KATHOLIEKE UNIVERSITEIT
LEUVEN

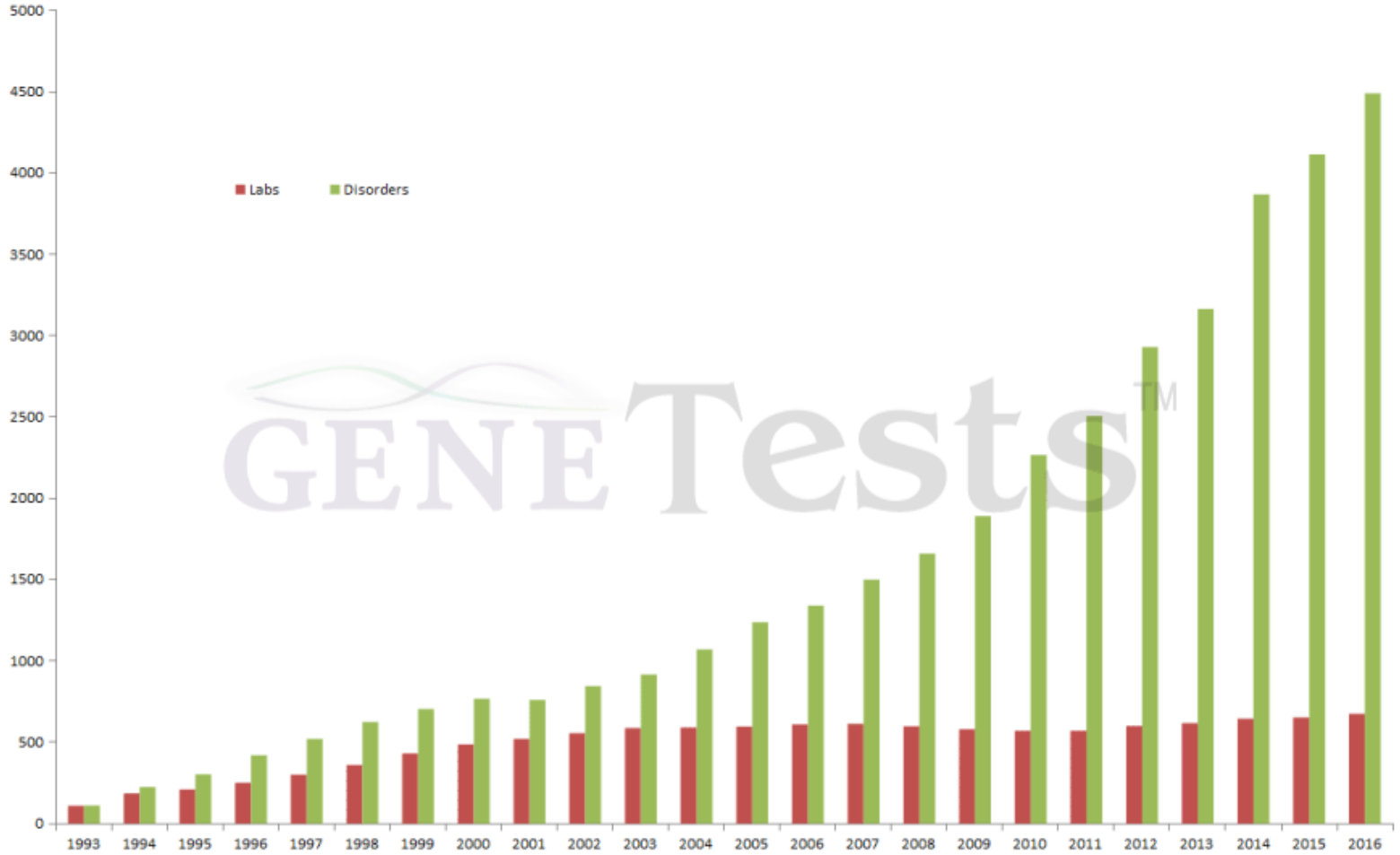
Genomic application in preimplantation and prenatal diagnosis

Joris Vermeesch

September 21, 2018

Joint meeting of International Federation of Fertiity Societies & Baltic fertility Society

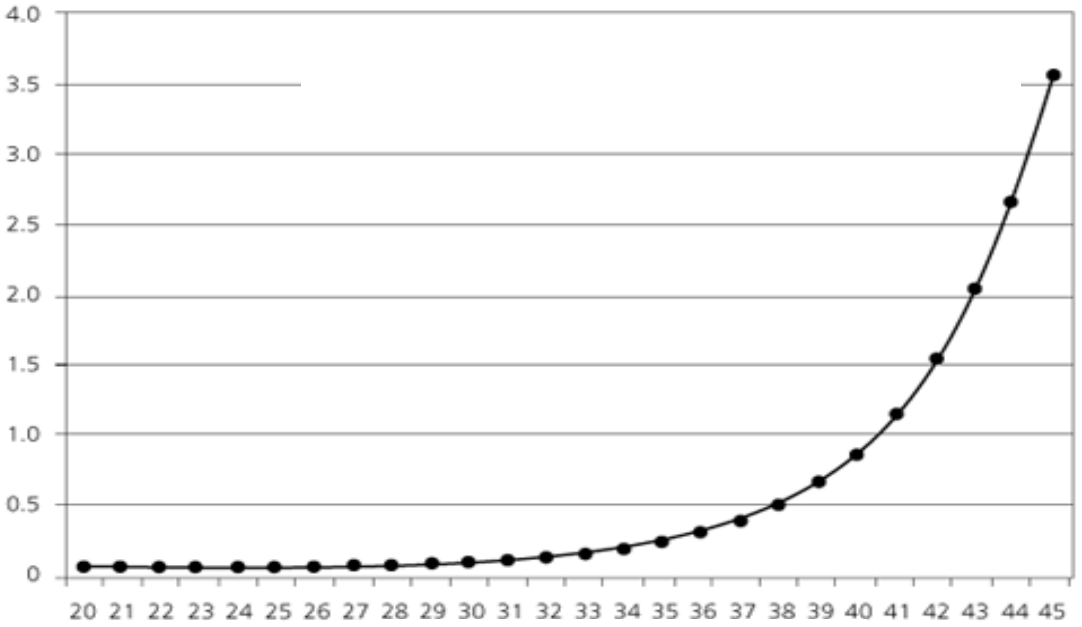
Ever more genetic disorders



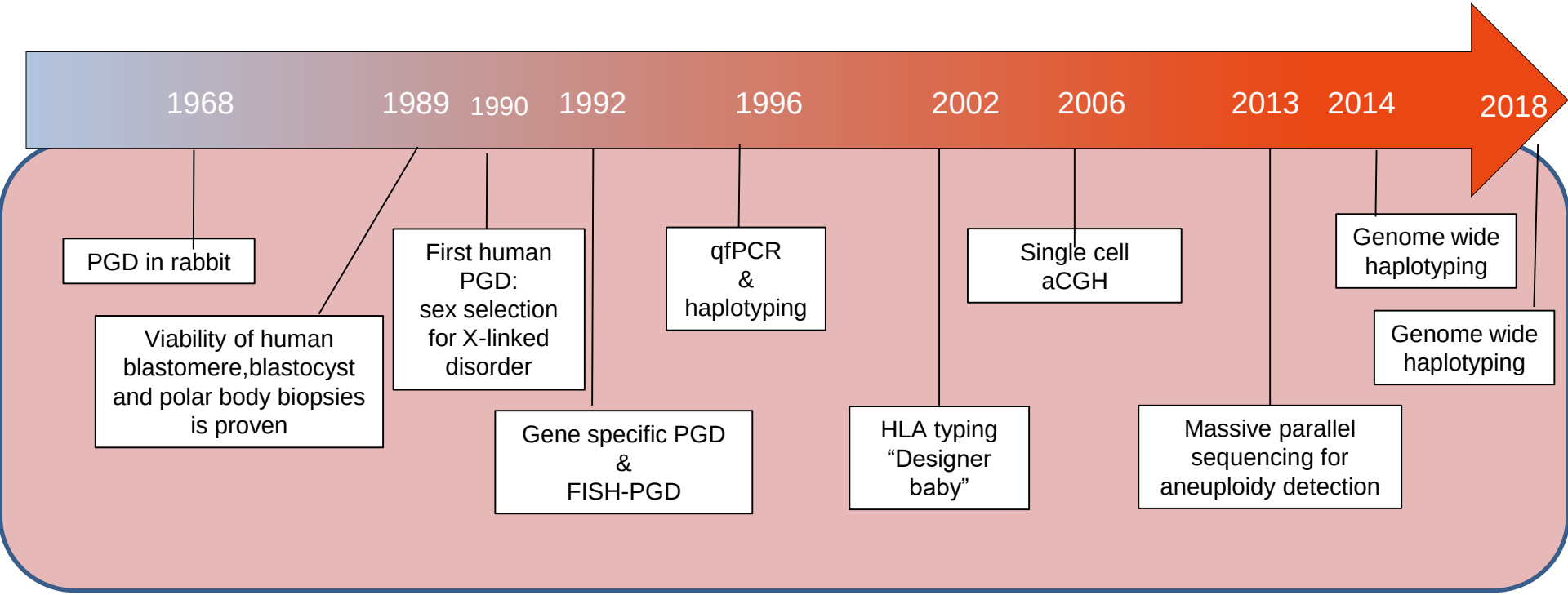
Risk for trisomy increases with maternal age

Trisomy 21
Down Syndrome

1/700 births



Key events in preimplantation genetic testing

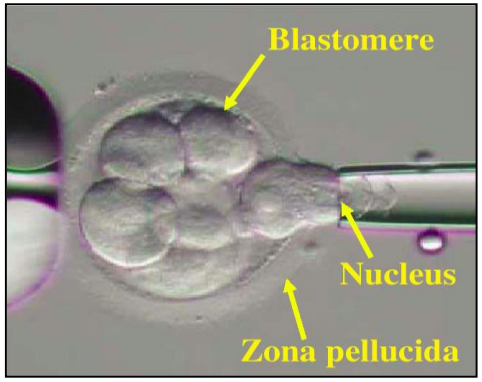


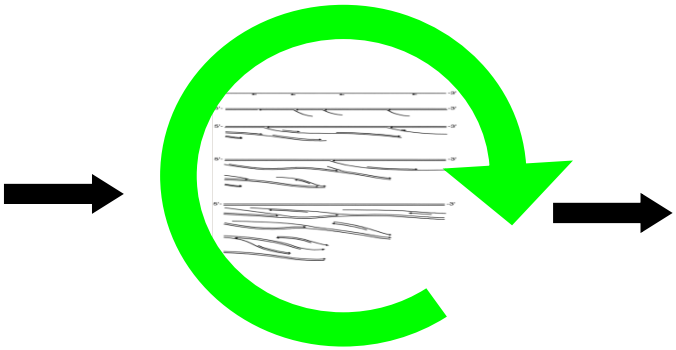
Vermeesch et al., 2016, Nature Review Genetics.

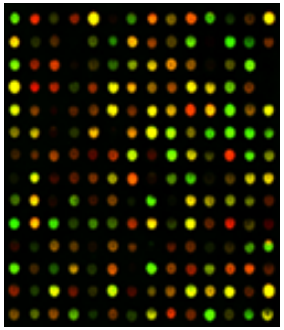
CAN WE DEVELOP A GENOME WIDE ANEUPLOIDY DETECTION METHOD FOR SINGLE CELLS?

AIM:GENERIC METHOD FOR PREIMPLANTATION GENETIC DIAGNOSIS?

Genome wide CNV profiling by arrays

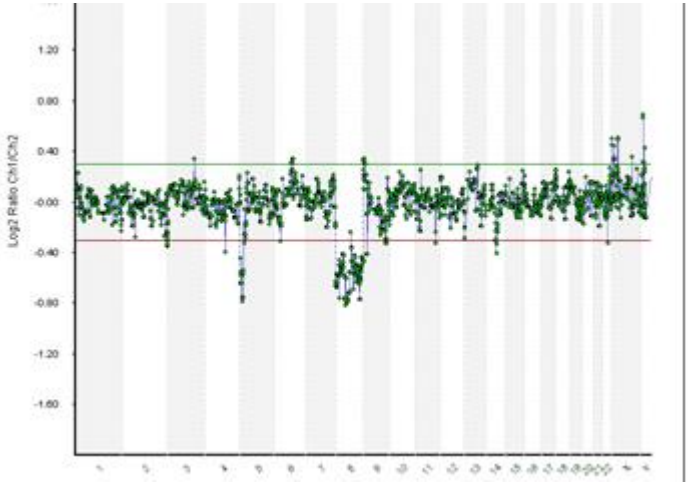






Genomi Phi
2.5 µg DNA

Single cell array CGH is generic method for genome wide aneuploidy detection

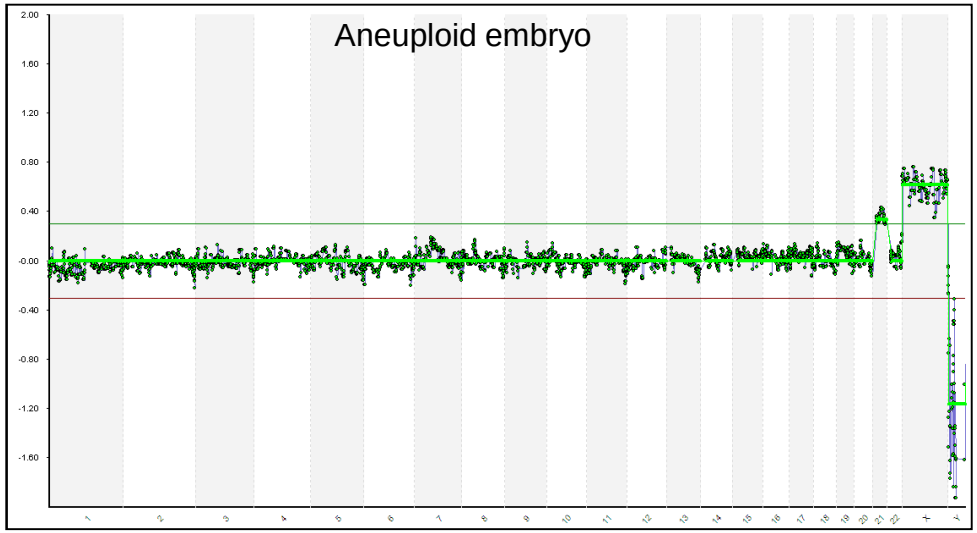


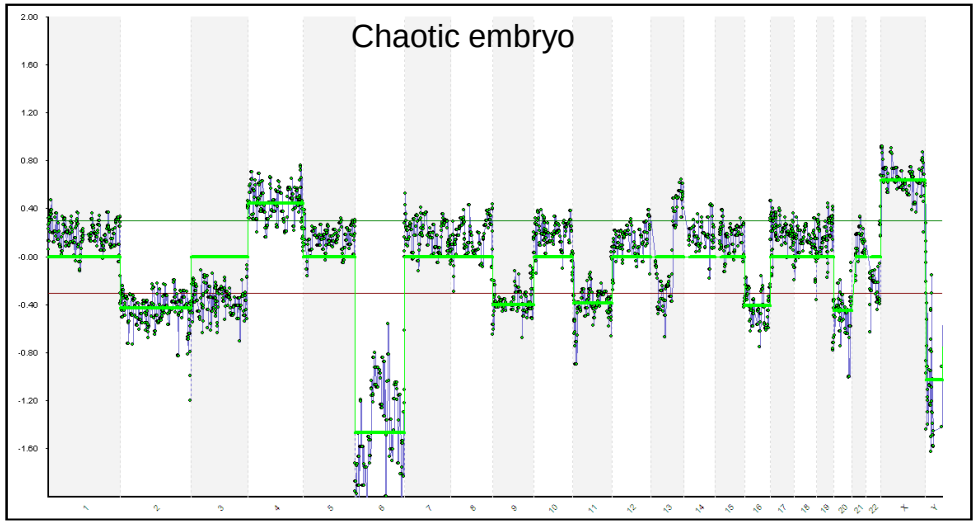
Nucleic Acids Research, 2006, Vol. 34, No. 9 **e68**
doi:10.1093/nar/gkl336

Single-cell chromosomal imbalances detection by array CGH

Cedric Le Caignec[1,2], Claudia Spits[2], Karen Sermon[2], Martine De Rycke[2], Bernard Thienpont[1], Sophie Debrock[4], Catherine Staessen[2], Yves Moreau[3], Jean-Pierre Fryns[1], Andre Van Steirteghem[2], Inge Liebaers[2] and Joris R. Vermeesch[1,*]

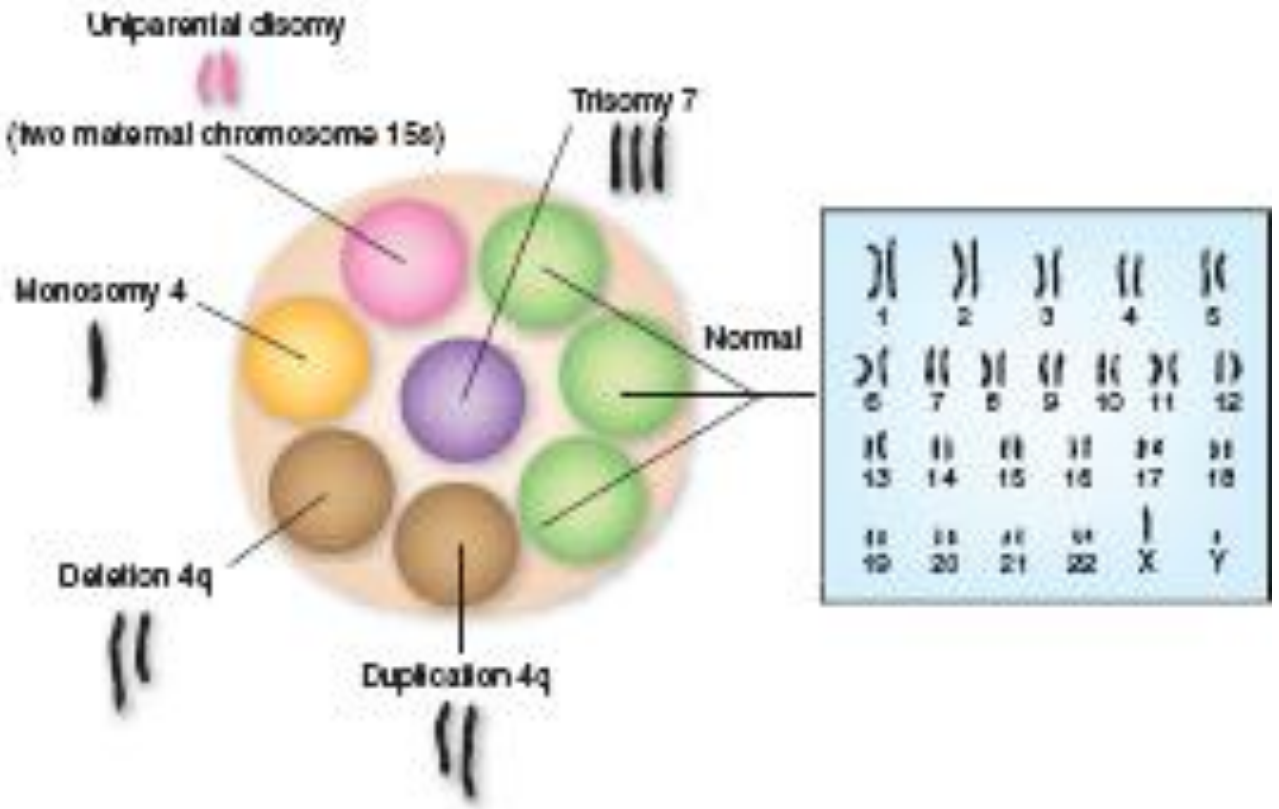
Great for preimplantation genetic screening





Cleavage stage embryos are chromosomally unstable

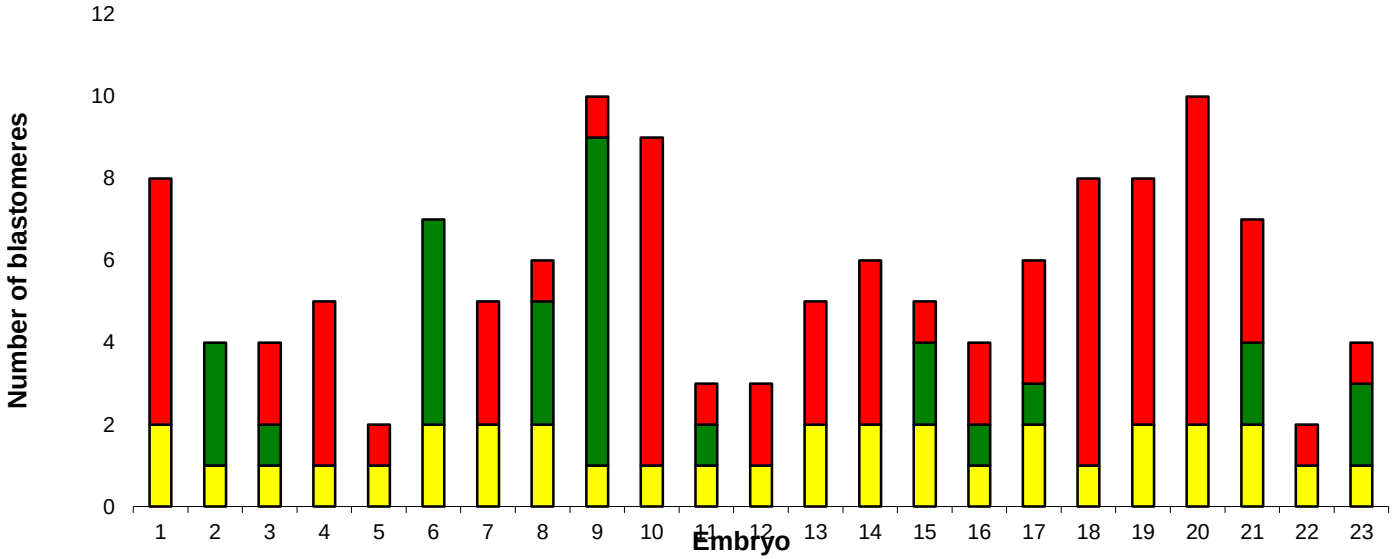
The embryo is chromosomally mosaic



Vanneste et al., Nature Medicine, 2009

Embryo’s are chromosomally unstable

- 2/23 (9%) : normal diploid in all cells
- 1/23 (4%) : diploid, but UPID
- 8/23 (35%) : mosaic diploid/aneuploid (4 embryos : ratio diploid/aneuploid > 1
- 12/23 (52%) : mosaic aneuploid (3 embryos : meiotic (same aberration in all cells)



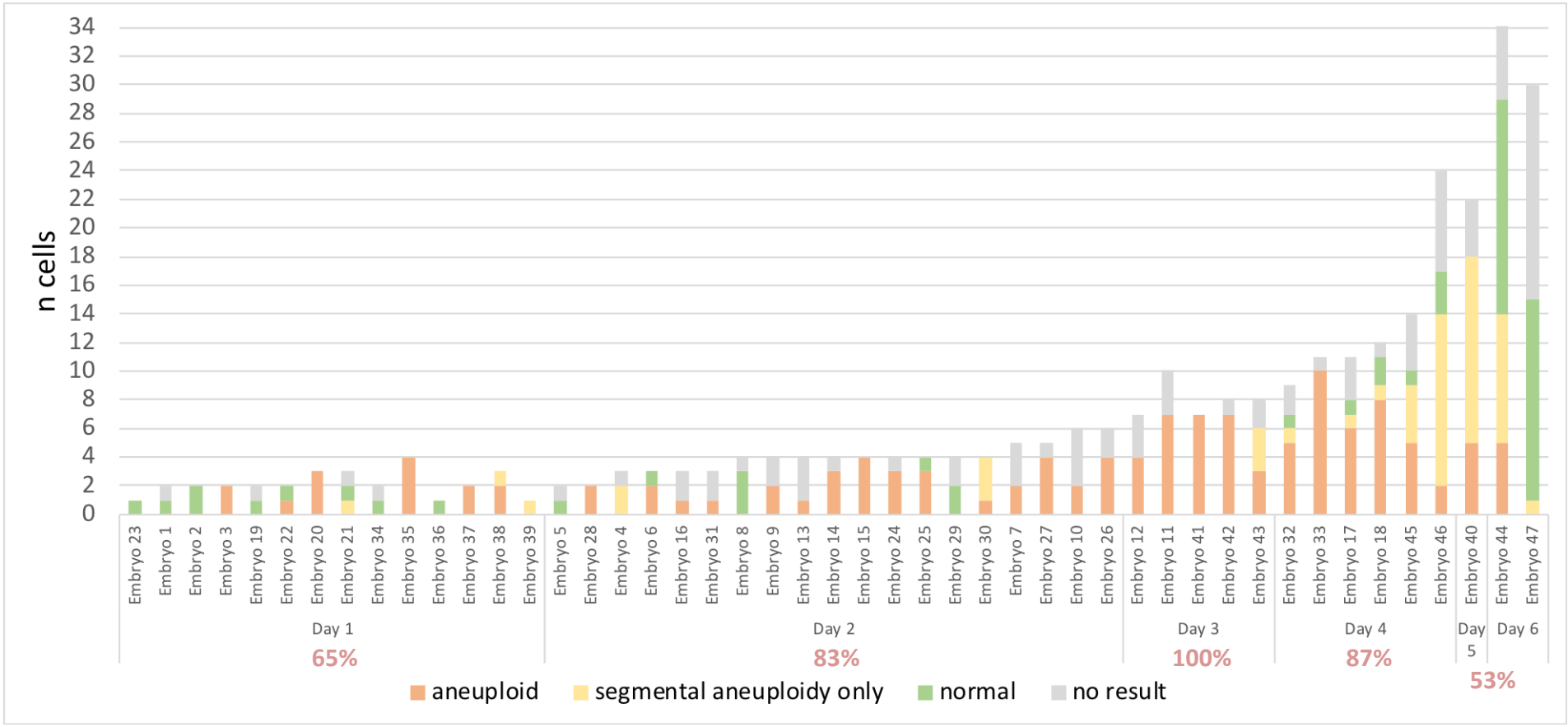
FISH **array : normal diploid** **array : abnormal**

Samples analysed

	Day 1	Day 2	Day 3	Day 4	Day 5/6	Total
Embryo stage						
N embryos	**14**	**19**	**5**	**6**	**3**	**47**
N cells sequenced	30	74	40	81	90	315
N cells passed DNA seq QC	**26**	**48**	**31**	**63**	**62**	**230**

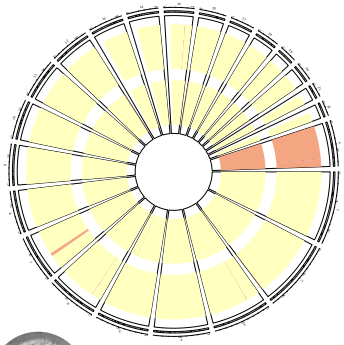
Fernandez et al., unpublished

Genomic composition of 47 embryos at single cell resolution

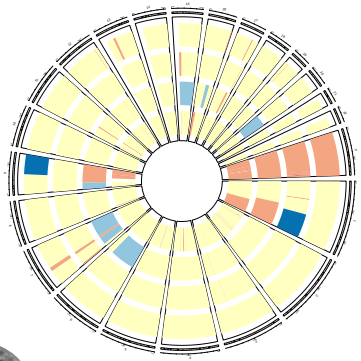


Fernandez et al., unpublished

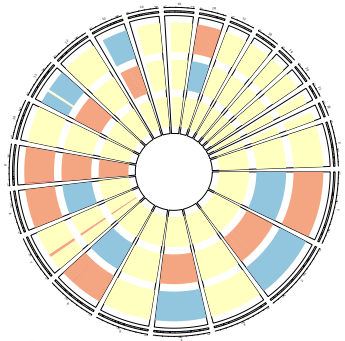
CNV profiles observed



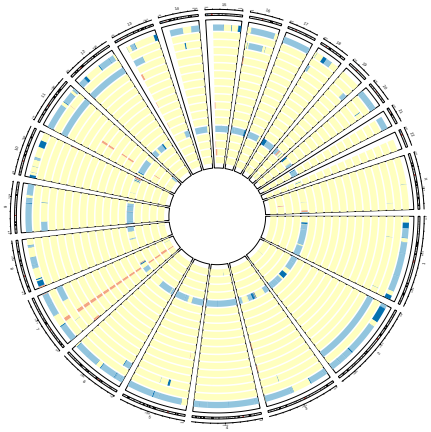
Normal diploid
Embryo 2 (2c Day 1)



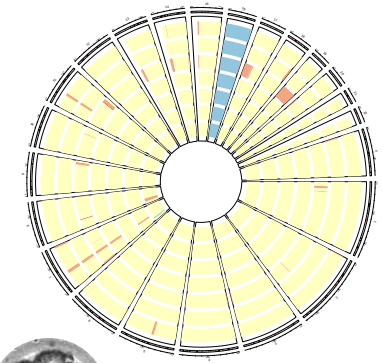
(Reciprocal) segmental aneupl.
Embryo 30 (4c Day 2)

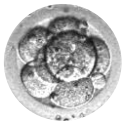


Reciprocal whole chromosome aneupl.
Embryo 20 (3c Day 1)

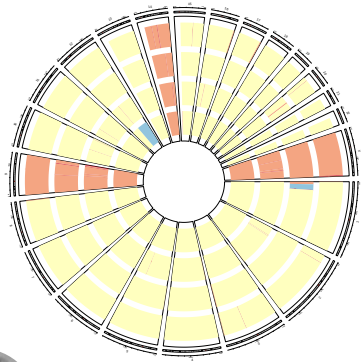


Genome-wide duplications
Embryo 47 (Day 6)

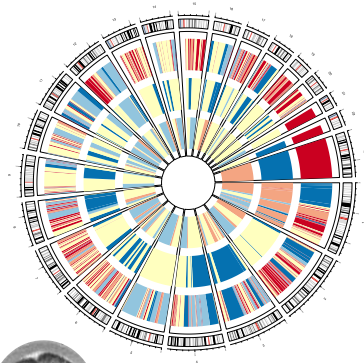




Meiotic trisomy
Embryo 41 (7c Day 3)



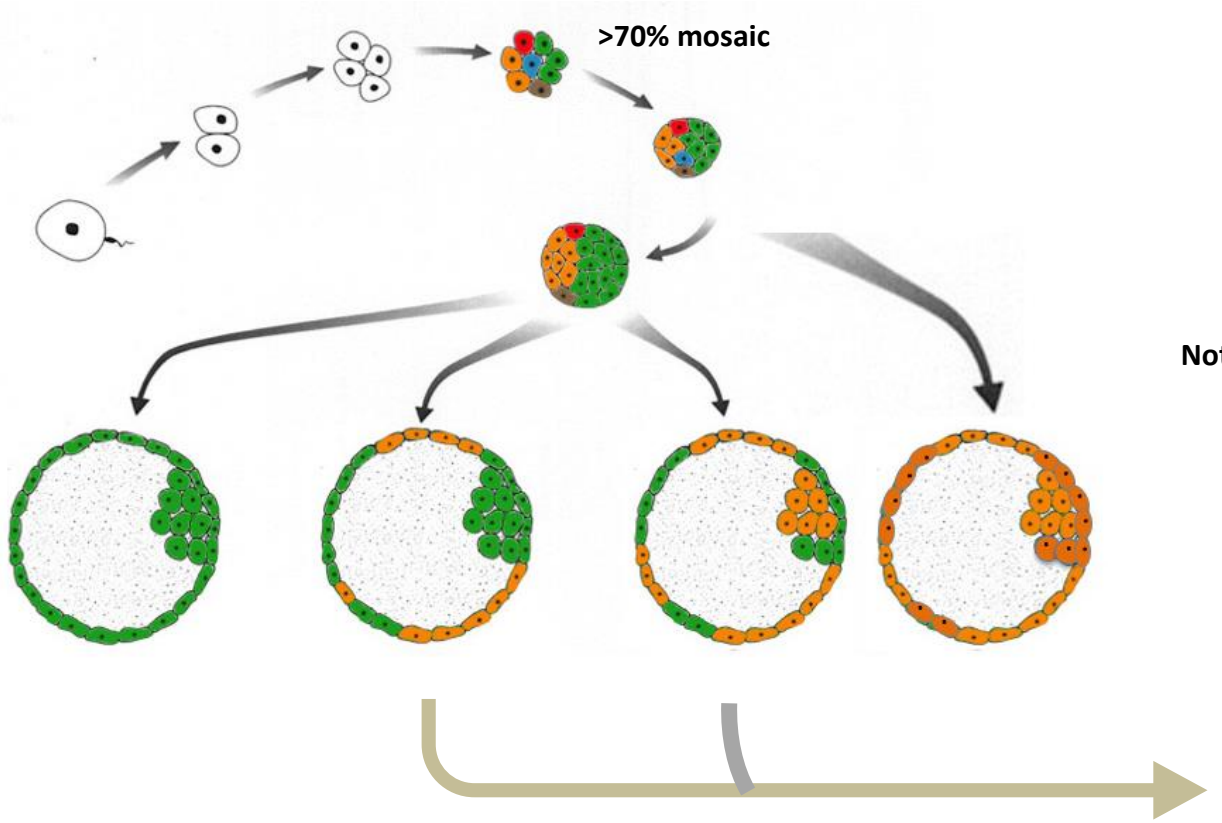
Meiotic monosomies
Embryo 12 (7c Day 3)



Chaotic CNV
Embryo 14 (4c Day 2)

Fernandez et al., unpublished

Survival of the fittest (cel)?
(or active response?)



Normal cell

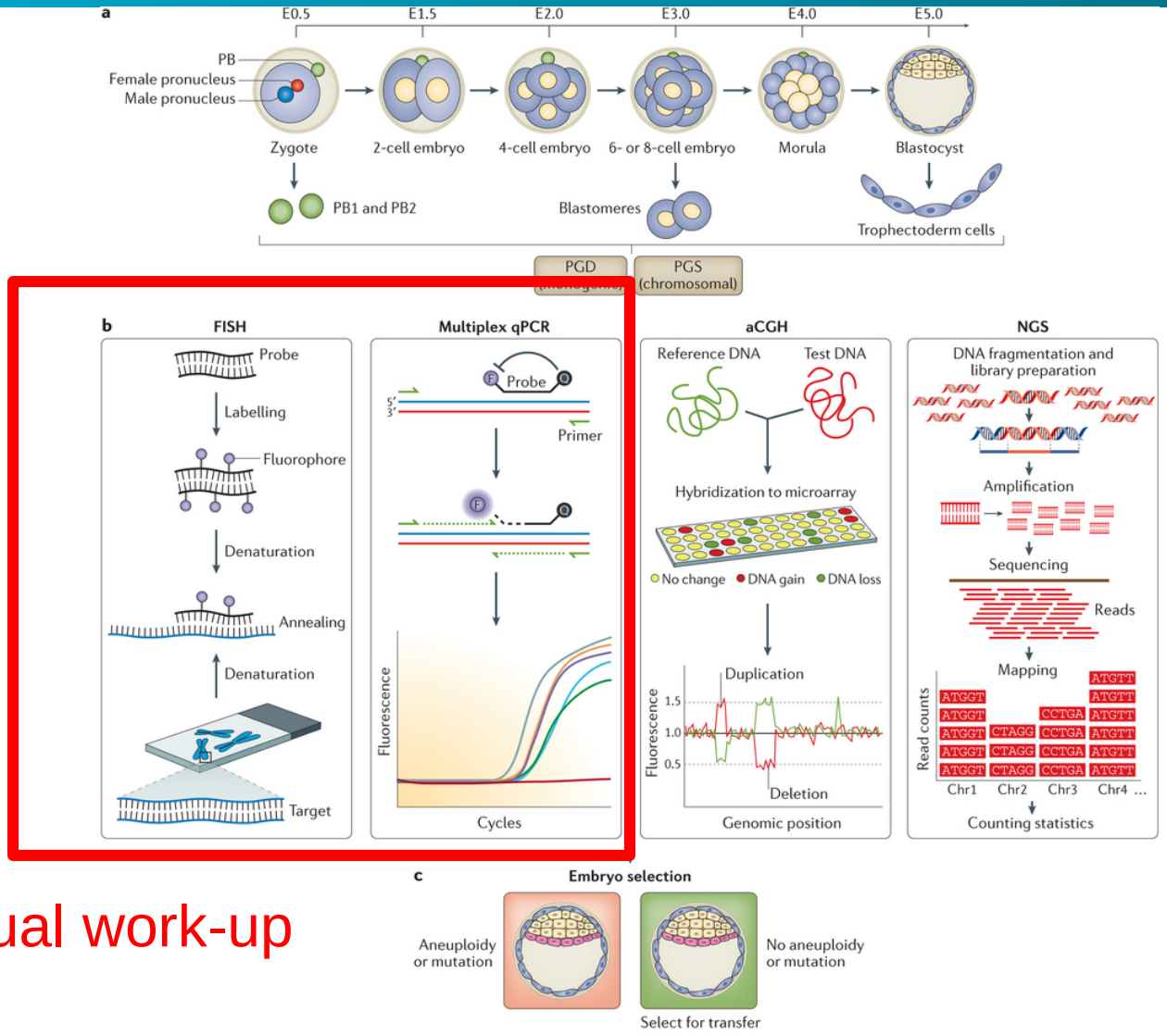
Aneuploid cell
Persistent until blastocyst stage

Aneuploid cells
Not persistent until blastocyst stage

Greco et al., 2015 N Eng J Med

CAN WE DEVELOP GENERIC METHOD FOR PREIMPLANTATION GENETIC DIAGNOSIS?

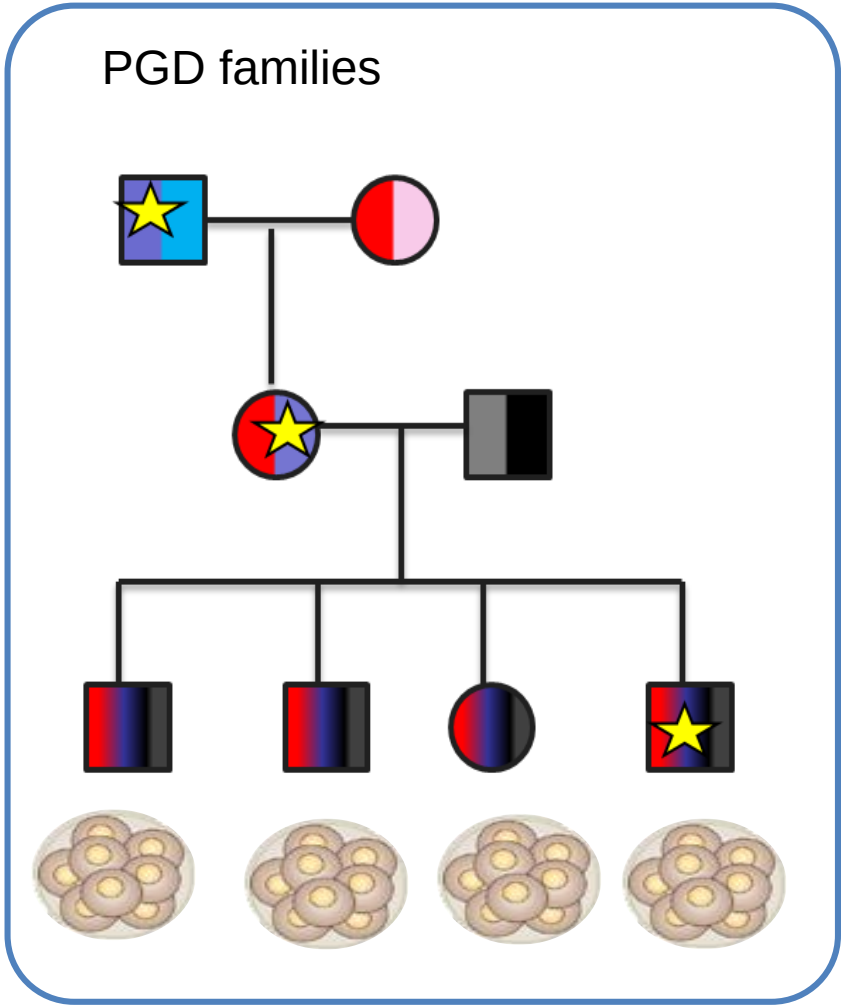
Preimplantatie genetische for single gene disorders



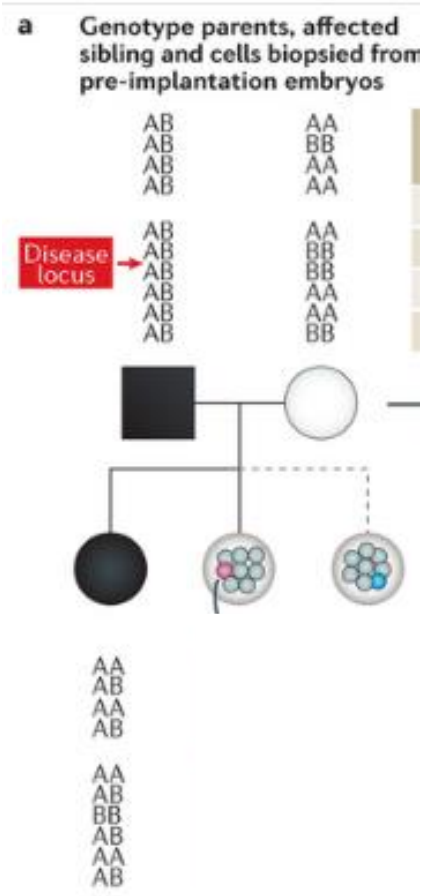
Individual work-up

Vermeesch JR., Voet T., Devriendt K. Nat Rev Genet. 2016.

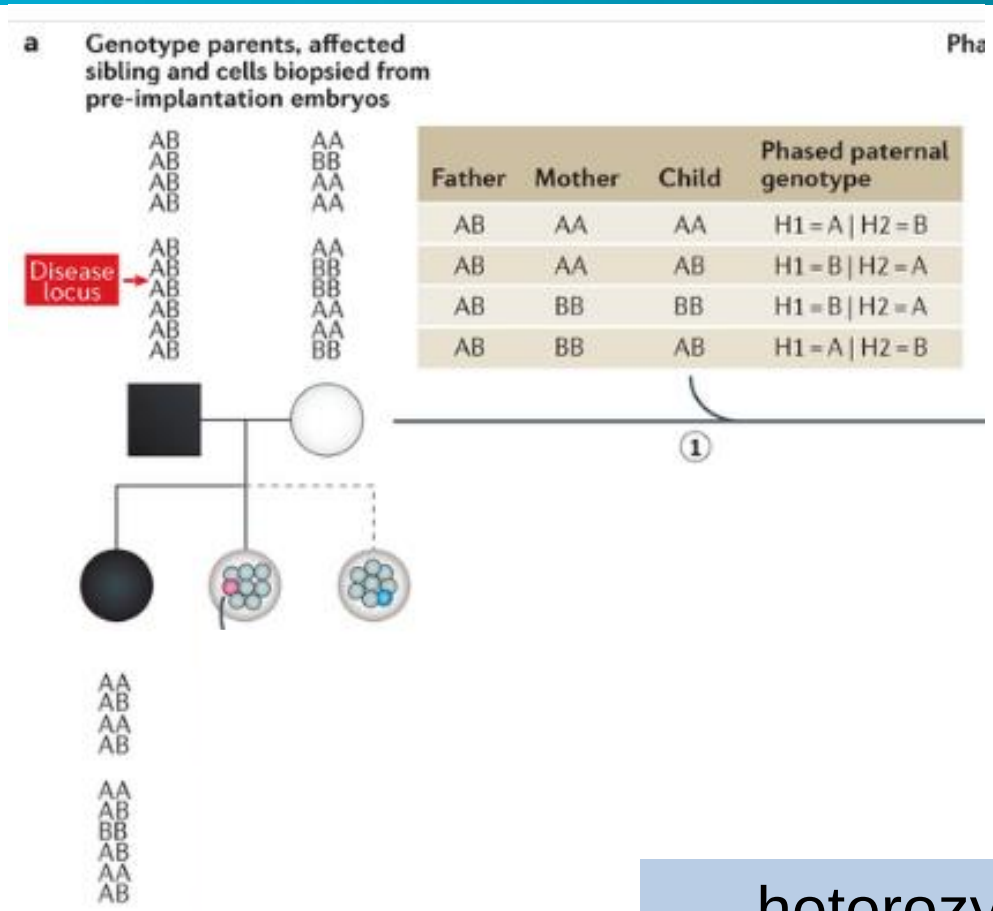
Avoid transmission of pathogenic genetic variants



Genotype parents and affected sib

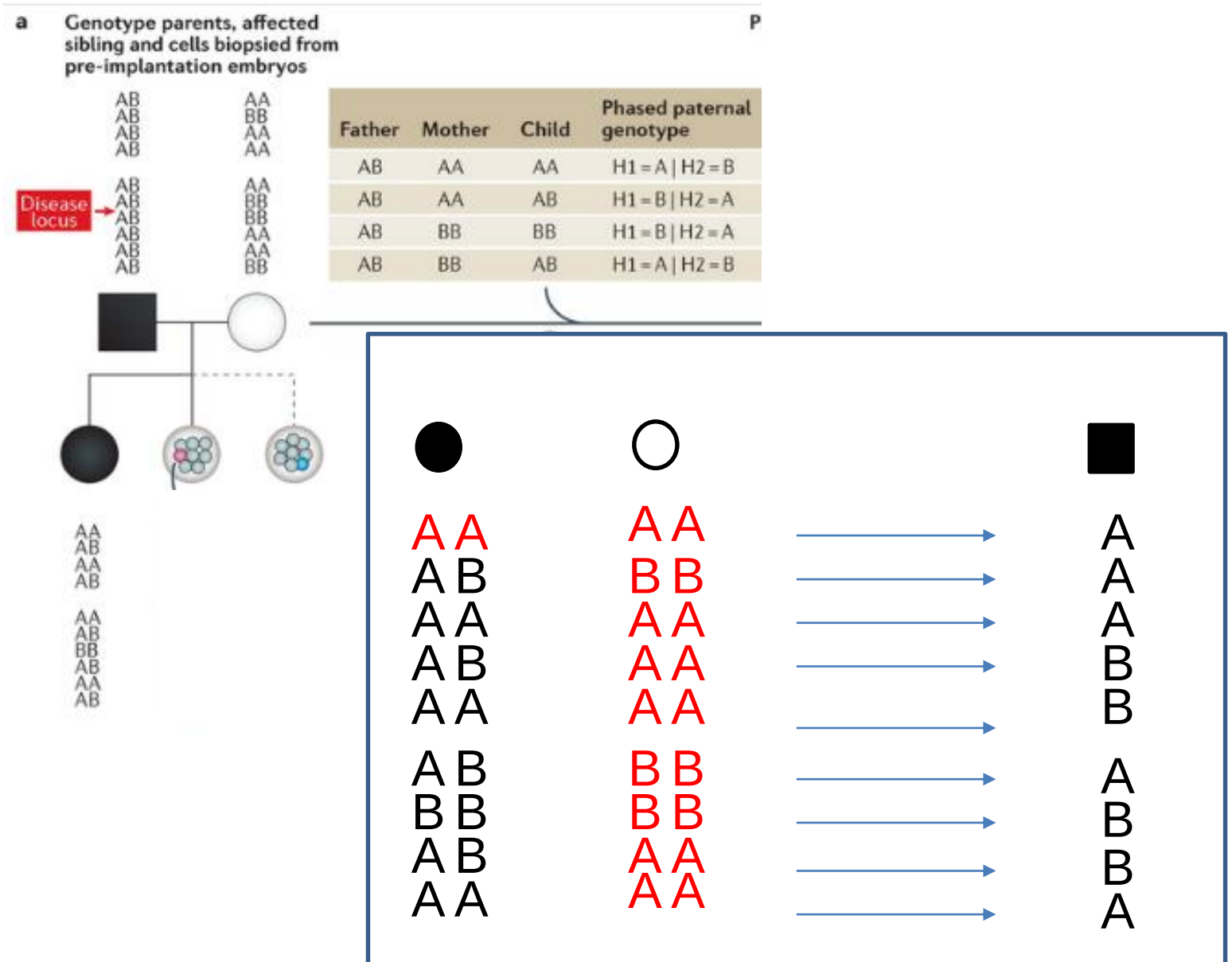


Genotype parents and affected sib

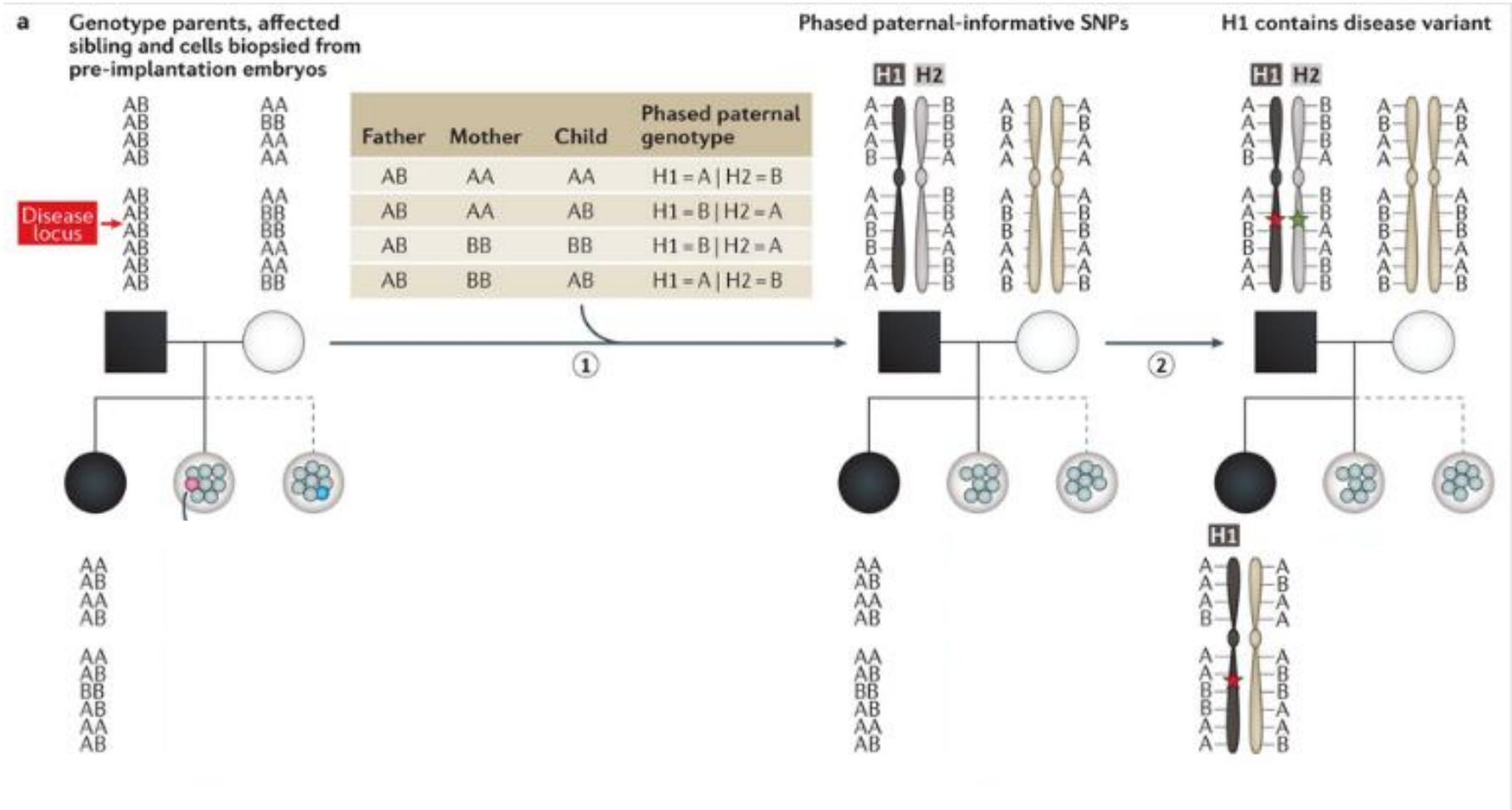


heterozygous in one parent and homozygous in the other parent

From genotype to haplotype



Genome-wide haplotyping as a generic method for PGD (karyomapping)



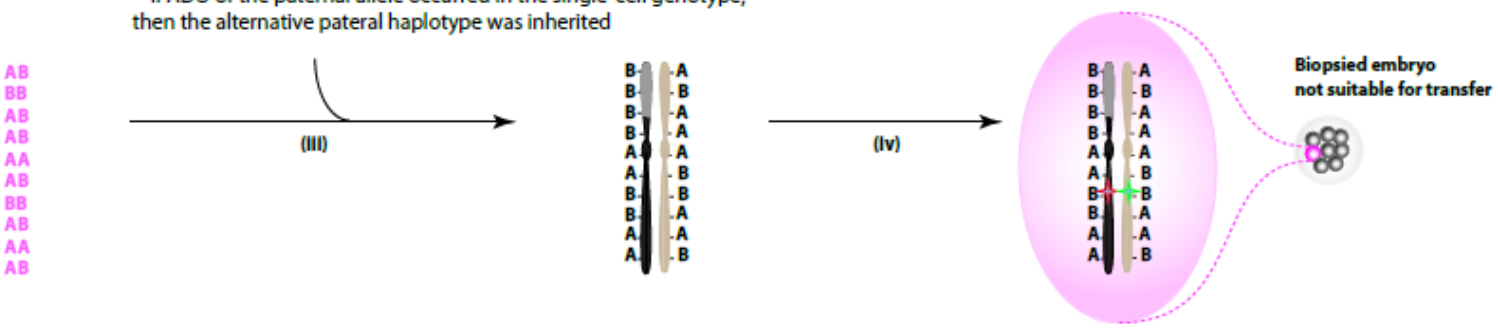
Zamani Esteki M., et al.. Am J Hum Genet 2015; 96(6):894-912
Vermeesch et al., 2016, Nature Review Genetics.

DISCRETE GENOTYPING

KARYOMAPPING

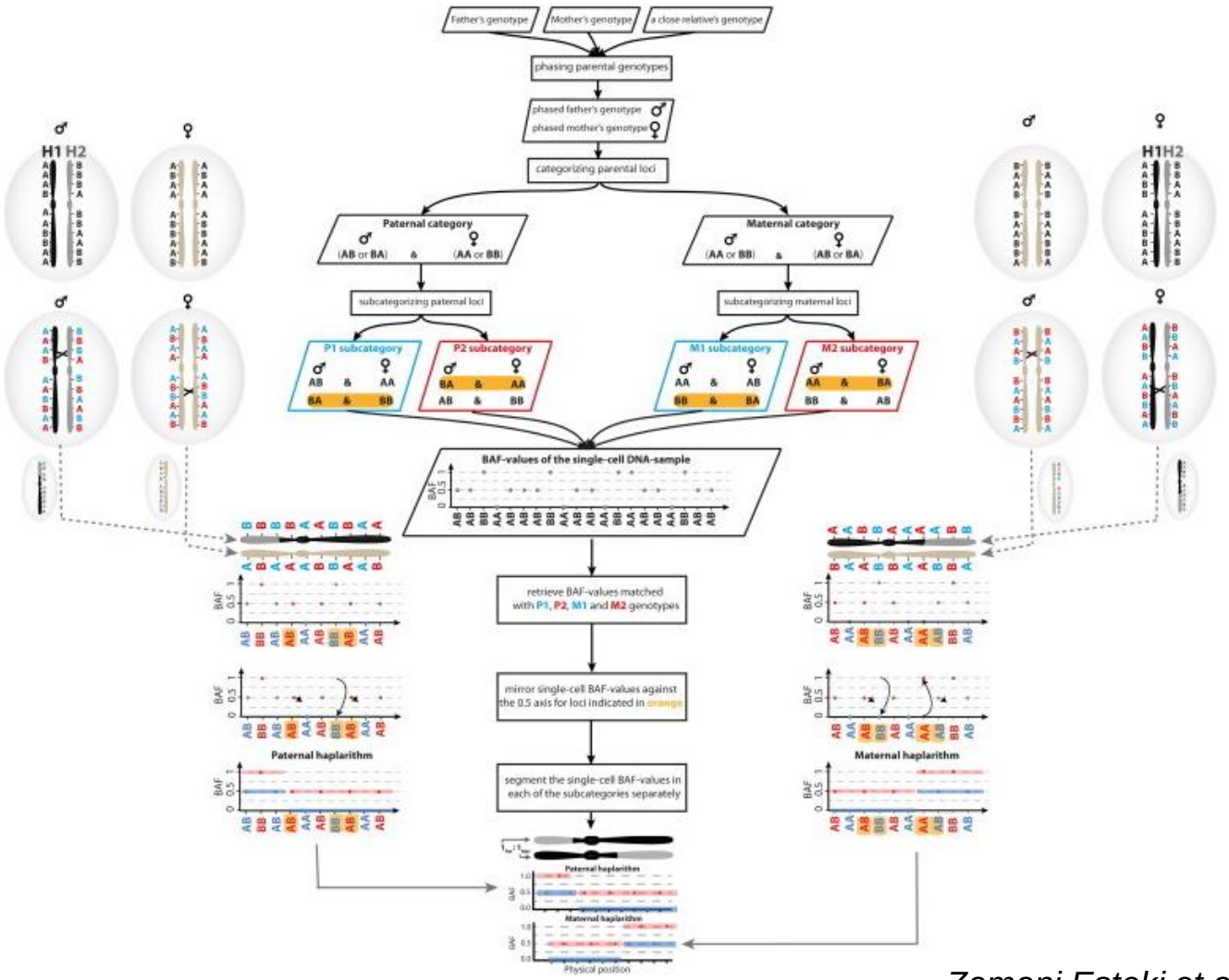
Phased paternal SNPs H1|H2	Phased maternal SNPs	Single-cell genotype	Inherited paternal haplotype by embryo
A|B	A|A	AA	H1=A ^
		AB	H2=B
		BB *	H2=B
A|B	B|B	BB	H2=B ^
		AB	H1=A
		AA *	H1=A
B|A	A|A	AA	H2=A ^
		AB	H1=B
		BB *	H1=B
B|A	B|B	BB	H1=B ^
		AB	H2=A
		AA *	H2=A

* ADO or loss of the maternal allele in the single-cell genotype
^ if ADO of the paternal allele occurred in the single-cell genotype, then the alternative pateral haplotype was inherited



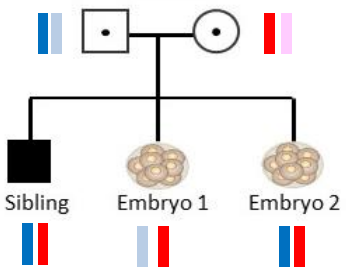
Concurrent genome-wide haplotyping and copy-number profiling of single cells

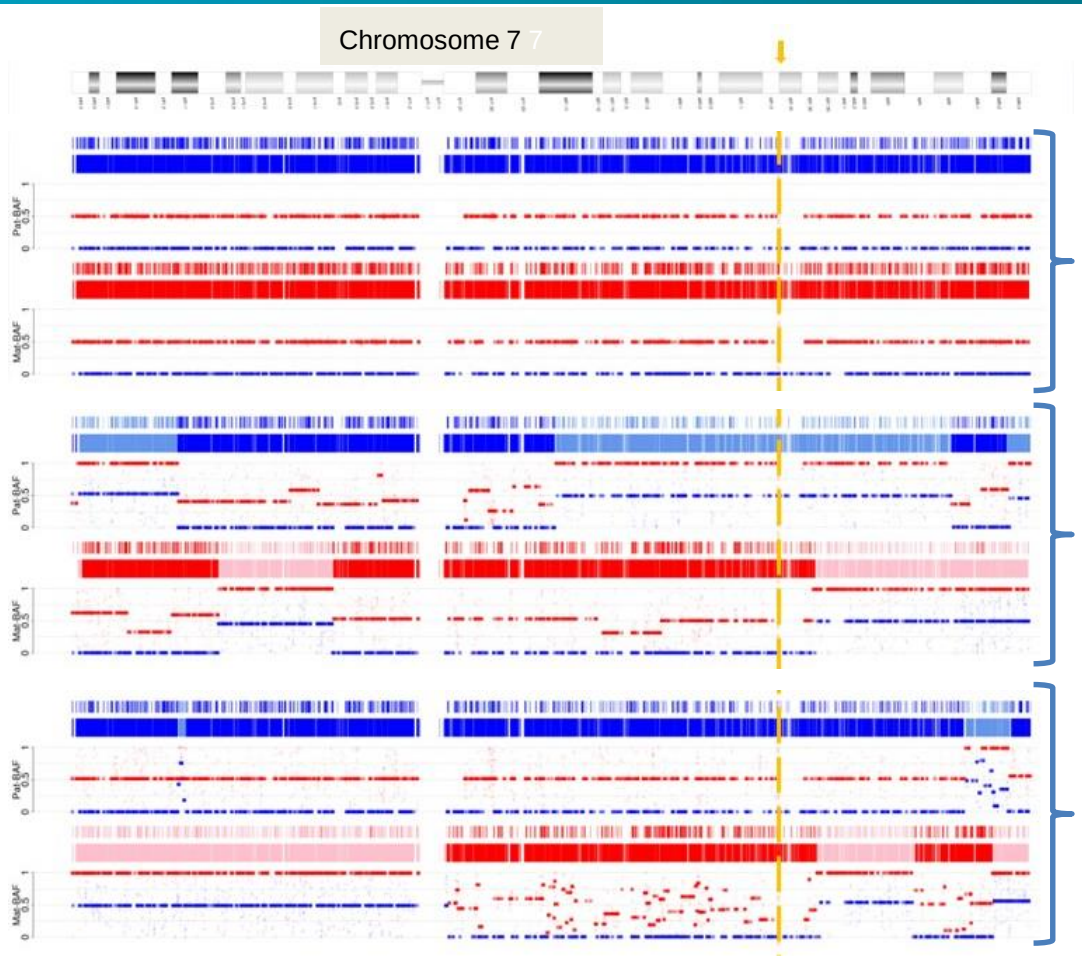
Haplarithmisis: computational workflow



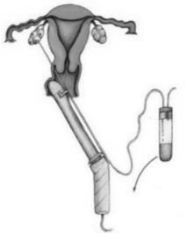
Zamani Esteki et al. AJHG. 2015.

siCHILD for CF – analysis by sibling

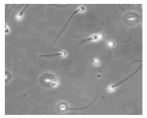




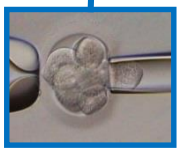
Current PGD scheme



Oocyte aspiration



Sperm processing

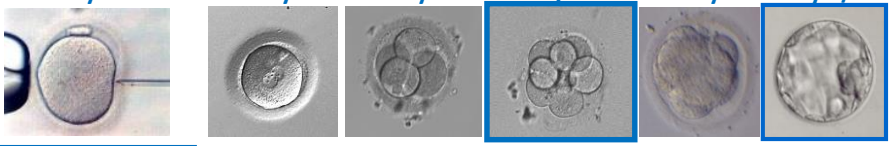


Embryo biopsy

Vitrification

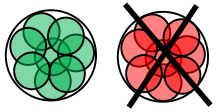
Genetic analysis: 1 month

Day 0 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5/6



ICSI

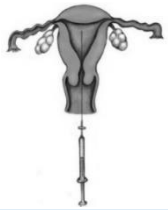
Following embryo development and morphology



Embryo selection

siCHILD

Thaw cycle



Embryo transfer

Indications

AD	ALK1	Rendu-Osler-Weber syndrome
	BMP2	Pulmonale Arterial Hypertension
	BRCA1	Breast and ovarian cancer
	BRCA2	Breast and ovarian cancer
	C9ORF72	Amyotrophic lateral sclerosis 1
	DMPK	Myotonic dystrophy 1
	EDAR	Ectodermal dysplasia 10A
	EXT1	Exostoses, multiple, type 1
	FSHD	Facioscapulohumeral muscular dystrophy 1
	HTT	Huntington disease
	KCNH2	Long QT syndrome 2
	KRT16	Palmoplantar keratoderma, nonepidermolytic, focal
	Lamine A/C	Cardiomyopathy, dilated, 1A
	MYBPC3	Cardiomyopathy, hypertrophic, 4
	NF1	Neurofibromatosis, type 1
	NOTCH1	Cardiopathy
	PMP22	Charcot-Marie-Tooth disease, type 1A
	RET	Hirschsprung disease
	SPG4	Spastic paraplegia 4
	STK11	Peutz-Jeghers syndrome
	TP53	Li-Fraumeni syndrome
AR	CFTR	Cystic fibrosis
	F5	Factor V deficiency
	HBB	Thalassemia, beta
	RPGRIP1	Leber congenital amaurosis 6
	SMN1	Spinal muscular atrophy
	STIL	Microcephaly 7, primary

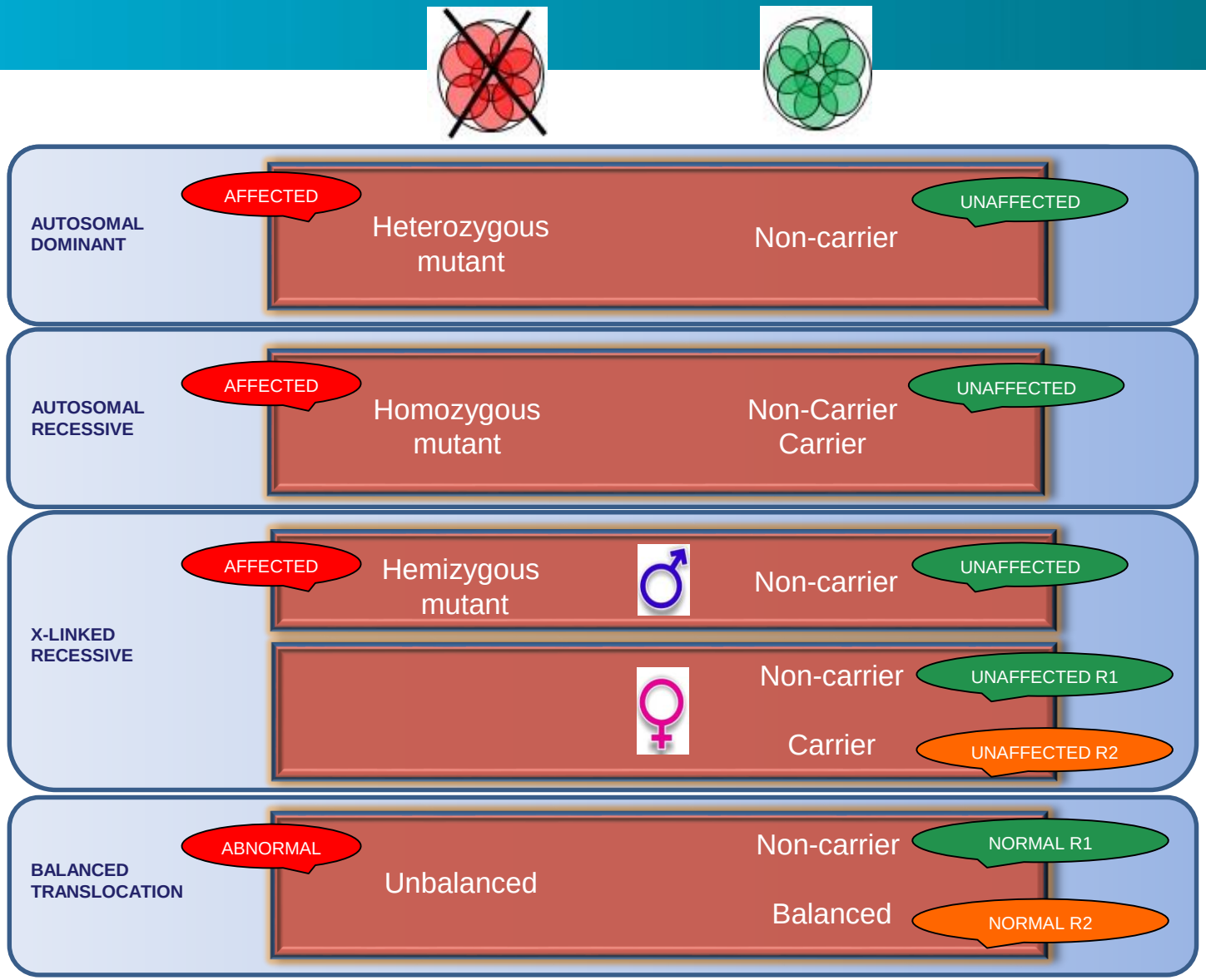
*60 families, some families multiple indications

XLD	EBP	Chondrodysplasia punctata
	FMR1	Fragile X syndrome
XLR	ABCD1	Adrenomyeloneuropathy, adult
	F8	Hemophilia A
	F9	Hemophilia B
	PIGA	Multiple congenital anomalies-hypotonia-seizures syndrome 2
	RP3	Retinitis pigmentosa 3

chromosomal	dup(10q24) dup(22q11) t(16;17) t(2;15) t(18;20)

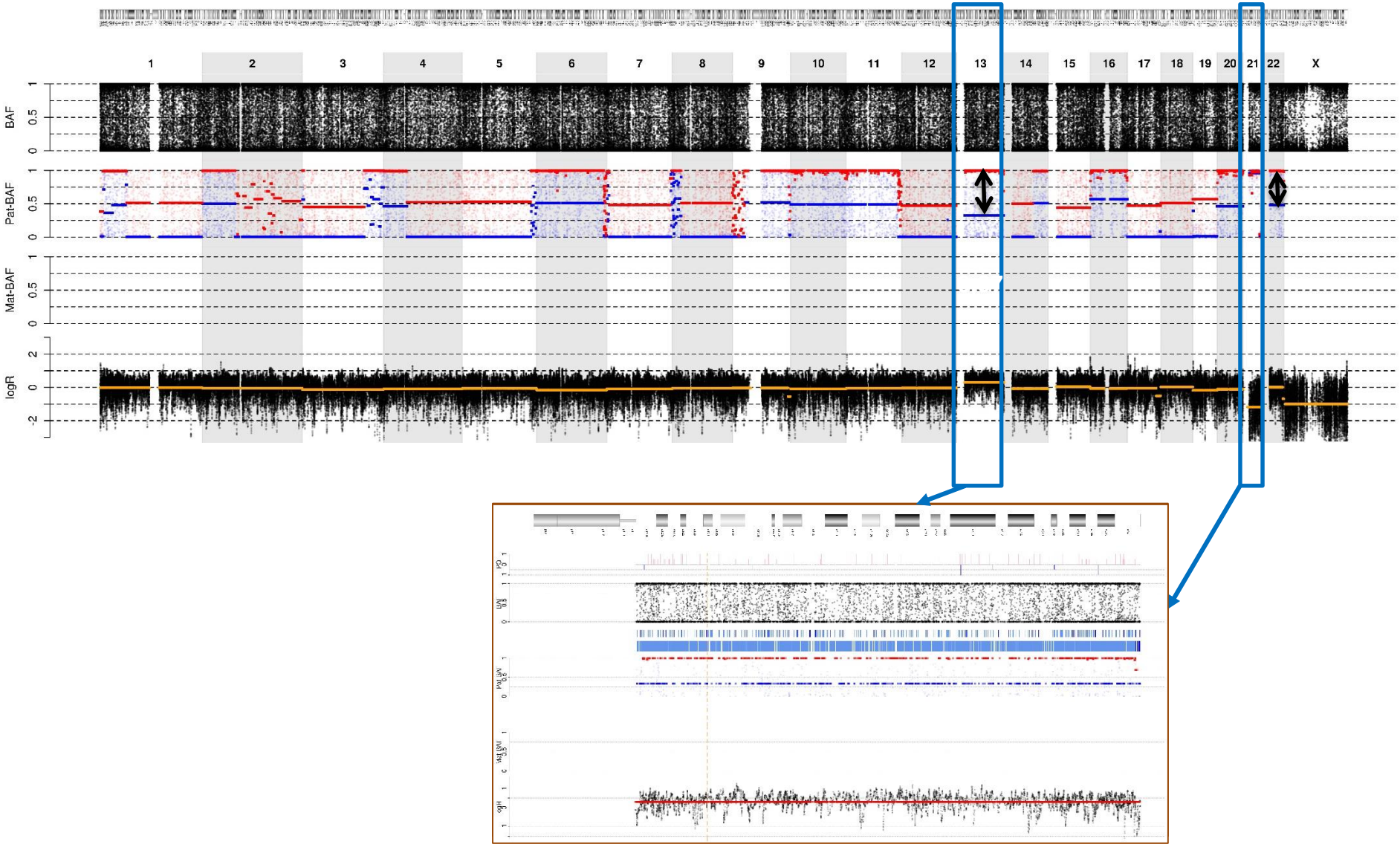
Dimitriadou. et al. Human Reproduction, 2016

Haplotype-gebaseerde classificatie

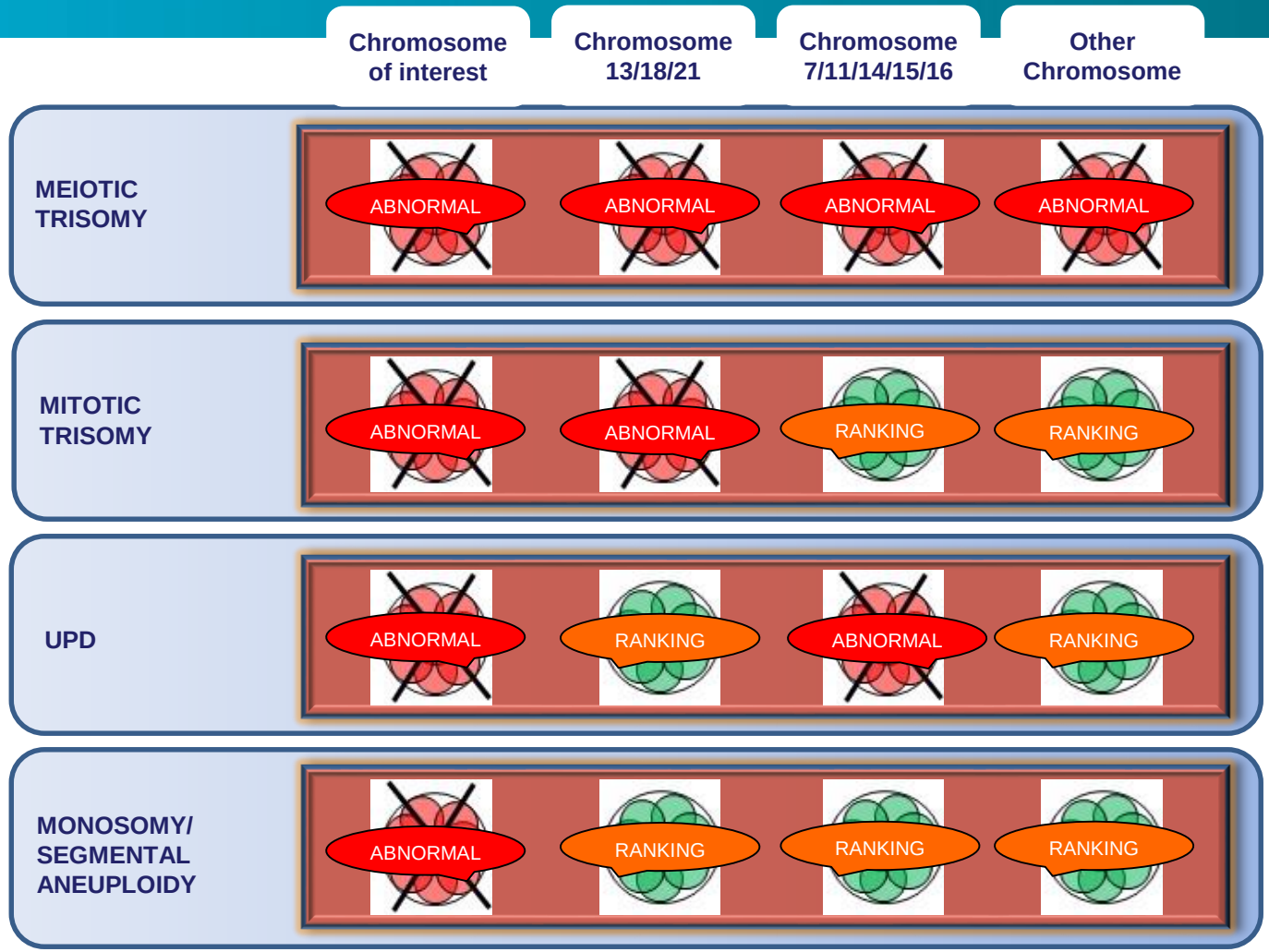


Dimitriadou E., et al. Principles guiding embryo selection following genome-wide haplotyping of preimplantation embryos. Hum Reprod. 2017;32(3):687-697.

Genoom-wijde aneuploidie en UPD detectie



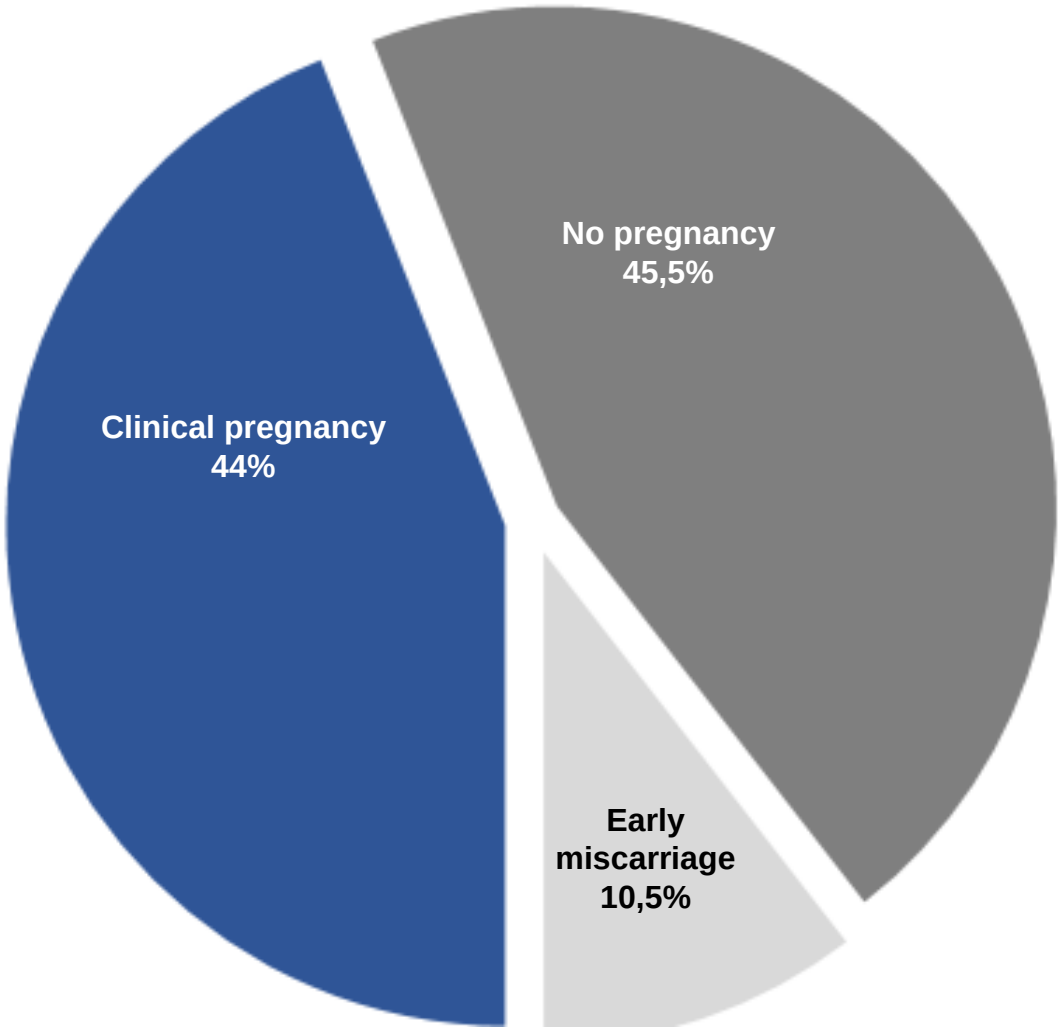
Genome-wide classificatie



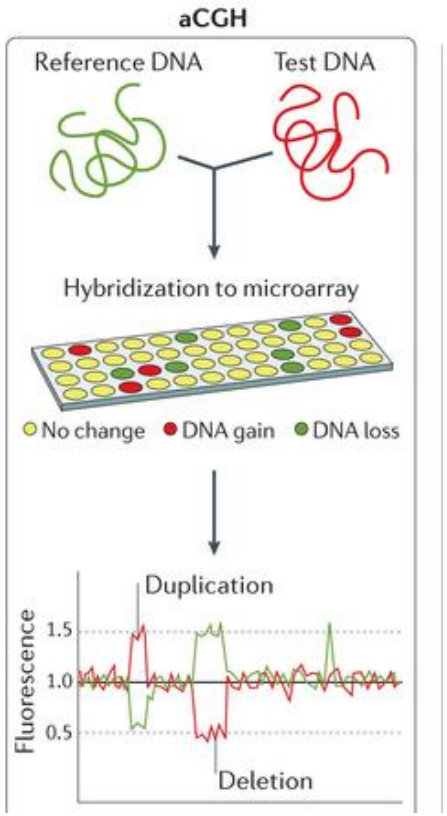
Dimitriadou E., et al. Principles guiding embryo selection following genome-wide haplotyping of preimplantation embryos. Hum Reprod. 2017;32(3):687-697.

Clinical outcome of haplotyping-based PGD

70% of cycli embryo transfer
(30% no unaffected embryos)



From arrays to sequencing



Preimplantation Genetic Testing

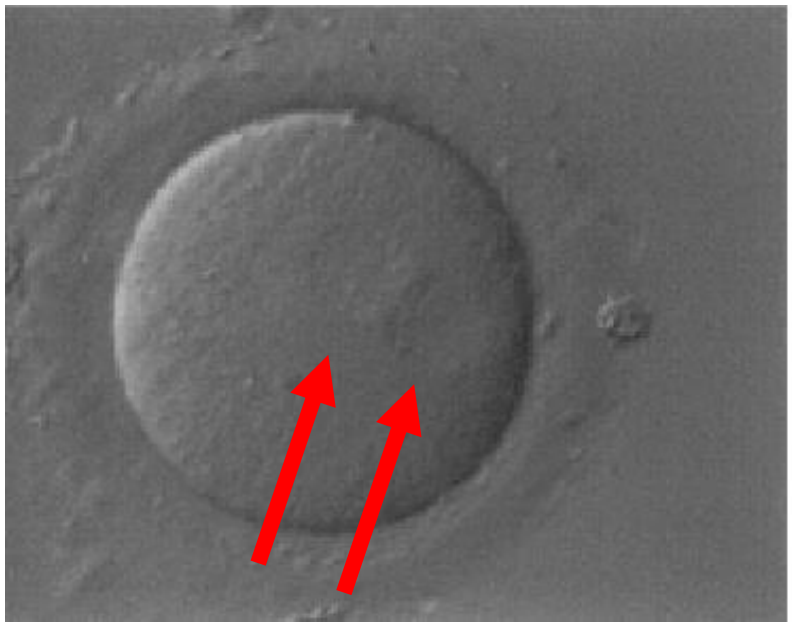
Agilent OnePGT Solution

Agilent OnePGT solution enables comprehensive insights for every IVF transfer with a single genome-wide NGS workflow integrating preimplantation genetic testing for single gene disorders (PGT-M), translocations (PGT-SR), and aneuploidy testing (PGT-A) including verified automatic data analysis software with built-in QC metrics.

Broadening the scope

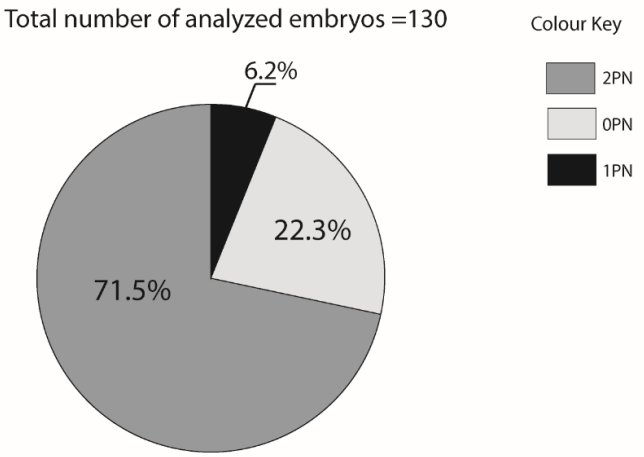
Improving IVF success rate

0 and 1 PN embryo's are discarded

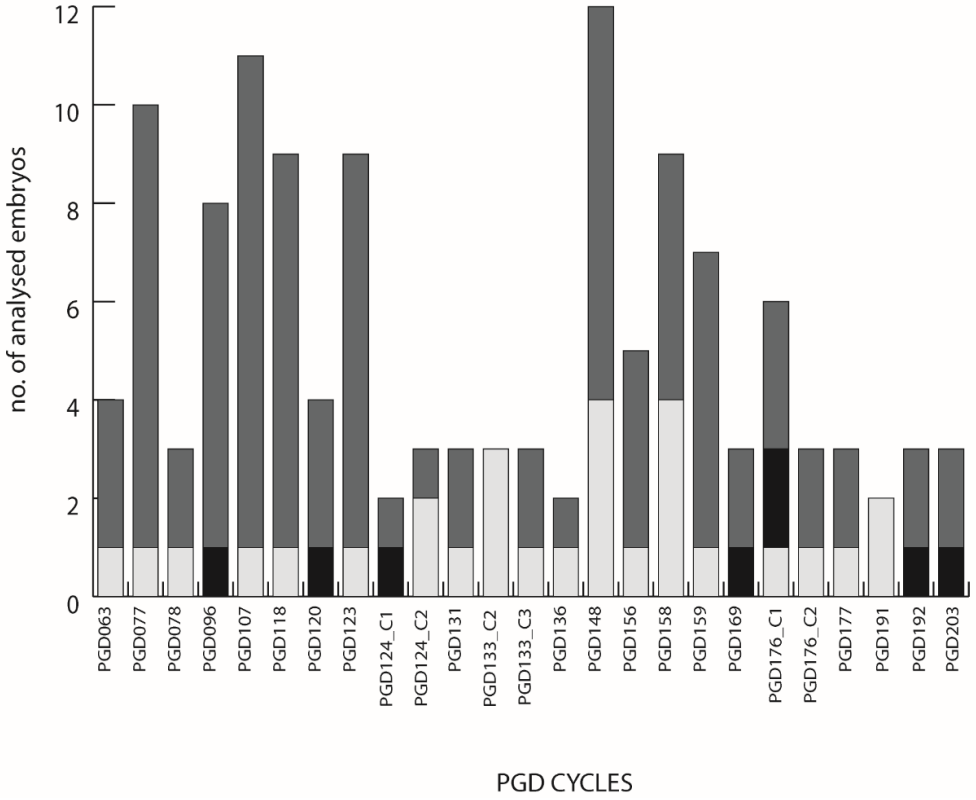


Proportion of 0 and 1 PNs currently discarded in IVF

A

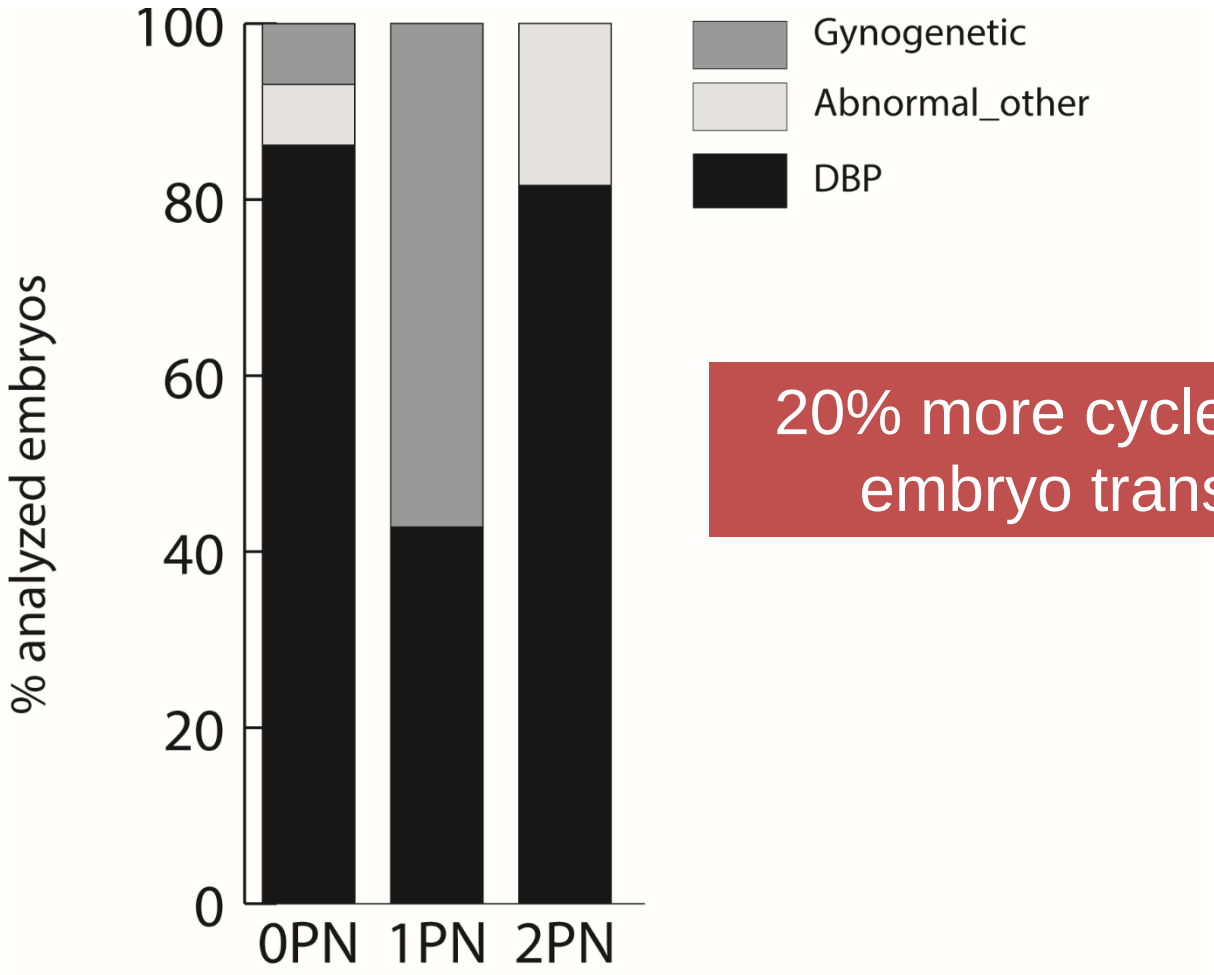


B



Destouni A. et al., under review.

The majority of O and 1 PNs are normal diploid



20% more cycles with embryo transfer

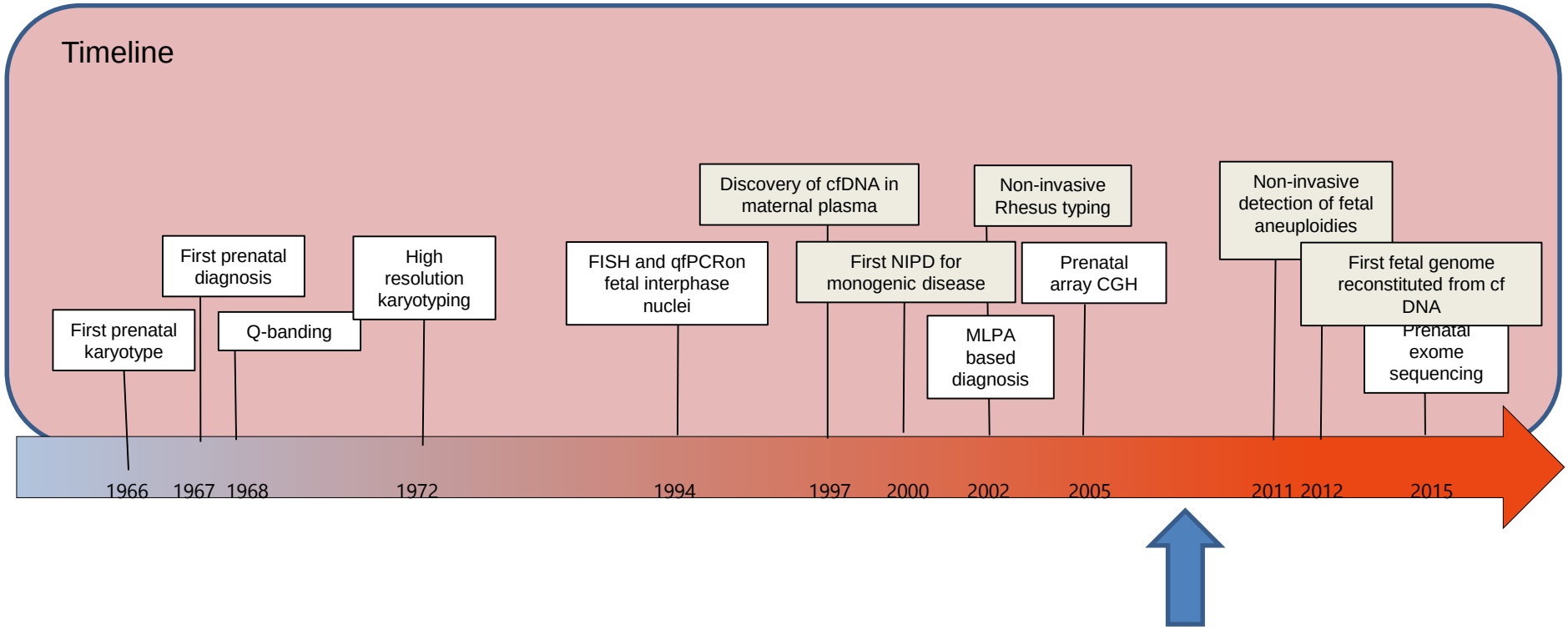
Destouni A. et al., under review.

Take home messages

- Preimplantation genetic diagnosis is now possible for all genetic lesions

- Genome wide haplotyping improves
  - Number of embryos to be transferred
  - Embryo selection

Key events in prenatal genetic testing



sequencing

Detection of cell free fetal DNA

THE LANCET

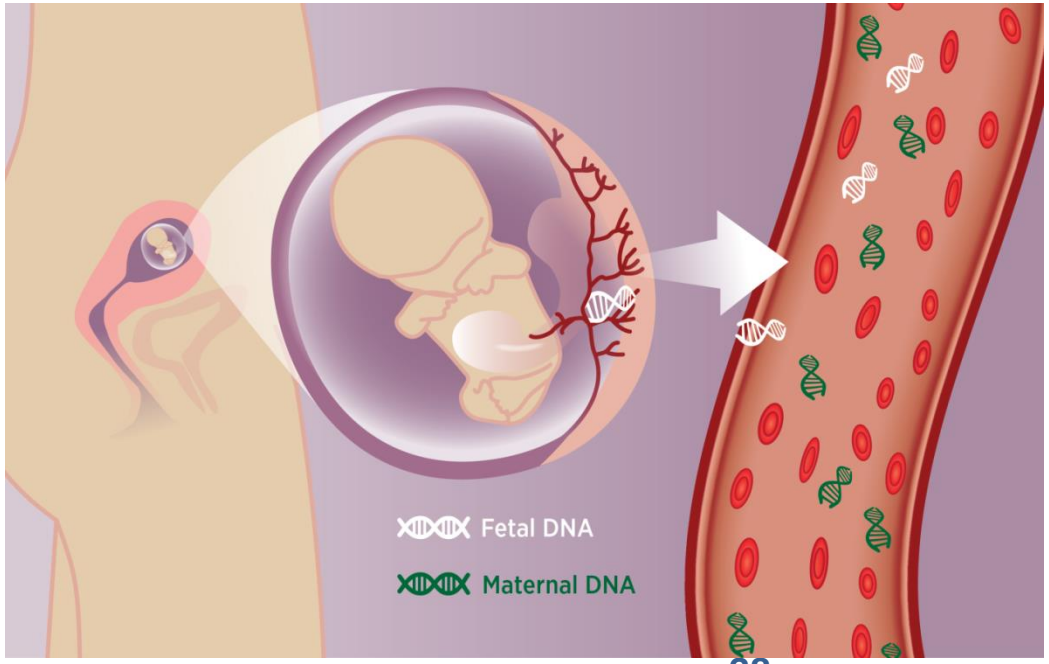
Early report **1997**

Presence of fetal DNA in maternal plasma and serum

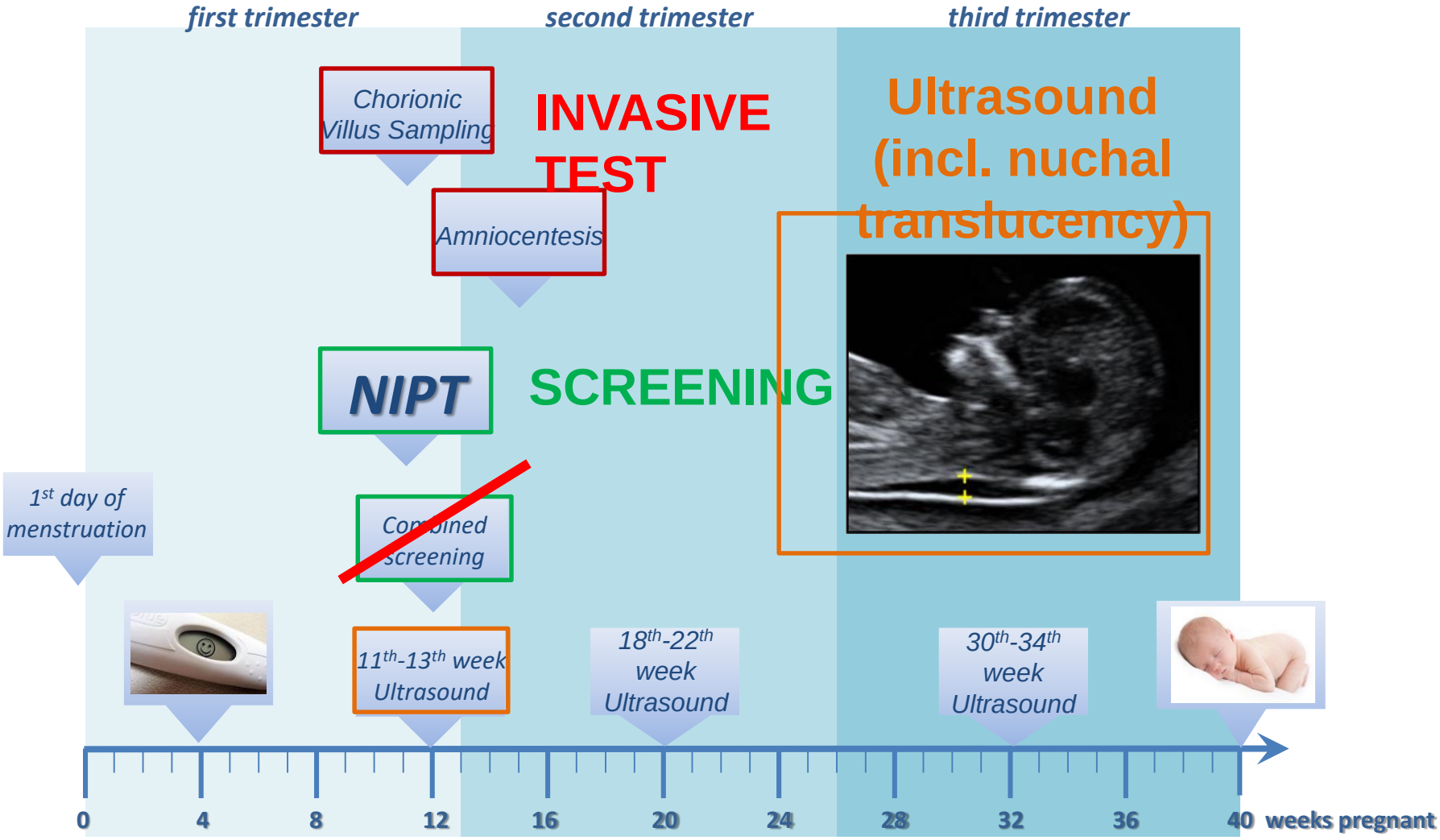
Y M Dennis Lo, Noemi Corbetta, Paul F Chamberlain, Vik Rai, Ian L Sargent, Christopher W G Redman, James S Wainscoat

cf DNA cell-free DNA
cff DNA cell-free fetal DNA

Origin:
(Apoptotic) trophoblasts



All women get NIPT in Belgium
Reimbursed by health care system



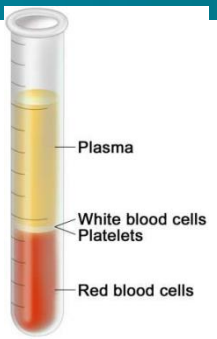
Non-invasive prenatal diagnosis

Trisomy 13, 18 & 21

NIPT - Leuven

Counselling

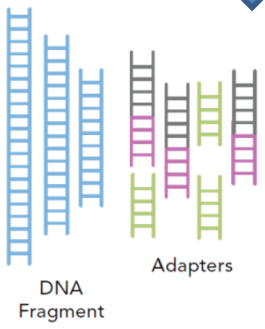
Blood sampling
'cfDNA tubes'



Plasma isolation

Cell-free DNA extraction

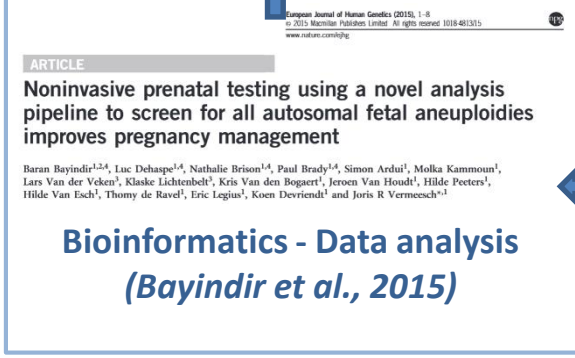
mother + fetus



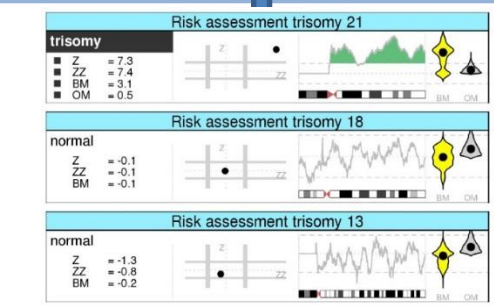
Library preparation

Hiseq® 2500/4000 Sequencing

Pool 23 samples
>7 million reads



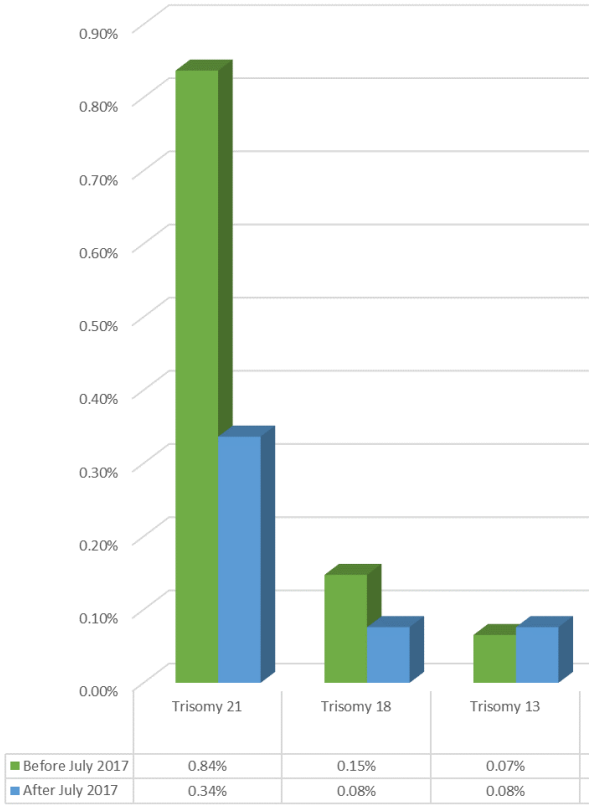
Bioinformatics - Data analysis
(Bayindir et al., 2015)



Risk assessment

REIMBURSED
01 July 2017
260€

NIPT for trisomy 13, 18, 21: The Belgian experience



2017-2018 77400 tests

Common trisomies detected by NIPT	# samples	% of total NIPT
Trisomy 21	261	0.34%
Trisomy 18	59	0.08%
Trisomy 13	59	0.08%

Non-invasive prenatal diagnosis

Beyond trisomy 13, 18 & 21

NIPT – Findings

9 mei 2016

Belgian Advisory Committee on Bioethics

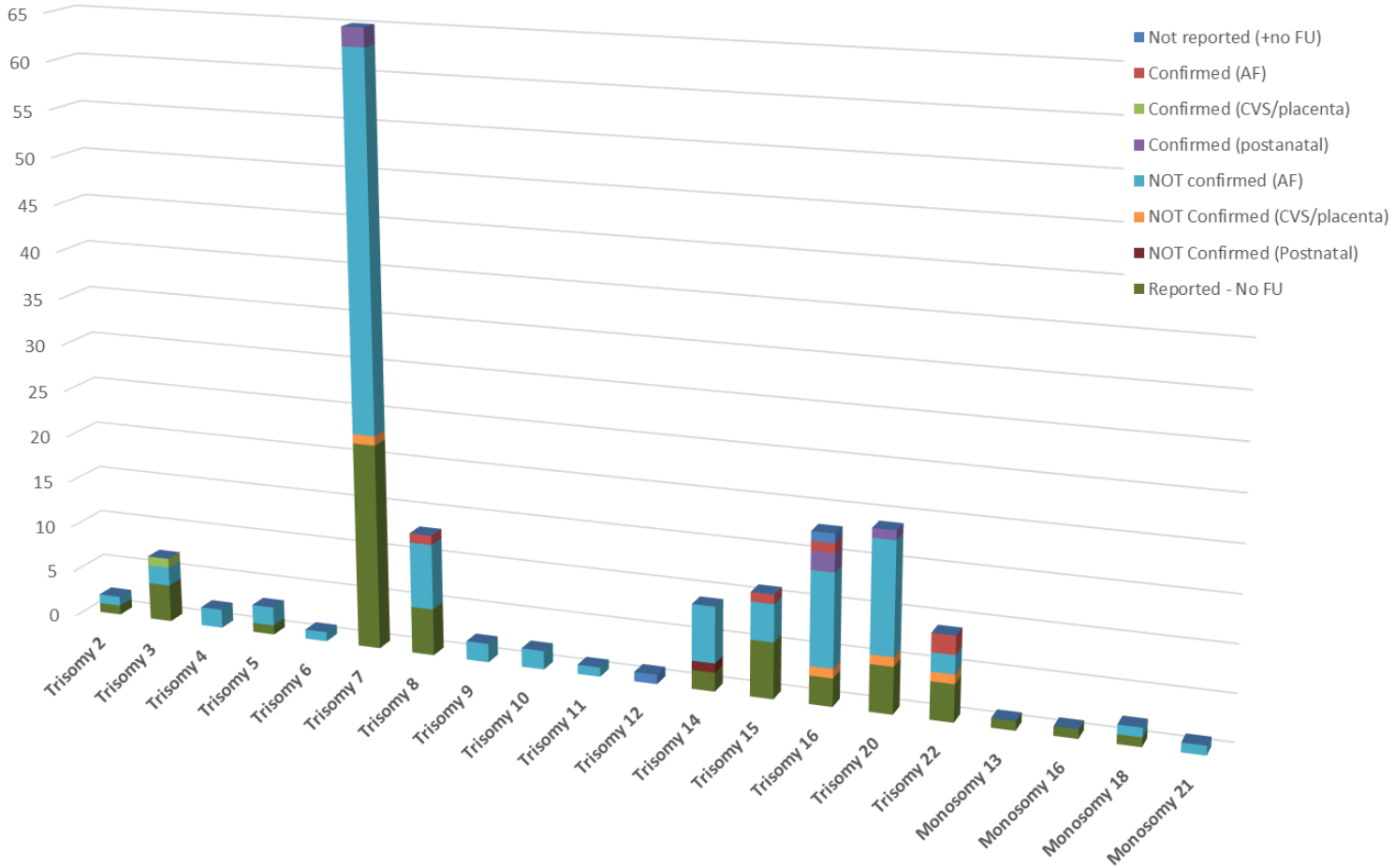
Advies nr. 66 - niet-invasieve prenatale diagnostiek (NIPT)

Advies van 9 mei 2016 betreffende de ethische uitdagingen gesteld door de niet-invasieve prenatale diagnostiek (NIPT) voor trisomie 21, 13 en 18

<u>Note on incidental findings (NIPT):</u>

"When this information can lead to preventive or therapeutic interventions, it is important to disclose this information to the patient within in a **Clinical Genetics** setting. Not reporting these findings can be considered a serious negligence."

Other fetal aneuploidies

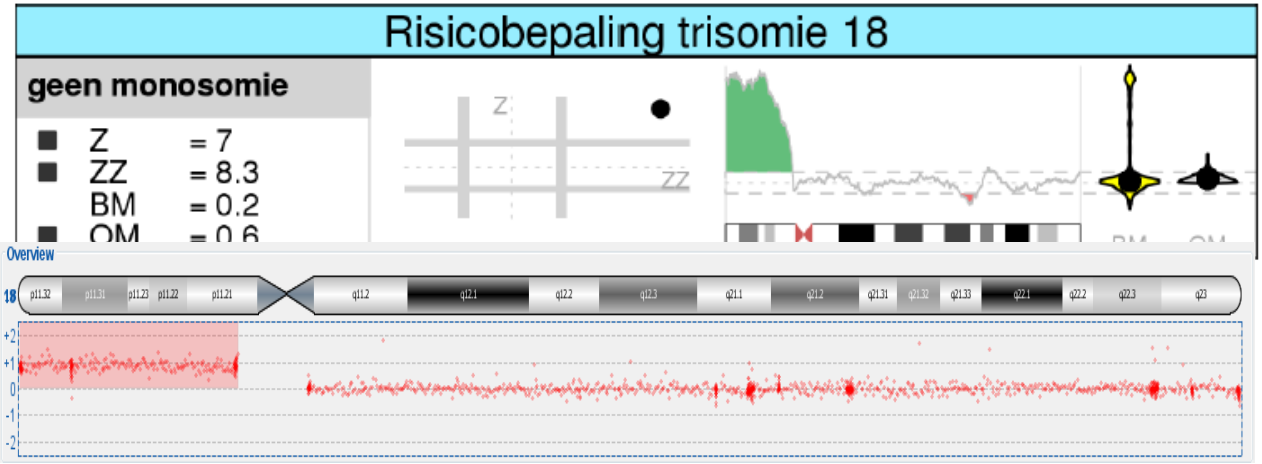


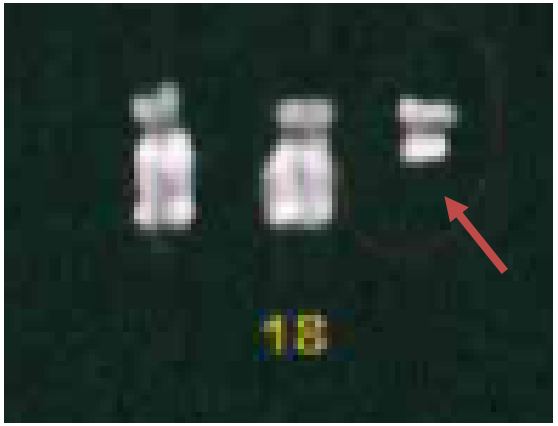
Detection of those aneuploidies allows for the detection of fetal UPD, low grade mosaicism
This analysis can also explain IUGR

SEGMENTAL CHROMOSOMAL IMBALANCES

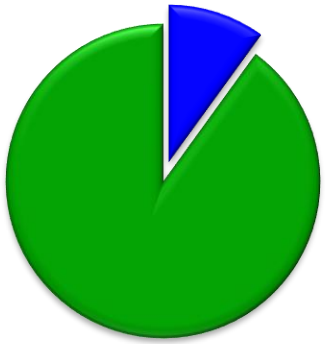
Fetal segmental imbalances

Isochromosome 18





Clinically relevant maternal aberrations



cf DNA cell-free DNA

= 5-20% **fetal** + 80-95% **maternal**

Clinically relevant
NIPT MATERNAL segmental aneuploidy

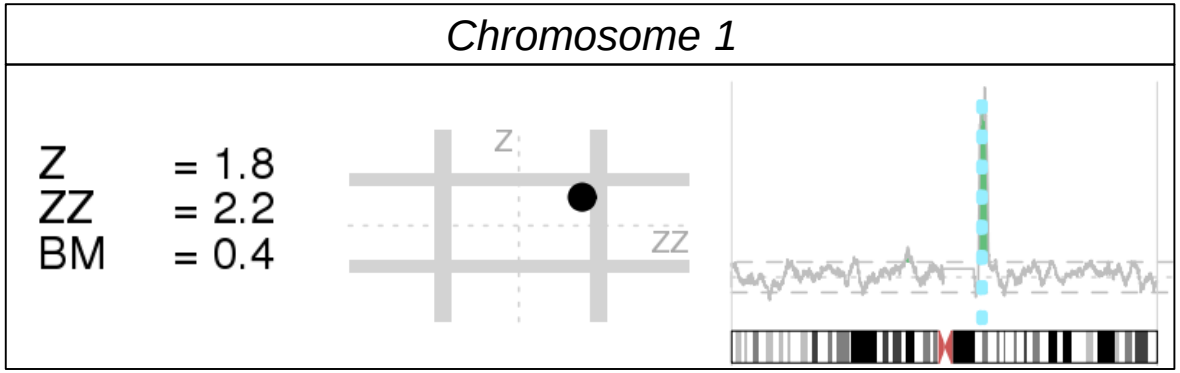
Genetics inMedicine | ORIGINAL RESEARCH ARTICLE

Accuracy and clinical value of maternal incidental findings during noninvasive prenatal testing for fetal aneuploidies

Nathalie Brison, PhD[1], Kris Van Den Bogaert, PhD[1], Luc Dehaspe, PhD[1], Jessica M.E. van den Oever, PhD[1], Katrien Janssens, PhD[2], Bettina Blaumeiser, MD, PhD[2], Hilde Peeters, MD, PhD[1], Hilde Van Esch, MD, PhD[1], Griet Van Buggenhout, MD, PhD[1], Annick Vogels, MD, PhD[1], Thomy de Ravel, MD, PhD[1], Eric Legius, MD, PhD[1], Koen Devriendt, MD, PhD[1] and Joris R. Vermeesch, PhD[1]

MATERNAL susceptibility CNVs

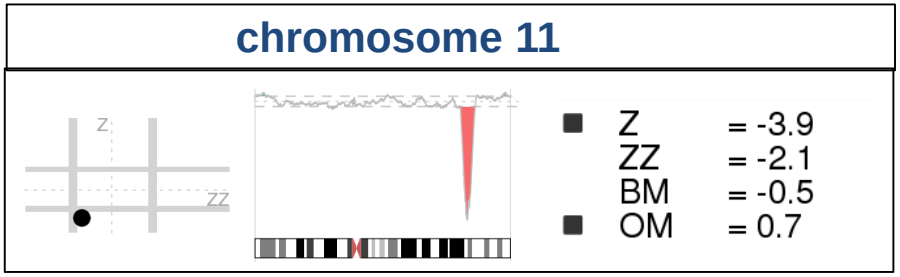
Origin	Clinical implication	Reported?
	1. Highly penetrant disorders with validated evidence on phenotype	YES
	2. Susceptibility loci with reduced penetrance and/or variable expression	NO

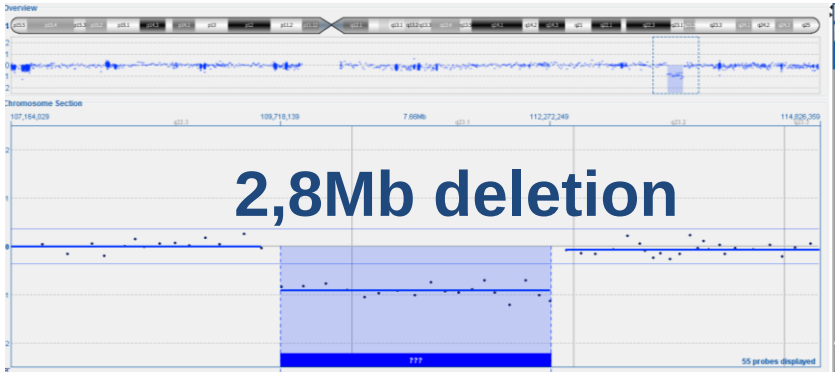


1q21.1 microduplication

MATERNAL segmental aneuploidy

Origin	Clinical implication	Reported?
Maternal IF	**1.** Highly penetrant disorders with validated evidence on phenotype	YES
	2. Susceptibility loci with reduced penetrance and/or variable expression	NO
	3. CNVs causing late-onset genetic disorders but still asymptomatic in the mother	YES





American College of Medical Genetics and Genomics

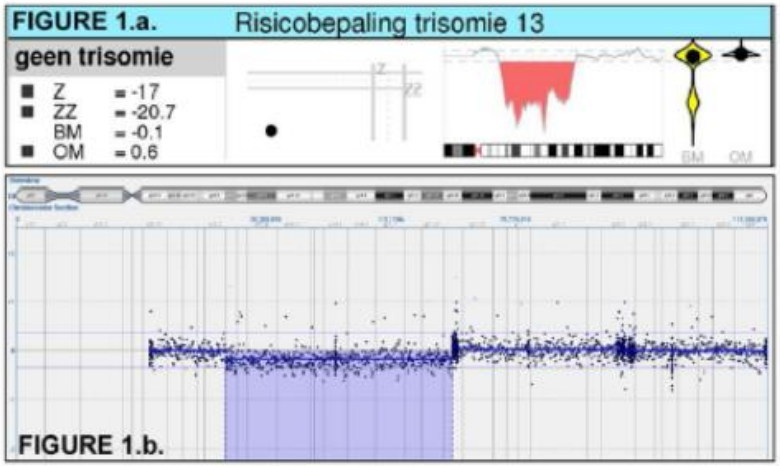
ACMG STATEMENT | Genetics inMedicine

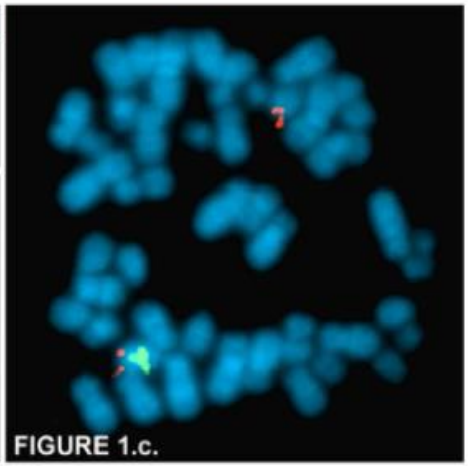
Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics

Phenotype	MIM disorder	PMID Gene Reviews entry	Typical age of onset	Gene	MIM gene	Inheritance[a]	Variants to report[b]
Hereditary paraganglioma-pheochromocytoma syndrome	168000 (PGL1)	20301715	Child/adult	*SDHD*	602690	AD	KP and EP
	601650 (PGL2)			*SDHAF2*	613019		KP
	605373 (PGL3)			*SDHC*	602413		KP and EP
	115310 (PGL4)			*SDHB*	185470		

MATERNAL segmental aneuploidy potentially harmful for the fetus

Origin	Clinical implication	Reported?
Maternal IF	**1.** Highly penetrant disorders with validated evidence on phenotype	YES
	2. Susceptibility loci with reduced penetrance and/or variable expression	NO
	3. CNVs causing late-onset genetic disorders but still asymptomatic in the mother	YES
	4. CNVs with no consequence for the mother but, if inherited, potentially harmful for the fetus in current/future pregnancy	
	4a. Carrier of AR disease (except carrier freq. >1/50 [Connexin26, CFTR])	NO
	4b. Carrier X-linked recessive disorder	YES
	4c. Carriership of a mosaic CNV	YES





FISH & array CGH on maternal genomic DNA

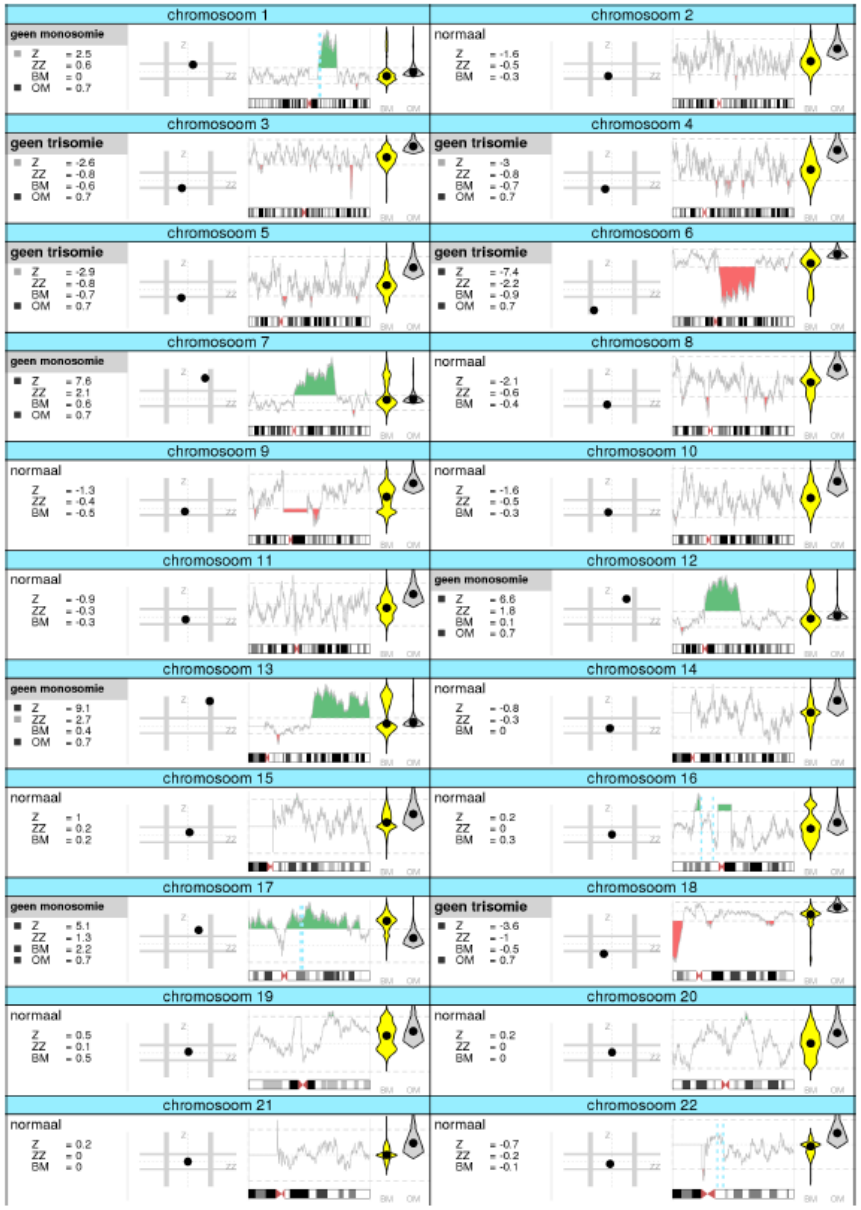
Partial monosomy 13 sized 34,73 Mb in 15-20% of cells

RISK FOR FUTURE PREGNANCIES (current pregnancy: fetus normal)

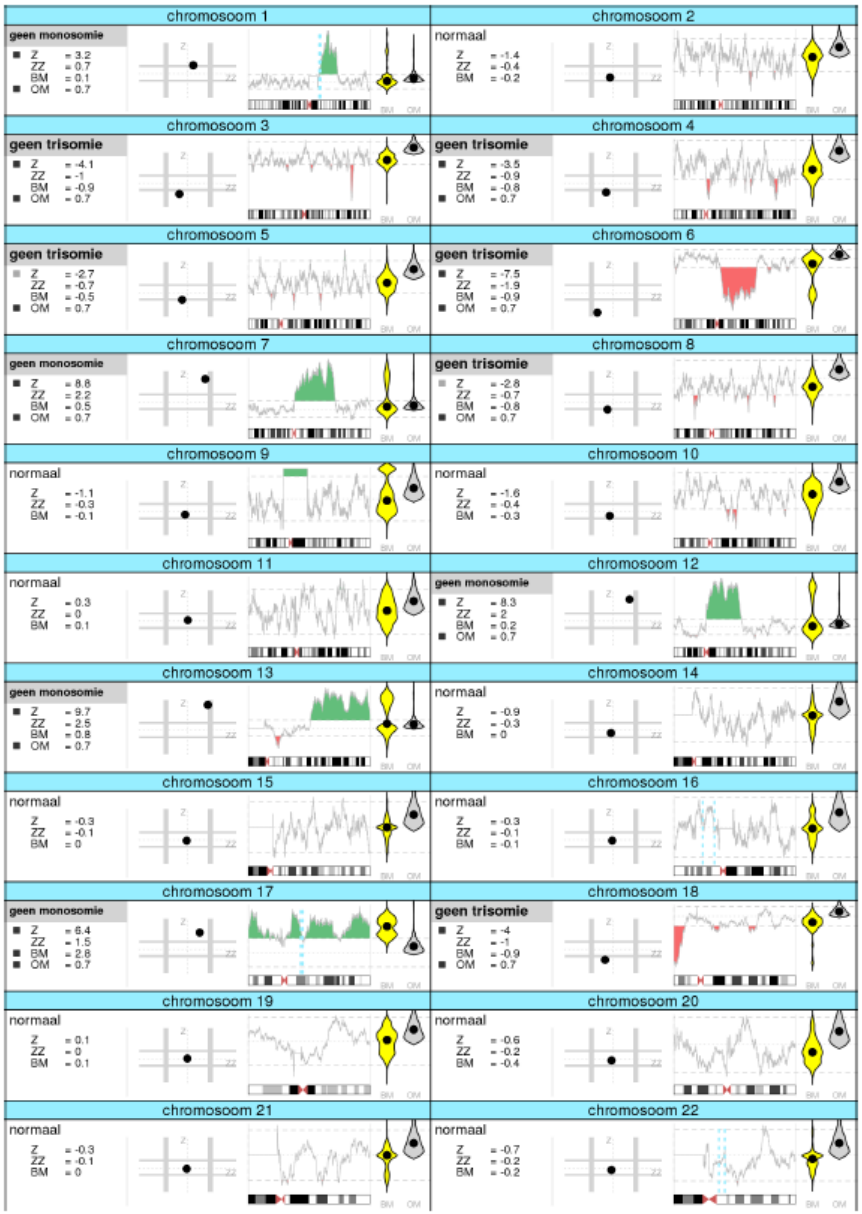
MATERNAL CANCERS

Reproducible aberrant GR profiles upon repeat sampling

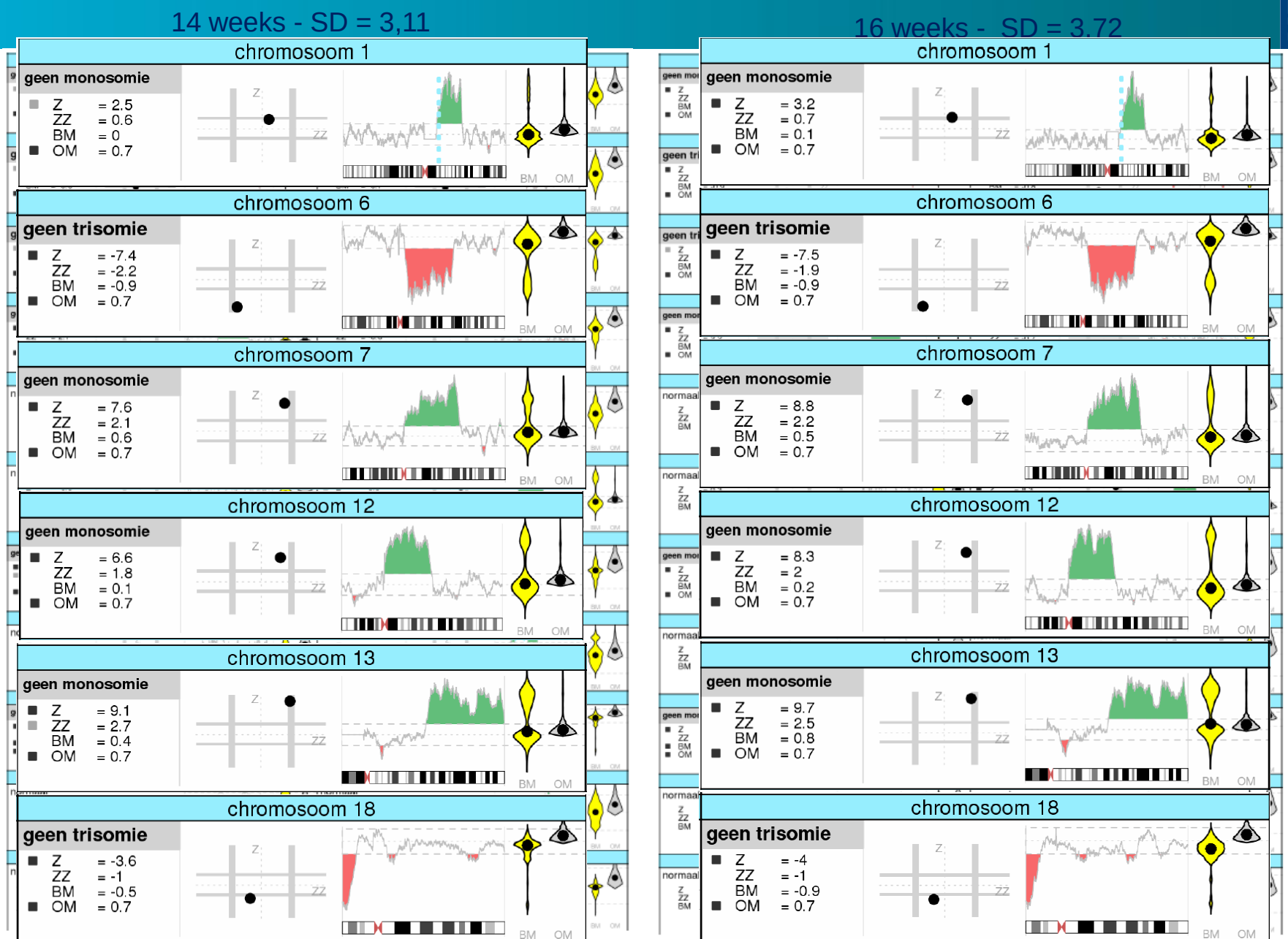
14 weeks - SD = 3,11



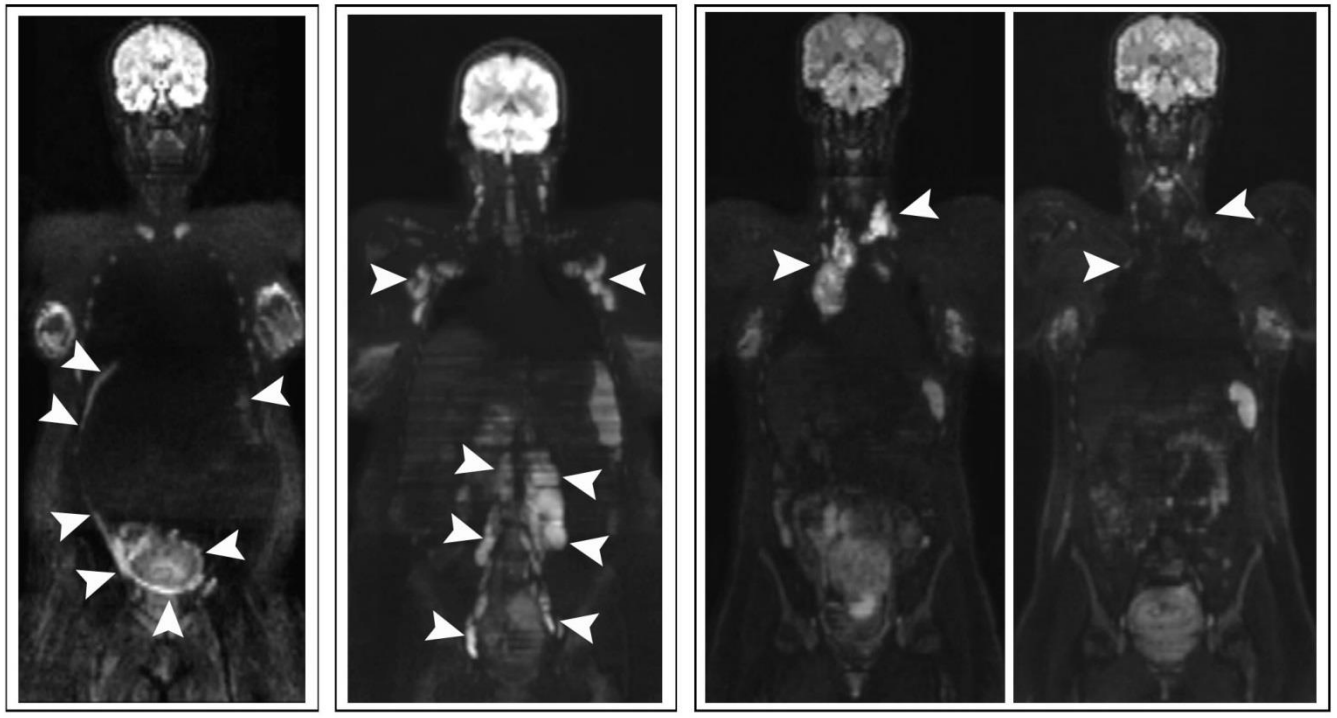
16 weeks - SD = 3,72



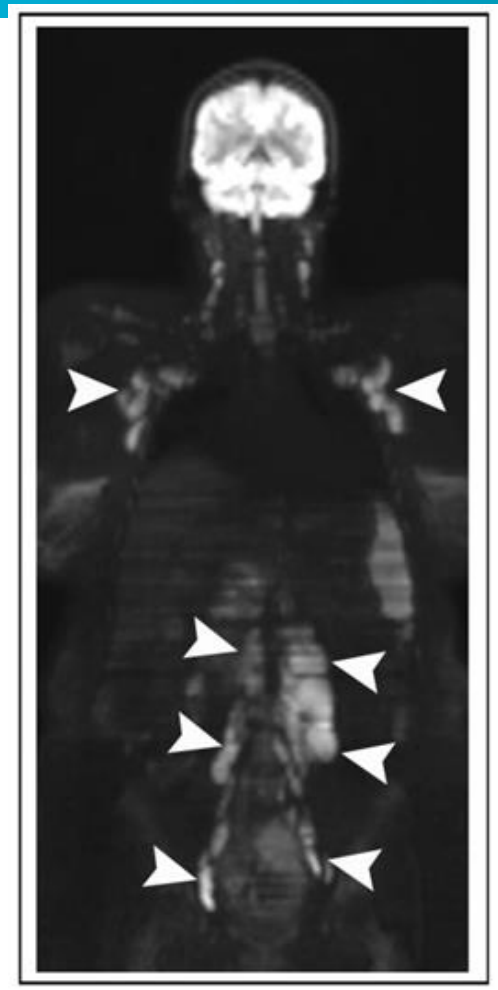
Reproducible aberrant GR profiles upon repeat sampling



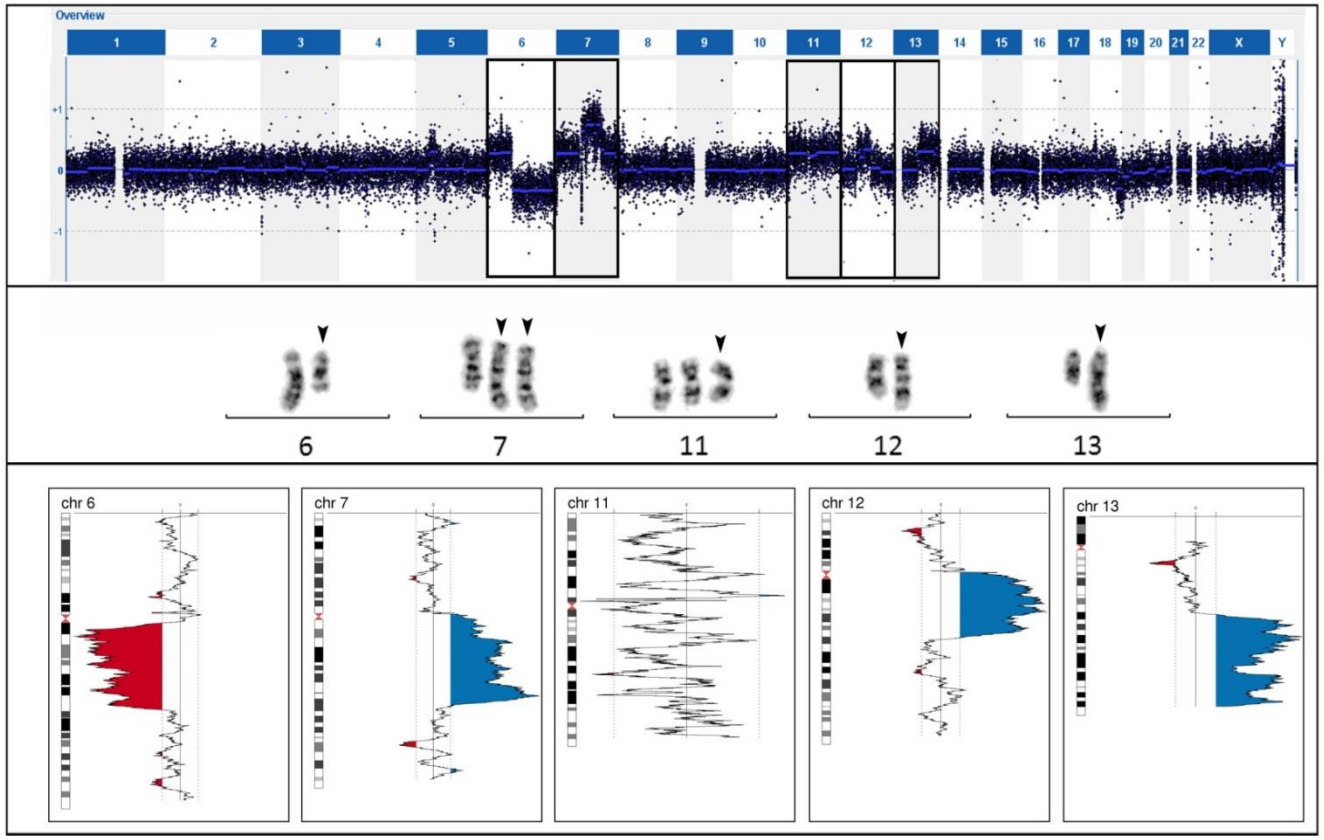
Whole body MRI



Patient follow-up: maternal cancer

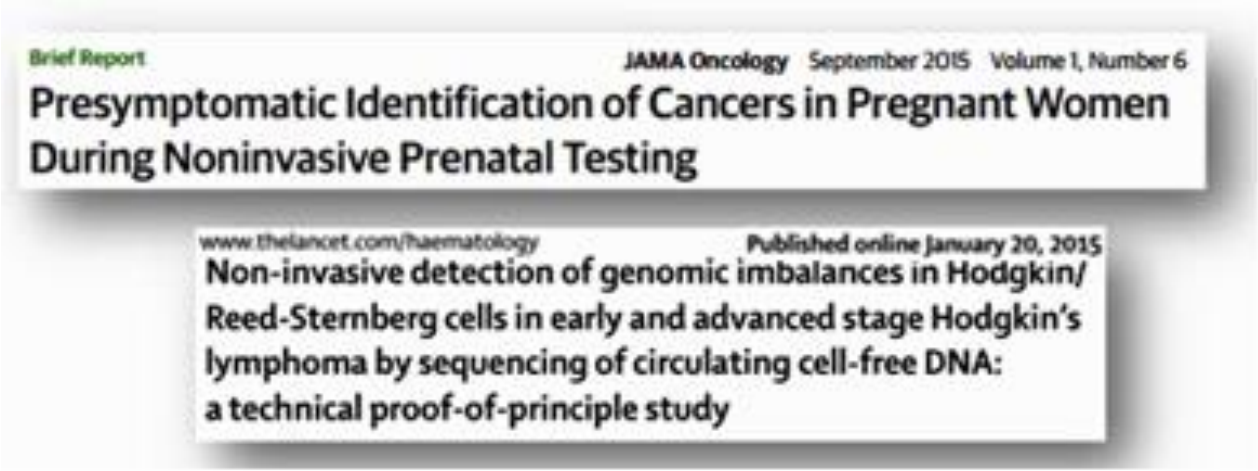


Supra-/Infradiaphragmatic lymphadenopathies



Diagnosis of follicular lymphoma (grade IIIa)

Maternal Cancers



Brief Report JAMA Oncology September 2015 Volume 1, Number 6

Presymptomatic Identification of Cancers in Pregnant Women During Noninvasive Prenatal Testing

www.thelancet.com/haematology Published online January 20, 2015

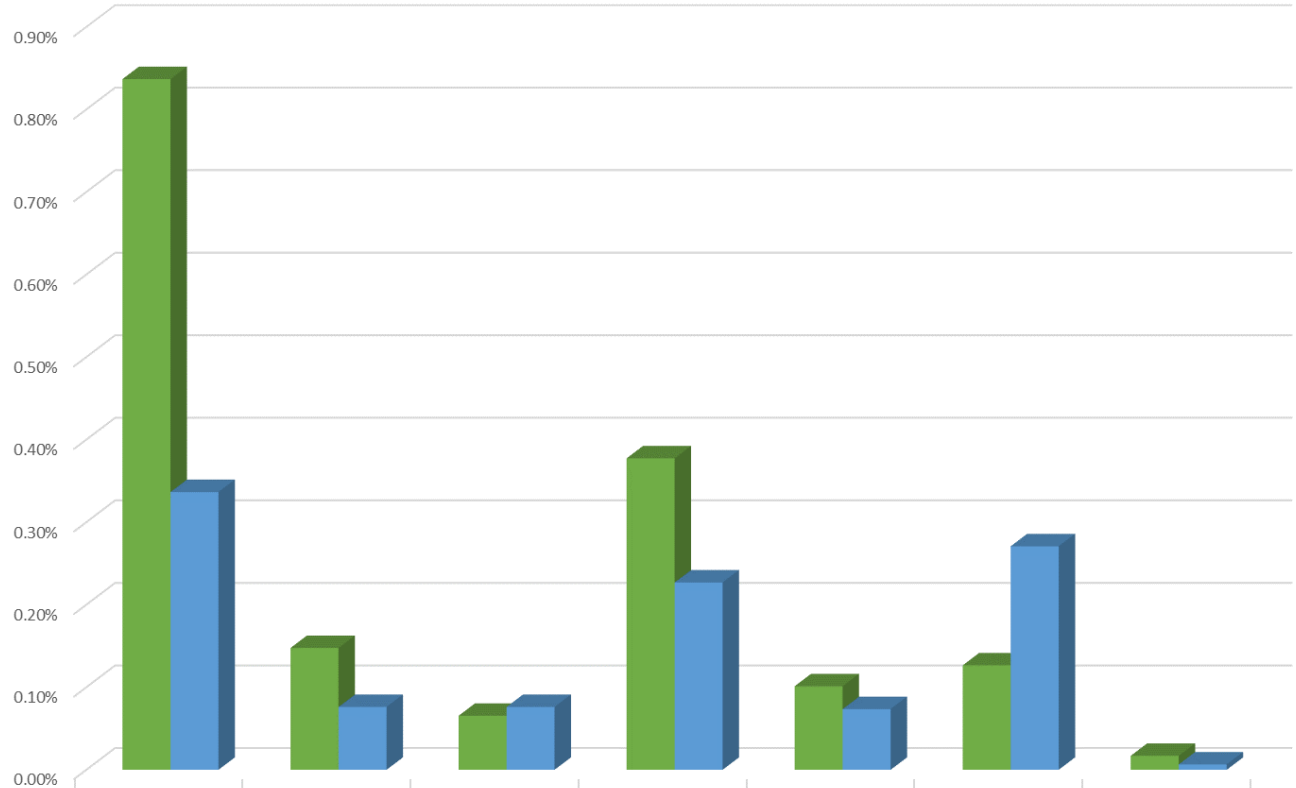
Non-invasive detection of genomic imbalances in Hodgkin/Reed-Sternberg cells in early and advanced stage Hodgkin's lymphoma by sequencing of circulating cell-free DNA: a technical proof-of-principle study

5/77400 tests in Belgium
(unpublished)

Centre	Confirmed in WBC mother (+ method)	Cancer diagnosis confirmed + Cancer type	Invasive fetal FU (+ result)	Newborn FU (+ result)
IPG	karyo+CGH on blood and ganglion	Hodgkin	ND	NA
IPG	karyo+FISH on bone marrow	Myelodysplasic syndrome	ND	NA
KUL	known cancer patient in other center	Cervix carcinoma with breast metastasis	ND	NA
KUL		Osteosarcoma	TOP	NA
KUL		Breast carcinoma	ND	NA

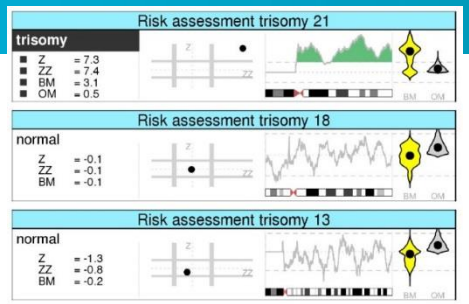
Incidence: 1/10000 pregnancies

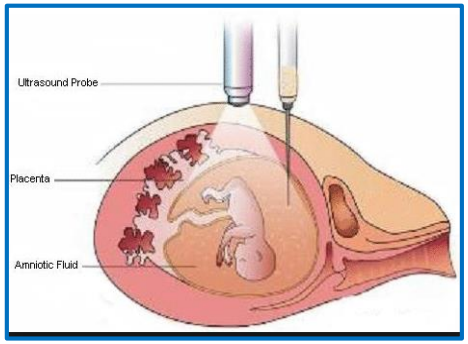
Reported anomalies in Belgium 1y after reimbursement



	Trisomy 21	Trisomy 18	Trisomy 13	Other fetal aneuploidies	Reported fetal segmental imbalances	Reported maternal IF	Maternal cancers
Before July 2017	0.84%	0.15%	0.07%	0.38%	0.10%	0.13%	0.02%
After July 2017	0.34%	0.08%	0.08%	0.23%	0.07%	0.27%	0.01%

Follow-up
Abnormal NIPT – normal invasive testing





GENETIC COUNSELLING

Consistent result

Pregnancy management

Inconsistent result

TOP or Birth

Follow-up: Additional material for genetic testing

Fetal biopsies

Placental biopsies

Conclusions

NIPT beyond common trisomies improves overal pregnancy management and enables true genomic medicine

Overall conclusions

Genomic medicines in preimplantation and prenatal diagnoses is a reality.

PGT and PND genomics provides personalised, family tailed medicine

SARM

WidenLife

Endometrial and Embryonic Genomics, Searching for Biomarkers in Assisted Reproduction

SymBioSys

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- Cindy Melotte
- Kris Van den Bogaert
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- Anneleen Boogaerts
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- Lia Margamuljana
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