‘Rischio residuo di anomalie cromosomiche dopo test di screening negativo per le maggiori aneuploidie’

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OUTLINES

- Discuss on the ‘a priori’ and ‘post-test’ or ‘residual risk’ after a negative test result
- Implications of the residual risk for pre-test counseling before screening test for common aneuploidies
- Support to women’s decision autonomy
  - Resources
  - Tools
  - Education

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Prenatal diagnosis of chromosome abnormalities

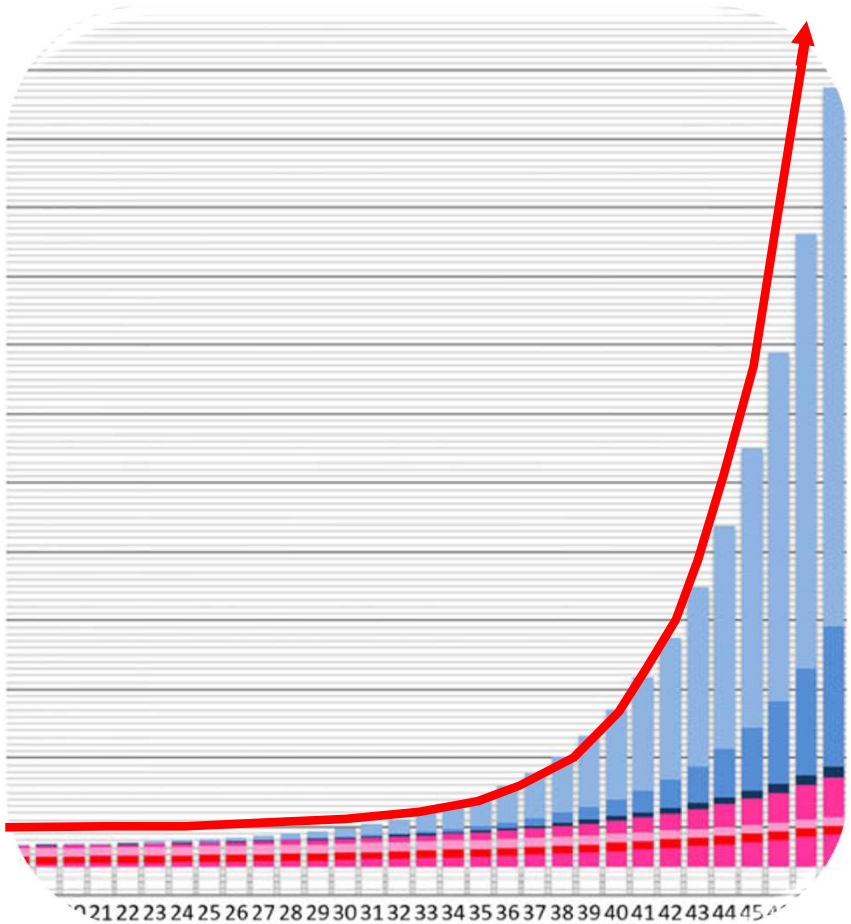
- The current standard test is the karyotype/CMA on fetal samples:
  - Chorionic villi sampling (CVS): 11-13wg
  - Amniotic fluid sampling (AF): 16-18wg

- AF and CVS are carried out for a variety of reasons:
  - fetal US abnormality/ies
  - previous affected fetus/child
  - parent carrier of a chromosome abnormality
  - …

- Main indication: diagnosis of fetal aneuploidies, primarily trisomy 21

MATERNAL AGE AND TRISOMIES

- An association between maternal age and trisomies: proneness of older oocytes to maternal meiosis I and II non-disjunction errors.



Hassold et al, Ann Hum Genet. 1980 Jul;44(Pt 1):29-36; Hassold T, Hunt PA, Sherman S. Curr Opin Genet Dev. 1993 Jun;3(3):398-403; Lamb et al Human Molecular Genetics, 1997, 1391–1399; Am. J. Hum. Genet. 76:91–99, 2005; Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155;

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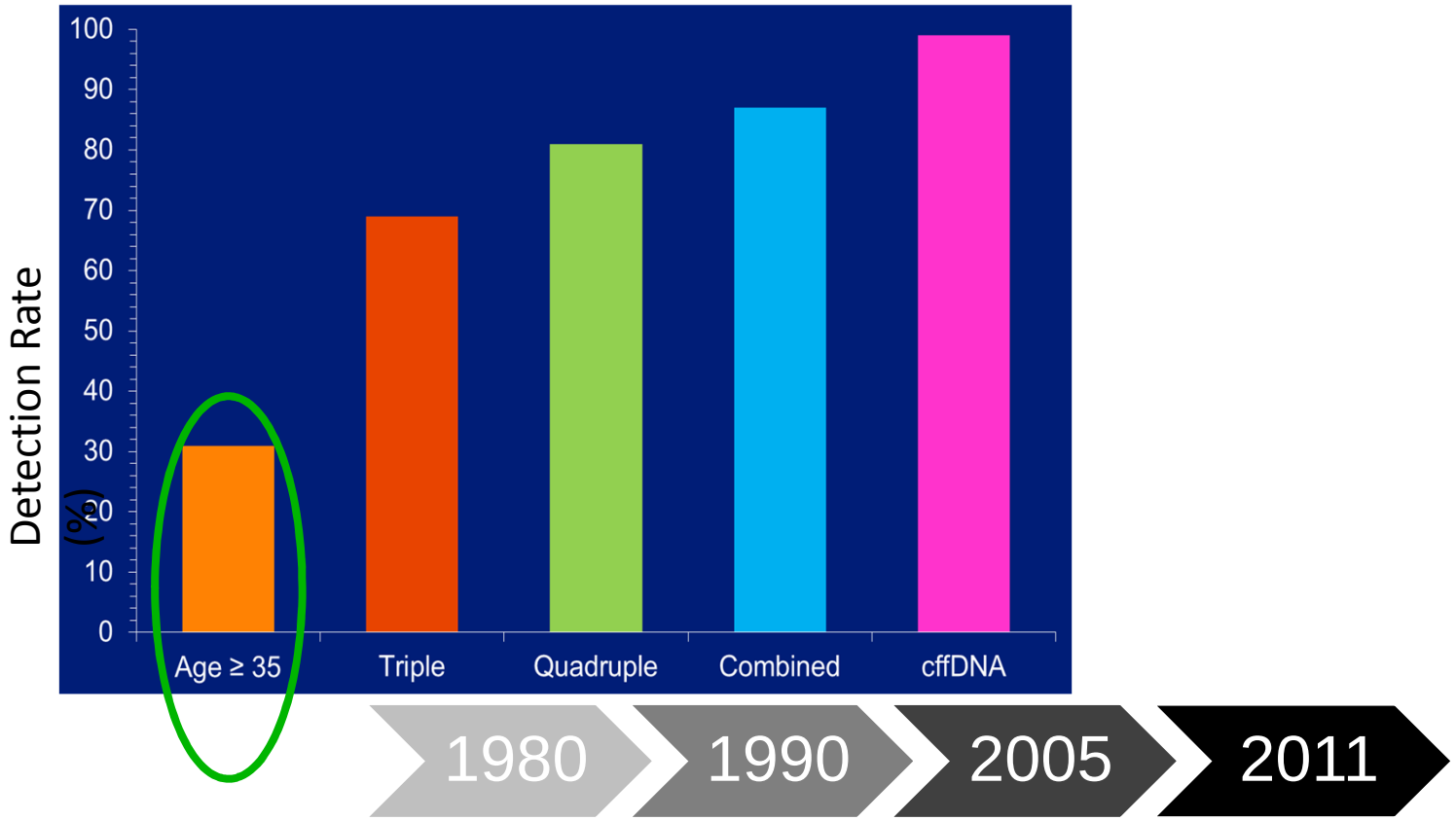
Screening programs for T21

- Developed starting from 70's: evolved considerably in the last few decades
- Recent developments in the cfDNA testing: DR ~99%; FPR<0.1%



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cfDNA testing performances: a meta-analysis

Type of aneuploidy	*number of studies n*	*trisomic cases n*	*non-trisomic cases n*	*Singleton pregnancies: weighted pooled*	
				DR	*FPR*
				(95% CI)	*(95% CI)*
T21	30	1,963	225,032	99.7%	0.04%
				(99.1-99.9)	*(0.02-0.08)*
T18	25	560	212,019	98.2%	0.05%
				(95.5-99.2)	*(0.03-0.07)*
T13	18	119	212,883	99.0%	0.04%
				(65.8-100)	*(0.02-0.07)*
45,X	23	36	7,677	95.8%	0.14%
				(70.3-99.5)	*(0.05-0.38)*
other SCA	11	17	5,383	100.0%	0.003%
				(83.6-100)	*(0-0.07)*
Type of aneuploidy				*Twin pregnancies: weighted pooled*	
				DR	*FPR*
				(95% CI)	*(95% CI)*
T21	8	24	1,111	100.0%	0%
				(95.2-100)	*(0-0.003)*

*peer-review studies reporting on clinical validation or implementation of maternal cfDNA testing in screening for aneuploidies, in which data on pregnancy outcome were provided for more than 85% of the study population (January 2011-31 December 2016)

Gil et al, Ultrasound Obstet Gynecol. 2017 Apr 11. doi: 10.1002/uog.17484. [Epub ahead of print]

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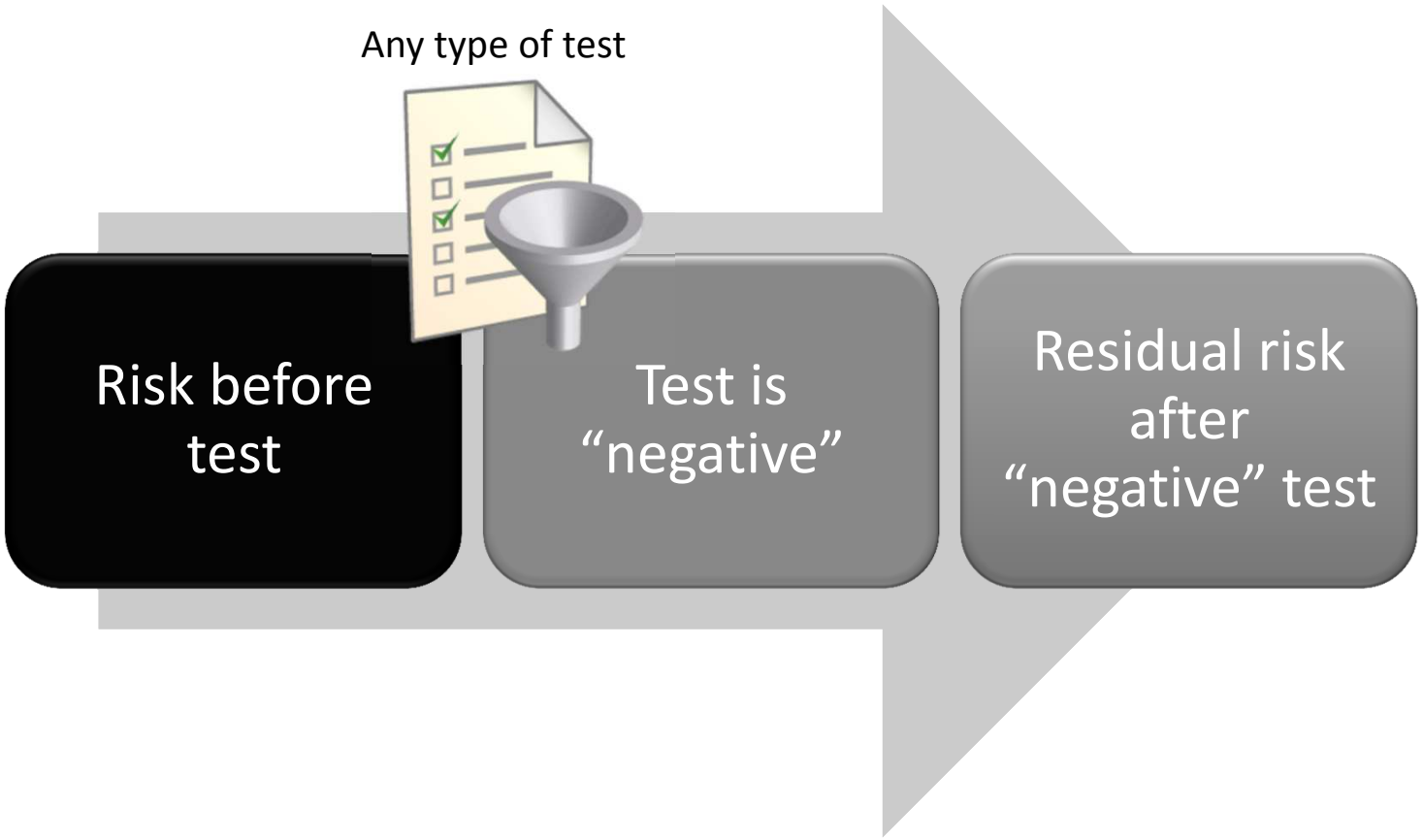
CfDNA TESTING CANNOT DETECT ALL FETAL CHROMOSOME ABNORMALITIES

CfDNA INFORMED CONSENT DISCLOSURES:

- Many fetal karyotype abnormalities cannot be identified
- Residual risk (RR) still remains
- It is crucial to provide accurate information on the actual rates of karyotype anomalies and RR at ***all maternal and gestational ages***

A priori and residual risk



Courtesy: Thomas J Musci

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Fetal chromosomal risks from previous studies

- Only for major aneuploidies that are obvious at birth (T21 and 18)
- Inferred the risk for chromosome abnormalities in women <35y at birth
- Not take into account sonography, which is now a routine tool in prenatal care
  - fetuses with anatomical abnormalities may have been included in these older datasets
  - skewed risk towards a higher range

Hook EB. Lancet. 1976 Jul 3;2(7975):33-4; Morris JK et al, Prenat Diagn. 2005 Apr;25(4):275-8; Morris JK et al J Med Screen. 2002;9(1):2-6; Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155

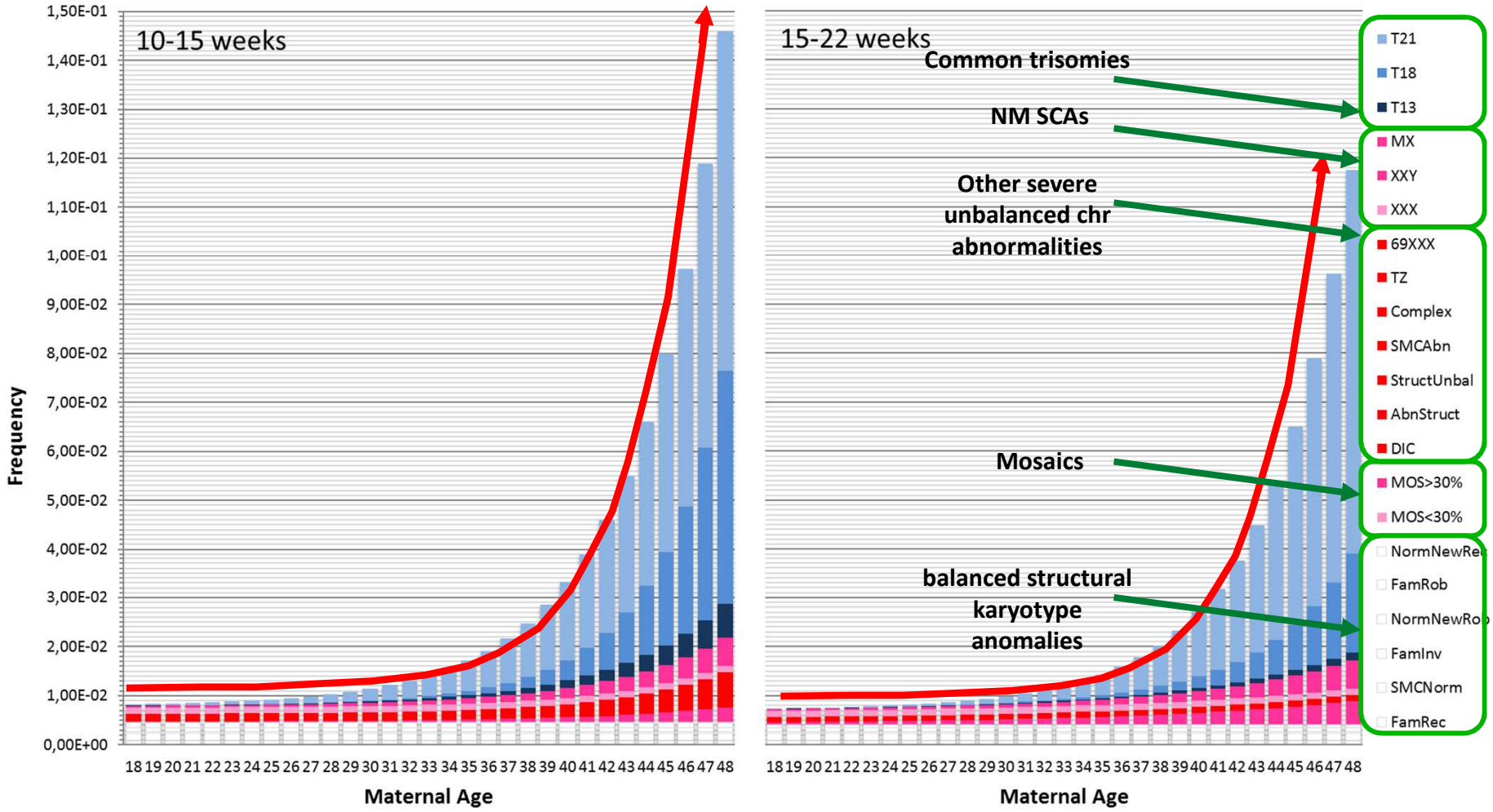
Determination of the fetal chromosomal risks stratified according to MA and GA

Enrolled population

- Unbiased retrospective analysis anonymized, database-stored cytogenetic diagnostic results on 129,263 samples of CVS (n=41,782) and AF (n=87,481);
- Indication: MA, anxiety or elective decision (≥35y and <35y)
  - NO other pretest risk factors aside from MA (no increased serum screening, negative family history)
  - NO obvious sonographic abnormalities detected prior to the procedure
- TOMA lab institutional review board approval (#0000015)

A PRIORI RISK OF A WOMAN TO CONCEIVE A CYTOGENETICALLY ABNORMAL FETUS

Stacked bar plot of the frequency of each chromosomal defect for each maternal age, and gestational age group.



Data on ~90,000 amniocand ~40,000 CVS,

Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155

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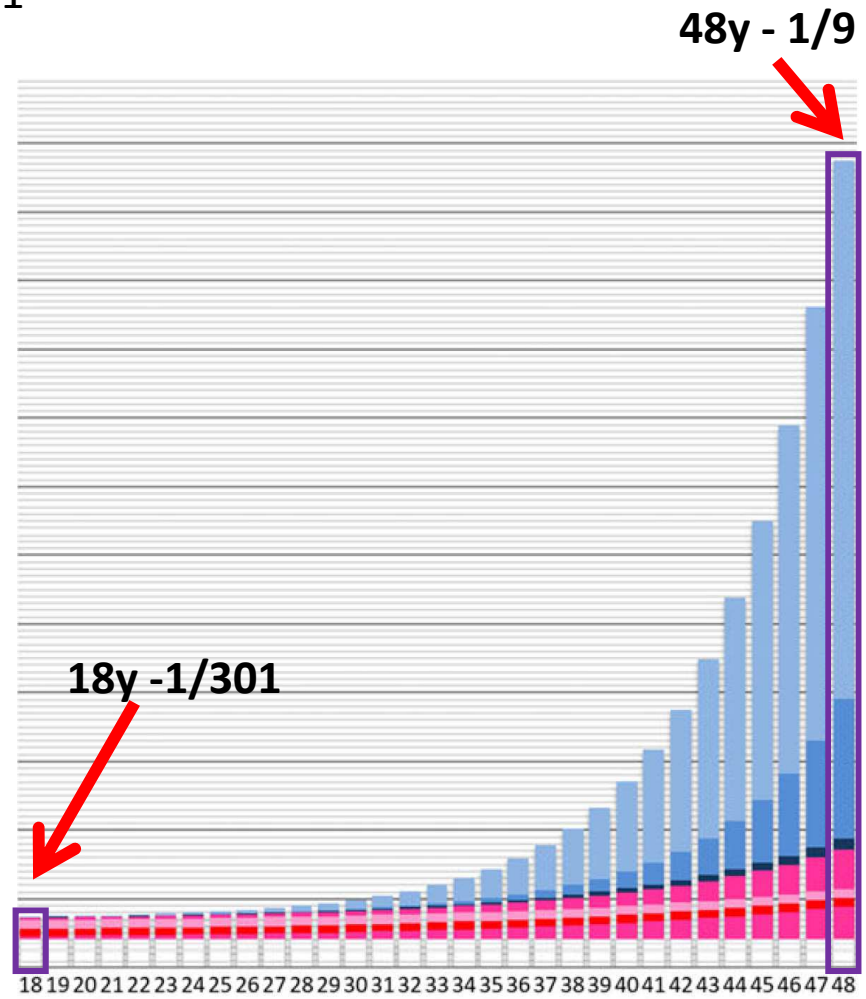
Effect of MA and GA on the a priori risk for fetal chr abnormalities

MA (years)	Risk for a cytogenetically visible genomic aberration <15w 1/X	≥15w 1/X
18	272	301
19	266	295
20	260	288
21	252	280
22	244	272
23	235	263
24	225	252
25	213	241
26	201	229
27	188	216
28	174	202
29	160	188
30	146	173
31	132	157
32	118	142
33	104	127
34	91	112
35	79	98
36	68	85
37	58	73
38	50	62
39	42	52
40	35	44
41	29	36
42	24	30
43	20	25
44	16	20
45	13	16
46	11	13
47	9	11
48	7	9

- Overall risk for cytogenetic abn at >15GA (including WHITE box)
  - 18y : 1/301
  - 48y: 1/9



Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155

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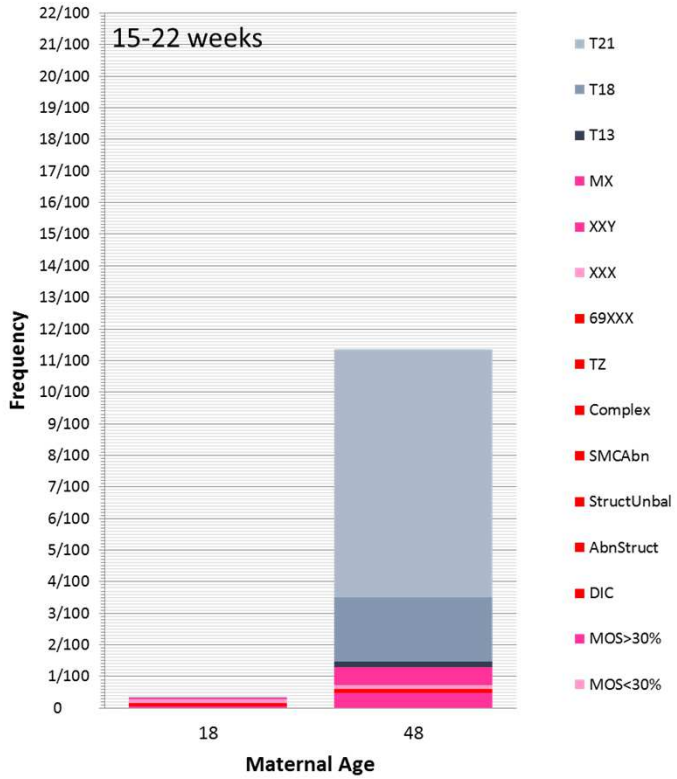
Effect of MA and GA on the a priori risk for fetal chr abnormalities

- The risk for common trisomies increases with MA
- In young women: risk is dominated by SCAs and other autosomal unbalanced rearr (red/pink)
- In older women: common trisomies dominate the risk (blue)



Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155

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Results

- Lower frequency of the common trisomies than reported from previous studies, in which sonographic findings were not available
- Frequency of chromosomal aneuploidies is significantly higher in earlier GA
  - CVS 2.63% (1100/41782) VS AF 1.82% (1596/87481); OR 0.6873 (95%CI 0.659-0.7428)

Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155

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Residual risk after a negative screening result

MA (years)	Risk for a cytogenetically visible genomic aberration		Proportion, of the risk for fetal T21, 18 or 13		Risk for disorders other than T21, 18 and 13		Proportion, of the risk for T21, 18 or 13 or SCAs		Risk for disorders other than T21, 18 and 13 and SCAs	
	<15w 1/X	≥15w 1/X	<15w %	≥15w %	<15w 1/X	≥15w 1/X	<15w %	≥15w %	<15w 1/X	≥15w 1/X
18	272	301	7.5	3.8	294	313	51.9	53.1	565	642
19	266	295	8.8	4.6	292	309	52.7	53.2	562	630
20	260	288	10.5	5.5	290	305	53.5	53.3	559	617
21	252	280	12.3	6.6	288	300	54.6	53.5	555	603
22	244	272	14.5	7.9	285	295	55.7	53.8	551	589
23	235	263	16.9	9.4	283	290	57.0	54.2	546	574
24	225	252	19.6	11.1	279	284	58.4	54.8	540	558
25	213	241	22.6	13.2	276	278	60.0	55.5	534	542
26	201	229	25.9	15.6	271	271	61.8	56.4	526	526
27	188	216	29.5	18.3	267	264	63.6	57.5	517	509
28	174	202	33.2	21.3	261	257	65.6	58.8	507	491
29	160	188	37.2	24.7	255	249	67.7	60.4	495	474
30	146	173	41.2	28.4	248	241	69.7	62.1	482	456
31	132	157	45.3	32.3	241	233	71.9	64.1	467	438
32	118	142	49.4	36.6	232	224	73.9	66.2	451	420
33	104	127	53.4	40.9	223	215	76.0	68.4	433	401
34	91	112	57.3	45.4	213	205	77.9	70.8	413	383
35	79	98	60.9	49.9	203	196	79.7	73.2	391	365
36	68	85	64.4	54.4	192	186	81.5	75.6	368	347
37	58	73	67.6	58.7	180	176	83.0	77.9	344	329
38	50	62	70.5	62.8	168	166	84.5	80.2	319	312
39	42	52	73.2	66.6	156	156	85.8	82.3	294	295
40	35	44	75.6	70.2	143	146	86.9	84.3	268	278
41	29	36	77.8	73.5	131	136	88.0	86.2	242	262
42	24	30	79.7	76.4	119	127	88.9	87.9	217	246
43	20	25	81.5	79.1	107	118	89.8	89.4	194	231
44	16	20	83.0	81.5	96	109	90.5	90.7	171	217
45	13	16	84.4	83.6	85	100	91.2	91.9	150	203
46	11	13	85.7	85.4	75	92	91.7	92.9	131	189
47	9	11	86.8	87.1	66	84	92.3	93.9	113	176
48	7	9	87.8	88.5	58	76	92.7	94.6	97	164

Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155

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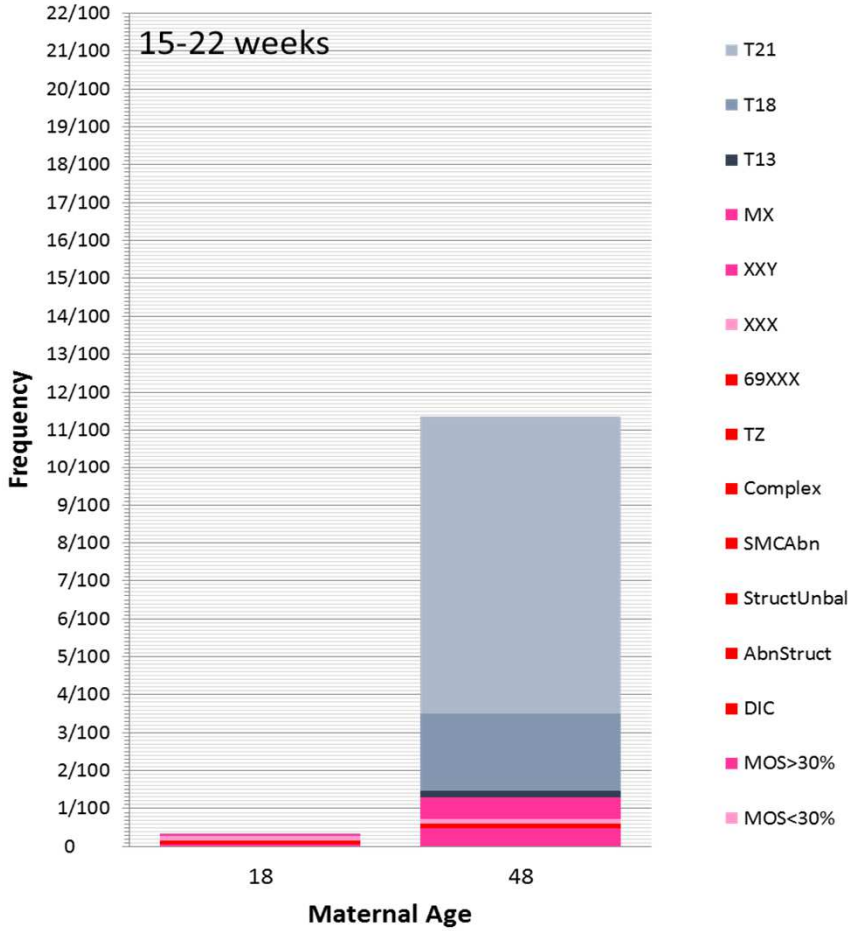
Effect of MA and GA on the residual risk for chr abnormalities not targeted by non-invasive screening strategies

- Clinically significant chromosomal abn ***other than*** T21,18,13,SCAs at >15GA:
  - 18y: 47% of the a priori risk
  - 48y: 5% of the a priori risk



Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155

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SUBMICROSCOPIC CHROMOSOME ABNORMALITIES

Newer technologies impact the epidemiology of fetal chr abn:

- pCNV or likely pCNVs prevalence (by CMA) in women with anatomically normal fetuses with normal karyotypes is 1.65% (1/61)
  - Clearly pCNVs: 0.5% - 95th% CI 0.2–0.8
  - pCNVs with variable expressivity: 0.6% – 95th% CI 0.3–1.1
  - Likely pVOUS: 0.6% – 95th% CI 0.3–1.1
- Lack of association with MA, serum screening analyte levels or GA
  - prevalence fixed at 1.65% (1/61) in all women

Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155; Wapner et al, N Engl J Med 2012;367(23):2175–2184

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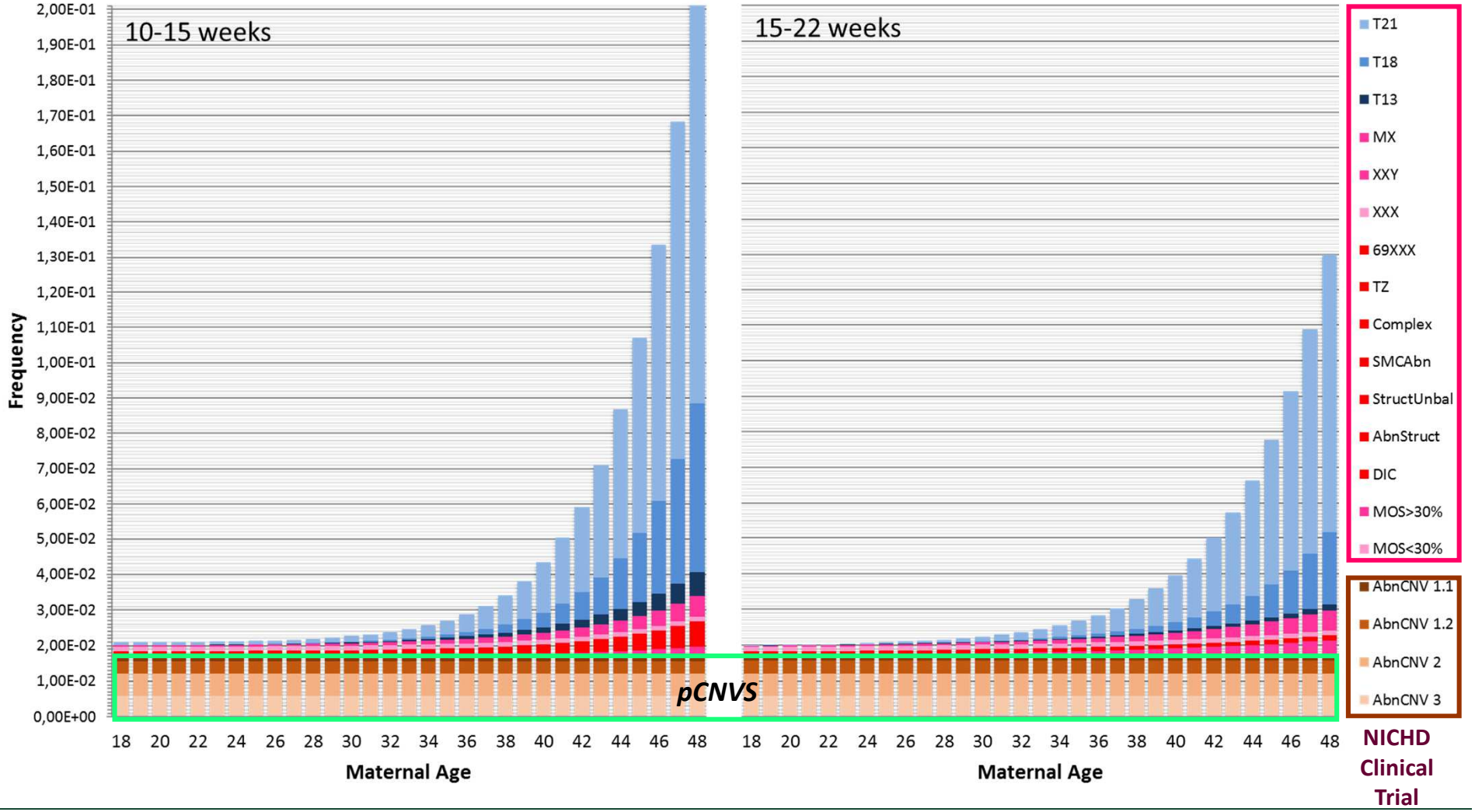
A PRIORI RISK OF A WOMAN TO CONCEIVE A CHROMOSOMICALLY ABNORMAL FETUS

Stacked bar plot of the frequency of each genomic defect for each maternal age, and gestational age group (no balanced rearr)

TOMA lab DataBase



NICHD Clinical Trial

Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155; Wapner et al, N Engl J Med 2012;367(23):2175–2184

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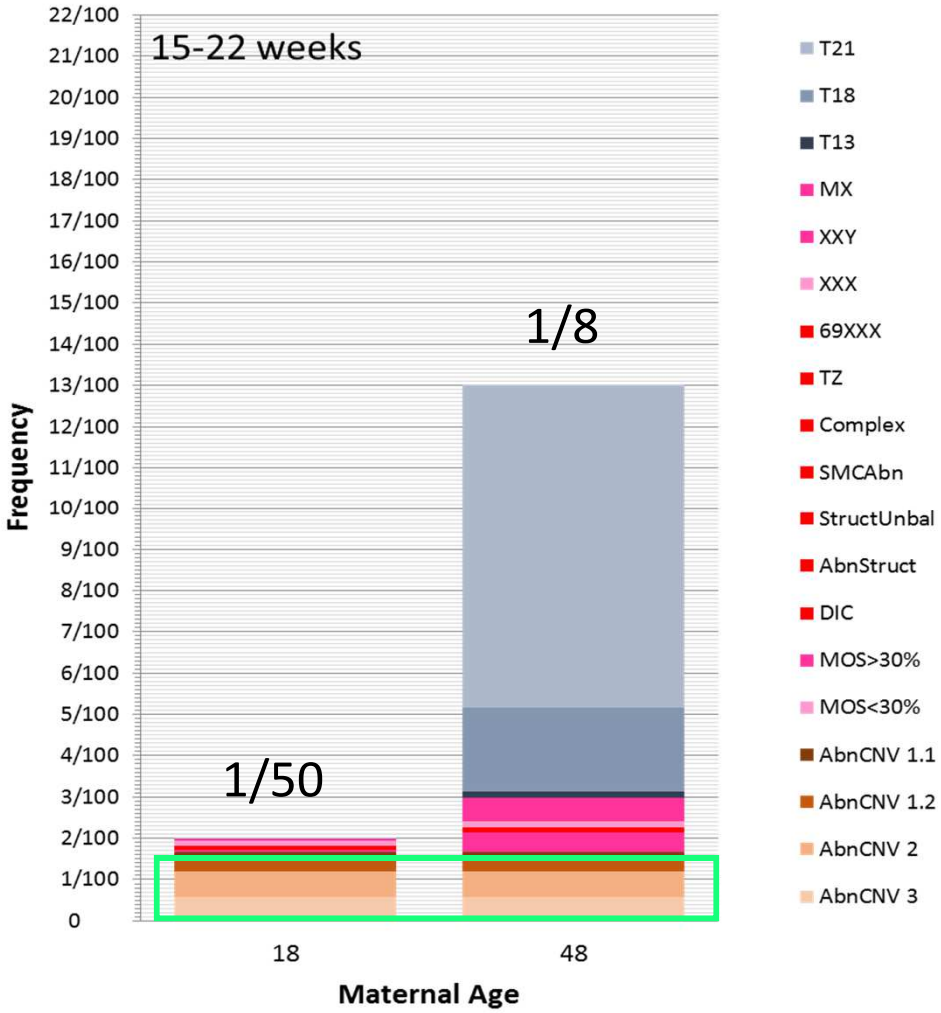
EFFECT OF MATERNAL AGE ON THE A PRIORI RISK



- In younger ages the non-age-dependent pCNVs dominate fetal risk
- CNVs represent the main component of the a priori risk for fetal genomic abnormalities in younger women:
  - 80% of the risk in 18y
  - 15% of the risk in 48y

Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155

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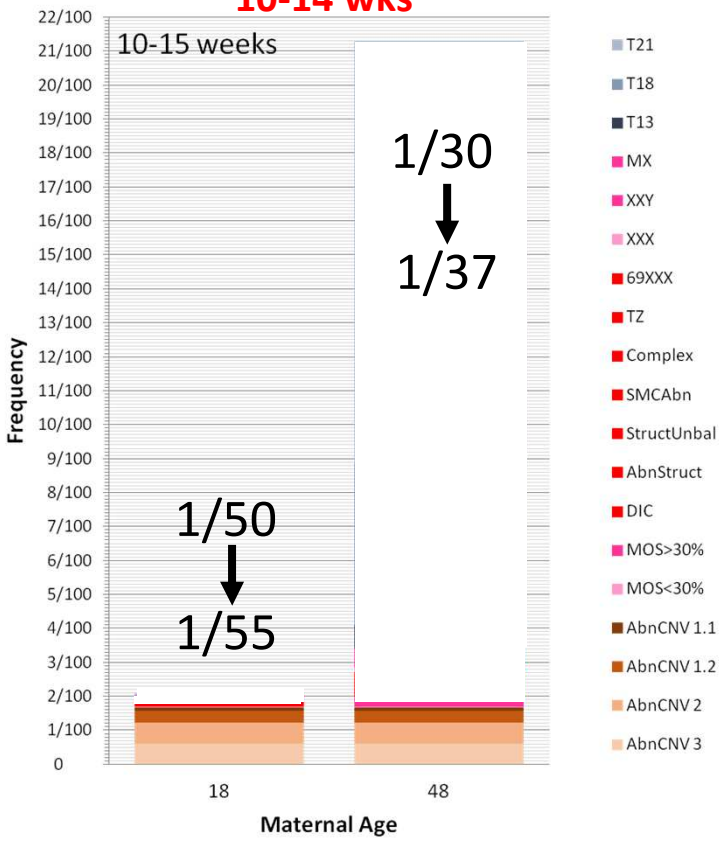
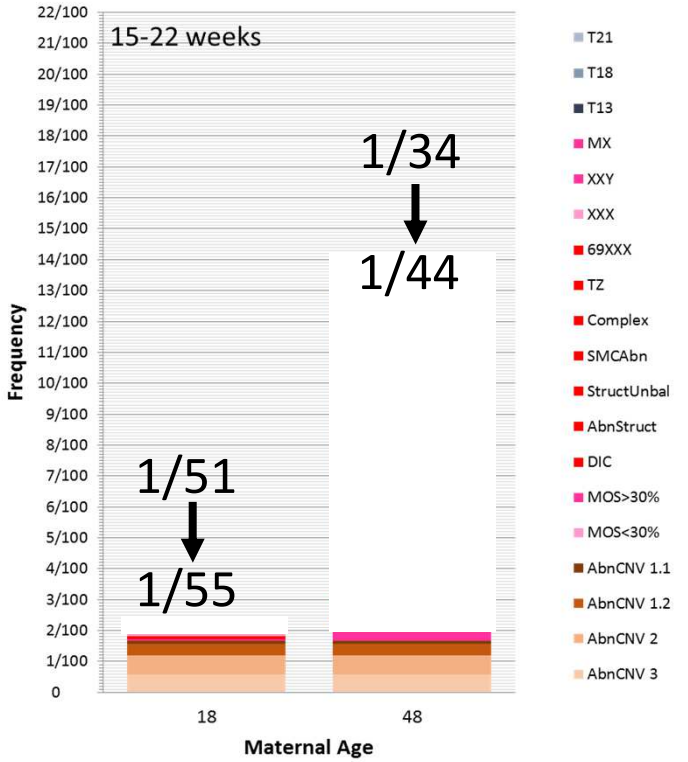
Effect of MA and GA on the residual risk for chr abnormalities not targeted by non-invasive screening strategies

- Residual risk for other 'off-target' clinically significant genomic abn
  - ***other than T21,18,13***
  - ***other than T21,18,13, homogeneous SCAs***

10-14 wks



15-22 wks



Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155

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PROPORTION OF DEFECTS DETECTED BY THE DIFFERENT TESTING STRATEGY

- After the exclusion of T21,18,13&SCAs, the RR for other pathogenic GENOMIC abnormalities is still consistent
  - Not so much different in young (~1/50) and old women (~1/40)
- Even excluding pCNVs with variable expressivity and likely pVOUS, a residual risk of 0.5% (95th% CI 0.2–0.8) for pCNVs with highly penetrant phenotypes still remains: ***level of risk to justify offering invasive testing***

Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155

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PROPORTION OF DEFECTS DETECTED BY THE DIFFERENT TESTING STRATEGY

'… Le gestanti che , per scelta personale , in assenza di una indicazione che conferisca loro un rischio " a priori" elevato per le microdelezioni/microduplicazioni, decidano di sottoporsi ad una diagnosi prenatale invasiva, dovrebbero essere informate dell'esistenza del CMA come tecnica di approfondimento diagnostico, ad integrazione del cariotipo fetale. La sua applicazione in questa popolazione dovrebbe rispondere all'obiettivo di ridurre il rischio di sindromi note da microdelezione/microduplicazione associate a fenotipi clinici gravi….'

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SUPPORTING WOMEN'S AUTONOMY IN PRENATAL TESTING

Perspective

AUGUST 10, 2017

Supporting Women's Autonomy in Prenatal Testing

Josephine Johnston, L.L.B., M.B.H.L., Ruth M. Farrell, M.D., and Erik Parens, Ph.D.

'Early and noninvasive fetal genetic sequencing is on the horizon. Such expanded prenatal testing could offer patients substantial benefits. But current practices in prenatal screening and the complex nature of genomic science and technology create the risk that these tests will be integrated into care without the robust, evidence-based informed consent processes necessary for respecting women's autonomy. If that happens, patients will be asked to decide whether to undergo invasive diagnostic testing and then to consider whether to terminate or continue their pregnancy without a full understanding of the results. …'

Johnston, Farrell, and Parens. NEJM 377;6 August 10, 2017

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HOW TO SUPPORT WOMEN'S AUTONOMY?

'The need for fully informed consent in prenatal screening and testing has never been more urgent. Meeting this need will require adoption of ***reimbursement policies and professional practice guidelines*** *that support clinicians in breaking with current routine practices, which too often involve dispensing with or failing to adequately carry out an informed consent process. It will also require* ***funding for development of approaches to pretest and posttest education and counseling*** *that empower patients to decide whether to be tested and what to do after receiving their results.' …*

'Only with these practices and policies in place can women's decisions about prenatal screening, diagnostic testing, and termination or continuation of pregnancy be truly free and informed.'

HOW TO SUPPORT WOMEN'S AUTONOMY?

Resources:

- Professional societies provide uniform educational materials for providers and women
  - Movies
  - Brochures
  - Slide decks
- Online and residential courses for providers
- Pratice with simulation
- Uniform informed consent (legally revised)

New tools:

- Movies for pretest counseling (@home)
- Apps and softwares to support calculation of RR during pretest counseling
  - Specific MA and GA
- Tele-counseling with recording of the informed consent
- Furum of professional societies on social media

New education strategies:

- Anticipation in preconceptional period
- Social media
- Family doctors
- Teens (reproductive risk education)

HOW TO SUPPORT WOMEN'S AUTONOMY?

Genetic Support Foundation

https://geneticsupportfoundation.org/

Perinatal Quality Foundation
Improving the Quality of Maternal Fetal Medicine Medical Services

https://www.perinatalquality.org/

Resources:

- GSF and PQF focus on improving the quality of communication regarding prenatal testing options

- The PQF educates obstetricians to help facilitate quality perinatal patient care. They have developed genetic education modules (GEM) for patients considering prenatal testing to help empower patients to make informed decisions.

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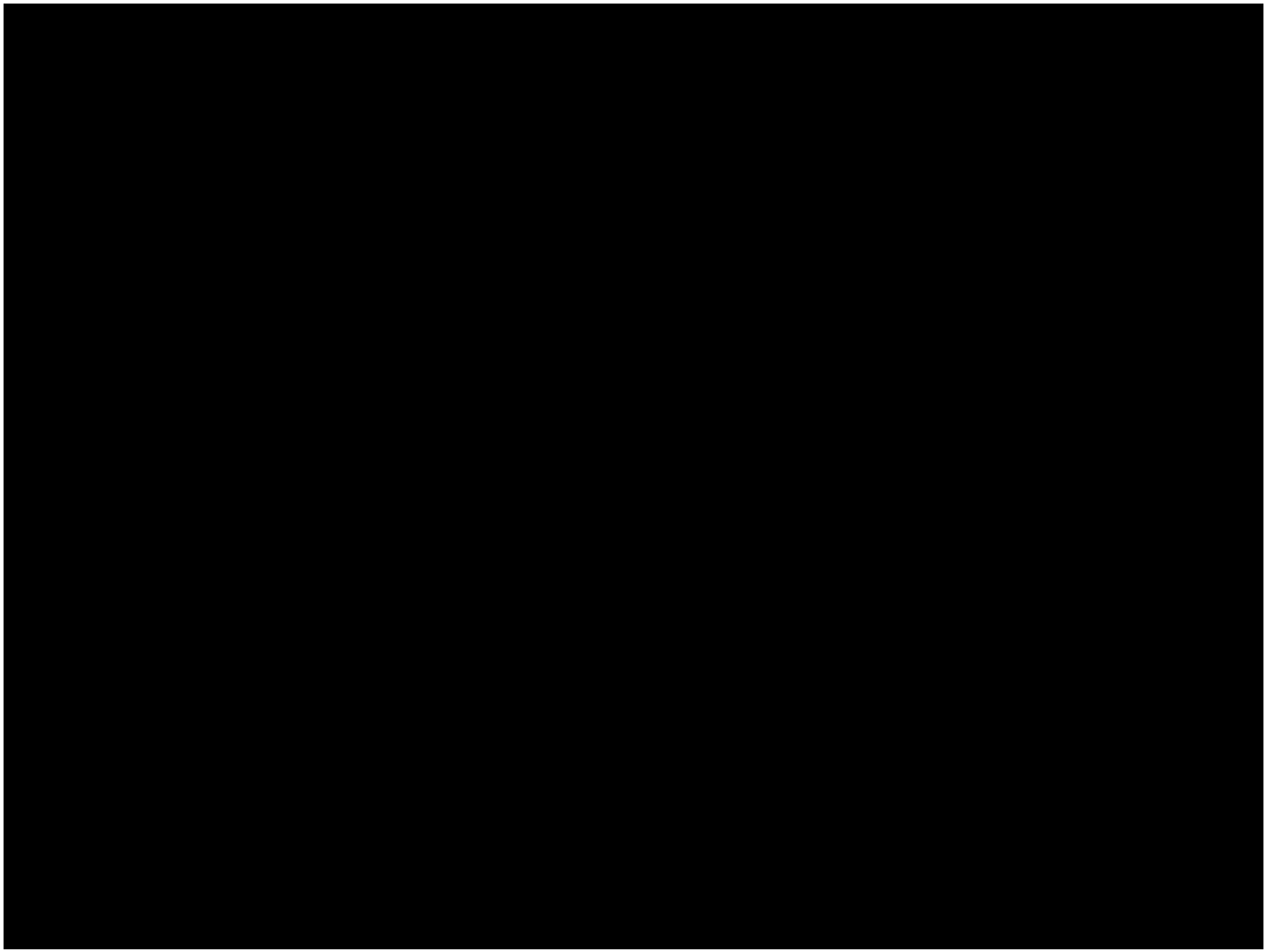
HOW TO SUPPORT WOMEN'S AUTONOMY?



HOW TO SUPPORT WOMEN'S AUTONOMY?

ISPD Global Updates (July/August 2017) – Genetic Counseling SIG

http://ispdhome.org/ISPD/Special_Interest_Groups/Genetic_Counseling/ISPD/SIGs/Genetic_Counseling.aspx?utm_source=Informz&utm_medium=Email&utm_campaign=eBlasts#GU817

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Susan Gross

Jose Ferreira Komal Bajaj

QUESTION

CfDNA vs. CVS in the high risk patients?

Increased a-priori risk for genetic abnormalities in pregnancies with U/S abnormalities

Type of genomic disorder	Type of test on CVS/AF	Resolution
Cytogenetic abnormalities	Karyotyping	>5-7Mb
Submicroscopic dels/dups	CMA	Kb--> <5Mb
Monogenic disorders	WES	bp
Imprinting disorders	Different molecular tests	Epigenetic

Fetal chromosome abnormalities in pregnancies with U/S abnormalities

TABLE I. HR + IR Chromosomal Abnormalities Detected in Chorionic Villi (CV) and in Amniotic Fluid (AF) According to the Indication for Invasive Prenatal Diagnosis

Chromosomal abnormality	<35 years	≥35 years	US fetal abnorm.	Bal. chr. abn. in a parent	sSMC or mosaic in a parent	Previous affected child or fetus	Increased risk at screening test	Confirmatory amniocentesis after CVS abn.	One or more spontaneous abortions	Chr. abn. in a relative	Other	Subtotal (no. cases)
CV												
Total number of cases analyzed	5,400	20,502	2,297	195	23	1,034	473	—	43	165	526	30,658
No. of HR + IR karyotypes	66	496	640	22	1	22	36	—	8	2	4	1,297
Frequency of HR + IR karyotypes (%)	1.22	2.42	27.86	11.28	4.35	2.13	7.61	—	18.60	1.21	0.76	4.23
AF												
Total number of cases analyzed	22,527	47,987	2,426	225	35	835	7,997	692	190	1,025	531	84,470
No. of HR + IR karyotypes	137	682	278	2	1	6	182	35	6	9	6	1,344
Frequency of HR + IR karyotypes (%)	0.61	1.42	11.46	0.89	2.86	0.72	2.28	5.06	3.16	0.88	1.13	1.59

US fetal abnorm., ultrasound fetal abnormalities; Bal. chr. abn., balanced chromosome abnormalities; sSMC, small supernumerary chromosome marker; CV, chorionic villi; AF, amniotic fluid; HR, high risk; IR, intermediate risk.

* OR 15.58, 95%CI 13.71-17.70

** OR 31.22, 95%CI 24.09-40.46

*** OR 8.98, 95%CI 7.76-10.39

**** OR 21.15, 95%CI 17.16-26.08

Grati et al, Am J Med Genet Part A 152A:1434–1442.

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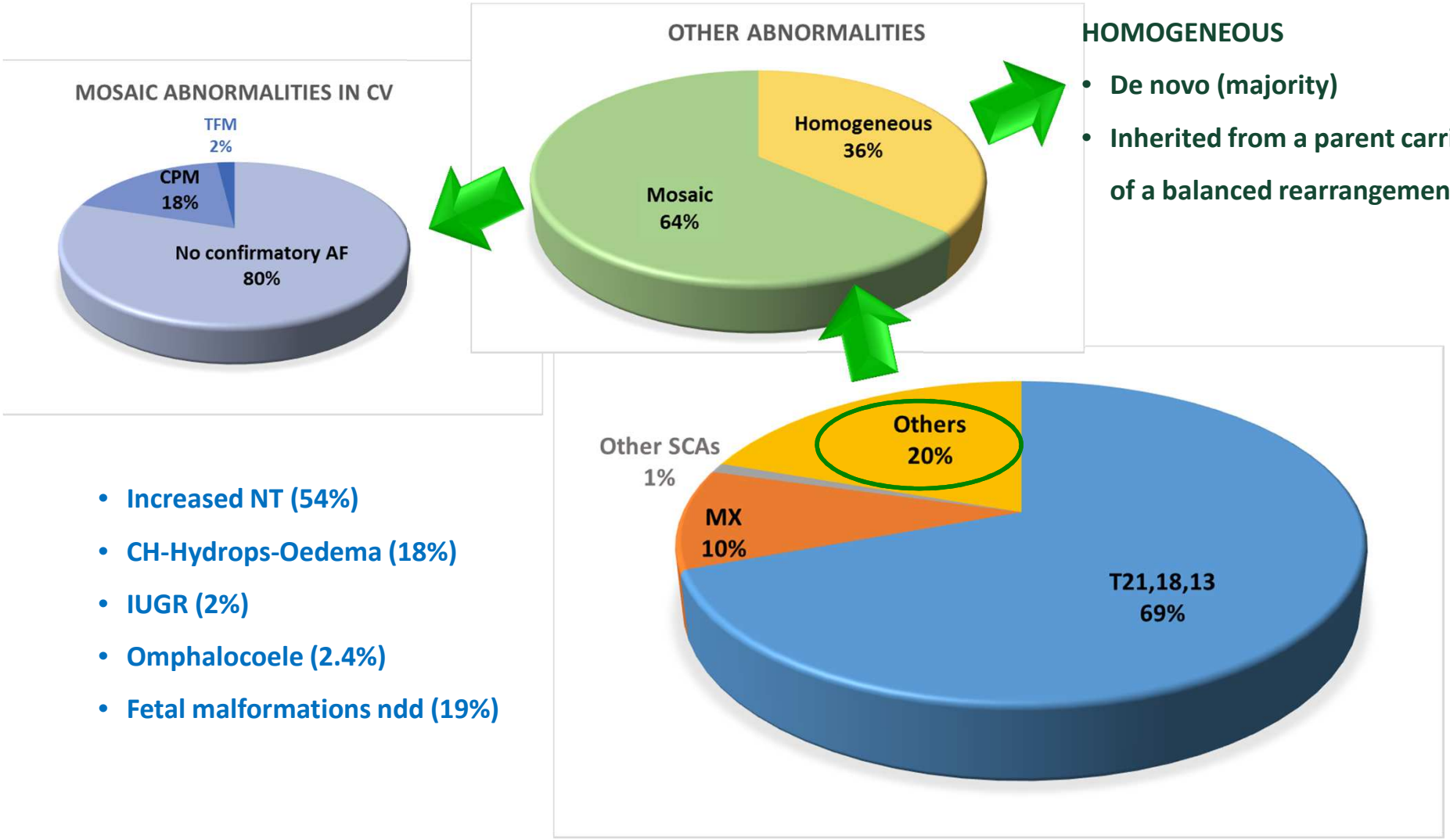
Chromosome abnormalities in CVS of pregnancies with U/S abnormalities



HOMOGENEOUS

- De novo (majority)
- Inherited from a parent carrier of a balanced rearrangement

- Increased NT (54%)
- CH-Hydrops-Oedema (18%)
- IUGR (2%)
- Omphalocoele (2.4%)
- Fetal malformations ndd (19%)

Grati et al, Am J Med Genet Part A 152A:1434–1442; Grati et al, Unpublished data

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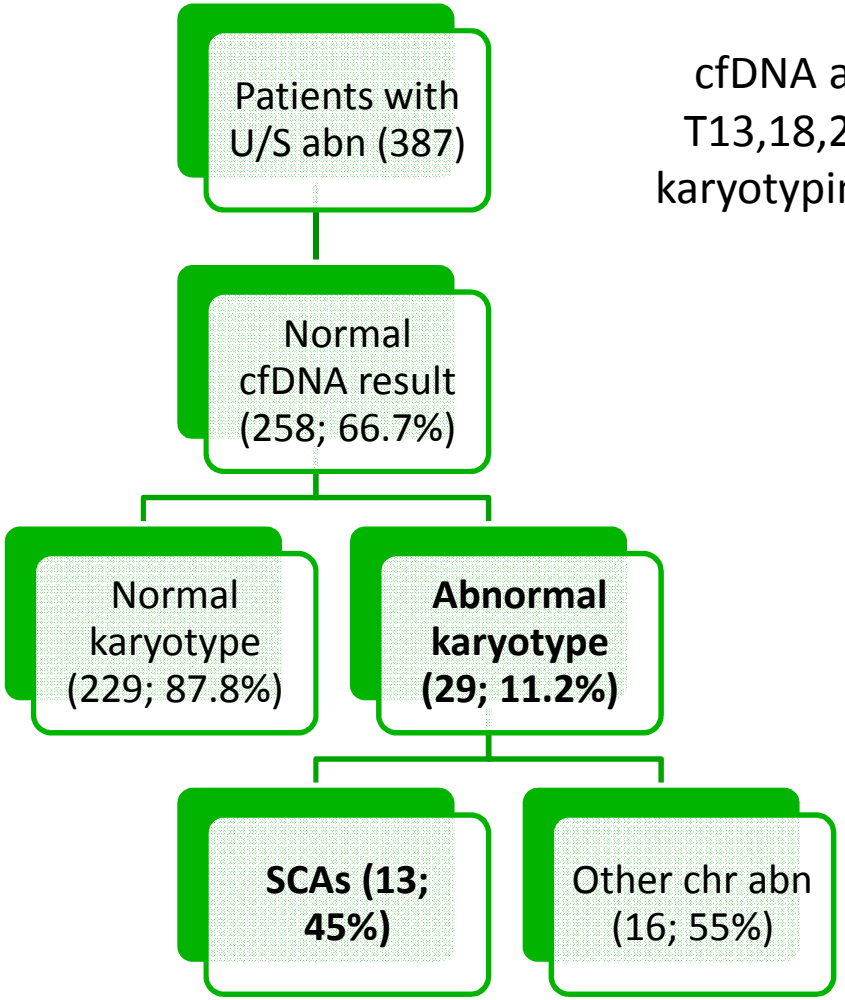
cfDNA TESTING IN FETAL ANOMALIES ON ULTRASOUND



cfDNA analysis for T13,18,21 only ***and*** karyotyping in parallel

Benachi et al. Obstet Gynecol. 2015 Jun;125(6):1330-7

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Increased a-priori risk for genetic abnormalities in pregnancies with U/S abnormalities

Type of genomic disorder	Type of test on CVS/AF	Resolution
Cytogenetic abnormalities	Karyotyping	>5-7Mb
Submicroscopic dels/dups	CMA	Kb--> <5Mb
Monogenic disorders	WES	bp
Imprinting disorders	Different molecular tests	Epigenetic

INCREMENTAL YIELD BY MICROARRAY WITH NORMAL FETAL KARYOTYPE

- **5.6% (95% CI 4.7-6.6) structural ultrasound anomaly restricted to one anatomical system and a normal karyotype**
- **9.1% (95% CI 7.5-10.8) poly-malformed fetuses**

	Isolated anomalies				
	Cardiac	**Resp**	**CNS**	**Facial**	**MSK**
Pooled prevalence (95% CI)	22/476 4.6% (2.7-6.5)	5/81 6.2% (0.9-11.4)	35/563 6.2% (4.2-8.2)	6/113 5.3% (1.2-9.4)	24/305 7.9% (4.8-10.9)

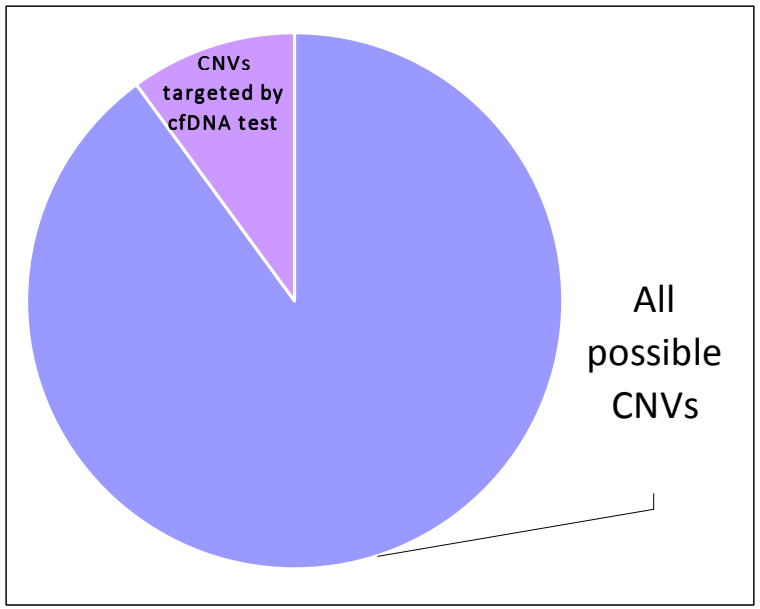
	Isolated anomalies				
	GIT	**Urogenital**	**NT >3.5 mm**	**Cystic hygroma**	**Total**
Pooled prevalence (95% CI)	7/105 6.7% (1.9-11.4)	9/153 5.9% (2.2-9.6)	5/162 3.1% (0.4-5.7)	12/262 4.6% (2.0-7.1)	**125/2220 5.6% (4.7-6.6)**

De Wit et al., UOG 2014

Toma

High post-test residual risk for fetal pCNVs

- 5-6 CNVs represent only a ***portion*** (~20%) of the overall pCNVs that can affect the fetus



False reassurance to patients – consistent residual risk

Wapner et al, NEJM 2012; Yaron et al, Obstet Gynecol. 2015 Nov;126(5):1095-9; Grati FR, Ultrasound Obstet Gynecol. 2016 May 31. doi: 10.1002/uog.15975

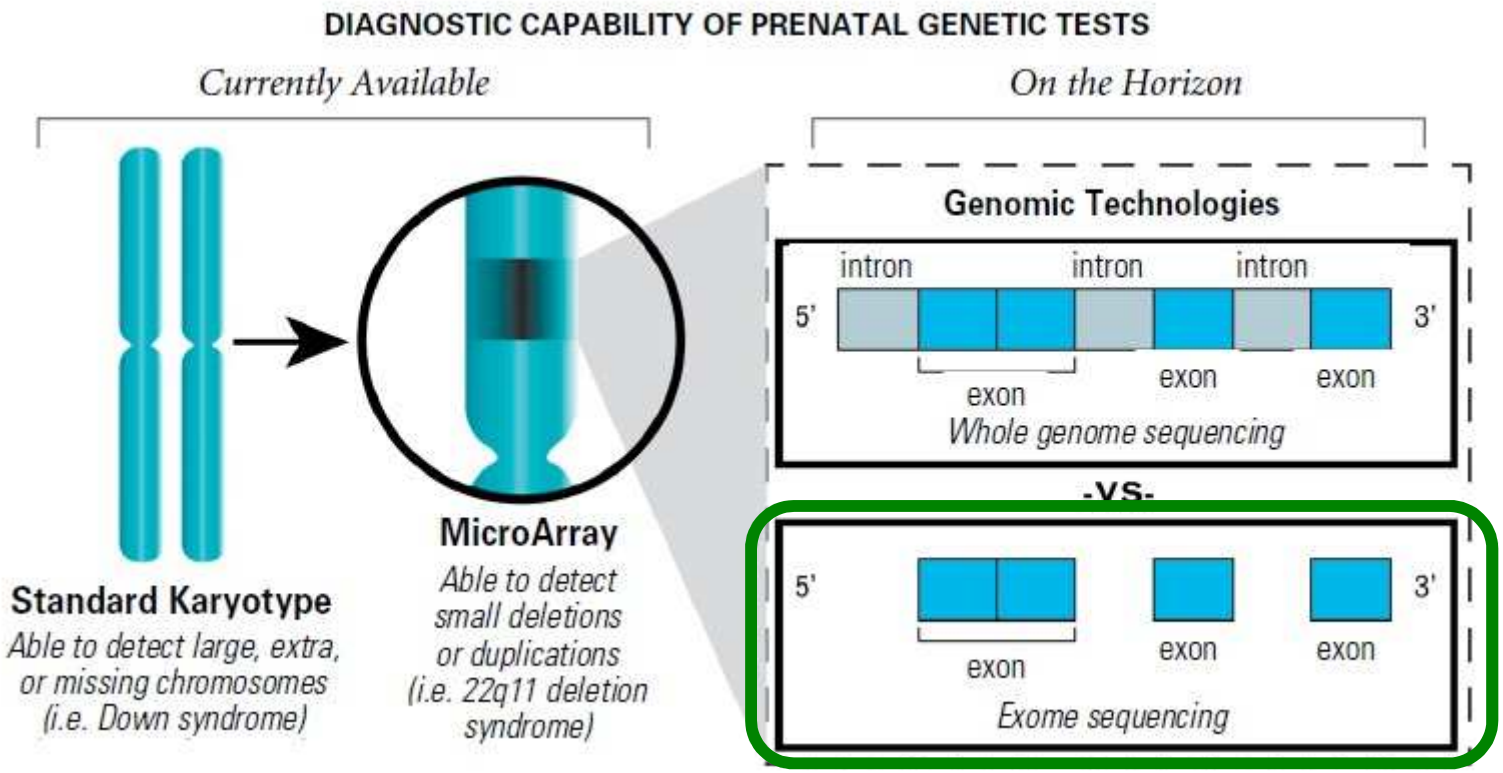
Toma

Increased a-priori risk for genetic abnormalities in pregnancies with U/S abnormalities

Type of genomic disorder	Type of test on CVS/AF	Resolution
Cytogenetic abnormalities	Karyotyping	>5-7Mb
Submicroscopic dels/dups	CMA	Kb--> <5Mb
Monogenic disorders	WES	bp
Imprinting disorders	Different molecular tests	Epigenetic

WHOLE EXOME SEQUENCING IN FETAL ANOMALIES ON U/S – ON THE HORIZON

- WES examines coding regions (exons) of the genome



Drury et al, Prenatal Diagnosis 2015, 35, 1010–1017; Pangalos et al, DOI 10.7717/peerj.1955; ACOG and sMFM Committee opinion Number 682, December 2016

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WHOLE EXOME SEQUENCING IN FETAL ANOMALIES ON U/S – ON THE HORIZON

- Aimed to identify the etiology for fetal U/S abnormalities
- Actually not recommended outside of the context of clinical trials
- Offered on research basis in some labs or for specific clinical indications in other labs (recurrent or lethal fetal anomalies)
- Limited published data on prenatal application of WES
- Monogenic diseases may be identified in up to 20-30% of fetuses with multiple anomalies suggestive of a genetic disorder for which karyotyping and CMA are normal
- Provide options of PGD or early prenatal diagnosis in a future pregnancy

Drury et al, Prenatal Diagnosis 2015, 35, 1010–1017; Pangalos et al, DOI 10.7717/peerj.1955; ACOG and sMFM Committee opinion Number 682, December 2016

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Increased a-priori risk for genetic abnormalities in pregnancies with U/S abnormalities

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Monogenic disorders	WES	bp
Imprinting disorders	Different molecular tests	Epigenetic

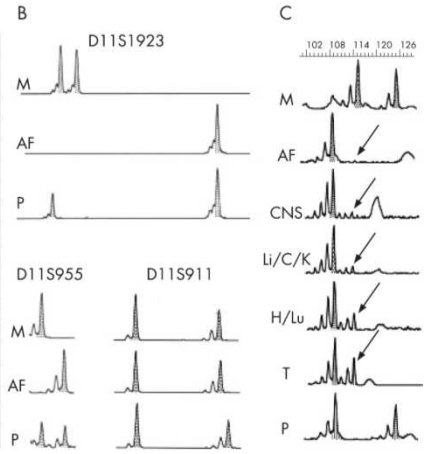
Normal fetal karyotype and normal CMA!!

Imprinting Syndromes and fetal U/S abnormalities

UPD11pat or other related imprinting defects

A

STR	M	AF	P	Result
D11S2071	2,3	1,1	1.2	isoUPDpat
D11S1363	2,3	1,1	1,3	isoUPDpat
D11S922	1,2	1,1	1,3	NI
D11S4046	1,2	4,4	3,4	isoUPDpat
D11S4088	2,4	3,3	1,3	isoUPDpat
D11S1923	2,3	4,4	1,4	isoUPDpat
D11S4146	2,3	2,2	1,2	isoUPDpat
D11S2345	1,1	1,1	1,1	NI
D11S988	2,3	1,1	1,3	isoUPDpat
D11S4181	1,1	1,1	1,1	NI
D11S1760	1,1	2,2	2,3	isoUPDpat
D11S1338	1,1	2,2	2,3	isoUPDpat
D11S995	1,1	2,2	1,2	isoUPDpat
D11S871	1,2	1,1	1,2	NI
D11S986	1,1	1,1	1,2	NI
D11S1303	1,3	1,2	1,2	no UPDpat
D11S4191	1,2	1,1	1,1	NI
D11S987	1,2	2,2	2,3	NI
D11S911	1,2	1,2	1,3	no UPDpat



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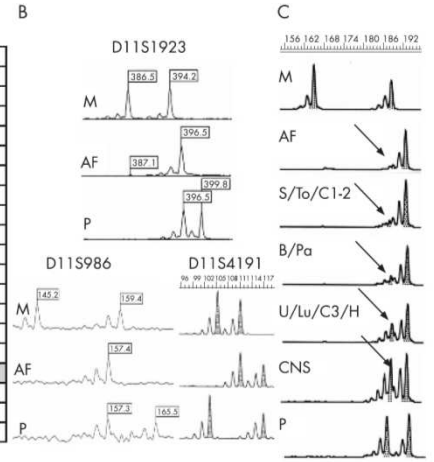
D11S995	1,1	1,1	1,2	NI
D11S871	2,3	3,3	1,3	NI
D11S986	1,3	2,2	2,4	isoUPDpat
D11S1303	1,2	2,3	2,3	no UPDpat
D11S4191	2,3	3,4	1,4	no UPDpat
D11S987	2,4	1,4	1,3	no UPDpat
D11S911	1,3	1,2	1,2	no isoUPDpat



Beckwith–Wiedemann syndrome (BWS) in fetuses with:

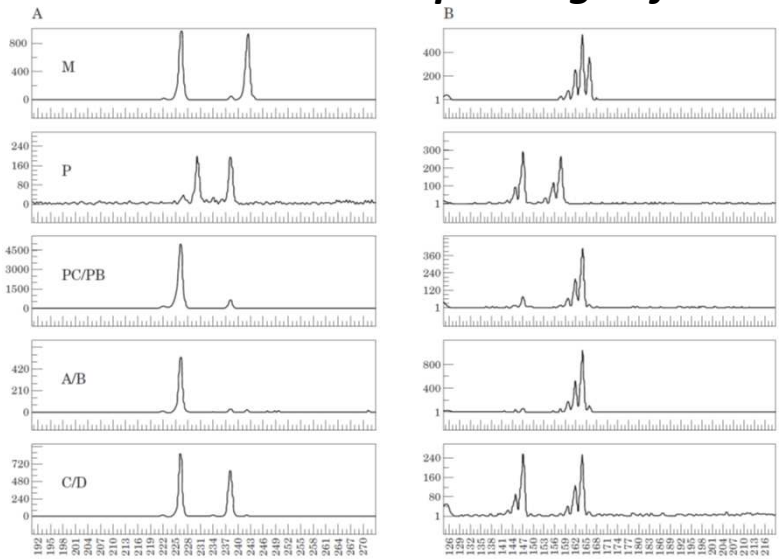
- isolated omphalocoele
- overgrowth
- polydramnios
- enlarged placenta
- distended abdomen
- visceromegaly
- macroglossia

Grati et al, J. Med. Genet. 2007;44;257-263

Toma

Imprinting Syndromes and fetal U/S abnormalities

UPD7mat or other imprinting defects



Silver Russell syndrome (SRS) in fetuses with:

- IUGR
- Micrognathia
- CHD
- clinodactyly
- Partial or total asymmetry

Miozzo, Grati et al, Placenta (2001), 22, 813–821; OMIM #180860

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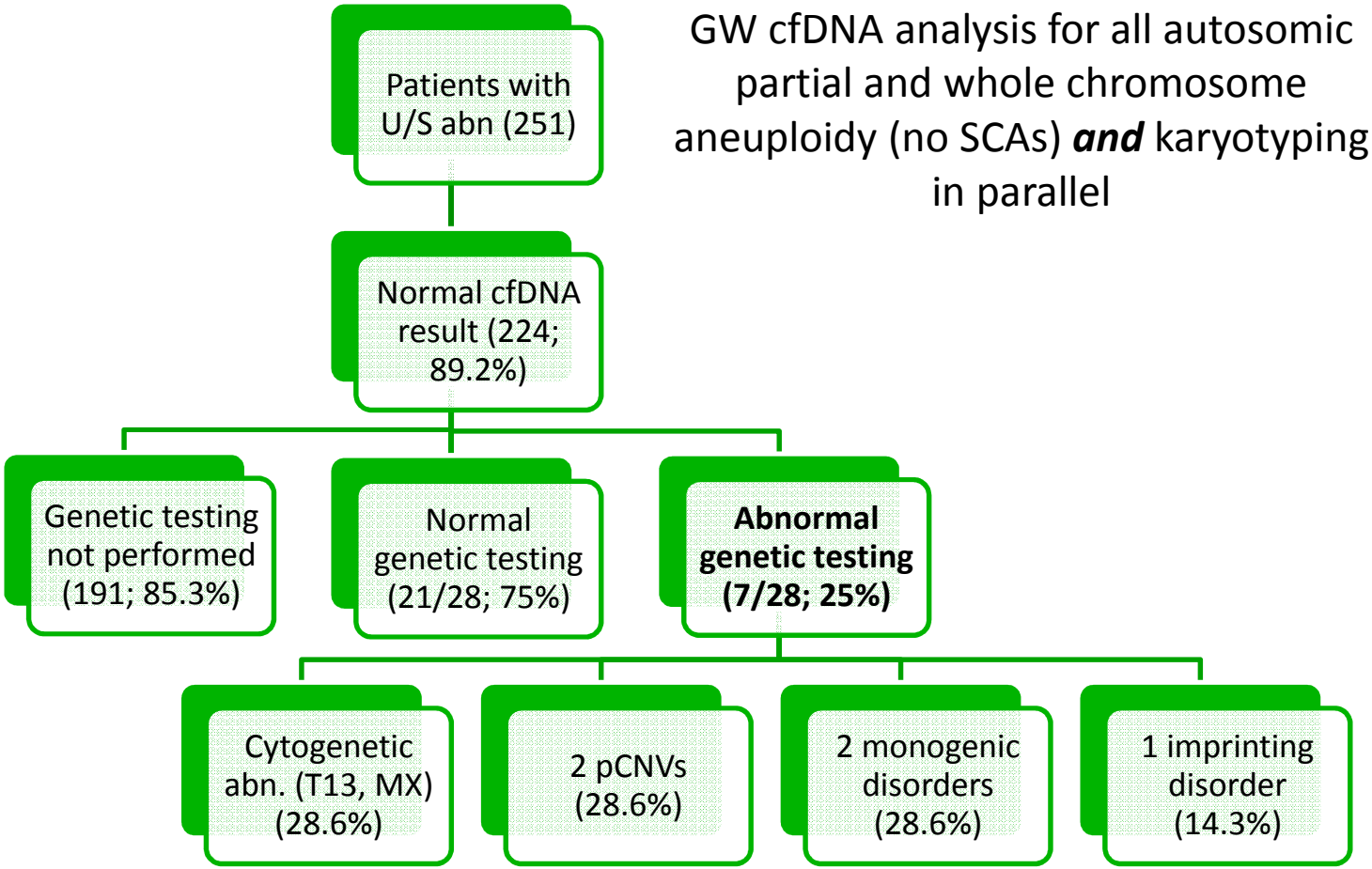
cfDNA TESTING IN FETAL ANOMALIES ON ULTRASOUND



GW cfDNA analysis for all autosomic partial and whole chromosome aneuploidy (no SCAs) ***and*** karyotyping in parallel

Beulen et al, Ultrasound Obstet Gynecol. 2016 Aug 12. doi: 10.1002/uog.17228

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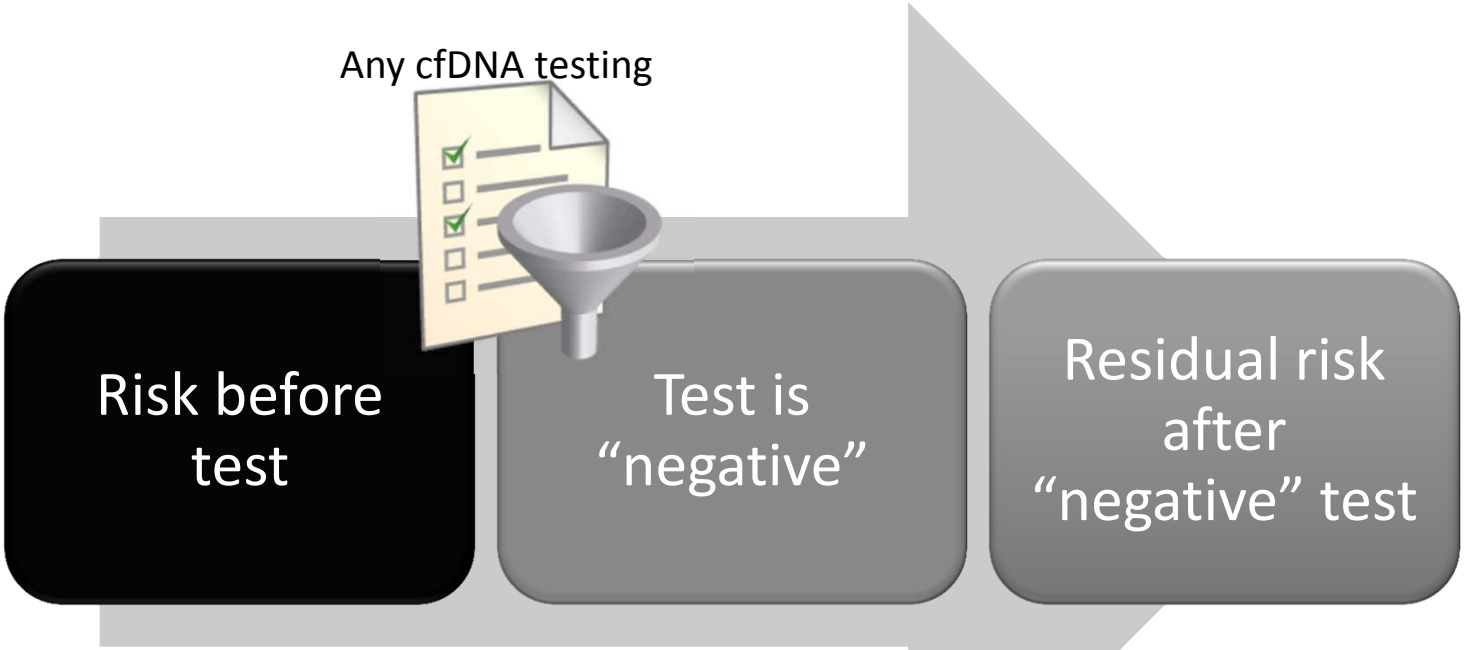
Residual Risk



Increased a priori risk for different types of genetic abnormalities

RR is dependent on:

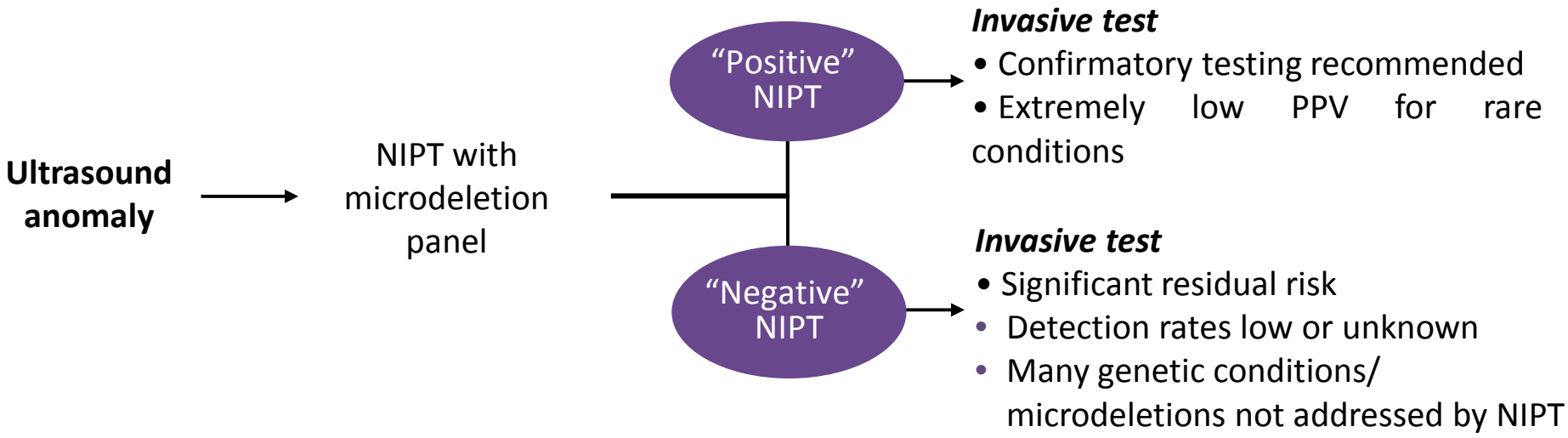
- detection rate of applied test
- genetic aetiology of the fetal malformation
  - 20-30% RR for cytogenetic imbalances
  - 6-9% pCNVs (submicroscopic)
  - 20-30% monogenic disorders
  - imprinting disorders

Courtesy: Thomas J Musci

Toma

Ultrasound abnormality: is there a role for NIPT?

Limited clinical utility in high risk cases

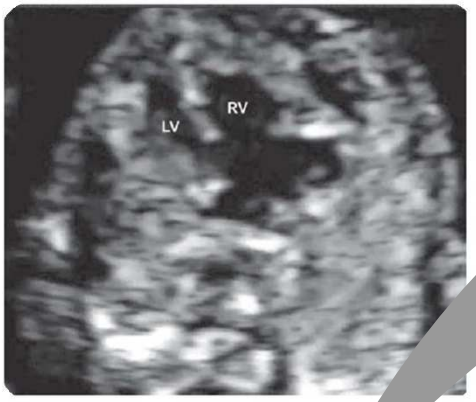


Potential for delayed diagnosis, additional cost, and anxiety for patients

Grati FR and Benn P, accepted reply letter to Fiorentino et al, Prenat Diagn. 2017 Jun;37(6):593-601; Courtesy: Thomas J Musci;

Toma

Ultrasound abnormality: is there a role for NIPT?



Differential Diagnosis

R/O chromosomal or genetic etiology

Patient counseling
Prognosis
Treatment options

Courtesy: Thomas J Musci

Toma

QUESTION

Which screening strategy?

COMPARISON OF DIFFERENT SCREENING STRATEGIES FOR THE DETECTION OF THE OVERALL FETAL CYTOGENETIC ABNORMALITIES AT BIRTH

Acronym	Screening stategy	First-tier test	Second-tier test	Third-tier test
FTS	Combined first trimester	Combined FTS	Karyotype if risk is ≥1/270	//

Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155; Grati et al, manuscript in preparation

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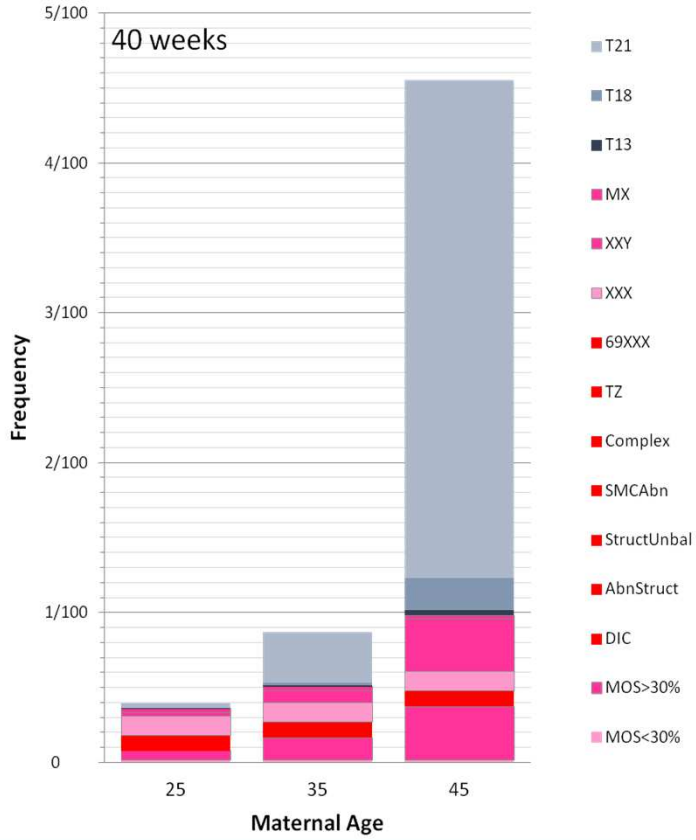
PROPORTION OF CHR DEFECTS DETECTED BY THE DIFFERENT TESTING STRATEGIES

- The distribution and prevalence of the chr abn are different at different MA
- Although two strategies may show approximately the same overall DR, one may favor the detection of a different subset of chr abn compared with another one



Grati et al, manuscript in preparation

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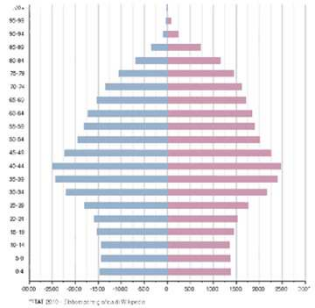
WHICH SCREENING STRATEGY?

Technological resources ***Budget*** ***Maternal age distribution and stratification*** ***Medical/scientific resources***

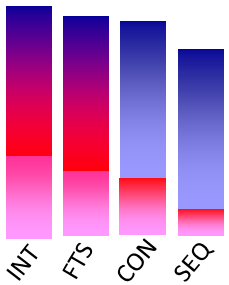





- ***Detection for large array of off-target chr abn***

At the cost of:

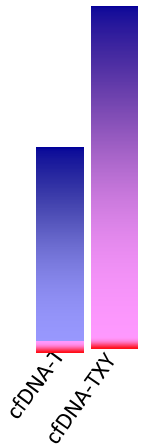
- a high residual risk for the targets of the test
- expenses for invasive procedures



- ***Low residual risk for the targets of the test***

At the cost of:

- a very low sensitivity for off-target chr abn
- expenses for cfDNA tests



Grati et al, manuscript in preparation

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False positive rate of screenings

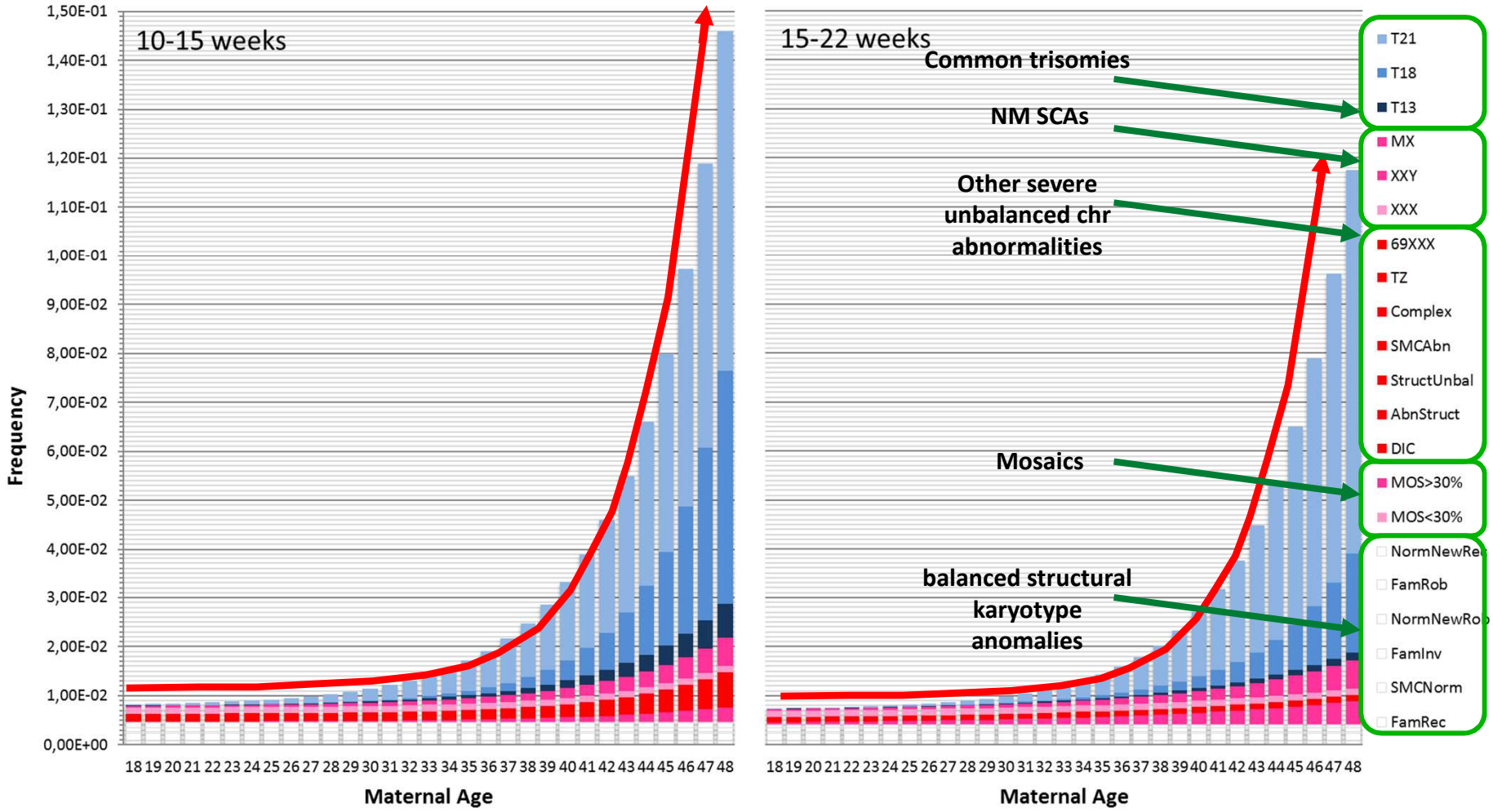
- Compared with traditional serum±ultrasound screening (TSS), cfDNA tests have a much lower FPR for T21,18,13
- The higher FPR of TSS was often considered a limitation
- Distinct advantage with TSS due to NT's ability to pick up additional chromosomal abnomalies ('off-target') in addition to the higher reflex invasive testing rate
- AIM: present detection rates of ***all*** (target and off-target) fetal karyotype abnormalities at birth by different screening strategies including cfDNA test and TSS

Grati FR et al, manuscript in preparation; Norton et al, Obstet Gynecol 2014;124:979–86; Syngelaki et al, Fetal Diagn Ther 2014;35:174–184

Toma

A PRIORI RISK OF A WOMAN TO CONCEIVE A CYTOGENETICALLY ABNORMAL FETUS

Stacked bar plot of the frequency of each chromosomal defect for each maternal age, and gestational age group.



Data on ~90,000 amniocand ~40,000 CVS,

Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155

Toma

PROPORTION OF THE CHROMOSOMAL DEFECTS OCCURRING AT BIRTH DETECTABLE BY DIFFERENT TESTING STRATEGIES

Methods

- ***Prior risks*** for each defect derived from TOMA lab Dataset of ≈130K prenatal dx on CVS (n=43K) and AF (n=87K) with an indication of AMA, anxiety and elective decision (reported by clinicians)
- ***Fetal loss rate*** at birth for T13,18,21°
- ***Sensitivities and specificities*** for common aneuploidies and triploidy abstracted from the published literature:
  - ***Serum screenings*** for T21,18,13, MX, triploids: from prior seminal studies* (5% cumulative FPR)
  - ***cfDNA testing:*** 0.13% cumulative FPR for T21,18,13; 0.273% cumulative FPR for T21,18,13+SCAs^
- ***Sensitivity*** for other karyotype abnormalities correspond to the FPR of TSS or cfDNA tests
- ***No result rate*** with cfDNA testing of 1%: the DR for all chr abnormalities was adjusted downward as a 1% of 'no result' cases by cfDNA are actually undetected karyotype abnormalities

*Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155; *Cuckle et al, Clin Biochem. 2015;48(15):932-41; ^ Gil et al, Ultrasound Obstet Gynecol. 2017 Apr 11. doi: 10.1002/uog.17484; *Nicolaides K, Prenat Diagn 2011; 31: 7–15; *Sonek and Cuckle Ultrasound Obstet Gynecol 2014; 44: 621–630; °Nicolaides K, American Journal of Obstetrics and Gynecology (2004) 191, 45e67*

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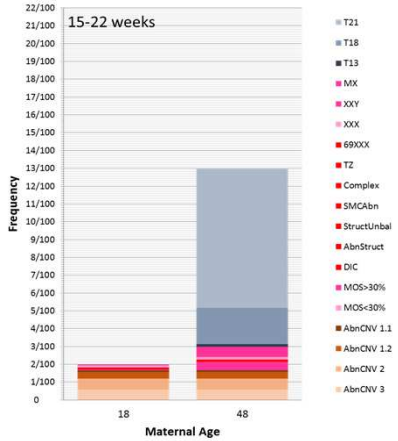
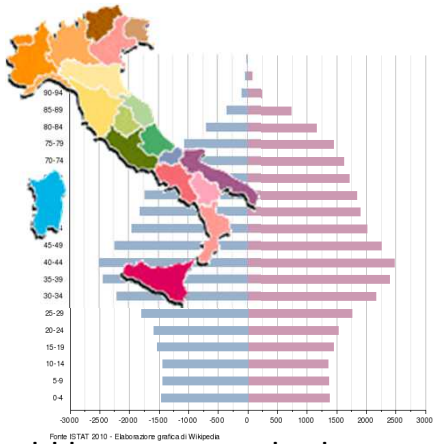
THE ROLE OF ‘MATERNAL AGE’ TODAY



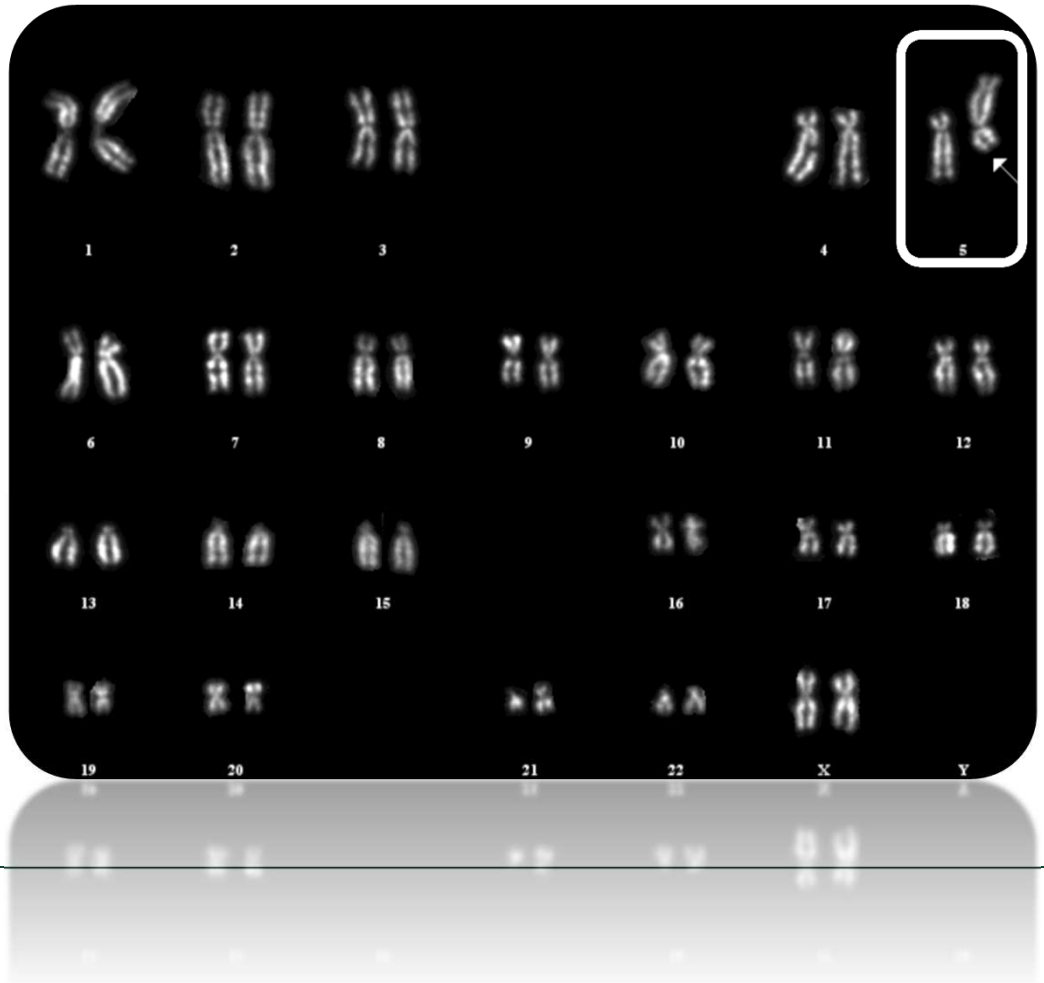


Maternal age should have a central role to rationalize resources to obtain the most efficient cost-benefit

Toma

Fetal chromosome abnormalities in pregnancies with U/S abnormalities

Encephalocoele→ 46,XX,rec(5)dup(5q)inv(5)(p15.2q32)



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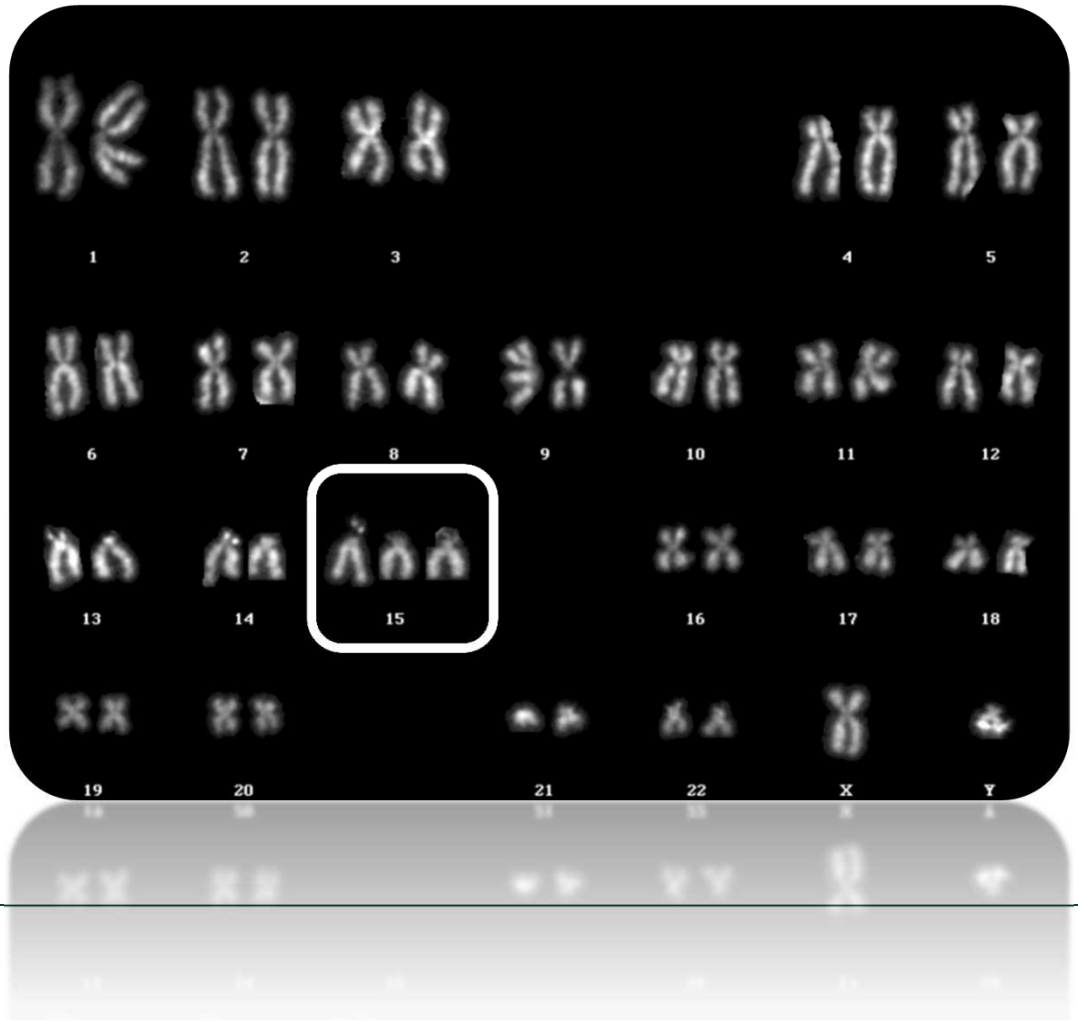
Fetal chromosome abnormalities in pregnancies with U/S abnormalities

Ventriculomegaly→ 47,XY,+15



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Fetal chromosome abnormalities in pregnancies with U/S abnormalities

Hydrocephaly→69,XXY

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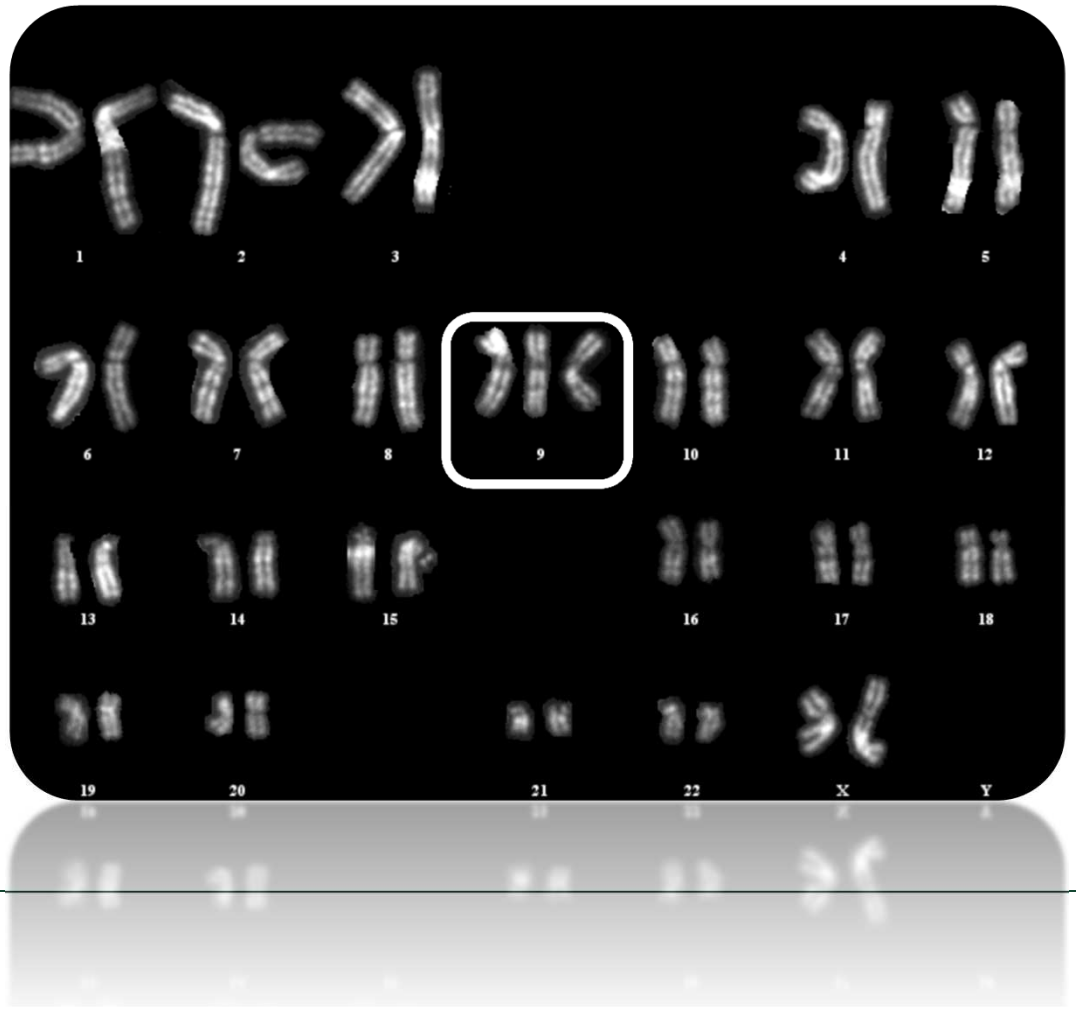
Fetal chromosome abnormalities in pregnancies with U/S abnormalities

Anencephaly→ 47,XX,+9



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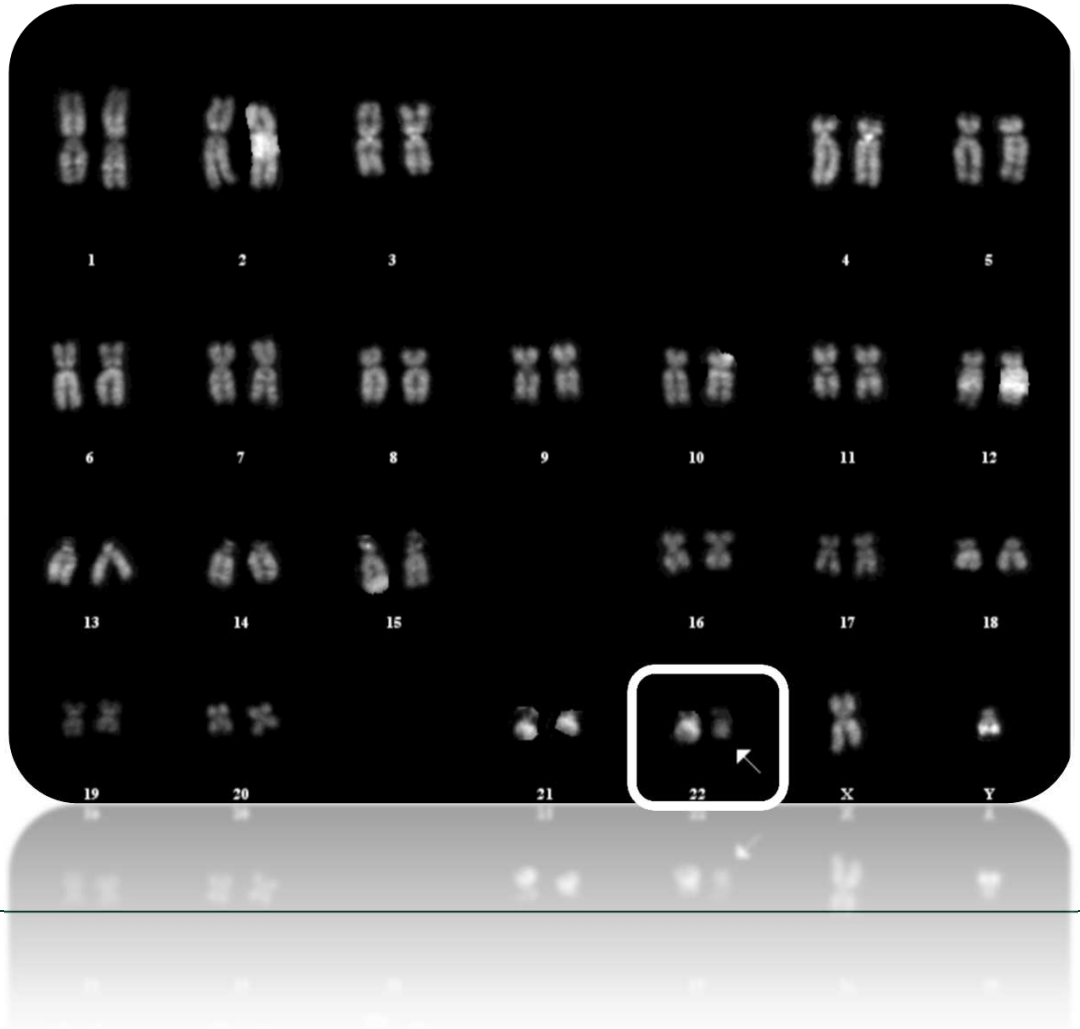
Fetal chromosome abnormalities in pregnancies with U/S abnormalities

Polymalformed fetus (ndd)→ 46,XY,r(22)(p11.2q13.3)



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INCREMENTAL YIELD BY MICROARRAY WITH NORMAL FETAL KARYOTYPE

Fetuses with CHD ± extracardiac defects (systematic meta-analysis)

- Pooled analysis: 7.0% (95% CI, 5.3–8.6%) incremental yield by CMA (excluding 22q11 microdeletion cases);
- **Incremental yield increases to 12%** (95% CI, 7.6–16%) when 22q11 deletion cases were included
- Stratified analysis: incremental yield
  - 3.4% (95%CI 0.3–6.6%) for isolated CHD
  - 9.3% (95%CI, 6.6–12%) when additional extracardiac malformations were present

Jansen et al, UOG 2015

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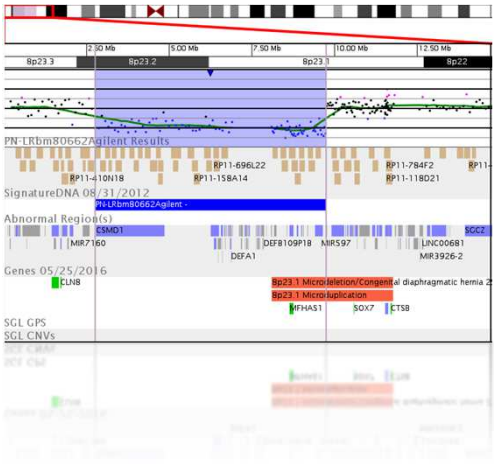
ROLE OF CMA IN ANATOMICALLY ABNORMAL FETUSES AND NORMAL KARYOTYPE



• 1 in every 20 anatomically abnormal fetuses with a normal karyotype shows a submicroscopic CNV that explains its phenotype and provides prognostic information

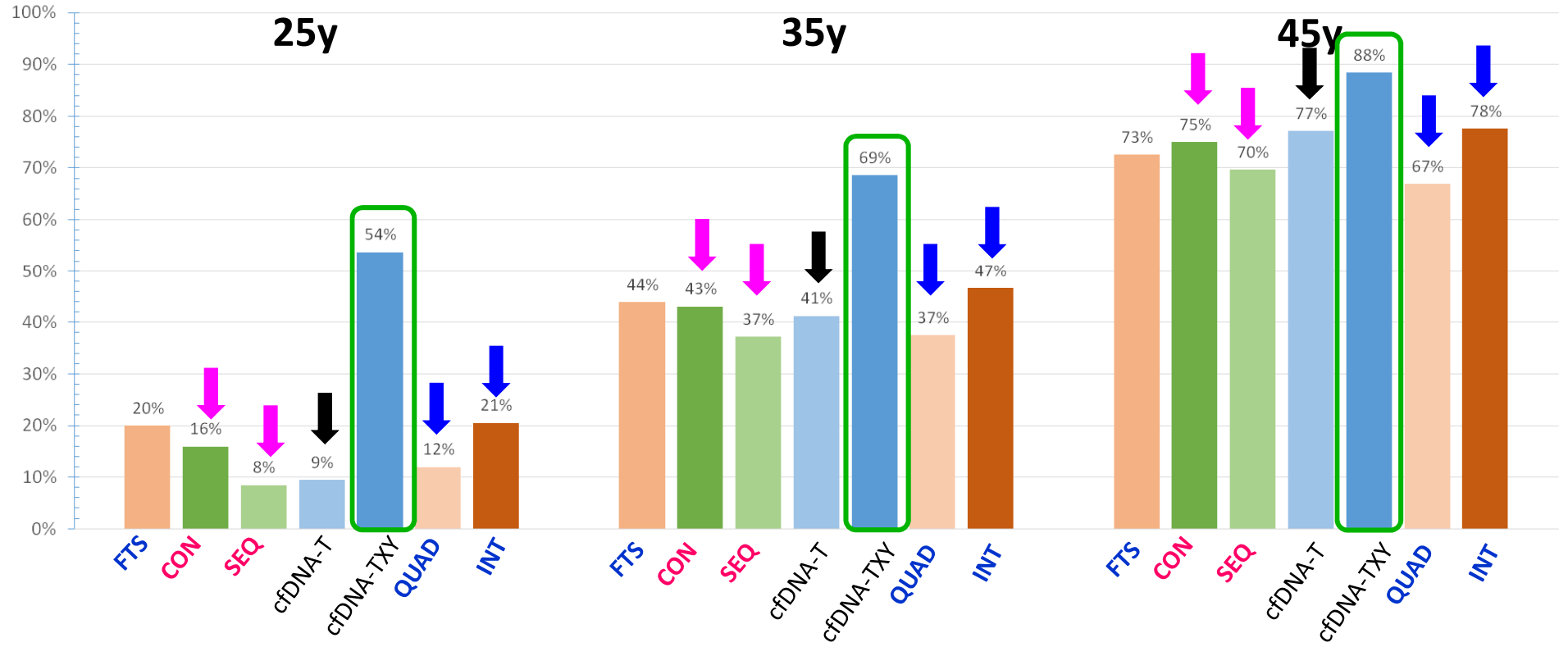
• Professional societies recommend prenatal invasive diagnosis with CMA as first-tier test on AF/CVS in CHD^

- Many different submicroscopic and monogenic causes for CHD
- Association between CHD and neurodevelopmental delay

De Wit et al., UOG 2014; ^ISUOG consensus statement, Ultrasound Obstet Gynecol. 2016 Nov 27. doi: 10.1002/uog.17324

Toma

PROPORTION OF THE CHROMOSOMAL DEFECTS OCCURRING AT BIRTH DETECTABLE BY DIFFERENT TESTING STRATEGIES



- ***cfDNA-TXY*** has the highest DR at all MA;
- ***SEQ*** has the lowest DR, approximating the ***QUAD*** only at older MA: with ***SEQ*** the second-tier cfDNA-T drops down by 40-folds (from 5% to 0.13%) the FPR of the strategy, thereby reducing the likelihood of finding other off-target chr abn
- ***CON*** is always better than ***SEQ*** thanks to the larger population performing follow up karyotyping
- Among TSS, ***INT*** has the highest DR, at the cost of a late GA reporting
- ***cfDNA-T*** equals or is better than ***CON*** or ***FTS*** only at older MA, when trisomies dominate the risk

Ferreira, Grati FR et al, Prenat Diagn. 2016 Dec;36(12):1146-1155; Grati et al, manuscript in preparation

Toma