Mosaicism in prenatal diagnosis: from NIPT to amniocytes investigation

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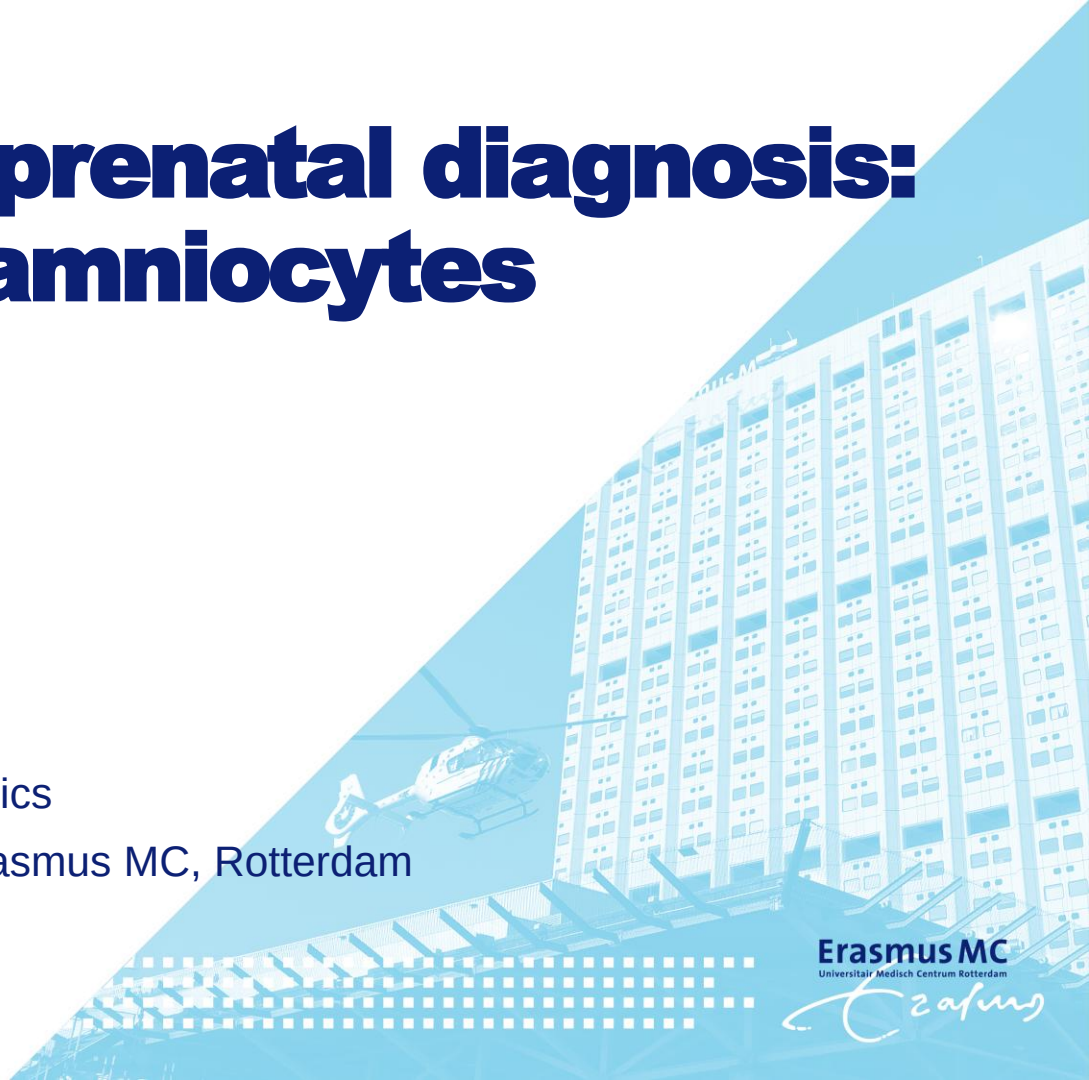
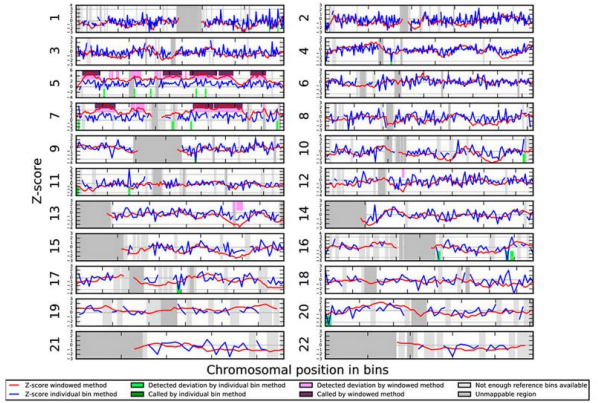


Current challenge - Genotype First Approach

- Large scale genotype first screening (NIPT)
- Phenotypic variability is larger than expected
- There may be more mosaic cases without phenotypic consequences in general population
- Abnormal results in “phenotypically normal fetus” – prediction of the (post partum) phenotype
- VUS interpretation is more challenging in absence of abnormal phenotype and in case of NIPT results they may indicate a presence of another abnormal cell line



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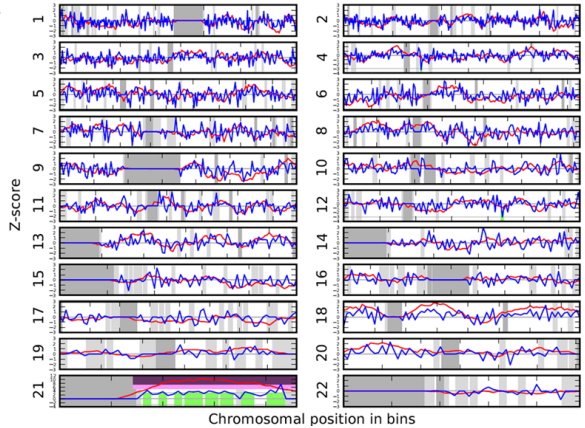
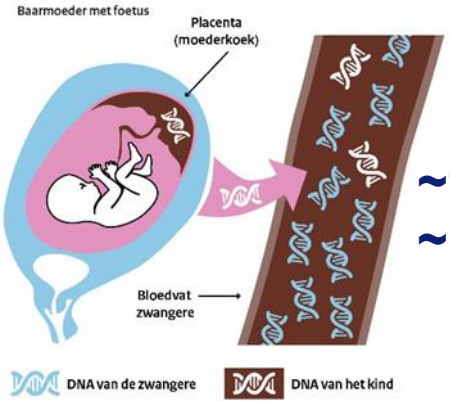
NIPT = test of cell-free DNA in maternal plasma

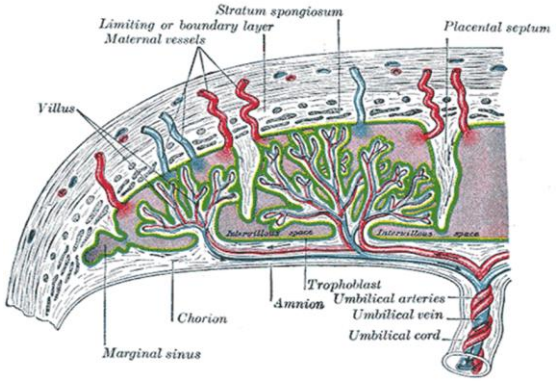
At this moment we use
VeriSeq2 (resolution~ 7Mb) and
Wisecondor (resolution ~10/20Mb)



shotgun
massively parallel
sequencing
WGS with
coverage ~ 0.2x
10-30mln reads per sample



~90% maternal
~10% fetal



Count number of reads per bin (1 Mb) on all autosomes and compare with reference bins (WISECONDOR)

NIPT = test of placental cell-free DNA

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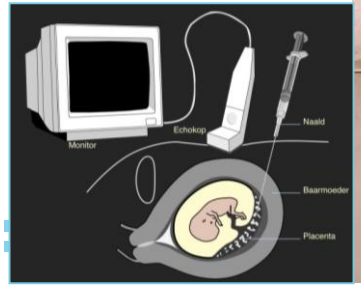
Fetal material

Chorionic villi biopsy
(CVS)
10-14 weeks

Miscarriage risk 0.2%
(Akolekar et al., 2015)

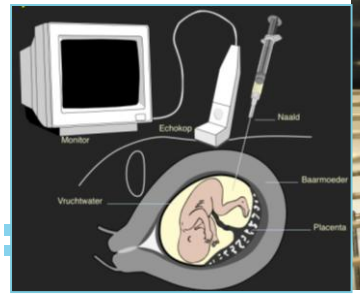
1-2% mosaic, *mostly confined placental mosaicism (± 85%)*

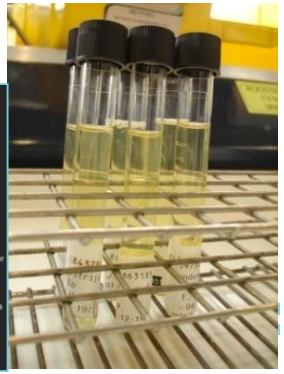


Amniocentesis
>16 weeks

Miscarriage risk 0.1%
(Akolekar et al., 2015)

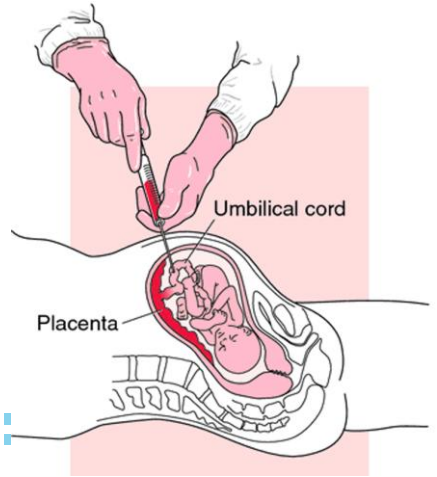
0,2% mosaic *(Hsu et al., 1992)*



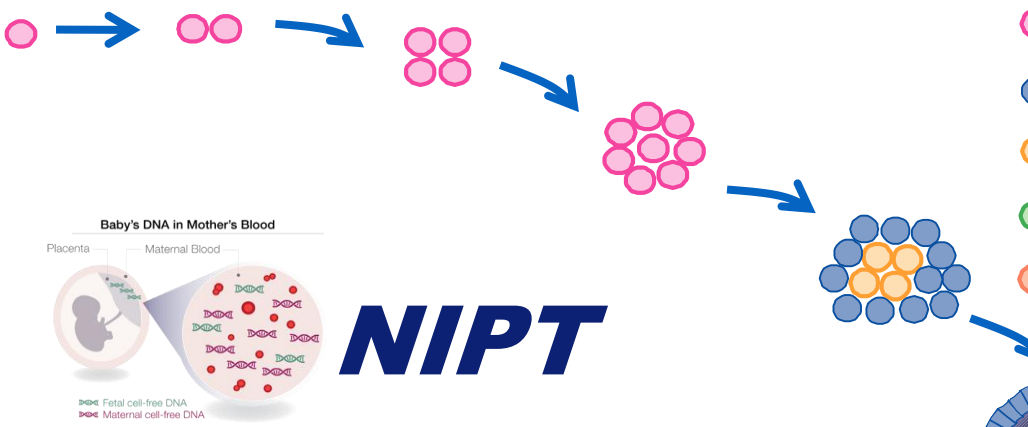


Cordocentesis
~18 weeks

Miscarriage risk 0.6%
(Tanvisut et al., 2020)



Prenatal investigations – tissue origin



NIPT

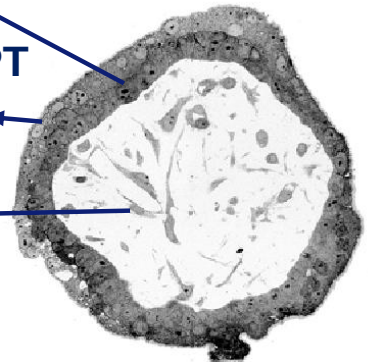
Cytotrophoblast

→ **STC ~ NIPT**

Syncytiotrophoblast

Mesenchymal core

→ **LTC**



CVS

AF

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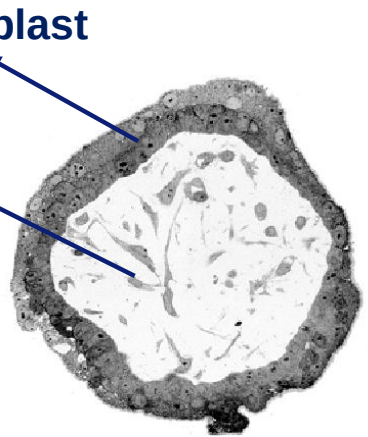
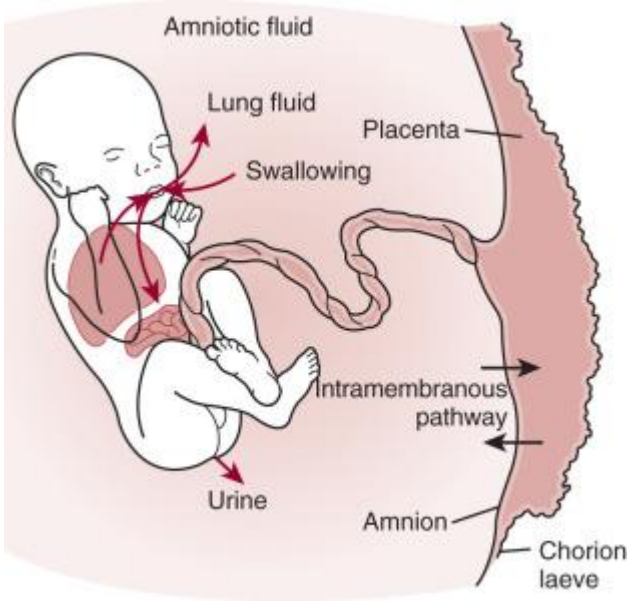
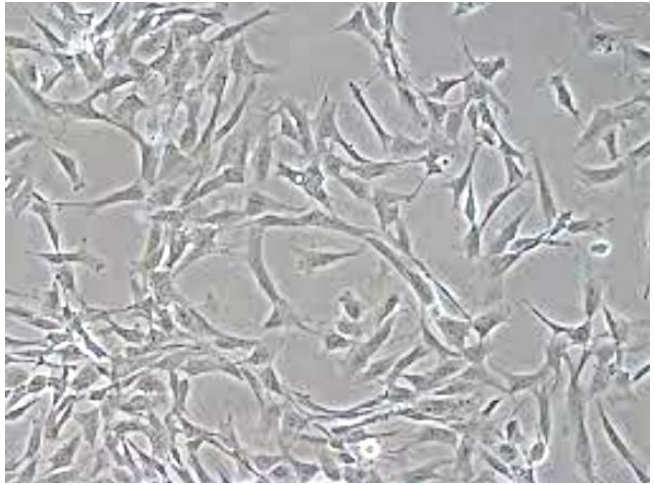
CV – separate before DNA isolation



- 2x wash in PBS
- Incubate 1 hour in **Trypsin/EDTA (0.05%)** in a CO2 incubator at 37˚ C.
- Re-suspend – trophoblast cells will separate from the mesenchymal core
- Transfer the core (villi) to a new dish, wash with PBS
- Transfer to a tube and add collagenase **(Collagenase II 600U/ml medium)**
- Incubate 1 hour in a CO2 incubator at 37˚ C
- Mix and Centrifuge 10 min. 2000 rpm
- Re-suspend pellet in culture medium

Prenatal investigations – tissue origin





Amniocentesis

heterogeneous population that includes both cell type derived from fetal membranes and of the fetus itself

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Types of mosaic

Chorionic villi: ***1-2%,*** *mostly confined placental mosaicism (± 85%)*

Amniotic fluid: ***0,2 %*** *(Hsu et al., 1992)*

	Affected tissues			% of conceptuses	
Type of mosaic	**Cytotrophoblast**	**Mesenchyme**	**AF cells**	**Study 1***	**2***
Confined placental mosaicism					
CPM-I	Abnormal†	Normal	Normal	0.80	0.59
CPM-II	Normal	Abnormal	Normal	0.31	0.68
CPM-III	Abnormal	Abnormal	Normal	0.16	0.17
Total CPM				1.28	1.44
Fetal mosaicism					
TFM-IV	Abnormal	Normal	Abnormal	0	0.02
TFM-V	Normal	Abnormal	Abnormal	0.08	0.09
TFM-VI	Abnormal	Abnormal	Abnormal	0.12	0.10
CFM	Normal	Normal	Abnormal	0.02	–

West JD, Everett CA. Preimplantation chromosomal mosaics, chimaeras and confined placental mosaicism. Reprod Fertil. 2022 Apr 5;3(2):R66-R90

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cfDNA based NIPT

Discordant positive NIPT

Discordant positive NIPT

Discordant negative NIPT

i(21q)

	Affected tissues			% of conceptuses	
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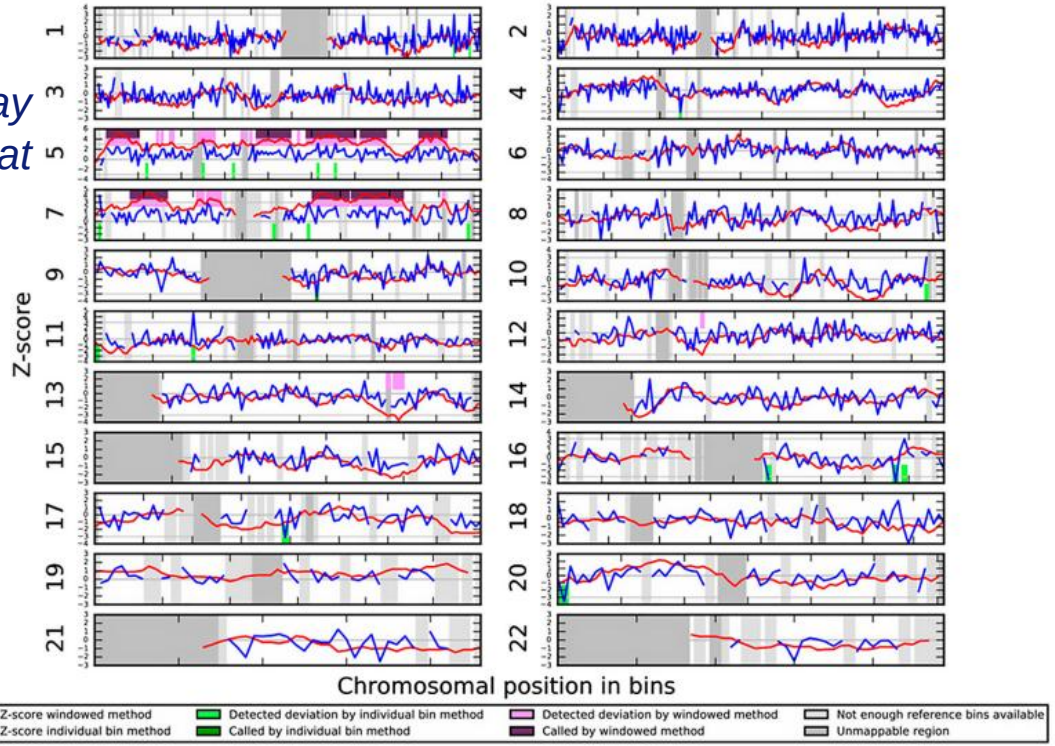
“False” positive NIPT ?

***NIPT: trisomy 5 and trisomy** 7*

Chorionic villi sampling (CVS):

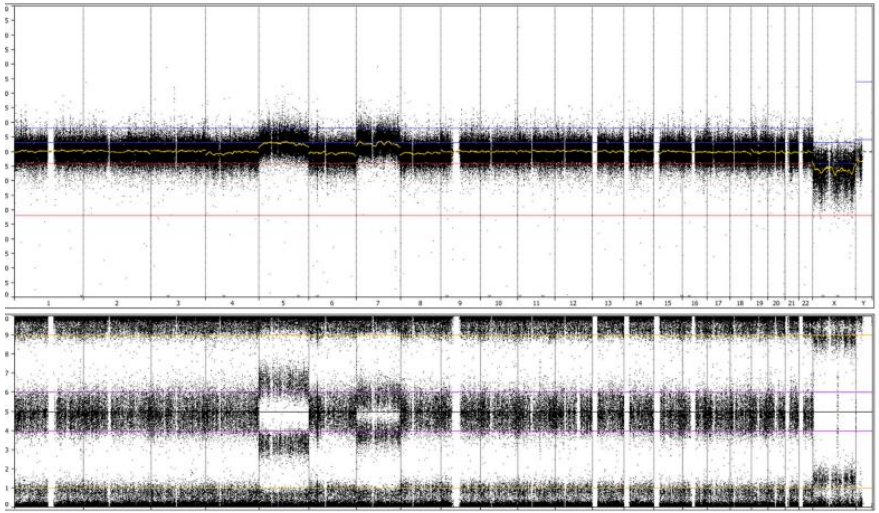
- *Cytotrophoblast - normal SNP array*
- *Mesenchymal core - normal SNP array*
- *Maternal genomic DNA from buffy coat - normal SNP array*

False positive NIPT?
Technical error??
Sample swap??



Placenta mosaicim

Placenta mosaicism-
abnormal CTB in 1 out of 4 biopsies



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DOI: 10.1002/pd.5766

RESEARCH LETTER

PRENATAL DIAGNOSIS WILEY

Noninvasive prenatal testing as compared to chorionic villus sampling is more sensitive for the detection of confined placental mosaicism involving the cytotrophoblast

Diane Van Opstal[1] | Geerke M. Eggenhuizen[2] | Marieke Joosten[1] | Karin Diderich[1] | Lutgarde Govaerts[1] | Robert-Jan Galjaard[1] | Attie Go[2] | Maarten Knapen[2] | Marjan Boter[1] | Wai Y. Cheung[1] | Nicole van Koetsveld[1] | Stefanie van Veen[1] | Walter G. de Valk[1] | Fernanda Jehee[1] | Femke de Vries[1] | Iris Hollink[1] | Lies Hoefsloot[1] | Malgorzata Srebniak[1]

[1]Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands

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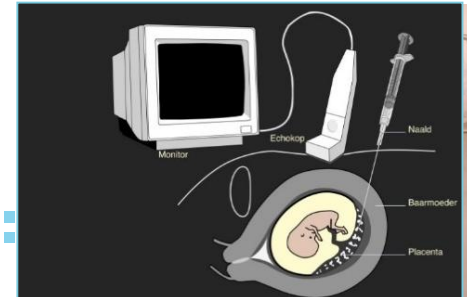
Email: a.vanopstal@erasmusmc.nl



REVIEW ARTICLE

Check for updates

Rare autosomal trisomies detected by non-invasive prenatal testing: an overview of current knowledge

Lore Lannoo[1], Khaila van Straaten[2], Jeroen Breckpot[3], Nathalie Brison[3], Luc De Catte[1], Eftychia Dimitriadou[3], Eric Legius[3], Hilde Peeters[3], Ilse Parijs[3], Olga Tsuiko[3], Leen Vancoillie[3], Joris Robert Vermeesch[3], Griet Van Buggenhout[3], Kris Van Den Bogaert[3], Kristel Van Calsteren[1] and Koenraad Devriendt[3]✉

T2, T9, T12, T15, T16, T22

Table 1. Summary of characteristics of the different RAT's included in this review.

	Relative frequency (CI 95%)[a]	Absolute frequency[b]	% meiotic	Risk of fetal trisomy[c]	False negative amniocentesis	Fetal blood sampling	Consequences of CPM	Outcome fetal trisomy	Test for UPD
T2	2.4% (95% CI 1.6–3.7)	0.0059% 1/17010	NIPT: mainly mitotic	ASC: 4/11 (36%) (95% CI 11–69) GOP: 1/6 (16.7%) (95% CI 0.4-64)	not described	No data to support FBS	° increased risk IUGR ° correlated to T2M level	° 18/21 (86%) abnormal outcome ° 9/21 (46%) major malformations ° no correlation to levels of T2M	not indicated
T3	5.1% (95% CI 3.8–6.7)	0.012% 1/8151	CVS: mainly mitotic	All: 0/13 (0%) (95% CI 0–25) GOP: 0/12 (0%) (95% CI 0–26)	not described	No data to support FBS	° possible risk of IUGR	° 3/5 normal development > 1 yr ° favorable in absence of mult. malformations ° insufficient data to correlate to T3M level	not indicated
T7	30.5% (95% CI 27.8–33.5)	0.073% 1/1368	mainly mitotic	All: 2/163 (1.2%) (95% CI: 0.2–4.4) GOP: 0/137 (0%) (95% CI: 0–2.6)	not described	No data to support FBS	° elevated risk of birth weight below 2.3rd centile (RR 5) (95% CI 2.6-9.8)	° favorable outcome ° low incidence of malformations (renal) ° intellectual development normal ° no correlation to levels of T7M	1% (1/109) risk UPD7mat (Silver-Russel syndrome)

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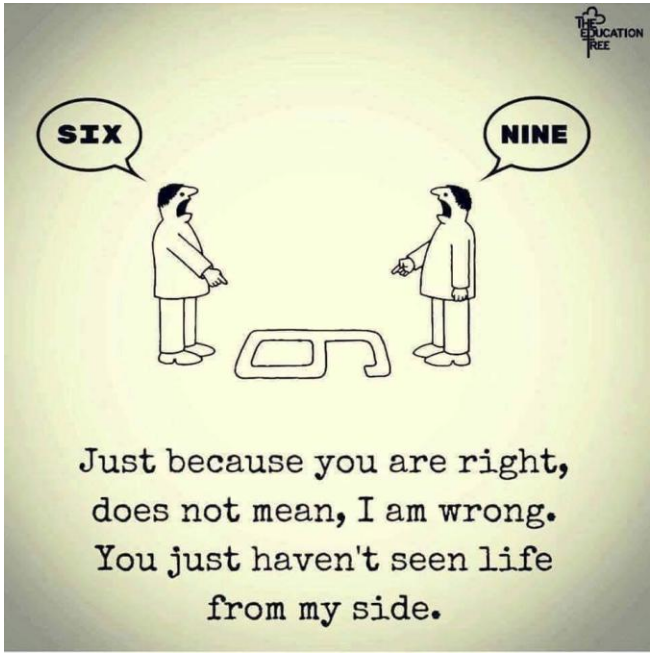
Good prenatal diagnostic flow provides early diagnosis

We are so proud of the innovations in genetic testing in prenatal diagnosis.

We are efficient. We perform less invasive tests and to ensure high diagnostic yield, we carefully select patients indications to perform appropriate tests.

We work with high through put techniques = We perform more tests, more patients can be helped!

How to implement the testing to serve our patients needs?



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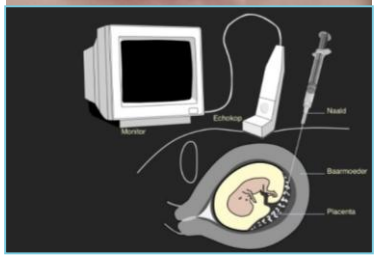
Advanced Maternal Age 42 (risk 1:54)

2005

Chorionic villi sampling at 11 weeks

Karyotyping in Short Term Culture – STC results within 2-3 days

Down syndrome – **diagnosis at *~11.3 weeks***



2017

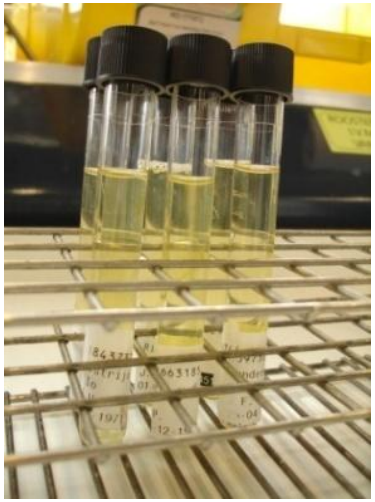
AMA– no indication for invasive prenatal diagnosis

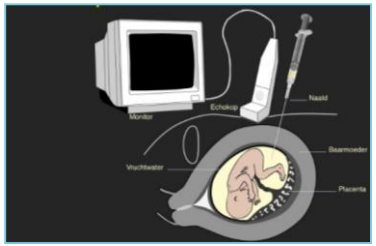
NIPT results at 12.2 wk

"After NIPT amniocentesis should be performed"

Amniocentesis at 15.4 weeks - QF-PCR and microarray

Non-mosaic Down syndrome – **diagnosis at *~16 weeks***





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Why can't we perform CVS after NIPT?

EXPERT REVIEW OF MOLECULAR DIAGNOSTICS, 2016
VOL. 16, NO. 5, 513–520
http://dx.doi.org/10.1586/14737159.2016.1152890

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SPECIAL REPORT

OPEN ACCESS

Cytogenetic confirmation of a positive NIPT result: evidence-based choice between chorionic villus sampling and amniocentesis depending on chromosome aberration

Diane Van Opstal and Malgorzata I Srebniak

Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, the Netherlands

ABSTRACT

It has been shown that in non-invasive prenatal testing (NIPT) there is a small chance of a false-positive or false-negative result. This is partly due to the fact that the fetal cell-free DNA present in maternal plasma is derived from the cytotrophoblast of chorionic villi (CV), which is not always representative for the fetal karyo
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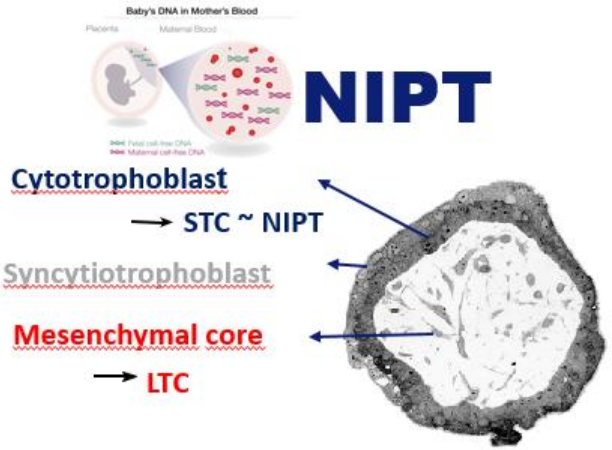
However, sinc
can be done f
for the prosp
investigations
confirmation

ARTICLE HISTORY
Received 13 November 2015
Accepted 8 February 2016
Published online

Table 3. Risk for a confirmatory amniocentesis after CVS for the different common aneuploidies based on the present study (of ACC CVS database [27]) and the single center study of Grati et al. [32].

Chromosome aberration	Present study N = 704	Grati et al. (2015) N = 1512
Trisomy 13	8.3%	22%
Trisomy 18	3.2%	4%
Trisomy 21	1.6%	2%



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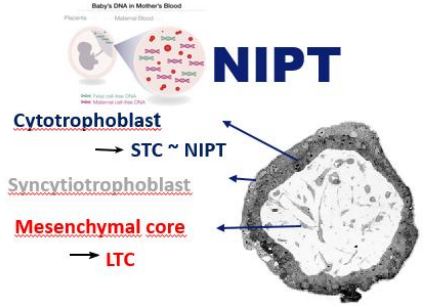

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ARTICLE HISTORY
Received 13 November 2015
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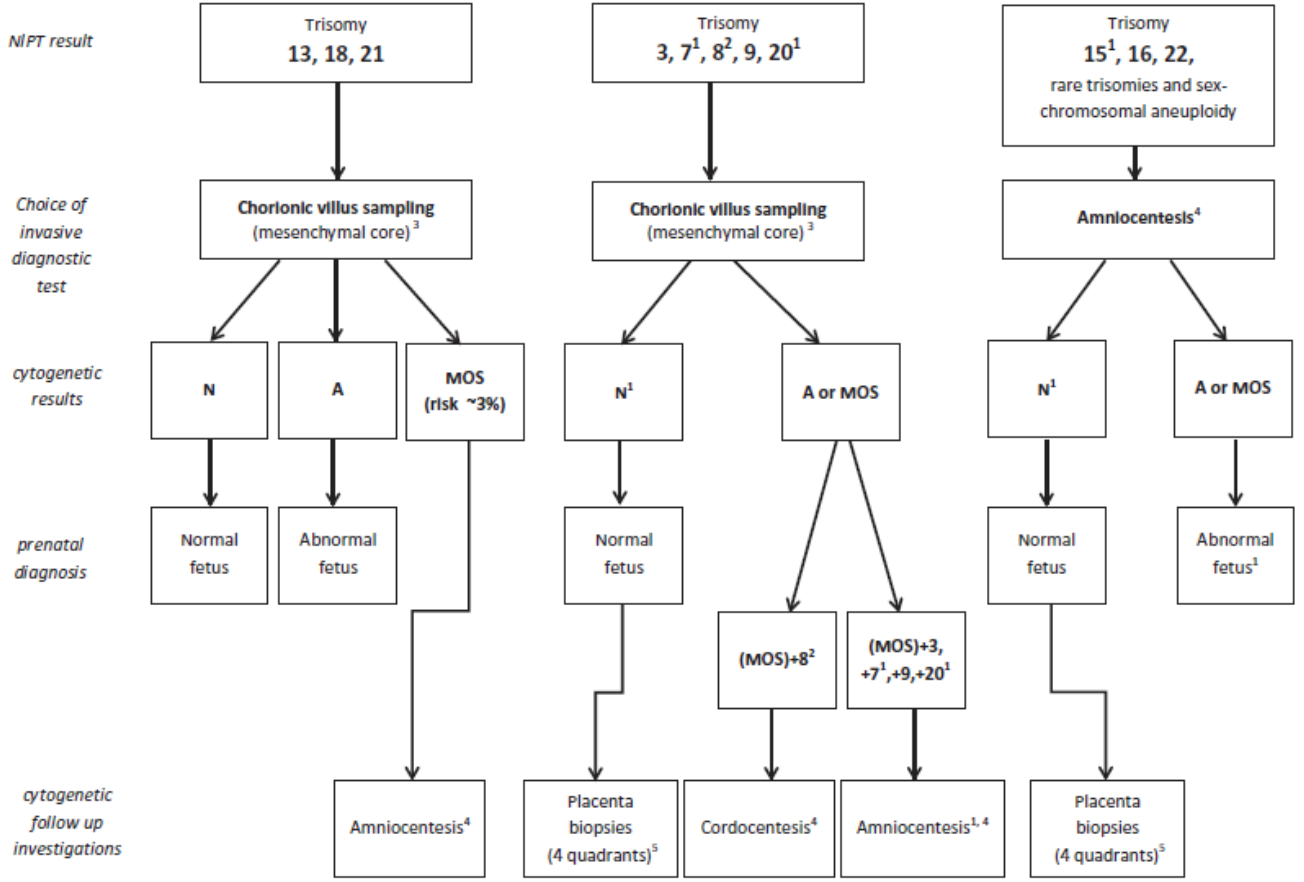
Published online
1 March 2016

KEYWORDS
NIPT; non-invasive prenatal diagnosis; prenatal diagnosis; trisomy 13; trisomy 18; trisomy 21; autosomal trisomy; confined placental mosaicism; CPM; chorionic villi; amniocentesis



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70% patients chose early diagnosis by CVS
96% had definitive diagnosis by CVS

Total number of patients with high risk for T21, T18 or T13 (AF + CVS)
n= 395

Excluded:
AF (NIPT performed after 13.4 wk) n = 44
AF (NIPT performed elsewhere) n=9

AF
n= 101 ***(29%)***

CVS
n= 241 ***(71%)***

2017-2022

retrospective data analysis

follow up studies in AF and CVS after NIPT showing increased risk for trisomy 21, 18 or 13

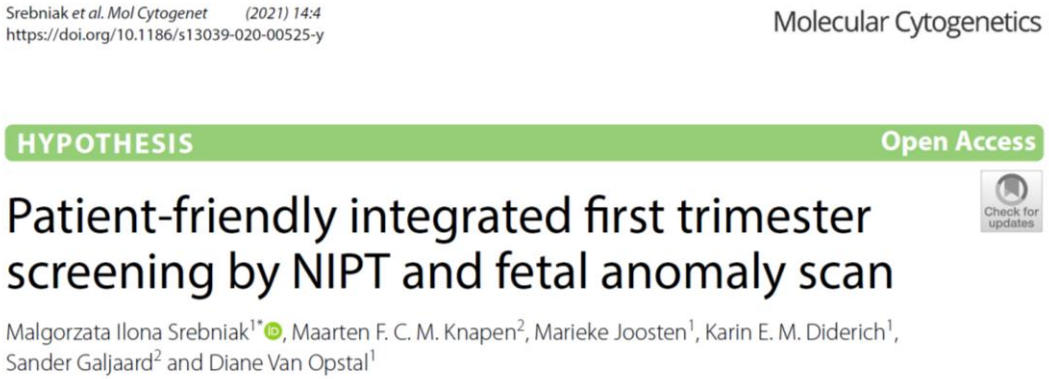
Abnormal NIPT result	T13	T18	T21	T13/18/21
Final report based on CVS (separate cytotrophoblast and mesenchymal core analysis)	**85%**	**100%**	**97%**	**96%**

New integrated prenatal screening

Genotyping first or phenotyping first?

NIPT possible at 10 wks

Fetal Anomaly Scan optimal at 13-14 wks



Srebniak *et al. Mol Cytogenet* (2021) 14:4
https://doi.org/10.1186/s13039-020-00525-y

Molecular Cytogenetics

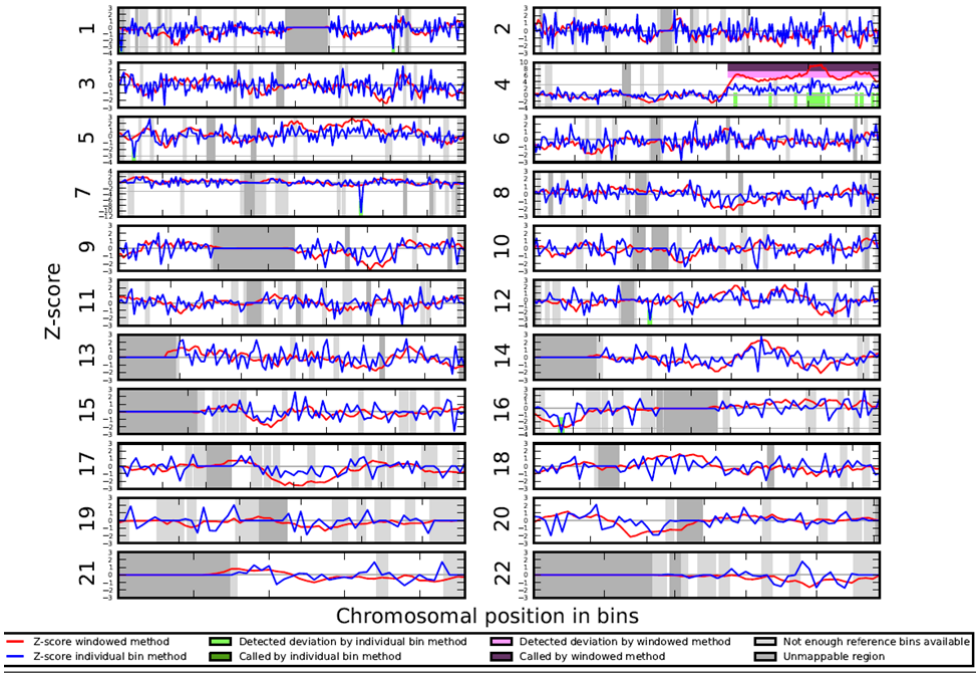
HYPOTHESIS Open Access

Patient-friendly integrated first trimester screening by NIPT and fetal anomaly scan

Malgorzata Ilona Srebniak[1*], Maarten F. C. M. Knapen[2], Marieke Joosten[1], Karin E. M. Diderich[1], Sander Galjaard[2] and Diane Van Opstal[1]

Both NIPT and early ultrasound detect a comparable percentage of aberrant cases and each component has an important impact, but cannot fully substitute the other.

NIPT: 4q terminal gain



Which follow up test should be done?

FISH 4qter probe?
Karyotyping?
QF-PCR?
Microarray?
Sequencing?

WISECONDOR

Straver et al., 2014,
Nucleic Acids Res. 2014 Mar; Vol. 42, No. 5 e31
doi:10.1093/nar/gkt9922(5):

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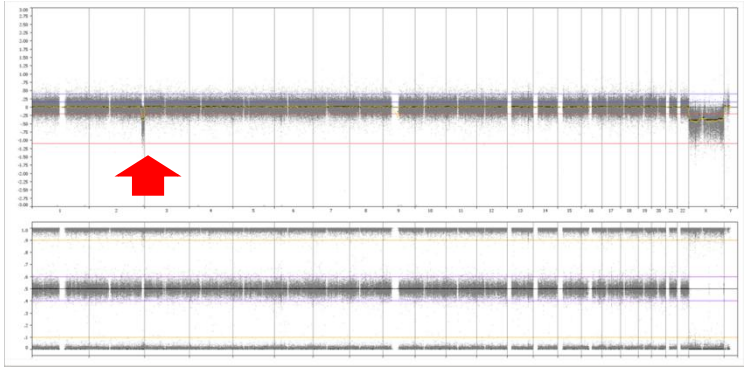
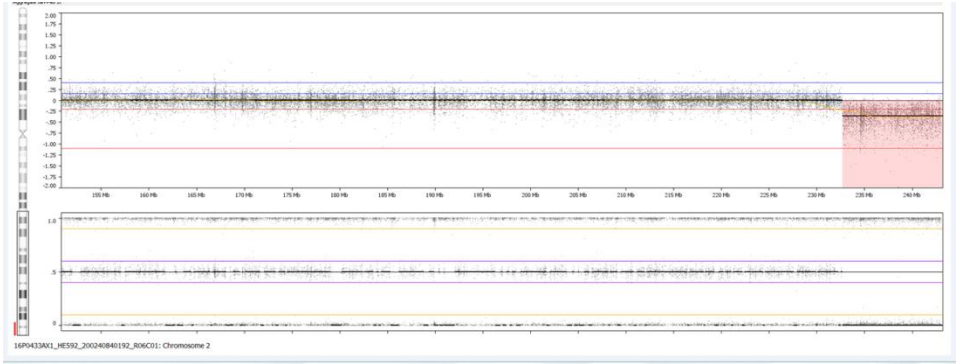
Amniocentesis: 2qter deletion





10 Mb deletion: 46,XY,del(2)(q37.1)
~2q37 deletion syndrome

Parents normal karyotypes

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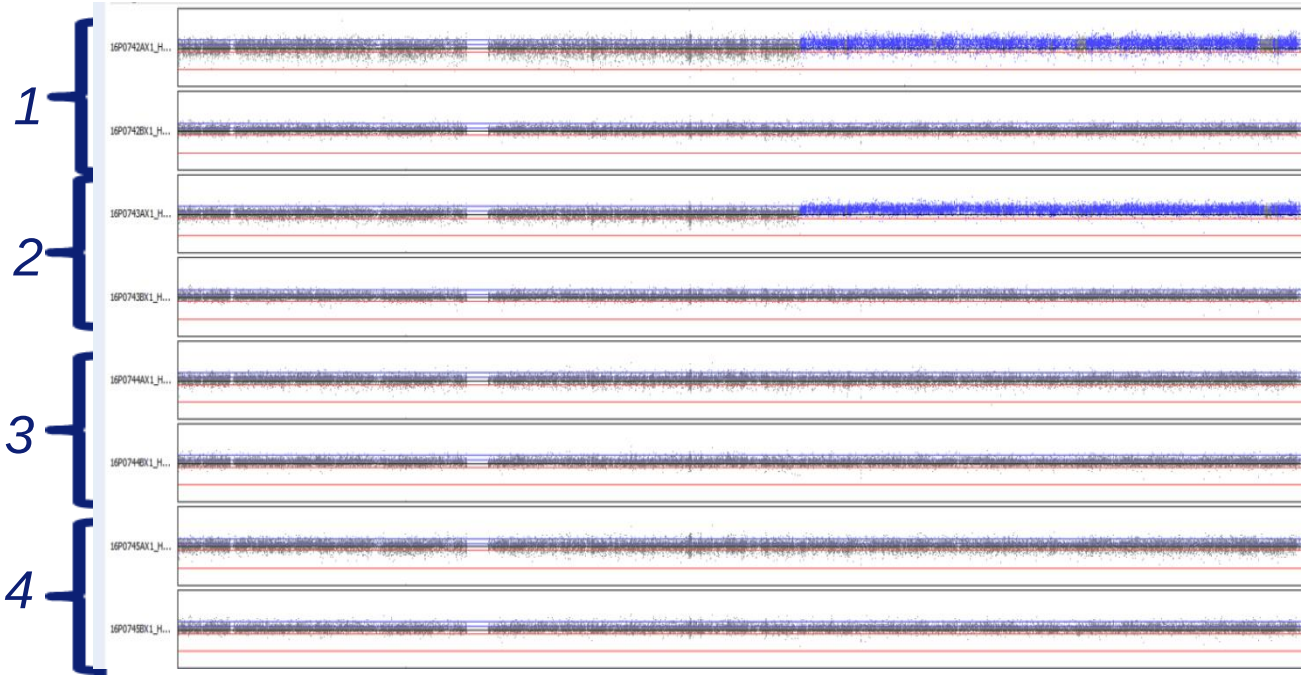
Placenta: chromosome 4



85 Mb duplication 2/4 biopsies (CTB) → ***NIPT confirmed***

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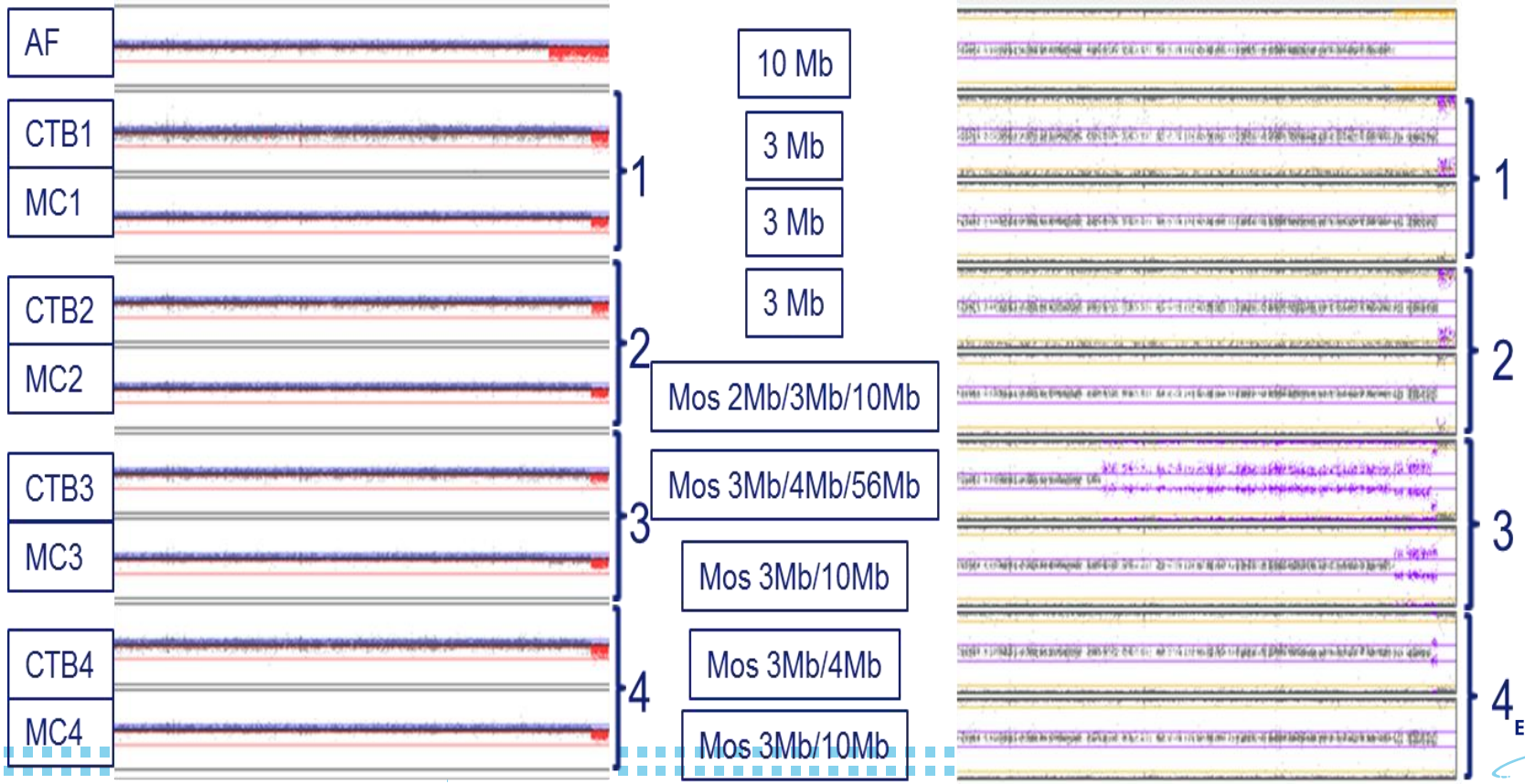
Placenta: chromosome 2



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Are these very exceptional cases? Whole genome follow up test needed!

Received: 19 April 2019 | Revised: 3 July 2019 | Accepted: 15 July 2019
DOI: 10.1002/pd.5531

ORIGINAL ARTICLE

WILEY PRENATAL DIAGNOSIS

Placental studies elucidate discrepancies between NIPT showing a structural chromosome aberration and a differently abnormal fetal karyotype

Diane Van Opstal[1] | Stefanie van Veen[1] | Marieke Joosten[1] | Karin E.M. Diderich[1] | Lutgarde C.P. Govaerts[1] | Joke Polak[1] | Nicole van Koetsveld[1] | Marjan Boter[1] | Attie T.J.I. Go[2] | Dimitri N.M. Papatsonis[3] | Krista Prinsen[2] | Lies H. Hoefsloot[1] | Malgorzata I. Srebniak[1]

Do not use targeted technique for genome wide NIPT follow up.

Conclusion: Our study shows that targeted cytogenetic investigations for confirmation of NIPT showing a microscopically visible structural chromosome aberration should be avoided, since another aberration, even a submicroscopic one or one involving another chromosome, may be present in the fetus.

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Zuffardi at al.

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Review

The embryo battle against adverse genomes: Are de novo terminal deletions the rescue of unfavorable zygotic imbalances?

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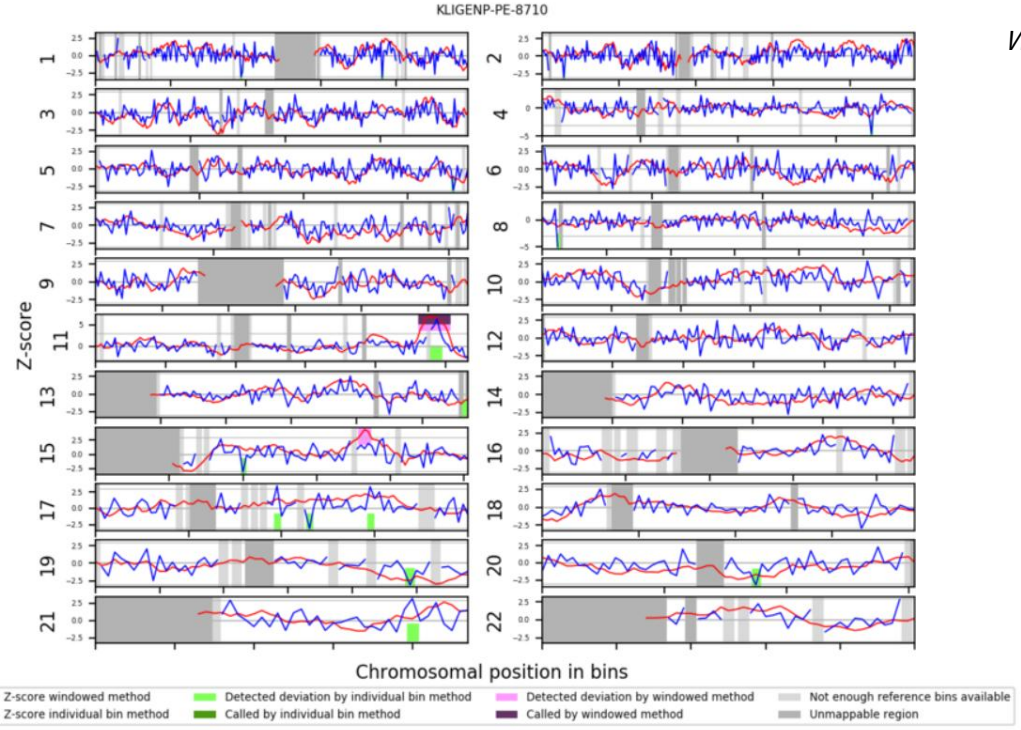
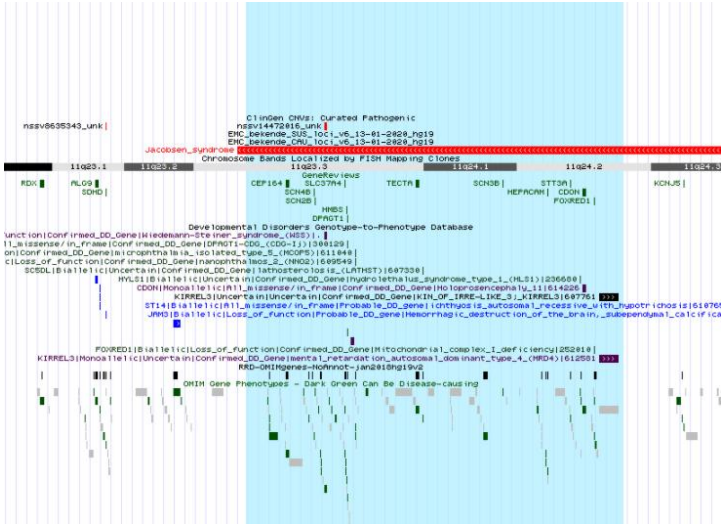
Report only (likely) pathogenic findings



Windowed, bin test: ***11Mb*** *chr11:116000000-127000000, z= 5.636*



To report or not to report?

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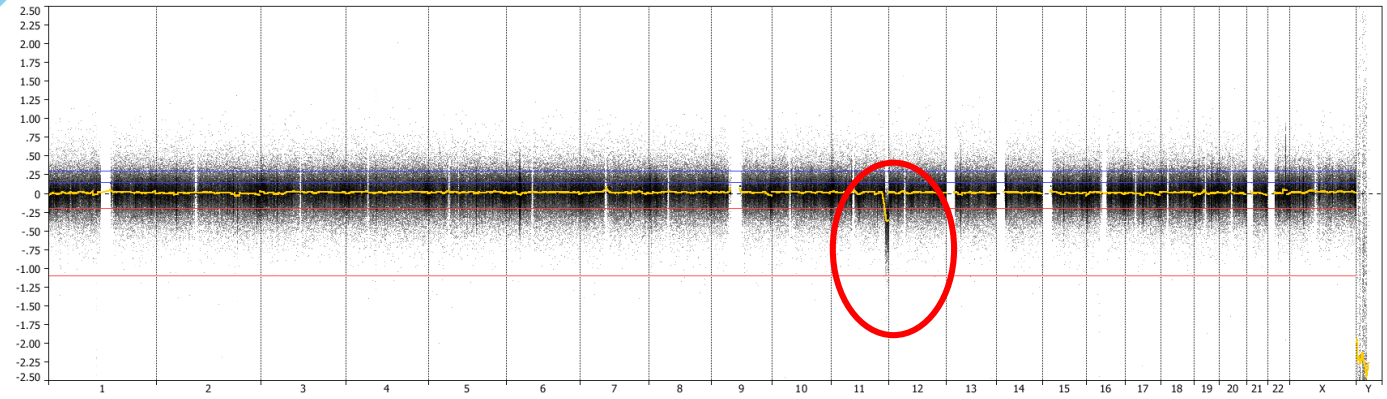
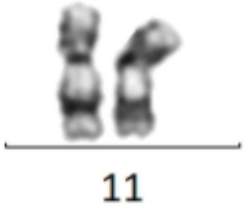
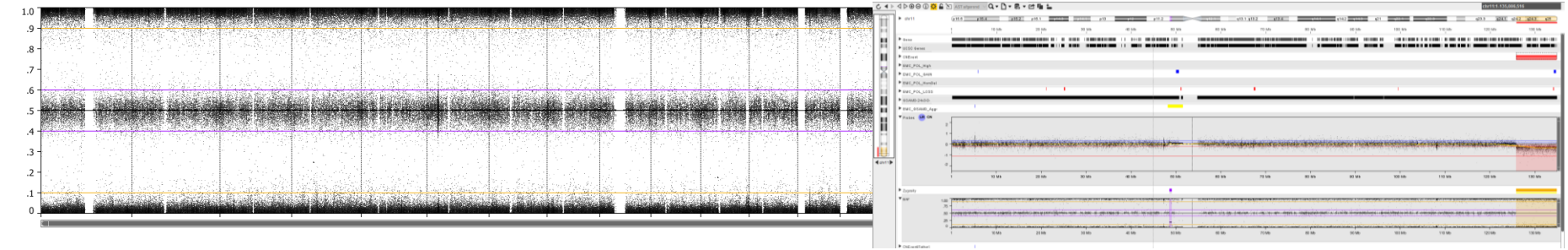
Amniocentesis at 15 3/7 weeks







*de novo terminal loss van ca. 9 Mb in band 11q24.2q25 associated with **Jacobsen syndrome.***

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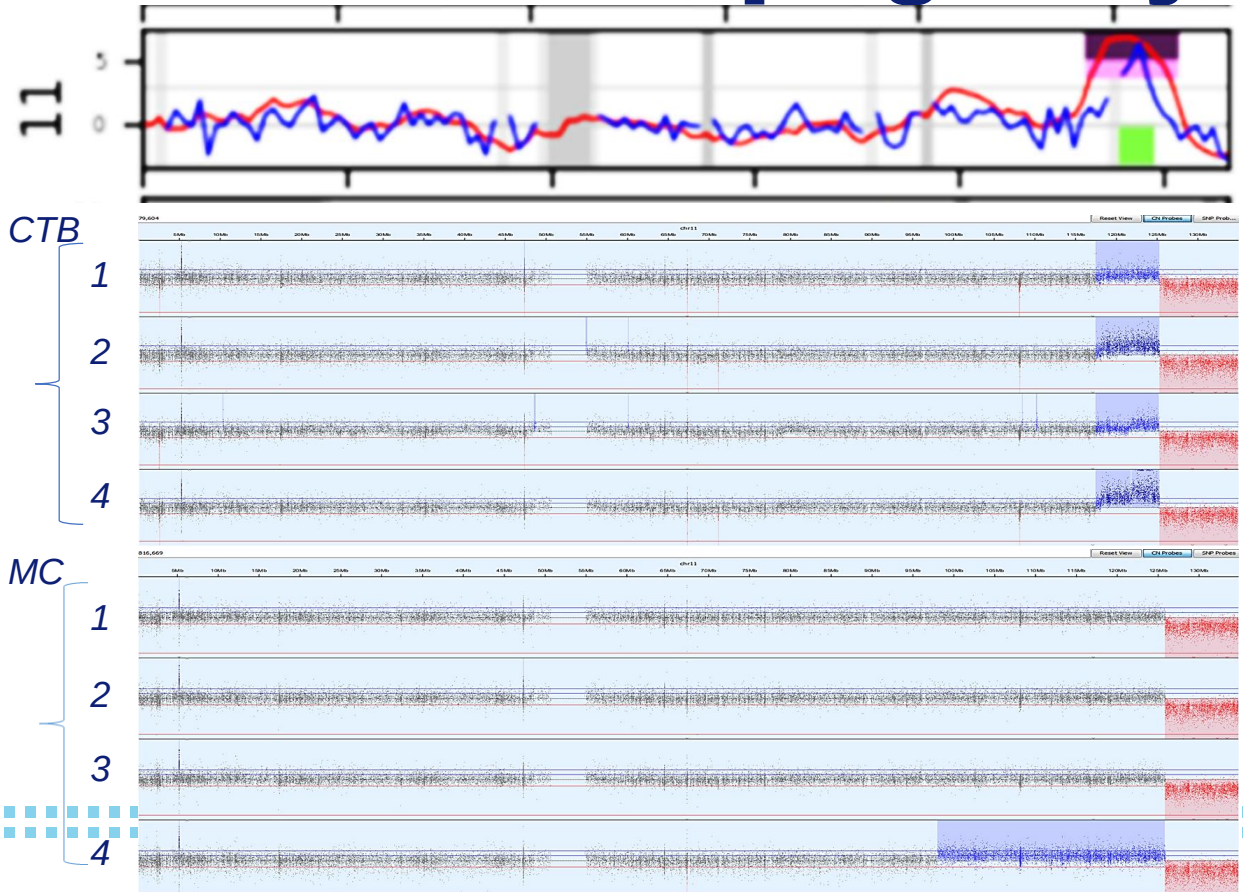
Placenta investigation after temination of pregnancy



Cytotrophoblast

mos loss 10Mb
mos gain ~8 Mb

Mesenchymal core

Loss 9Mb

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Pseudo-mosaicism versus true mosaicism

Pseudo-mosaicism Type A – one cell in a colony

Pseudo-mosaicism Type B – all cells of one single colony abnormal

Pseudo-mosaicism Type C – multiple colonies in the same culture dish

True mosaicism – two or more abnormal colonies in at least two independent in situ culture dishes.

Indications for extensive work-up (24 cell clones excluding the original dish with abnormal colony)

- Autosomal trisomy involving chromosomes 2, 5, 8, 9, 12, 13, 14, 15, 16, 18, 20, 21, 22 (SCo, MCo)
- Unbalanced structural rearrangement (MCo)
- Marker chromosome (MCo)

Indications for moderate work-up (12 cell clones excluding the original dish with abnormal colony)

- Autosomal trisomy involving a chromosome 1, 3, 4, 6, 7, 10, 11, 17, 19 (SCo, MCo)
- Unbalanced structural rearrangement (SCo) * Marker chromosome (SCo)
- Extra sex chromosome (SCo, MCo)
- 45, X (SCo, MCo)
- Balanced structural rearrangement (MCo)
- Monosomy (other than 45,X) (SCo, MCo)

SCo: single colony/single dish; MCo: multiple colonies/single dish

No additional work-up

- Balanced structural rearrangement (SCo)
- Break at centromere with loss of one arm (SCo)
- All single cell abnormalities

(Hsu et al., 1992; Hsu and Benn, 1999)

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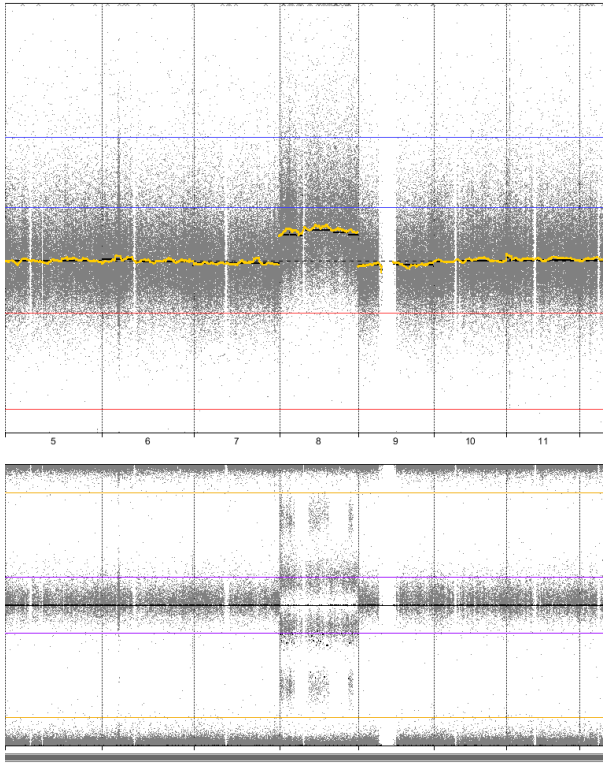
Take home message

- **NIPT is more sensitive** for placental mosaicism than CVS
- Analysis of mesenchymal core of **CV can be offered** as a follow up in case of T21, T18, T13
- Most patients choose CVS as it gives a high probability for early final results (96%)
- Be aware of **mosaicism in case of a structural aberration** found with NIPT – offer amniocentesis and whole genome follow up test
- Be aware that low percentage mosaicism in amniotic fluid may derive from **extraembryonic cells**



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