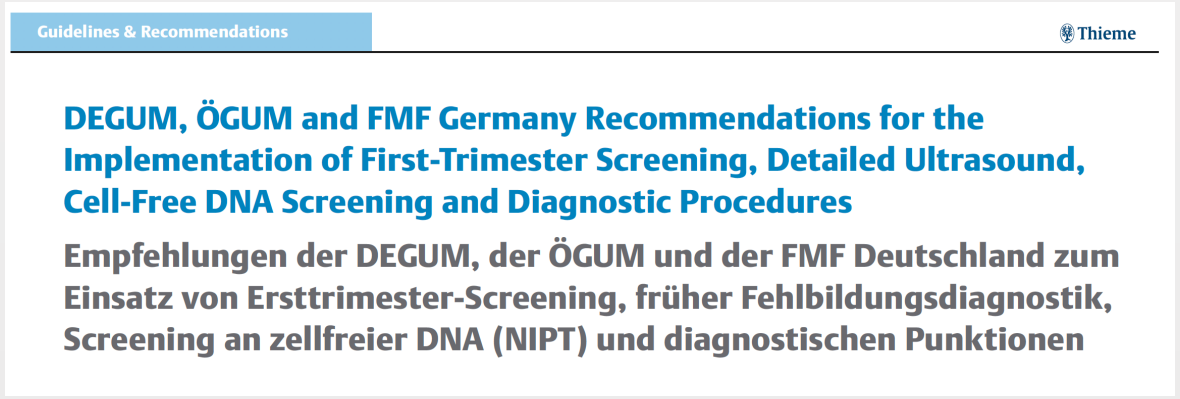
Srebniak 2018 UOG

CMA as primary tool after cFTS

Comb FTS trisomy risk	% of total	% of total abnormalities	Tris 21,18,13 and SCA	CMA abnormality
>1:50	24%	17%	87%	4%
1:50 - 1:100	24%	9%	25%	50%
1:100 - 1:300	52%	5%	31%	50%
>1:300	100%	9%	55%	29%

„Traditional“ aneuploidies in cFTS risk >1:50
Pathogenic CNV more present in risk 1:100-1:300

Vogel 2018 UOG



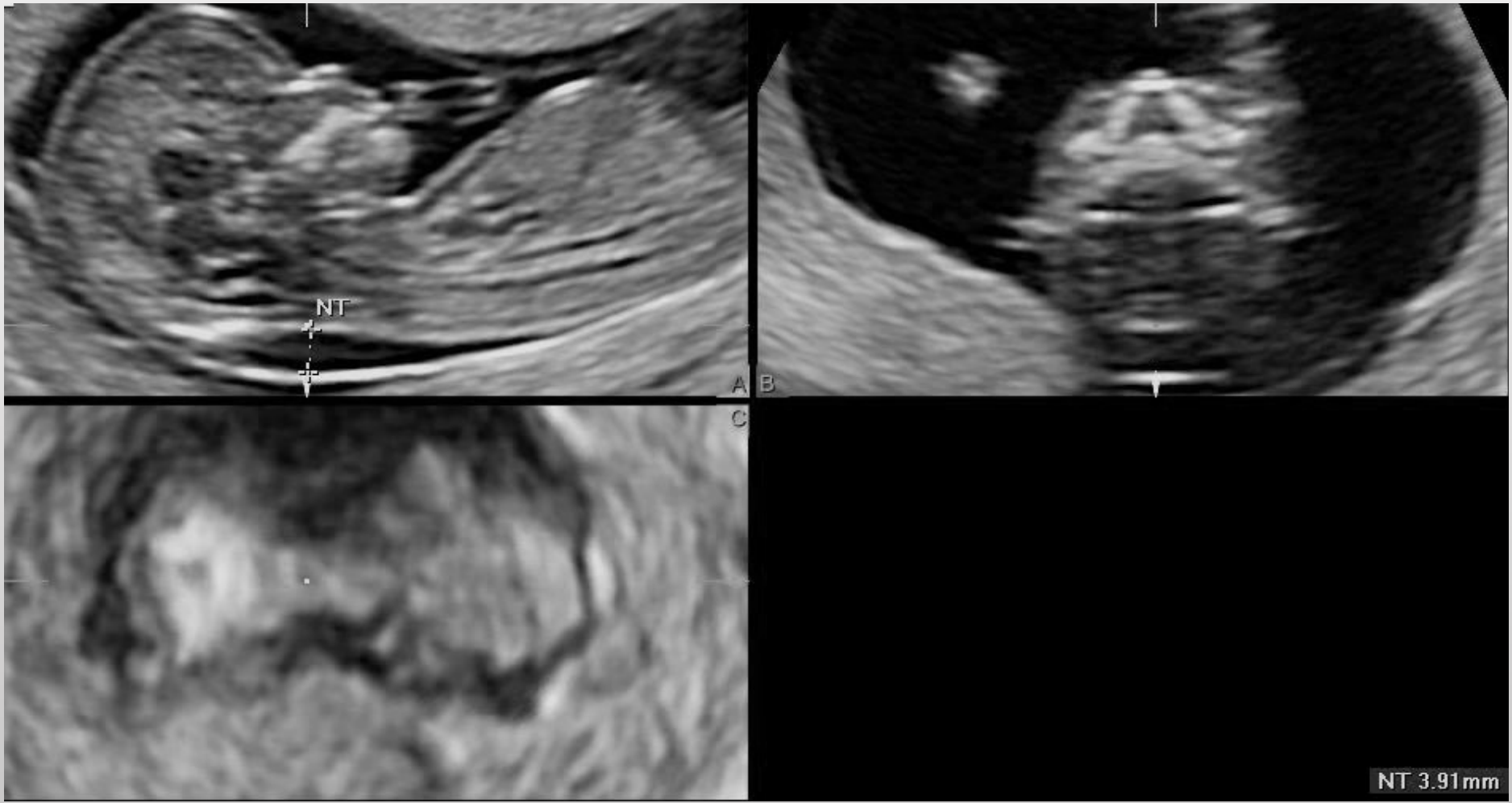
Discuss diagnostic procedure in case of ...

- Fetal malformations
- Early growth restriction
- Nuchal translucency > 95th percentile
- Increased FTS risk
- PAPP-A <0,2 MoM or fßHCG <0,2 or > 5MoM
- Abnormal cfDNA findings
- Wishes of the pregnant woman

Kozlowski et al 2018 EJU

SSL	50 mm	60 mm	70 mm	80 mm
Median (mm)	1,4	1,7	1,9	2,0
95. Perz. (mm)	2,2	2,4	2,6	2,8

NT- Percentilen (gerundet nach Nicolaides 2007)



Diagnostische Punktionen

Ultrasound Obstet Gynecol 2016; **47**: 38–44
Published online in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.15820

Risk of fetal loss associated with invasive testing following combined first-trimester screening for Down syndrome: a national cohort of 147 987 singleton pregnancies

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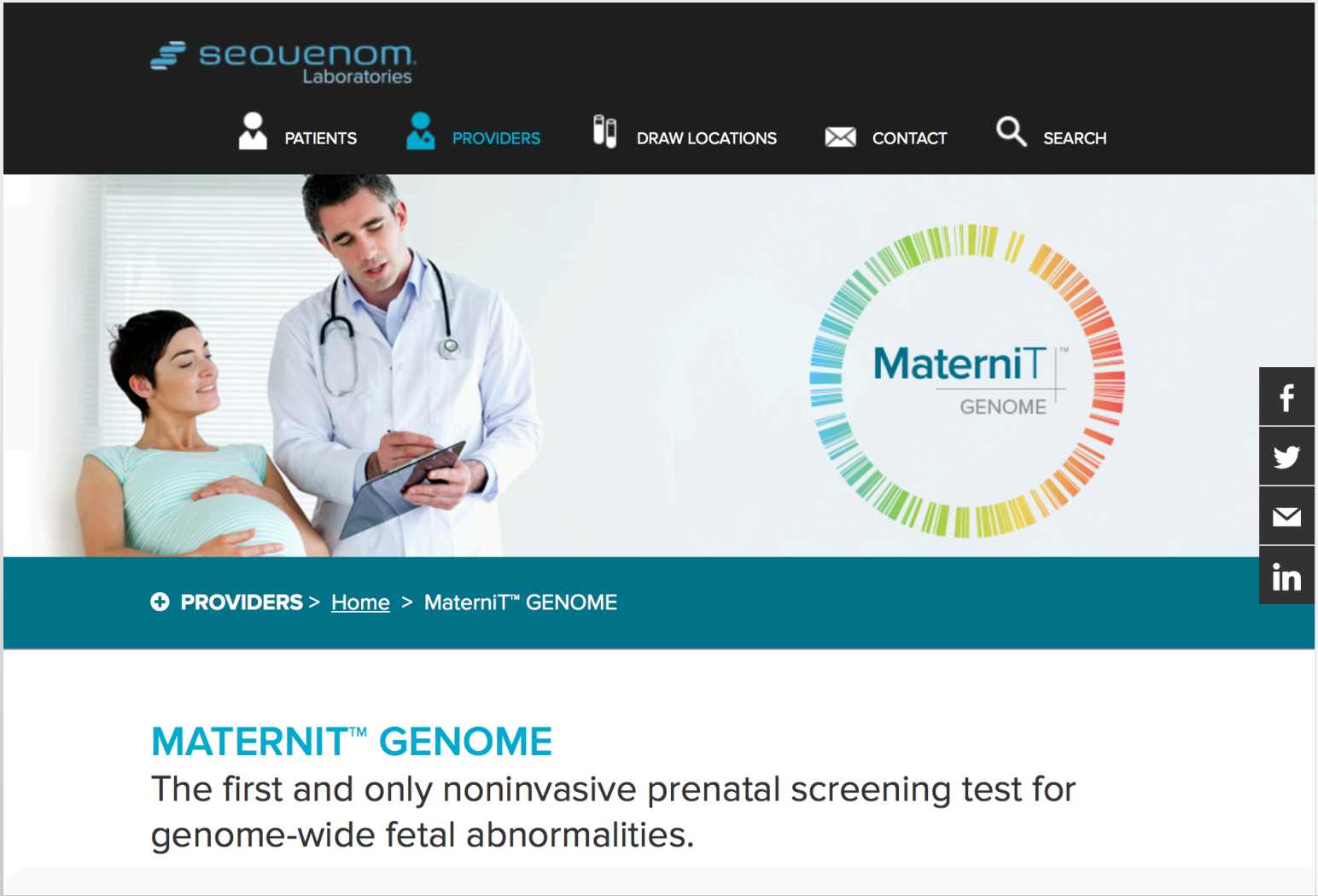
KEYWORDS: amniocentesis; chorionic villus sampling; combined first-trimester screening; fetal loss; invasive prenatal testing; miscarriage; procedure-related risk; stillbirth

Conclusion *Neither CVS nor AC was associated with increased risk of miscarriage or stillbirth. These findings indicate that the procedure-related risk of CVS and AC is very low.*

Genom wide screening



PRENATAL DIAGNOSIS

ispd International Society for Prenatal Diagnosis

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DOI: 10.1002/pd.5161

WILEY PRENATAL DIAGNOSIS

REVIEW

Challenges in non-invasive prenatal screening for sub-chromosomal copy number variations using cell-free DNA

Henna V. Advani[1] | Angela N. Barrett[1] | Mark I. Evans[2,3] | Mahesh Choolani[1]

TABLE 2 Commercially available microdeletions

Syndrome	MaterniT21 PLUS ESS	Verifi PLUS	Panorama	NIFTY
22q11.2 (DGS)	√	√	√	X
5p (cri-du-chat)	√	√	√	√
15q (PWS/AS)	√	√	√	√
1p36del	√	√	√	√
Wolf-Hirschhorn	√	√	X	X
Langer-Giedion	√	X	X	X
Jacobsen	√	X	X	√
Van der Woude	X	X	X	√
DGS2	X	X	X	√
16p12	X	X	X	√
2q33.1	X	X	X	√

Advani 2017 Prenat Diagn

Advani 2017 Prenat Diagn

TABLE 1 The prevalence and phenotypes of subchromosomal aneuploidies currently screened for by commercial providers using NIPS

Name	Position of deletion	Frequency	Phenotype	Refs
DiGeorge syndrome (DGS)	22q11.2	1:1000	Cardiac abnormalities; thymic aplasia; immune conditions; endocrine, genitourinary, and gastrointestinal problems; developmental delay.	46
1p36 deletion	1p36	1:5000	Developmental delay; dysmorphic craniofacial features; hypotonia; seizures; congenital heart defects.	47
16p12.2-p11.2 syndrome	16p12.2-p11.2	<1:1,000,000	Developmental delay; speech impairment; gastrointestinal problems; hypotonia; cardiovascular abnormalities; seizures; dysmorphic craniofacial features.	48,49
Angelman syndrome (AS; deletion of maternal allele)	15q11.2-q13	1:12 000–20 000	Severe developmental delay; speech impairment; ataxia; happy demeanor and excessive laughter.	50
Prader-Willi syndrome (PWS; deletion of paternal allele)	15q11.2-q13	1:10 000–30 000	Hypotonia; feeding difficulties in early infancy; obesity; hypogonadism; short stature; behavioural difficulties.	51
Cri-du-chat syndrome	5p	1:15 000–50 000	Developmental delay; microcephaly; dysmorphic features; hearing defects; short statures; ADHD; a high pitched, cat-like cry.	52
Wolf-Hirschhorn syndrome	4p16	1:20 000–50 000	Craniofacial dysmorphia (prominent forehead, hypertelorism, wide bridge of nose continuing to the forehead); seizures; developmental delay; intellectual disability.	53
Jacobsen syndrome	11q del	1:100 000	Developmental delay; growth restriction; congenital heart defects gastrointestinal, genital, skeletal and nervous system anomalies; dysmorphic features.	54
Van der Woude syndrome	1p32-p41	1:35 000: 100 000	Cleft lip and/or cleft palate, hypodontia, cerebral abnormalities, heart diseases, risk of developmental delay.	55
DiGeorge syndrome 2 (DGS2)	10p13-p14	1:200 000	Many overlapping features with DiGeorge syndrome: heart malformations, renal malformations, hypocalcaemia.	56,57
Langer-Giedion syndrome (Tricho-rhino-phalangeal syndrome)	8q	< 1: 1 000 000	Sparse, de-pigmented hair; short stature; mild to moderate intellectual disability; hearing impairment; joint pains; extoses.	58,59
2q33.1 deletion syndrome	2q33.1	Unknown	Learning difficulties; growth retardation; dysmorphic features; sparse hair; cleft palate.	60,61

Relevance of studies about microdel

Prospective studies define in advance

- Thesis – „cfDNA detects del 22q“
- Group – „all low risk pregnant women in 2018 who choose NIPT and agree to participate “
- Criteria for exclusions – „structural abnormalities in US“
- Outcome – „all newborns del 22q checked“

Screening 1p36, 5p, 15q, 22q11.2

Clinical outcome of subchromosomal events detected by whole-genome noninvasive prenatal testing

J. Helgeson[1], J. Wardrop[1], T. Boomer[1], E. Almasri[1], W. B. Paxton[1], J. S. Saldivar[1], N. Dharajiya[1], T. J. Monroe[2], D. H. Farkas[3], D. S. Grosu[1] and R. M. McCullough[1]*

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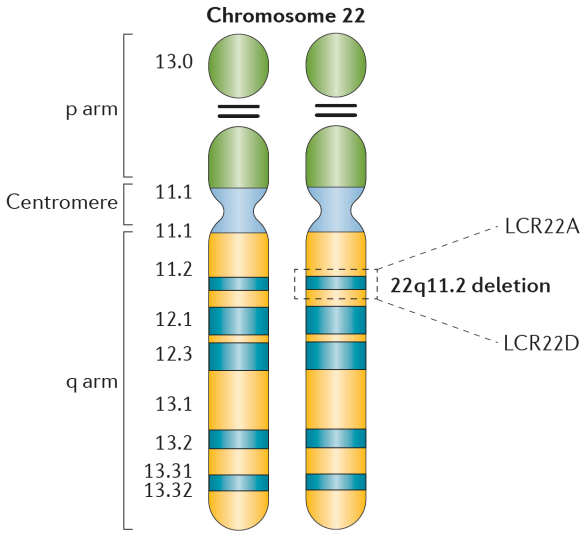
*Correspondence to: R. M. McCullough. E-mail: rmccullough@sequenom.com



n=175.393 Sequenom cfDNA-Screening
1p36, 5p, 15q, 22q11.2 del

22q11.2 32 (0,02%) positive
20 maternal (1:8.770)
12 fetal (1:14.616)

Helgeson 2015 Prenat Diagn

Ultrasound Obstet Gynecol 2016; 47: 177–183
Published online 5 January 2016 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/uog.15754.

Clinical experience with single-nucleotide polymorphism-based non-invasive prenatal screening for 22q11.2 deletion syndrome

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del 22q11.2

SNP Array n=20.776 Prevalence 1:950

95 (0,5%) positive

11 true positive fetal

50 (0,2%) false positive

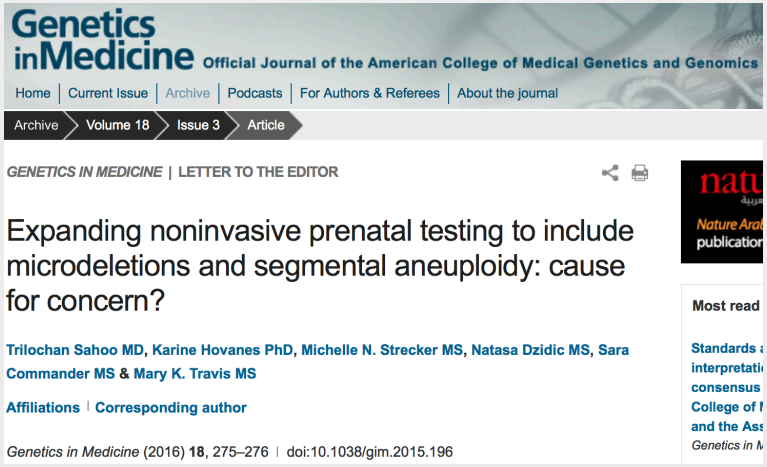
34 no outcome

2 maternal deletions

PPV=18%

Post study change of coverage: PPV 18% ➡ 42%

Gross 2016 UOG



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Archive > Volume 18 > Issue 3 > Article

GENETICS IN MEDICINE | LETTER TO THE EDITOR

Expanding noninvasive prenatal testing to include microdeletions and segmental aneuploidy: cause for concern?

Trilochan Sahoo MD, Karine Hovanes PhD, Michelle N. Strecker MS, Natasa Dzidic MS, Sara Commander MS & Mary K. Travis MS

Affiliations | Corresponding author

Genetics in Medicine (2016) 18, 275–276 | doi:10.1038/gim.2015.196

Most read

Standards a interpretati consensus College of M and the Ass Genetics in M

Sahoo 2016 Genet Med

Table 1 Invasive testing results for cases with microdeletions or segmental aneuploidies reported by NIPT

	Array/karyotype results			
	Concordant (6)	Discordant (17)		Concordant + structural abnormality (3)
Abnormal NIPT results (26)		No.	Description (discordant results)	
22q11.2 Deletion (7)	2	5	All normal	0
5p Deletion (6)	1	5	All normal	0
1p36 Deletion (4)	1	3	All normal	0
4p Deletion (1)	0	1	Normal	0
8q24 Deletion (1)	NA	NA	NA	NA
15q Deletion (1)	1	0		
9p Duplication (1)	0	0		1 (idic 9p)
13q Deletion (1)	1	0		
18p Deletion + 18q deletion (1)	0	1	14-Mb deletion: 18p11.32p11.21	
Trisomy 18q (1)	0	0		1, 18p11.21q23(14,419,130-78,077,248)x3; 46,XY,der(13;18)(q10;q10),+18
21q Partial deletion (1)	0	1		Duplication 21q11.2-q21.1 (9.2 Mb), ins(14;21)(p11.2;q11.2q21.1)
No result for chromosome 13 (1)	0	1	ROH chromosome 13/?UPD13	

idic, isodicentric; NA, data not available; NIPT, noninvasive prenatal testing; ROH, region(s) of homozygosity.

PLOS ONE

RESEARCH ARTICLE

Validation of a SNP-based non-invasive prenatal test to detect the fetal 22q11.2 deletion in maternal plasma samples

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Abstract

Table 1. Clinical information for affected samples.

No.	Deletion Syndrome	MA (Years)	GA (Weeks)	FF (%)	Procedure for Sample Procurement	DiagnosticTest	Deletion Size (Mb)	Time Between Invasive Test and Blood Draw (Days)	NIPT Call	Deleted Haplotype
1	22q11.2	N/A	22.0	13.3	Amnio	CMA	2.55	15	High risk	Paternal
2	22q11.2	19	15.6	11.7	CVS	mmPCR	≥2.91	0*	High risk	Paternal
3	22q11.2	20	30.4	15.4	Amnio	FISH	N/A	53	High risk	Maternal
4	22q11.2	21	21.5	39.7	Amnio	CMA	2.55	14	High risk	Maternal
5	22q11.2	35	13.3	19.4	CVS	FISH	N/A	6	High risk	Maternal
6	22q11.2	31	20.2	7.9	Amnio	CMA	2.55	0*	High risk	Maternal
7	22q11.2	35	25.1	8.0	Amnio	CMA	3.15	15	Low risk	N/A
8	22q11.2	30	16.2	21.4	Amnio	FISH	N/A	6	High risk	Maternal
9	22q11.2	31	14.0	9.6	CVS	FISH	N/A	2	High risk	Paternal
10	22q11.2	31	37.4	19.3	Amnio	CMA	2.55	109	High risk	Maternal

Amnio, amniocentesis; CMA, chromosomal microarray; CVS, chorionic villus sampling; FF, fetal fraction; FISH, fluorescence in situ hybridization; GA, gestational age; MA, maternal age; mmPCR, massively-multiplexed polymerase chain reaction; N/A, not available, NIPT, non-invasive prenatal testing.

*Blood draws were performed prior to procedures.

9/10 true positive

9 abnormal US

1/390 false positive

Ravi 2018

Screening for microdeletions / CNVs

TABLE 1 Recurrent microdeletion syndromes with prevalence and deletion size

Syndrome	Location	Estimated Prevalence	Deletion Size	% ≥3 Mb
DiGeorge	22q-	1/2,000	1.5-3 MB	85%
Wolf-Hirschorn	4p-	1/50,000-1/20,000	1.9-3.5 Mb	97%
Cri du Chat	5p-	1/50,000	10-30 Mb	99%
Prader-Willi/Angelman	Del 15q11.2	1/20,000	5.0-6.0 Mb	70%
1p36 deletion	1p36-	1/5,000	1.5-10 Mb	85%
Miller Dieker	Del 17p13.3	~1/100,000	0.4-2.Mb	Very rare

TABLE 2 Sensitivity, false positive rate and positive predictive value for copy number variants detected by cell free DNA

Study	Cohort	Sample Details	Sensitivity	False-Positive Rate	Positive Predictive Value
Zhao (2015)[15]	18 maternal plasma with known CNVs	CNV size 3 to 40 Mb	94.4%	N/A	N/A
Wapner (2015)[10]	358 maternal plasma and 111 maternal plasma spiked	"Normals" not confirmed by microarray	97.8% of known 22q- (including artificial samples)	0.76% for 22q- 0.24% for 5p-	3.8% 17%
Lo (2015)[16]	31 maternal plasma with known CNVs	CNVs 3 to 40 Mb genome wide (3 maternally inherited)	64.5% (83% ≥6 Mb, 20% <6 Mb)	0.4%	N/A
Gross (2016)[11]	20,776 maternal plasma screened with NIPT	22q- only reported, 30% LTFU, "normals" not confirmed by chromosomal microarray	Unknown	0.4%, 22q- only reported	18% overall 89% in fetuses with ultrasound anomalies 4.9% when normal ultrasound
Li (2016)[17]	Maternal plasma, 11 with known CNVs and 99 normal on microarray	CNVs 1.4 to 38 Mb across the genome	61% (90% ≥5 Mb, 14% <5 Mb)	5%	71% high risk
Yin (2015)[18]	1,456 maternal plasma with microarray results. 78 abnormal	Semiconductor sequencing detected 56/78	71.8%	3.8%	50% high risk
Lefkowitz (2016)[19]	1166 reportable cases at high risk for aneuploidy	43 cases with a CNV >7 Mb or rare trisomy	97.7%	N/A	97% high risk
Helgeson (2015)[20]	175,393 maternal plasma screened by NIPT. Normals not confirmed by microarray	53 microdeletion positive, 20 maternally inherited	Not known	0.0017%	60%-100%

CNV, copy number variant; LTFU, lost to follow-up; N/A, not applicable.

Chitty 2018 Prenat Diagn

Screening for microdeletions / CNVs

ISPD 2017 MEETING ISSUE

WILEY PRENATAL DIAGNOSIS

Current controversies in prenatal c
prenatal screening should be used
abnormalities

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Funding information
NIHR Biomedical Research Centre

Abstract

Noninvasive prenatal testing (NI
clinically available since 2011. T
common aneuploidies trisomy 2
clinical laboratories have offere
chromosome abnormalities and
the sensitivity, specificity, and
performing prenatal screening via cfDNA for all chromosome abnormalities is discussed. At the time of the debate in 2017, the general consensus was that the literature does not yet support using this technology to screen for all chromosome abnormalities and that education is key for both providers and the patients so that the decision-making process is as informed as possible.

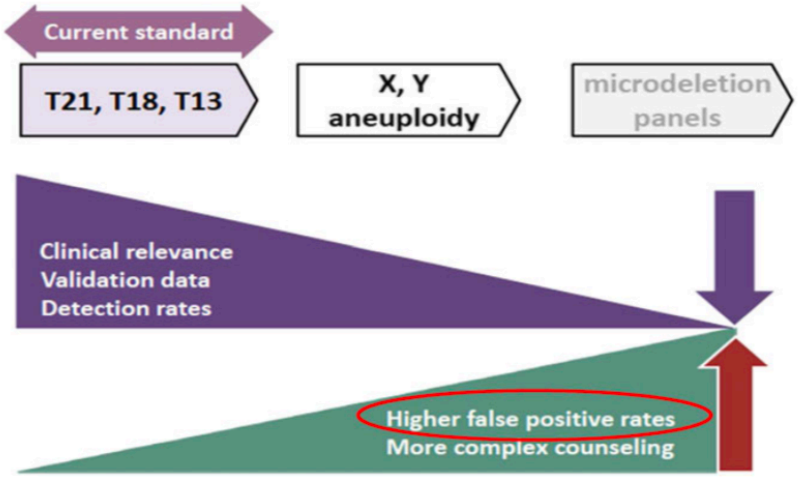

FIGURE 1 The effect of expanding noninvasive prenatal testing menus on test performance [Colour figure can be viewed at wileyonlinelibrary.com]

Chitty 2018 Prenat Diagn

Screening for microdeletions / CNVs

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ORIGINAL ARTICLE

WILEY PRENATAL DIAGNOSIS

Clinical experience of laboratory follow-up with noninvasive prenatal testing using cell-free DNA and positive microdeletion results in 349 cases

S. Schwartz[1] | M. Kohan[2] | R. Pasion[1] | P. R. Papenhausen[1] | L. D. Platt[3,4]

Deletion	screenpositive *	true positive CVS oder AC	PPV (%)
1p36	21	1	5
4p	6	1	17
5p	45	6	13
15q	80	5	6
22q11.2	183	12	7

* Pathologic Microarrays in 11 cases

Schwartz 2018 Prenat Diagn

Screening for single-gene diseases

vistara
SINGLE-GENE NIPT

Vistara identifies the risk for severe conditions that affect the skeletal, cardiac, and neurological systems

The non-invasive prenatal test (NIPT) that screens for single-gene mutations in cell-free fetal (placental) DNA

Vistara non-invasive prenatal screen

Vistara identifes risk for conditions that may have otherwise gone undetected until after birth or into childhood.
All conditions are inherited in an autosomal or X-linked dominant fashion, which means that if the mutation is present, the child will be affected by the condition and experience related symptoms.

Condition[1] *Gene(s)*	Clinical synopsis[2,3]	Cases caused by de novo mutations[2,3]	Ultrasound findings[2,3]			Clinical actionability	Detection rate for gene[1]
			None	Late gestation	Non-specific		
Achondroplasia *FGFR3*	The most common form of skeletal dysplasia; may cause hydrocephalus, delayed motor milestones, and spinal stenosis	80%		●	●	Labor and delivery management, monitor for spinal stenosis, early sleep studies to reduce risk of SIDS	>96%
Alagille syndrome *JAG1*	Affects multiple organ systems and may cause growth problems, congenital heart defects, and vertebral differences	50% to 70%	◐		◐	Symptom-based treatment	>79%
Antley Bixler syndrome *FGFR2*	A type of craniosynostosis; also causes premature fusion of the arm bones, blockage of the nasal passage, and permanently flexed or extended joints	more severe forms		●		Fetal MRI, avoid instrumented delivery, corrective surgery, monitor for hydrocephalus	>96%
Apert syndrome *FGFR2*	A type of craniosynostosis; also causes abnormal formation of the fingers, toes, and vertebrae, and other organ anomalies	more severe forms		●		Fetal MRI, avoid instrumented delivery, corrective surgery, monitor for hydrocephalus	>96%
Cardiofaciocu-taneous syndrome 1,3,4 *BRAF, MAP2K1, MAP2K2*	Causes abnormalities of the heart, face, skin, and hair; may cause developmental delays and intellectual disability	majority		●	●	Fetal echocardiogram	>96%
CATSHL syndrome *FGFR3*	Acronym stands for camptodactyly, tall stature, scoliosis, and hearing loss; may increase risk for intellectual disability	unknown	●			Early adoptionof sign language and behavioral intervention	>96%
CHARGE syndrome *CHD7*	Acronym stands for coloboma, heart defects, atresia of the choanae, retardation of growth and development, genital abnormality, ear abnormalities; may cause hearing loss, developmental delays,	majority	◐	◐	◐	Early referral to endocrinology, adoption of sign language, and behavioral intervention	>91%

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Carrier-Screening

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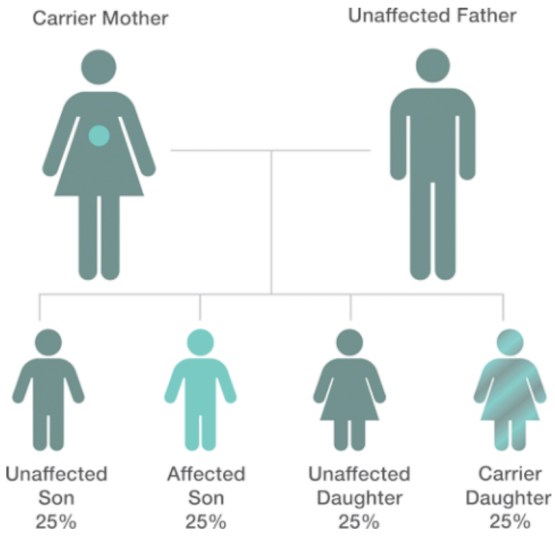
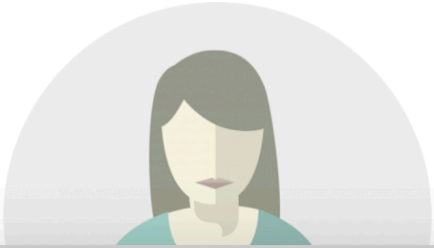
Inheritance: X-linked Conditions

Male or female gender comes from the “sex chromosomes” X and Y. Females have two copies of the X chromosome. Males have one X chromosome and one Y chromosome. This means that females have two copies of each of the genes on the X chromosome and males only have one copy of these genes. Some genetic diseases are caused by mutations in genes found on the X chromosome. These are called X-linked genetic diseases. Females can be carriers of X-linked diseases. This woman has one working copy and one non-working copy of an X-linked gene. Males are not typically carriers because they only have one X chromosome, so they will be either healthy or affected.

For X-linked genetic diseases, only the mother needs to be a carrier to have a chance of having an affected child. This woman’s chance with each pregnancy is 25% or 1 in 4 to have a son affected with the disease. She also has a 25% chance with each pregnancy to have a daughter who is a carrier. This woman also has a 75% chance to have a child who does NOT have the disease.





Recessive Conditions: Pre-Pregnancy

If you are a carrier for a specific recessive condition, your partner may want to have carrier screening for the condition ordered by a health care professional. Your doctor or a local genetic counselor can help decide which carrier screen is best for your partner. If your partner screens positive for the same condition that you are a carrier for, different reproductive options can be considered.

THE JOURNAL OF
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Practicability of prenatal testing using lectin-based enrichment of fetal erythroblasts

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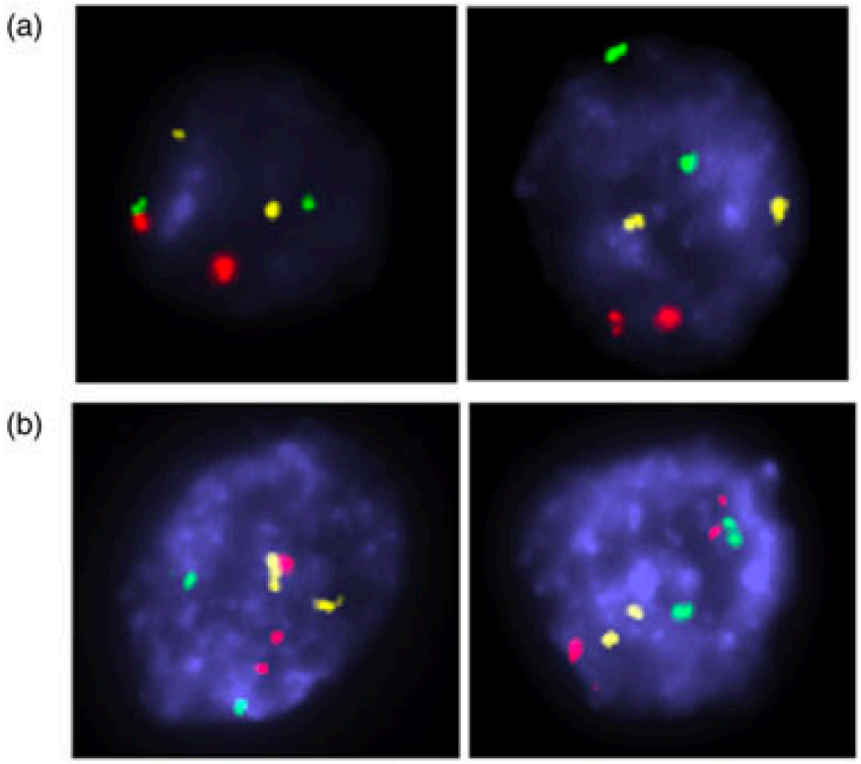

Figure 2 Fluorescence *in situ* hybridization images of cells obtained from the blood sample of the mother of a male fetus with trisomy 18 (three-color probe for chromosomes 13 [green], 18 [red], and 21 [yellow]). (a) Erythroblasts with two signals for chromosome 18. (b) Erythroblasts with three signals for chromosome 18.

Kanda 2016 J Obstet Gynaecol Res

ACS NANO

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ARTICLE

Imprinted NanoVelcro Microchips for Isolation and Characterization of Circulating Fetal Trophoblasts: Toward Noninvasive Prenatal Diagnostics

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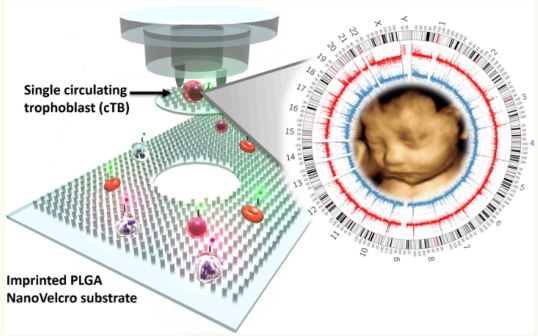
ABSTRACT: Circulating fetal nucleated cells (CFNCs) in maternal blood offer an ideal source of fetal genomic DNA for noninvasive prenatal diagnostics (NIPD). We developed a class of nanoVelcro microchips to effectively enrich a subcategory of CFNCs, *i.e.*, circulating trophoblasts (cTBs) from maternal blood, which can then be isolated with single-cell resolution by a laser capture microdissection (LCM) technique for downstream genetic testing. We first established a nanoimprinting fabrication process to prepare the LCM-compatible nanoVelcro substrates. Using an optimized cTB-capture condition and an immunocytochemistry protocol, we were able to identify and isolate single cTBs (Hoechst+/CK7+/HLA-G+/CD45−, 20 μm > sizes > 12 μm) on the imprinted nanoVelcro microchips. Three cTBs were polled to ensure reproducible whole genome amplification on the cTB-derived DNA, paving the way for cTB-based array comparative genomic hybridization (aCGH) and short tandem repeats analysis. Using maternal blood samples collected from expectant mothers carrying a single fetus, the cTB-derived aCGH data were able to detect fetal genders and chromosomal aberrations, which had been confirmed by standard clinical practice. Our results support the use of nanoVelcro microchips for cTB-based noninvasive prenatal genetic testing, which holds potential for further development toward future NIPD solution.



KEYWORDS: *noninvasive prenatal testing (NIPT), nanoVelcro assays, circulating trophoblasts, single-cell analysis, array comparative genomic hybridization (aCGH)*

The current gold standard for diagnosing fetal genetic abnormalities involves invasive procedures[1,2] such as amniocentesis (AC, >16 weeks of gestational age, GA) and chorionic villus sampling (CVS, 10−12 weeks of GA), by which fetal cells are harvested for karyotyping and genetic testing. These procedures provide accurate information for clinical decision making. However, concerns have been raised regarding their invasiveness and increased risk of miscarriage

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Trophoblast retrieval and isolation from the cervix (TRIC) for noninvasive prenatal screening at 5 to 20 weeks of gestation

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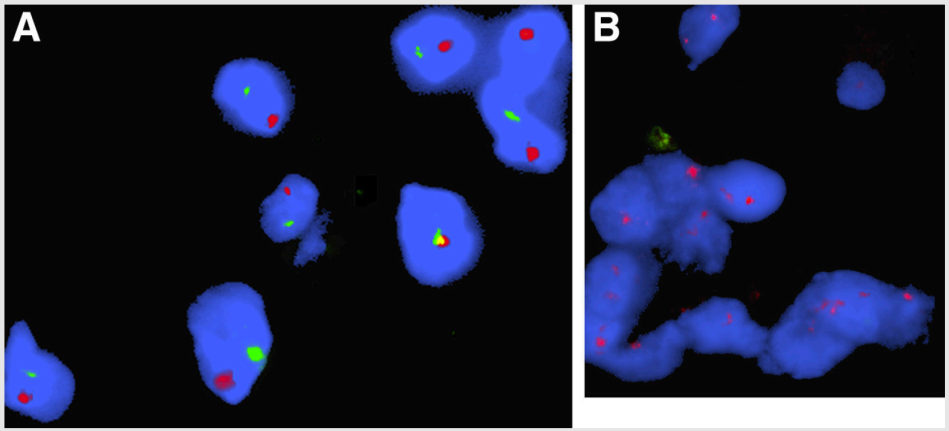
of Child Health and Human Developmer

Bolnick 2014

Fertility and Sterility®

FIGURE 2



FISH for X and Y chromosomes in trophoblast cells obtained by TRIC. Trophoblast cells in TRIC samples from pregnancies with a male (A) or female (B) fetus were labeled with probes for the DYZ1 satellite III on the Y chromosome (*green*) or the DXZ1 alpha satellite on the X chromosome (*red*). Nuclear chromatin is labeled with DAPI (*blue*). A pair of sex chromosomes is labeled over most nuclei. The green signal in B is due to nonspecific labeling of debris not associated with a nucleus.

Bolnick. Noninvasive testing with fetal cells. Fertil Steril 2014.

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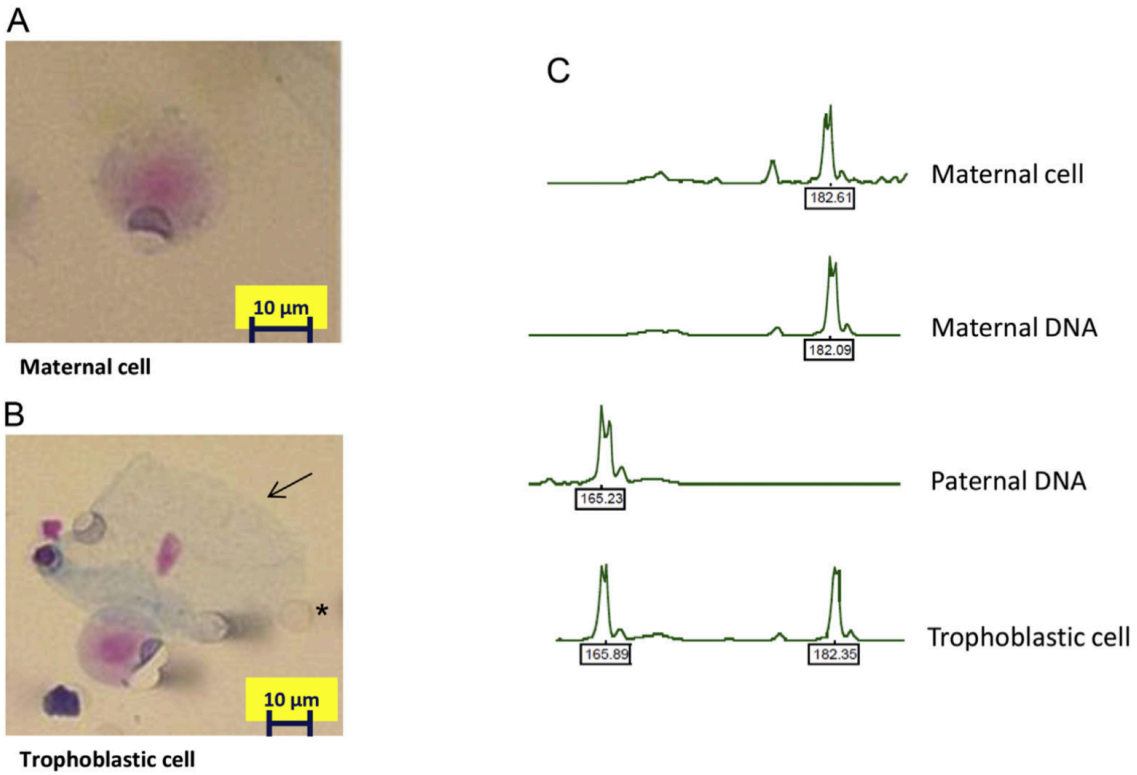
Cervical trophoblasts for non-invasive single-cell genotyping and prenatal diagnosis

I. Pfeifer [a, 1], A. Benachi [b, 1], A. Saker [a], J.P. Bonnefont [c], H. Mouawia [a], L. Broncy [a], R. Frydman [d], M.L. Brival [e], B. Lacour [f], R. Dachez [g], P. Paterlini-Bréchot [a, f, *]



Pfeifer 2016 Placenta