ALGORYTHMY POSTEPOWANIA SCREENING PRENATALNY ORAZ DIAGNOSTYKA GENETYCZNA

Malgorzata I. Srebniak
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THE GOAL OF PRENATAL GENETIC DIAGNOSIS

The goal is to inform pregnant on the genetic status of the fetus to allow **reproductive autonomy**

- Early diagnosis enables medical interventions
- More time to be prepared for a child with “extra needs”
- Early diagnosis facilitates future therapy
- Early diagnosis enables early elective termination of pregnancy
- Provides information on risks in future pregnancies

Reproductive autonomy is the power to make and act on decisions about reproduction

Making decisions is only possible when choices are available!

PREGNANCY MANAGEMENT IN NL

General population

Abnormal screening
High risk pregnancy

Abnormal diagnostic test

Midwife

NIPT screening
First trimester ultrasound
Second trimester ultrasound
Delivery

Gynecologist

First trimester ultrasound
Second trimester ultrasound
Delivery

Gynecologist University Center

(medical) NIPT testing
Invasive testing
Fetal therapy

Clinical Geneticist

Genetic testing

Gynecologist
Clinical Geneticist

Adjusted pregnancy management

Termination of pregnancy allowed < 24 weeks

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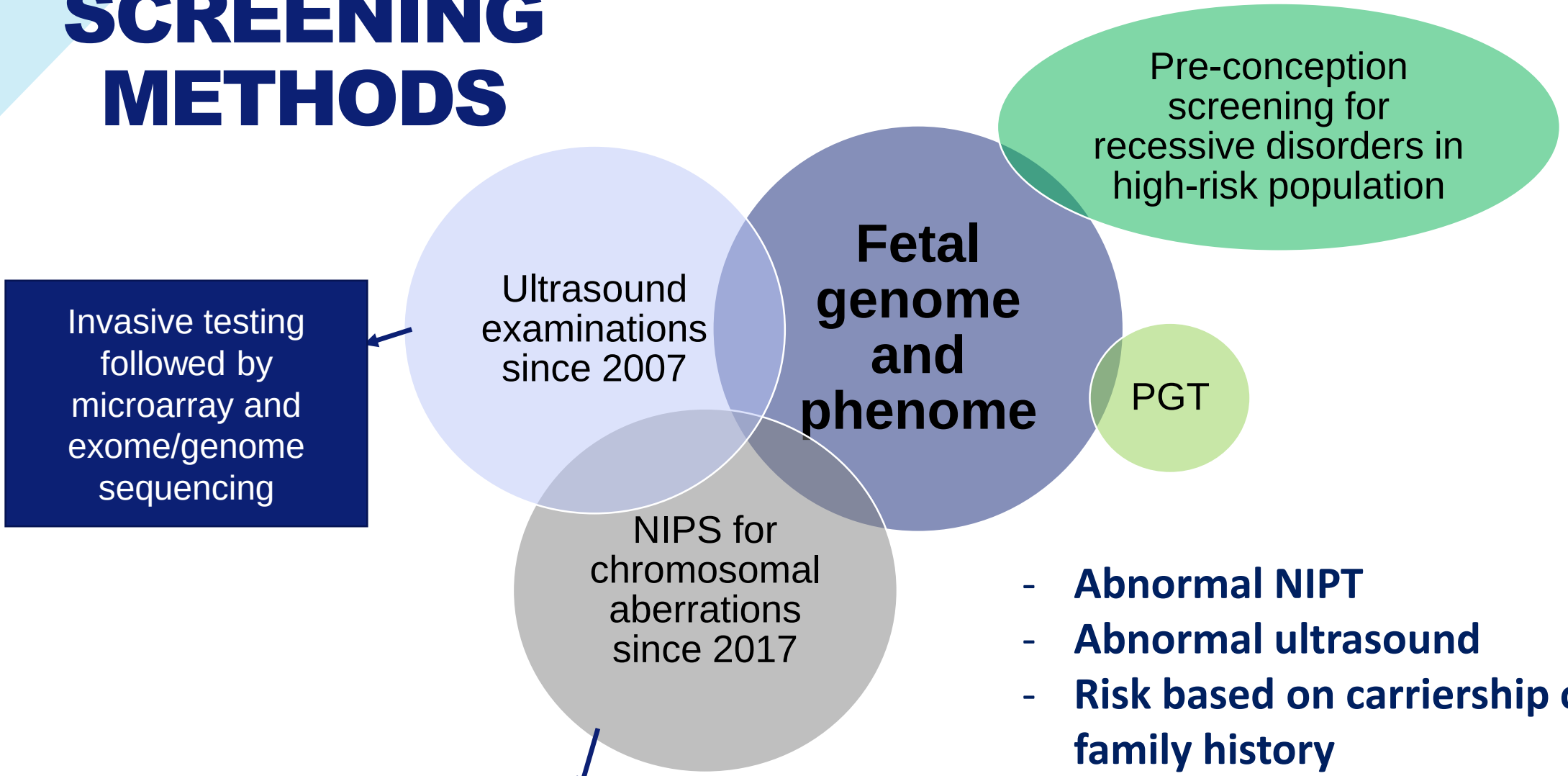
SCREENING METHODS



- **Abnormal NIPT**
- **Abnormal ultrasound**
- **Risk based on carriership or family history**
- **Patient undergoing an invasive procedure due to other indication**

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NEDERLANDSE VERENIGING VOOR OBSTETRIE EN GYNAECOLOGIE

Leidraad indicatiestelling prenatale diagnostiek

versie 7 februari 2019
aanpassing 10 juli 2019, aanpassing 21 februari 2023

1. Increased risk for genome anomalies
   - Previous pregnancy with genetic anomalies
   - Parent with a genetic disorder
   - Single gene disorder carrier status of the parent
   - Carrier status for chromosomal aberration
2. Abnormal NIPT
3. Ultrasound anomaly
4. Failed NIPT (2x)
5. Indication for cytogenomic test due to other indication for invasive test (e.g. seroconversion, hematological analysis etc.)
6. Pregnancy resulting from assisted reproduction by ICSI
7. Residual risk after preimplantation genetic test (PGT) procedure
8. Other special cases only after extensive pre-test counseling

ABNORMAL NIPT – WHAT'S NEXT?

Chorionic villi biopsy
(CVS)
10-14 weeks

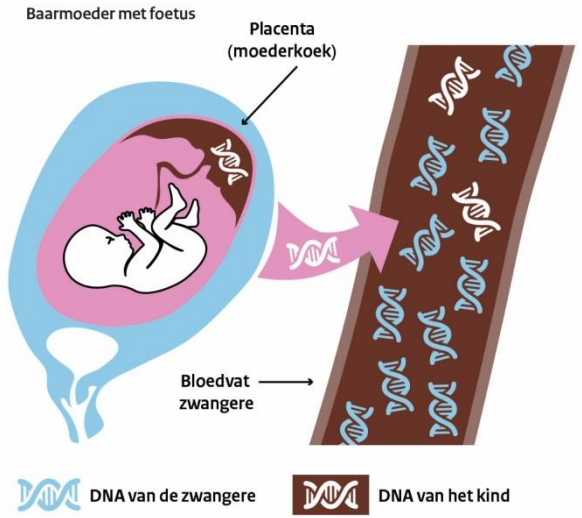
Miscarriage risk 0.2%
(Akolekar et al., 2015)

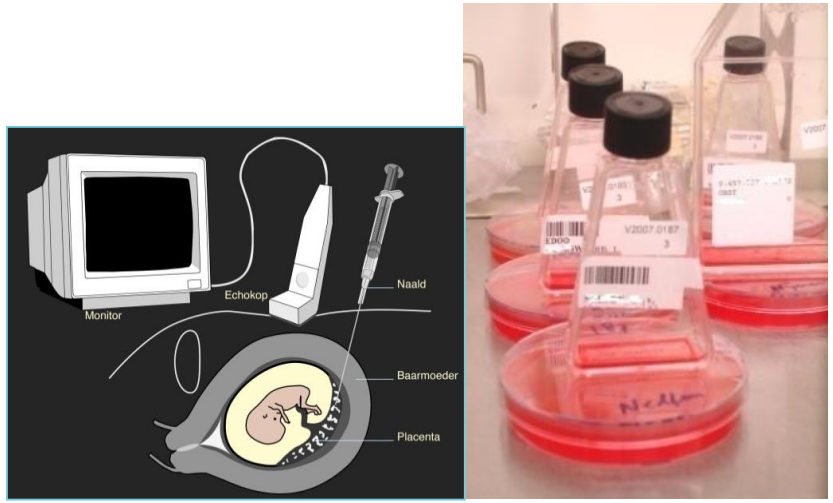
1-2% mosaic, mostly confined placental mosaicism (± 85%)

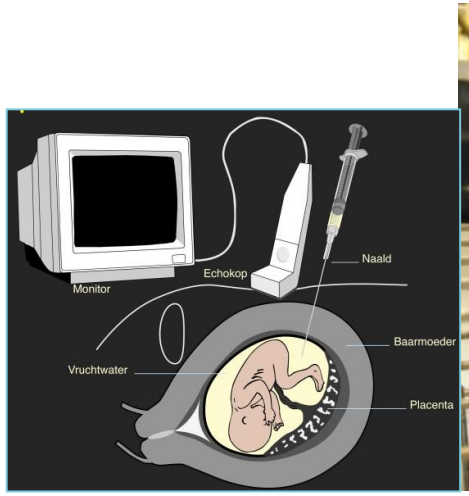
Amniocentesis
>16 weeks

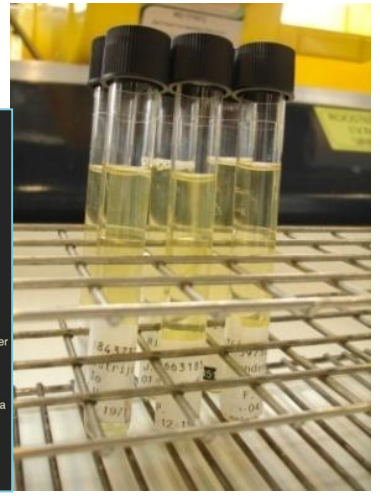
Miscarriage risk 0.1%
(Akolekar et al., 2015)

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









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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

Taylor & Francis
Taylor & Francis Group

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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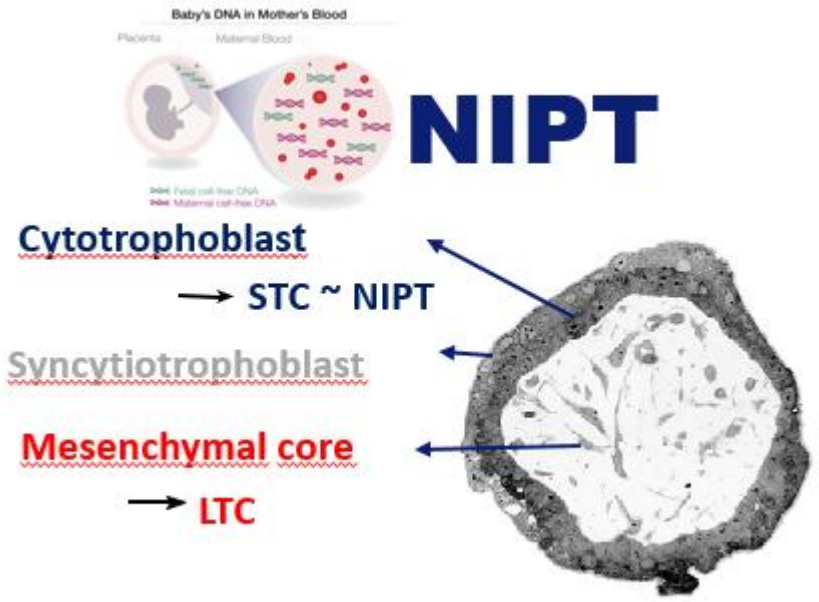
can be done f
for the prosp
investigations
confirmation

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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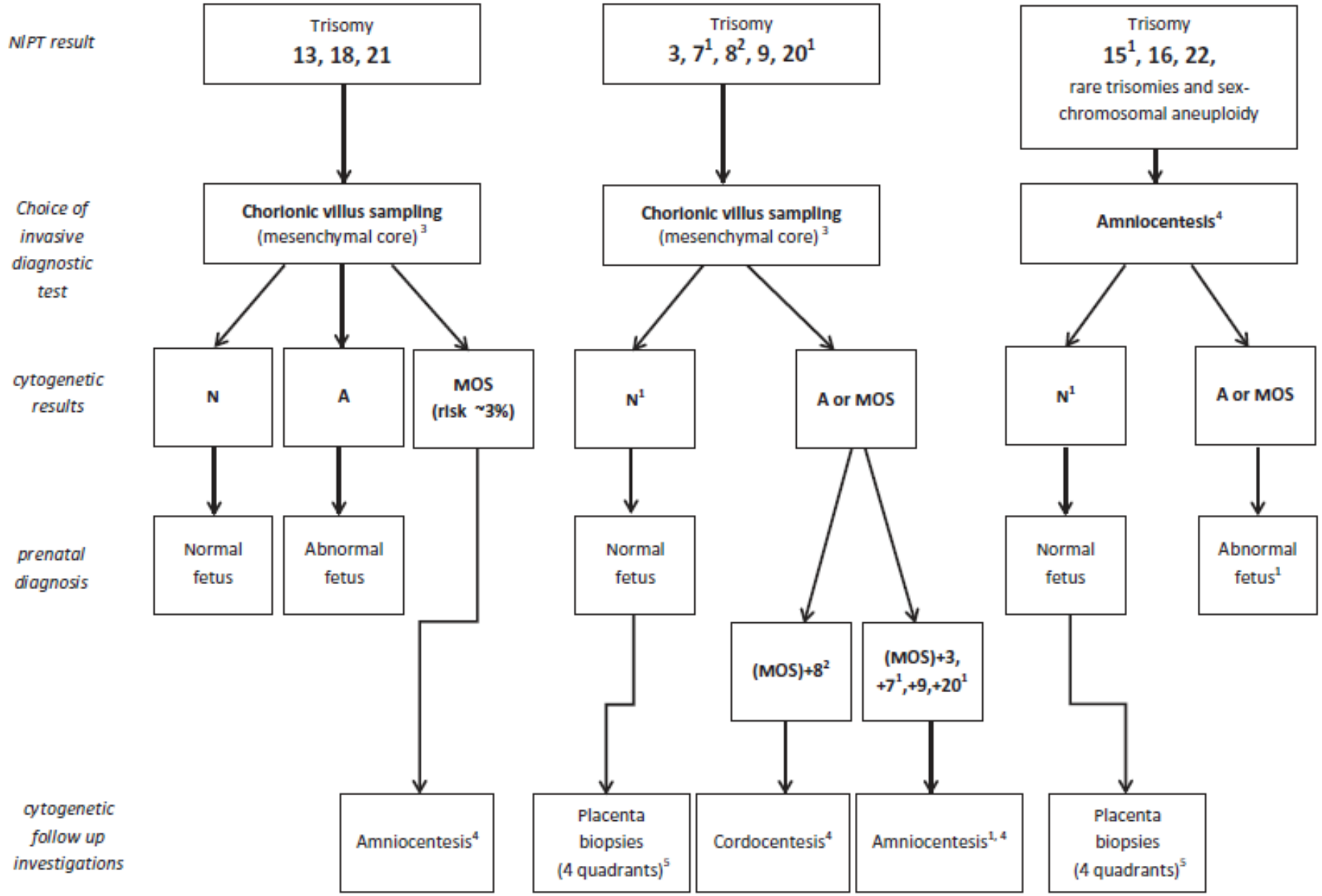
WHY CAN'T WE PERFORM CVS AFTER NIPT?



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RISK FOR SECONDARY AMNIOCENTESIS

Prenatal Diagnosis

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PRENATAL DIAGNOSIS

ORIGINAL ARTICLE OPEN ACCESS

Chorionic Villus Sampling for Rapid Confirmation of High-Risk NIPT Results for Trisomy 21, 18, and 13

Malgorzata I. Srebniak[1] | Marjolein Weerts[1] | Marieke Joosten[1] | Mark Drost[1] | Robert Jan Galjaard[1] | Vyne van der Schoot[1] | Myrthe van den Born[1] | Maarten F. C. M. Knapen[2] | Krista Prinsen[2] | Jerome M. J. Cornette[2] | Philip L. J. DeKoninck[2] | Dimitri Papatsonis[3] | Julia Spaan[3] | Anneke Dijkman[4] | Sabina de Weerd[5] | Attie T. J. I. Go[2] | Karin E. M. Diderich[1] | Diane Van Opstal[1]

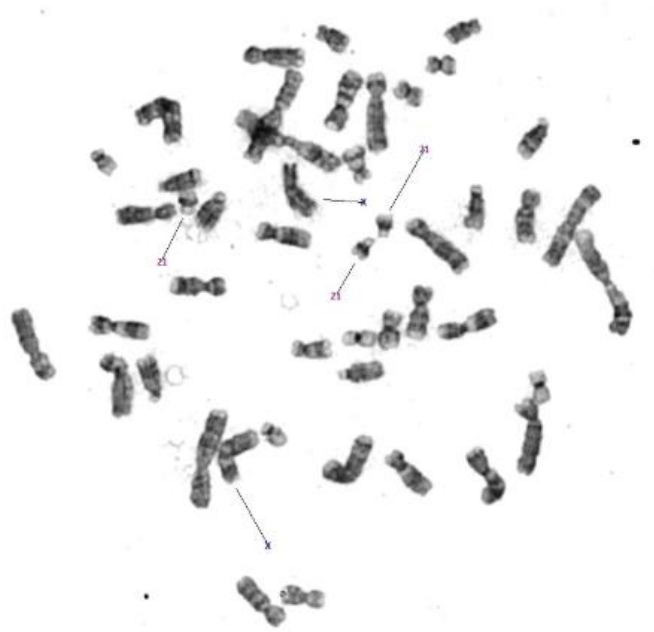
- What does this study add?
  - Our study showed that CVS provided conclusive results for 97.5%, 100%, and 86.5% of patients with high-risk NIPT results for trisomy 21, 18, and 13, respectively.
  - The risk for a secondary amniocentesis is the highest for trisomy 13 (13.5%) and lower for trisomy 21 (2.5%) and trisomy 18 (0%).

ABNORMAL NIPT – WHAT'S NEXT?

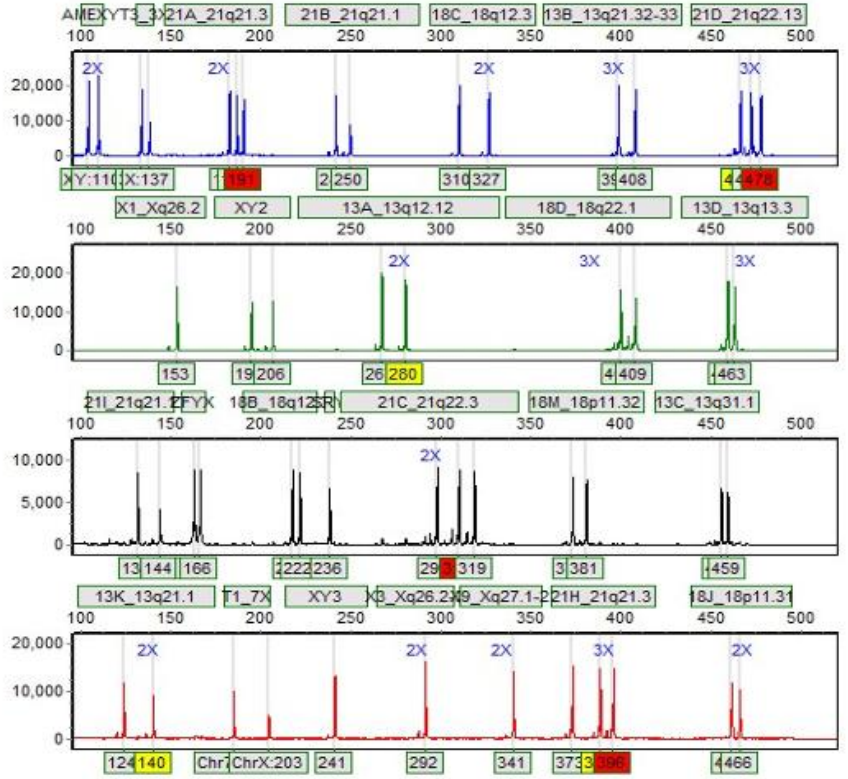
Trisomy 21, 18, 13 -> Rapid Aneuploidy Detection (RAD) by QF-PCR

If positive for T13 or T21 -> karyotyping

If positive for T18 -> no follow up



Marker	Alleles	Allele Length	Peak Area	Peak Ratio	Check
13A_13q12.1	2	267:280	47336:44919	1.05	
13B_13q21.3	2	399:408	36981:39567	0.93	x
13C_13q31.1	2	456:459	37848:35735	1.06	
13D_13q13.3	2	459:463	38265:34050	1.12	
13K_13q21.1	2	124:140	27155:22035	1.23	
18B_18q12.3	2	218:222	41382:40755	1.02	
18C_18q12.3	2	310:327	54413:49467	1.10	
18D_18q22.1	2	401:409	29673:28334	1.05	
18J_18p11.31	2	461:466	36190:31874	1.14	
18M_18p11.3	2	373:381	42049:40800	1.03	
21A_21q21.3	3 (1:1:1)	183:187:191	41283:38908:34831		
21B_21q21.1	3 (2:1)	242:250	82272:43810	1.88	
21C_21q22.3	3 (1:1:1)	298:310:319	23007:23971:23213		
21D_21q22.1	3 (1:1:1)	466:472:478	39160:40222:39243		
21H_21q21.3	3 (1:1:1)	373:388:396	27255:27433:27178		
21I_21q21.1	3 (2:1)	132:144	44472:22049	2.02	
AMEXY	2	X:104:Y:110	46047:49959	0.92	
SRY	1	236	32883		
T1_7X	3 (2:1)	Chr7:183:Chr?	45321:22601	2.01	
T3_3X	3 (2:1)	3:133:X:137	90933:46006	1.98	
X1_Xq26.2	1	153	88972		
X3_Xq26.2-3	1	292	39249		
X9_Xq27.1-2	1	341	38428		
XY2	2	195:206	53822:56801	0.95	
XY3	1	241	62453		
ZFYX	2	163:166	48390:47779	1.01	



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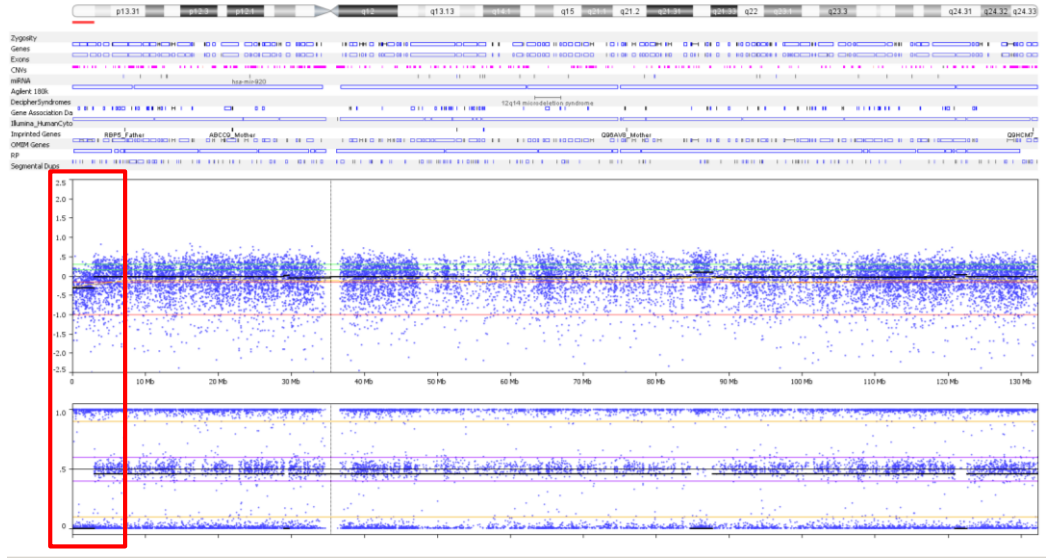
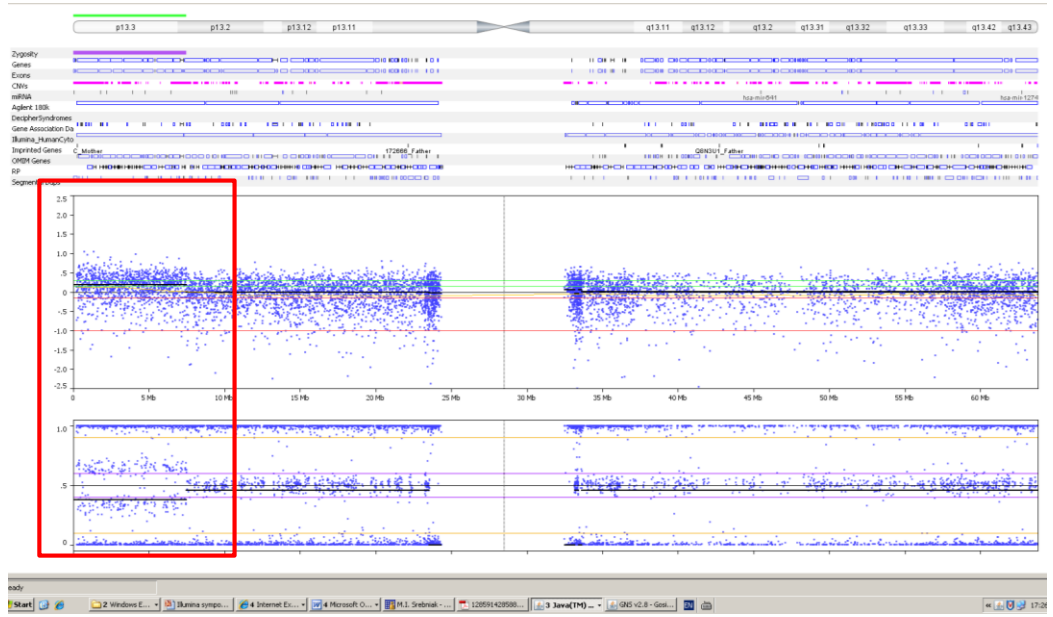
ABNORMAL NIPT – WHAT'S NEXT?

Structural aberration, (other trisomy) or normal QF-PCR

– genome-wide SNP microarray

- Structural anomalies (also elsewhere in the genome)
- Mosaic status
- Deletion rescue – segmental ROH (segUPD)
- Uniparental Disomy (UPD)
- Additionally detects: MCC and triploidy



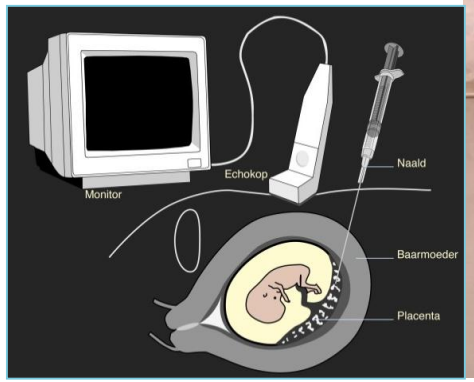


ABNORMAL ULTRASOUND – WHAT'S NEXT?

Chorionic villi biopsy
(CVS)
10-14 weeks

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

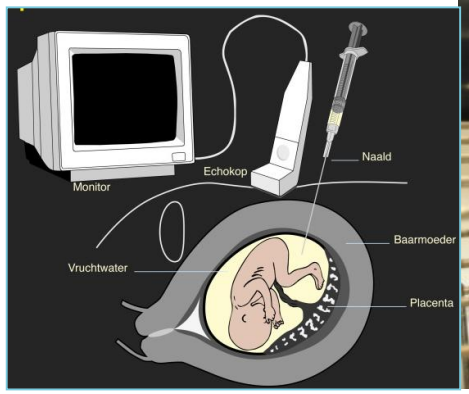
1-2% mosaic, mostly confined placental mosaicism (± 85%)

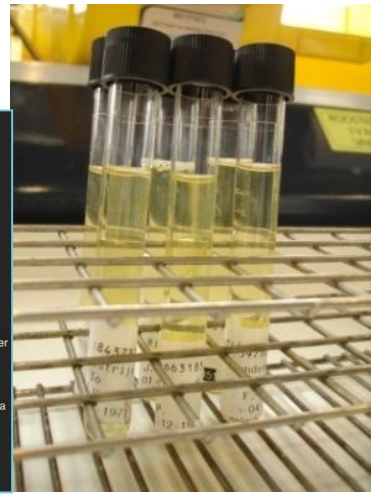


Amniocentesis
>16 weeks

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

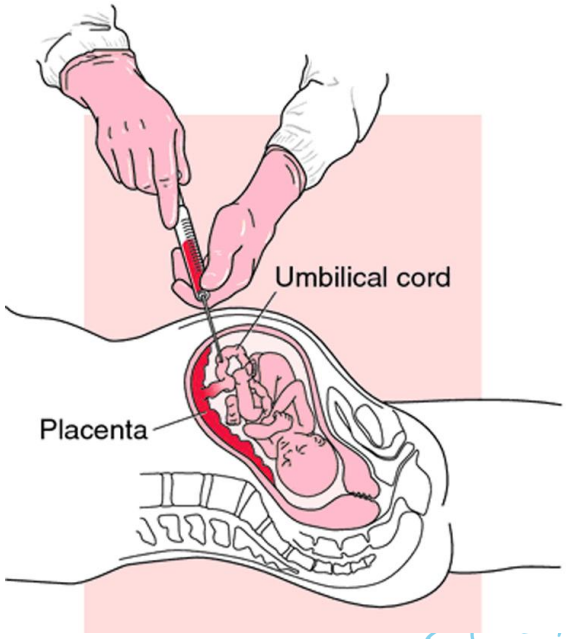
0,2% mosaic (Hsu et al., 1992)





Cordocentesis
~18 weeks

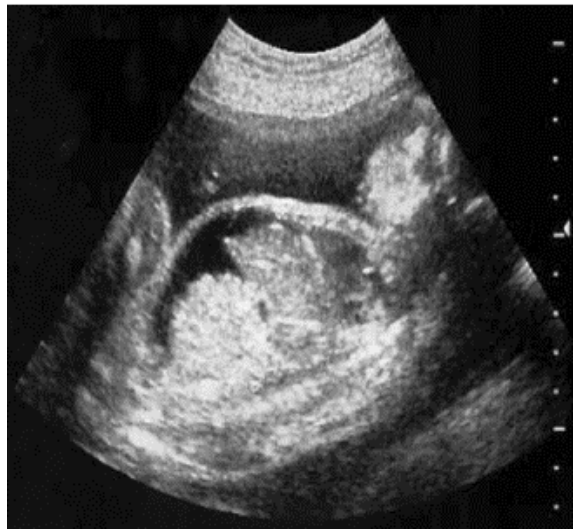
Miscarriage risk 0.6%
(Tanvisut et al., 2020)



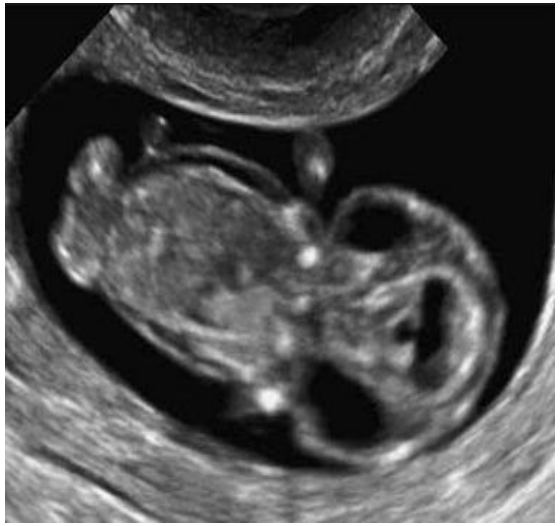
ABNORMAL ULTRASOUND – WHAT'S NEXT?

~70%	~30%
have already undergone NIPT	had no NIPT done

Always offer invasive testing!



Echogenic bowel



Hydrops

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Prenatal Diagnosis

WILEY

PRENATAL DIAGNOSIS

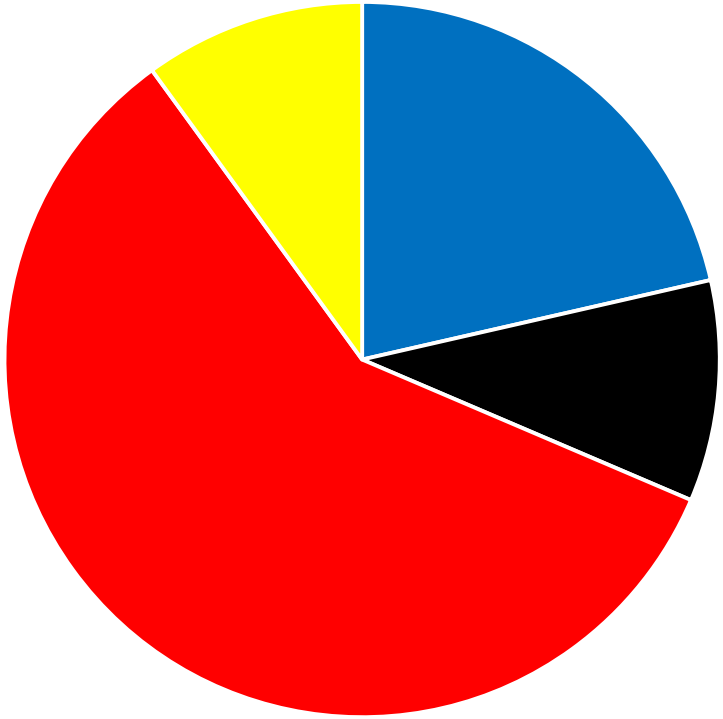
ORIGINAL ARTICLE OPEN ACCESS

Residual Risks of Fetal Chromosome Aberrations When Cell-Free DNA Prenatal Screening Is Normal: A Retrospective Study

Adriana I. Iglesias[1] | Diane Van Opstal[1] | Florentine F. Thurik[1] | Mark Drost[1] | Marjolein J. A. Weerts[1] | Marieke Joosten[1] | Karin E. M. Diderich[1] | Vyne van der Schoot[1] | Myrthe van den Born[1] | Robert-Jan H. Galjaard[1] | Stefanie van Veen[1] | Eveline Goedegebuur-Zwalua[1] | Sabina de Weerd[2] | Anneke Dijkman[3] | Dimitri Papatsonis[4] | Jérôme M. J. Cornette[5] | Sander Galjaard[5] | Maarten F. C. M. Knapen[5] | Krista Prinsen[5] | Attie T. J. I. Go[5] | Kyra E. Stuurman[1] | Malgorzata I. Srebniak[1]

Follow up in 46,007 women in 2017-2021

For fetuses with ultrasound anomalies, the residual risk for a pathogenic chromosomal aberration:
1:8 (13.3%) in the targeted-cfDNA group (T21, 18, 13)
1:12 (8.1%) in the genome-wide cfDNA group.



ABNORMAL ULTRASOUND – WHAT'S NEXT?

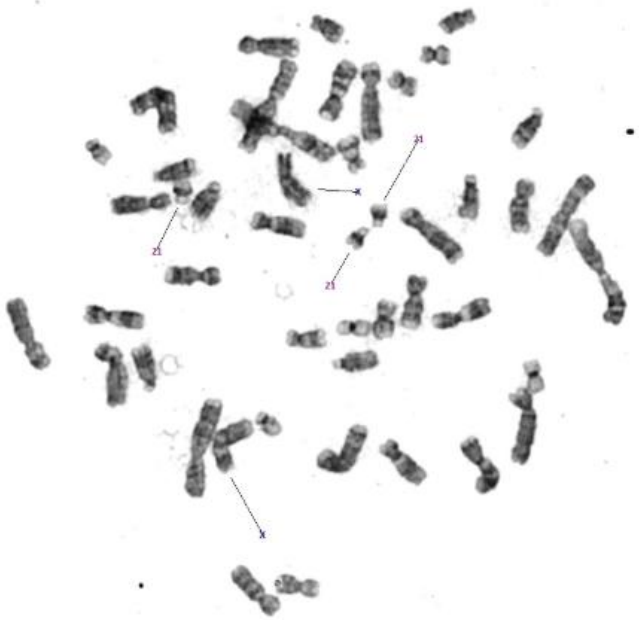
Ultrasound strongly suggestive for

Triploidy, X0, T21, 18, 13 -> QF-PCR

If positive for T13 or T21 -> karyotyping

If positive for T18 – no follow up

If negative -> SNP microarray (?mosaic)



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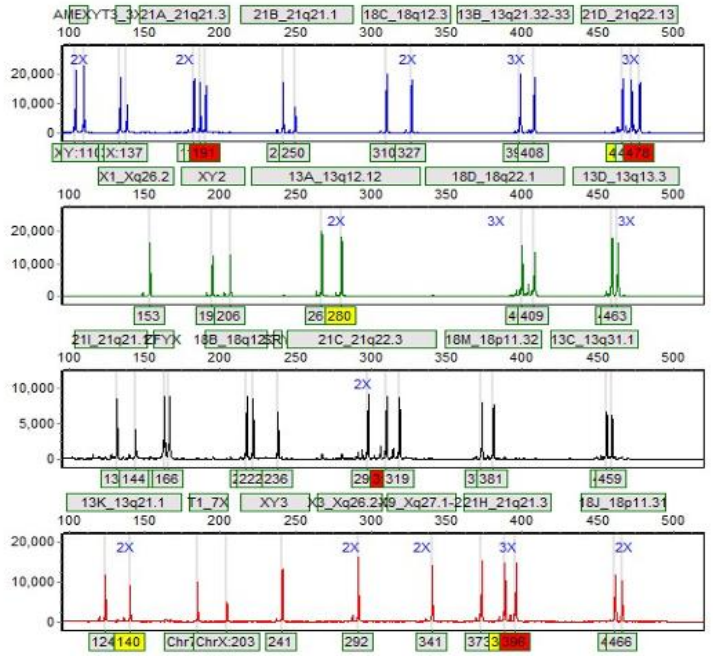

In case the result does not fit your very strong clinical suspicions – never give up asking questions!

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WHY ALWAYS TRIO ANALYSIS?

FETAL MATERIAL + PARENTAL EDTA BLOOD

Trio analysis facilitates prenatal diagnosis:

- rapid analysis - interpretation of (uncertain) results
- parental origin study – indication for follow up in one of the parents
- enables uniparental disomy (UPD) detection
- maternal cell contamination check
- sample swap controls in experiments
- rapid results in case of issues with other samples within the experiment

- direct sampling prevents anxiety by parents if their DNA is needed for the interpretation of the results

ULTRASOUND ANOMALY- WHAT NEXT?

SNP microarray (CMA)

- All patients undergoing invasive procedure

WES/WGS sequencing

- Patient with ultrasound anomalies except:
  - Isolated gastroschisis
  - Isolated VSD
  - Isolated Transversaal reductiedefect
  - Isolated Neural tube defect
  - Isolated unilateral hydronephrosis
  - Isolated CPAM (Congenital Cystic Pulmonary Malformation)
  - Isolated echogenic bowel (CF test only)

First trimester ultrasound anomaly

Pre-test counseling by fetal medicine specialist

Invasive testing

~1 wk

QF-PCR / CMA request

If normal referral for clinical genetics and WGS counseling

3-5 wks

WGS testing

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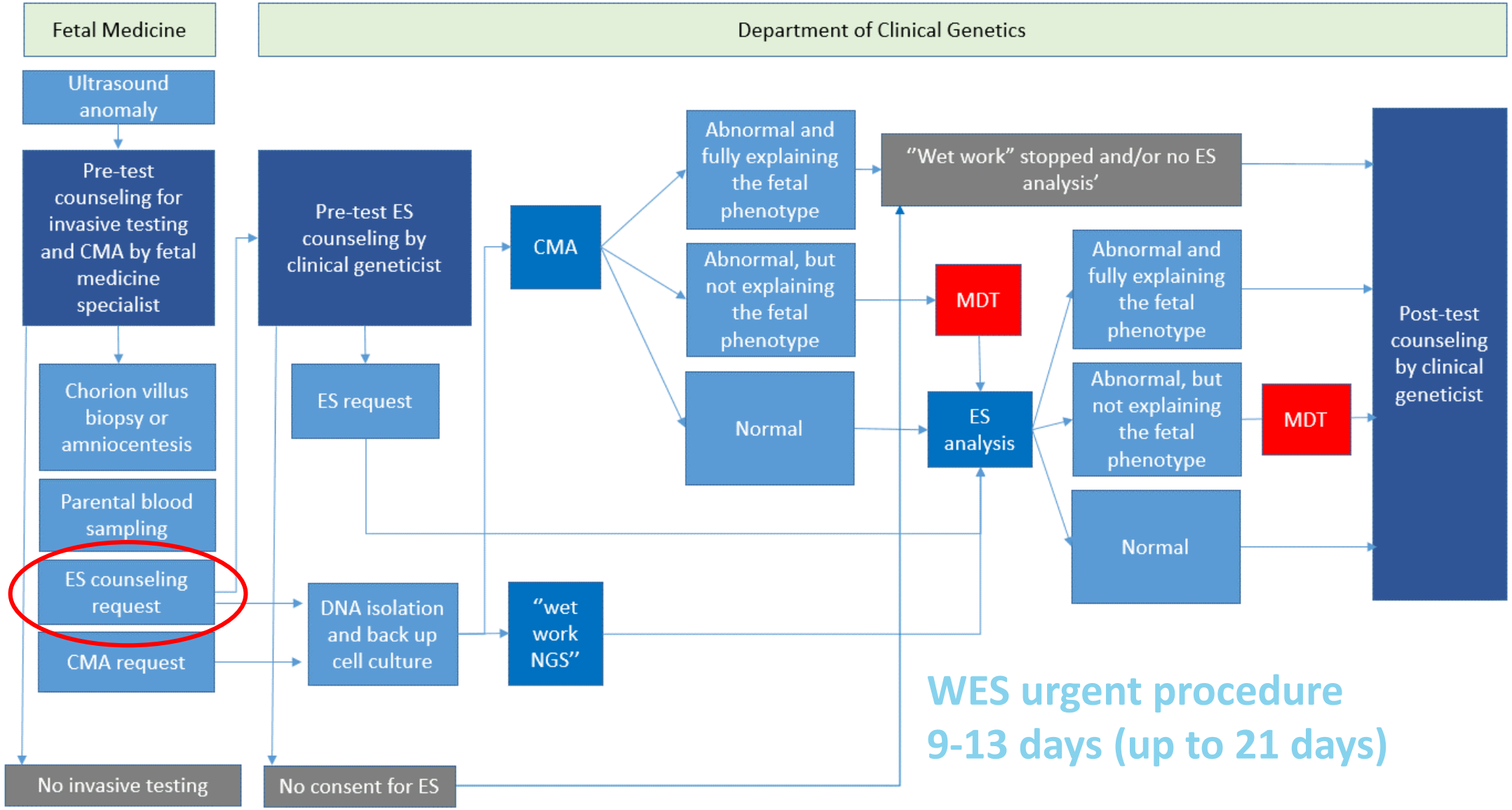
SECOND TRIMESTER ULTRASOUND



WES urgent procedure
9-13 days (up to 21 days)

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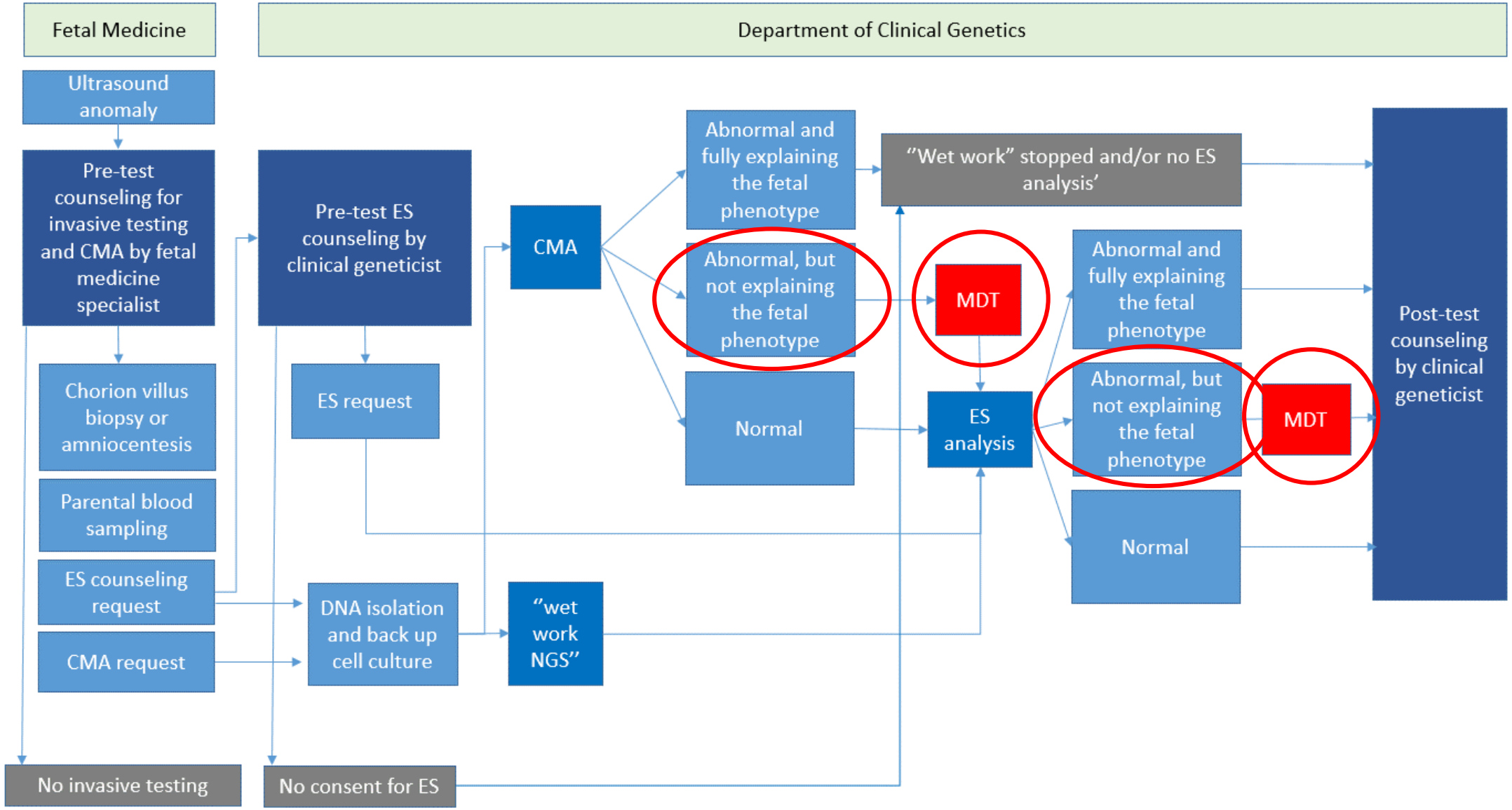
SECOND TRIMESTER ULTRASOUND



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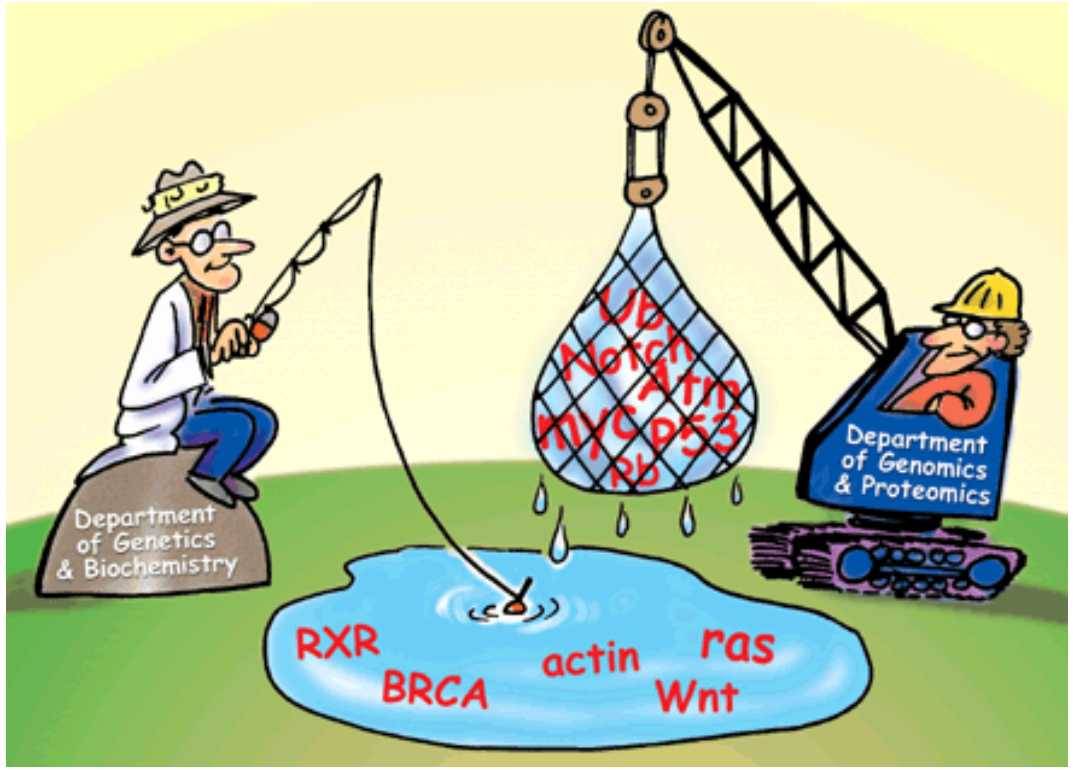
WHOLE EXOME SEQUENCING -PANEL ANALYSIS + HPO BOOST

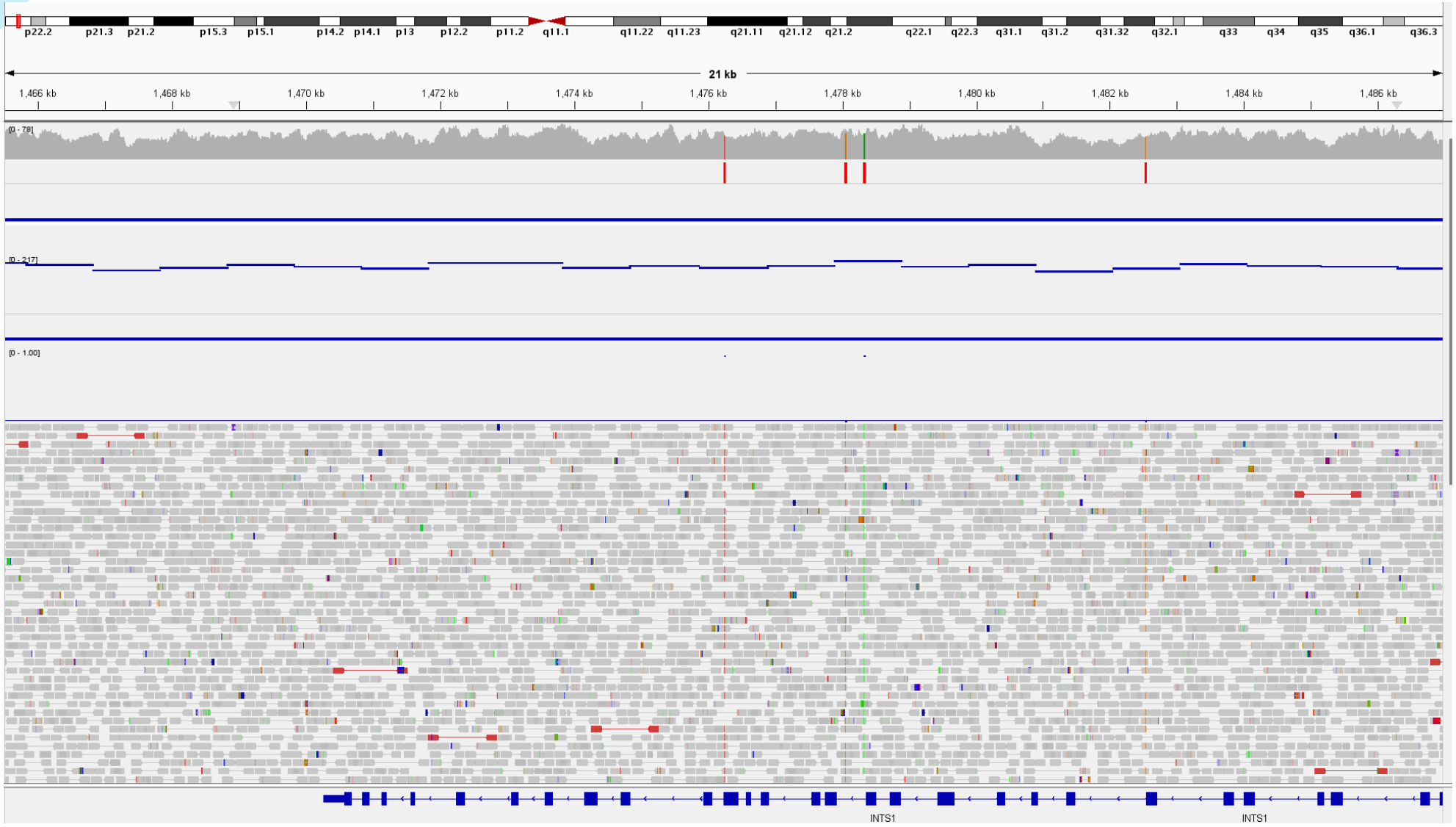
Prenatal gene panel – ~3500 genes associated with congenital anomalies and/or intellectual disability

General policy – do not report VUS (importance of post-test counseling)

Case 1 15.3 weeks
Ultrasound anomalies:
Anhydramnios
Low-set ears
Fetal nuchal edema
Bilateral renal agenesis
Abnormality of the kidney



WHOLE GENOME SEQUENCING



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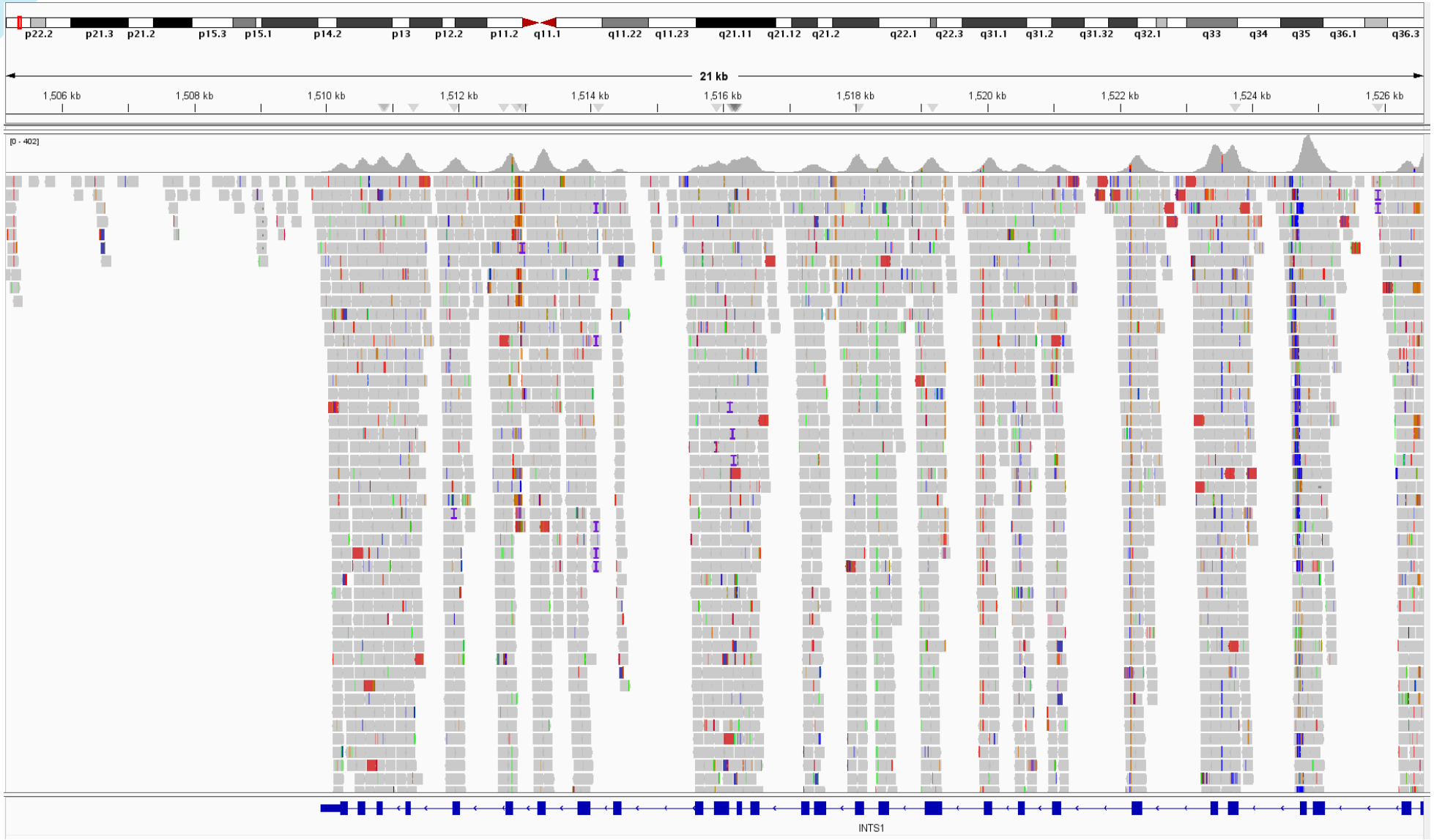
WHOLE EXOME SEQUENCING



min. conc.
3ng/ul

300ng

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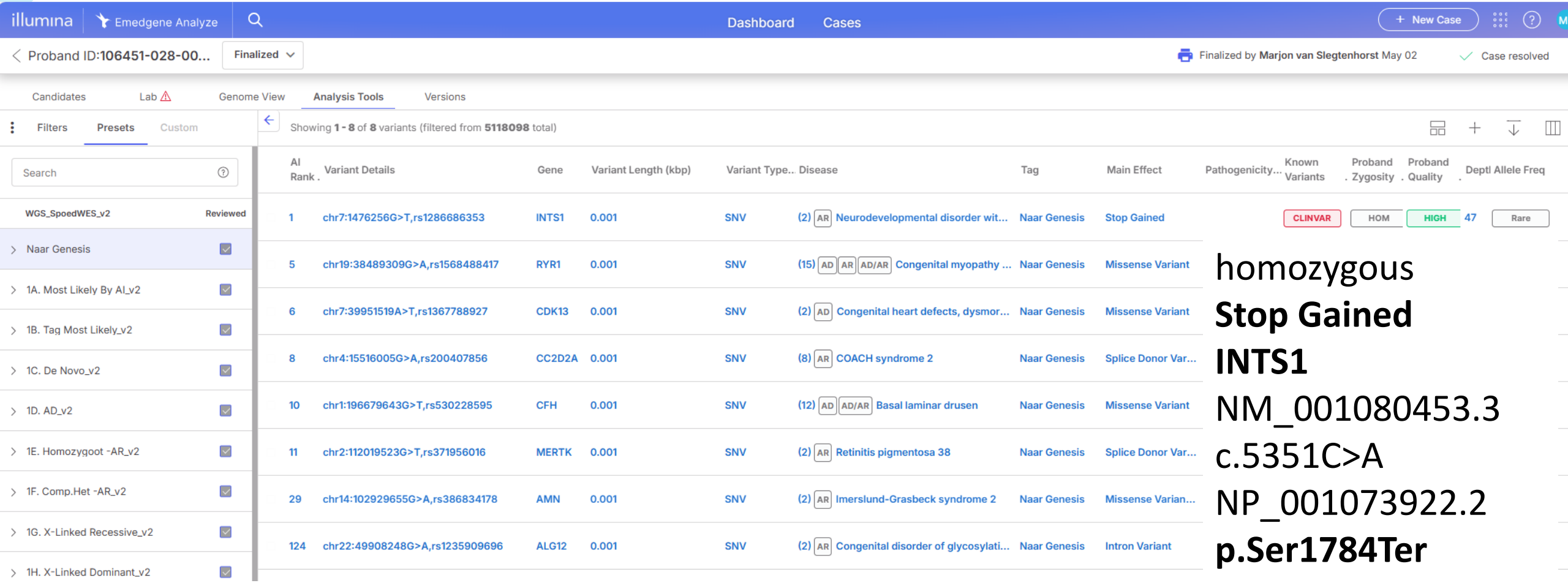
EMEDEGENE ANALYSIS



homozygous

Stop Gained

INTS1

NM_001080453.3

c.5351C>A

NP_001073922.2

p.Ser1784Ter

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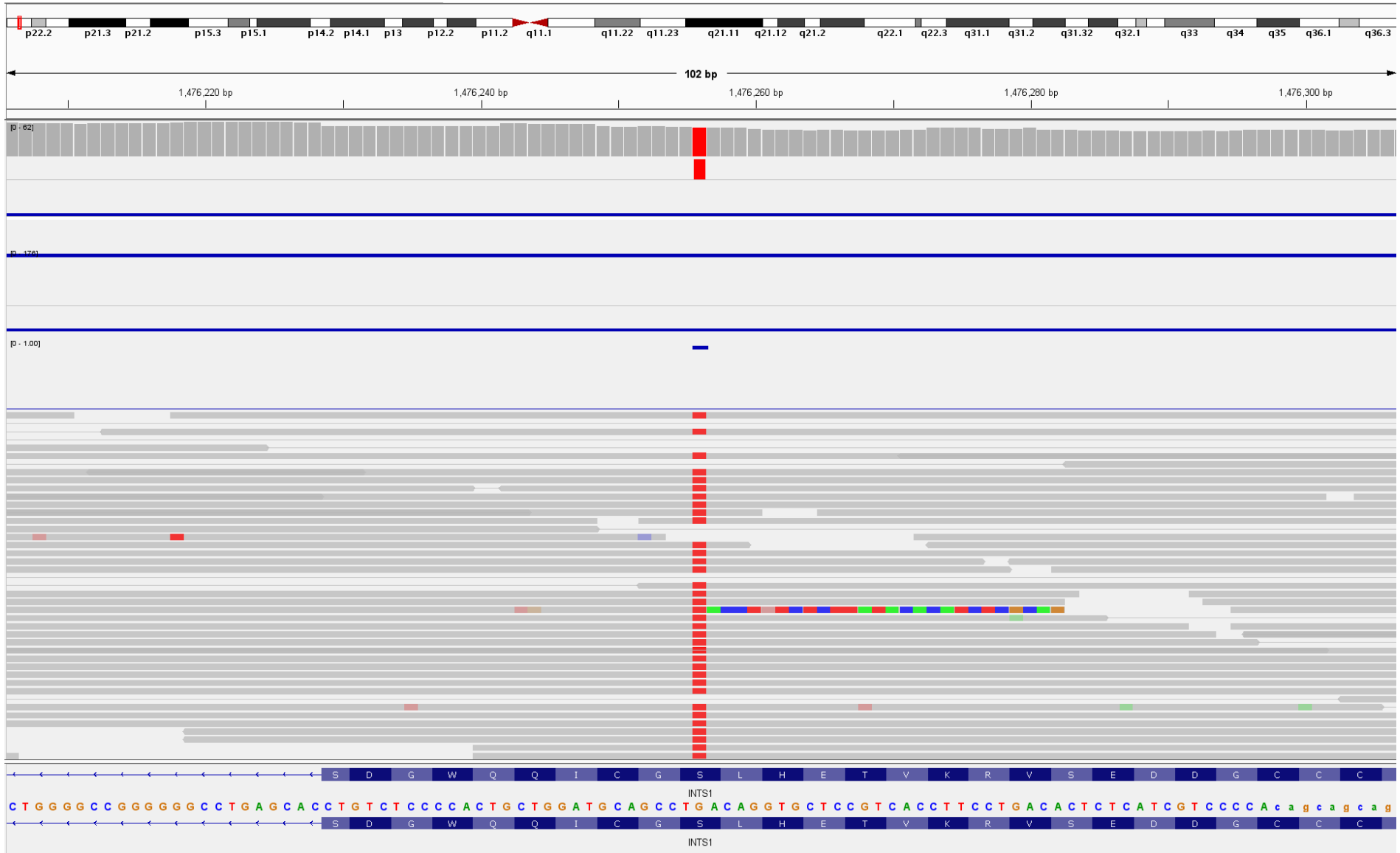
ULTRASOUND ANOMALY- WHAT NEXT?



INTS1?

Anhydramnios
Low-set ears
Fetal nuchal edema
Bilateral renal agenesis
Abnormality of the kidney

618571

NEURODEVELOPMENTAL DISORDER WITH CATARACTS, POOR GROWTH, AND DYSMORPHIC FACIES; NDCAGF

Phenotype-Gene Relationships

Location	Phenotype	Phenotype MIM number	Inheritance	Phenotype mapping key	Gene/Locus	Gene/Locus MIM number
7p22.3	Neurodevelopmental disorder with cataracts, poor growth, and dysmorphic facies	618571	AR	3	INTS1	611345

- Short stature
  Poor growth
  - Dolichocephaly
  - Dysmorphic facial features, variable
  - Frontal bossing
  - Full cheeks
  - Micrognathia
  - Abnormal philtrum
  - Low-set ears
  - Dysplastic ears
  - Cataracts
  - Myopia
  - Strabismus
  - Hypertelorism
  - Microphthalmia
  - Microcornea
  - Coloboma
  - Epicanthal folds
  - Small palpebral fissures
  - Slanted palpebral fissures
- Broad nasal bridge
  Downturned corners of the mouth

*Teeth (*Diastema, Dental crowding)
- Short neck
- Pectus excavatum
- Pectus abnormalities
- Cryptorchidism
- Renal abnormalities, variable (in some patients)
- Abnormal thumbs
- Overlapping toes
- Irregularly implanted toes
- Broad halluces
- Global developmental delay

- Impaired intellectual development
- Hypotonia
- Delayed walking, mild (2 to 3 years)
- Abnormal gait
- Poor or absent speech
- Cerebellar atrophy (in some patients)
- Autistic features

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CHALLENGES

Phenotype ➡ Genotype

- Imperfect screening (almost only structural anomalies)/ incomplete fetal phenotype, NIPT for only large aberrations
- Almost no knowledge on intellectual development
- Minor anomalies may turn major after birth
- Missed diagnoses although invasive sampling was done

Genotype ➡ Phenotype

- Screening and diagnostic boundaries are blurred
- Major problems: phenotypic variability and abnormal results in “phenotypically normal fetus” – prediction of the post partum phenotype
- Interpretation of mosaic karyotypes is more difficult in fetus without ultrasound anomalies
- VUS interpretation is challenging in absence of abnormal phenotype

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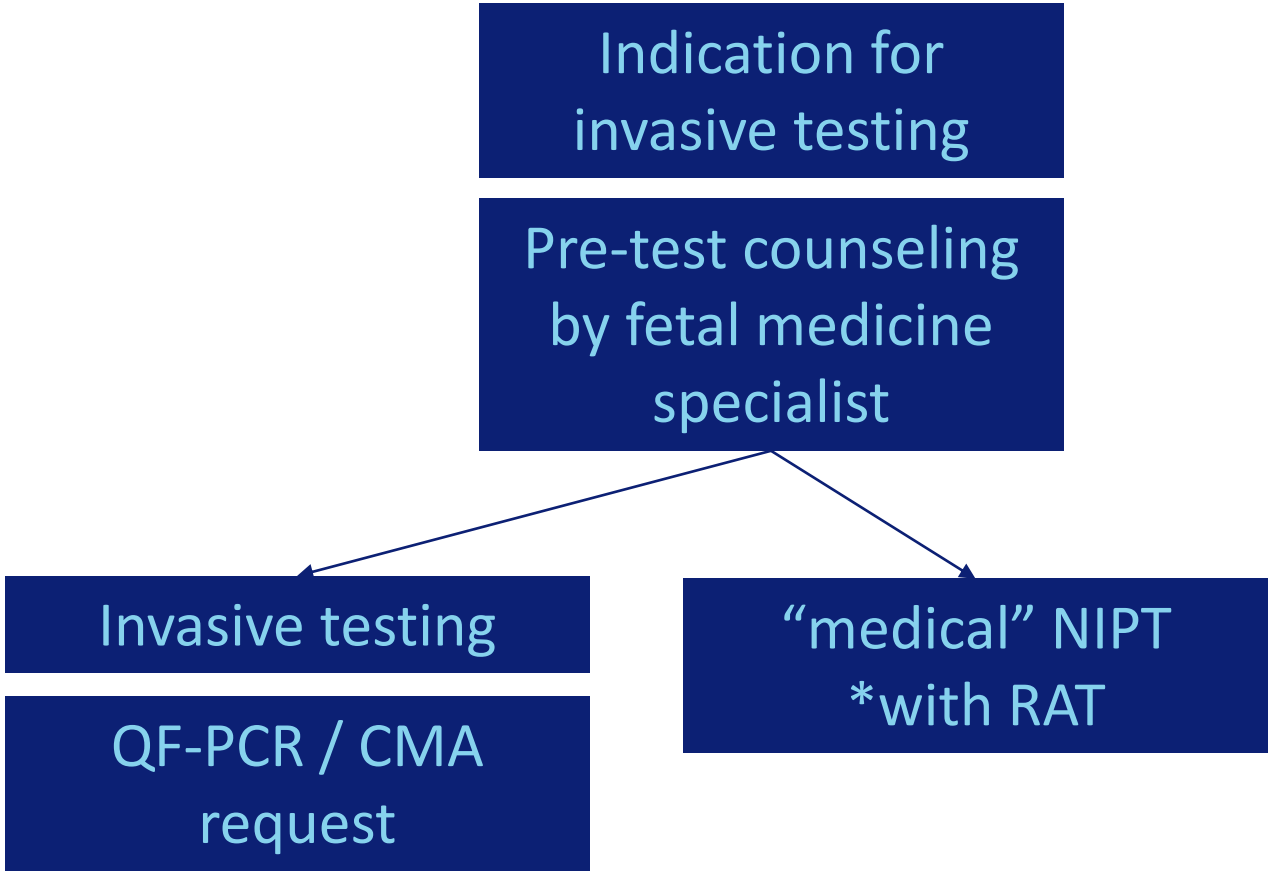
NIPT FOR MEDICAL INDICATIONS

- Recurrence risk for trisomy
- Recurrence risk for other chromosomal aberrations
- Patients declining invasive testing
  - Translocation carriers
  - Ultrasound anomalies
  - IUGR – suspicion of chromosome aberrations in the placenta



KARYOTYPING – as additional targeted test for the recurrence risk assessment

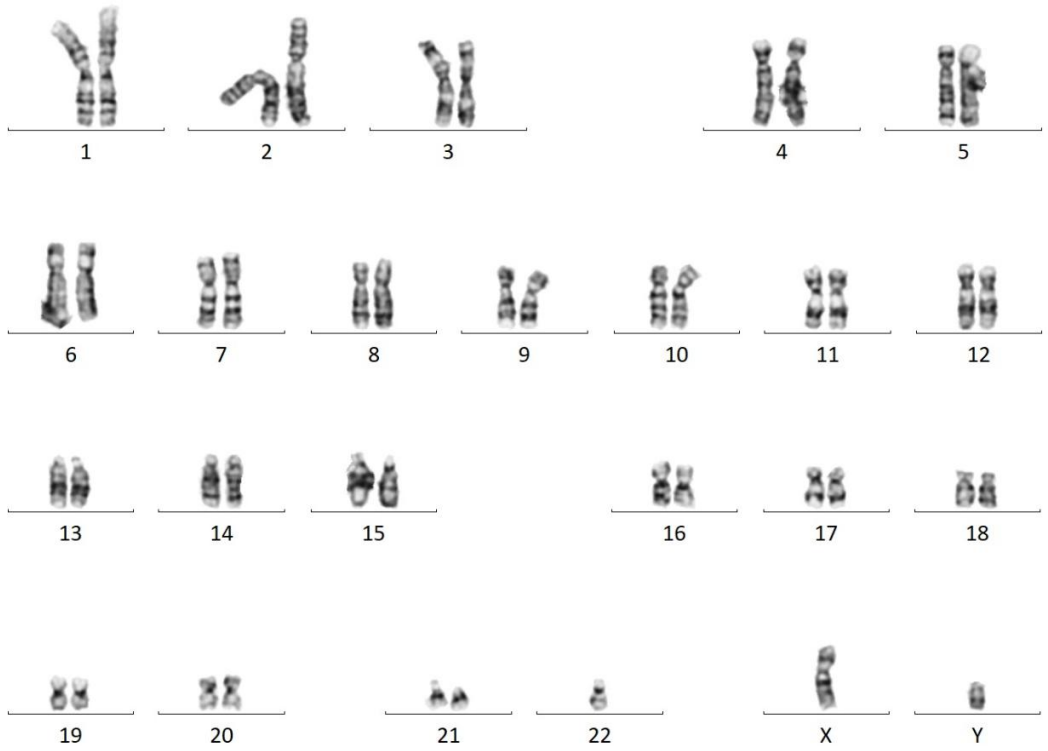
- after an abnormal array result suggesting the presence of an unbalanced translocation

- after detecting trisomy 13 or 21

- to exclude/detect parental balanced translocation/ inversion if array analysis excludes an unbalanced karyotype in case of parental balanced chromosomal aberration

- after an abnormal array result showing a large gain to specify the chromosome abnormality (duplication, insertion, marker chromosome etc.)

- to visualize complex abnormalities



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WHEN THE CHILD IS BORN?

Congenital anomaly

Pre-test counseling by neonatologist/ pediatrician

CMA request

CNV analysis request

If normal referral for clinical genetics and WGS counseling

WGS testing

CNV analysis in WGS data

SNV analysis in WGS data

WGS testing

All postnatal WGS – turn around time is 3-6 months

Intensive care unit (ICU) – WES/WGS most urgent results 21 days

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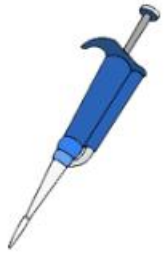
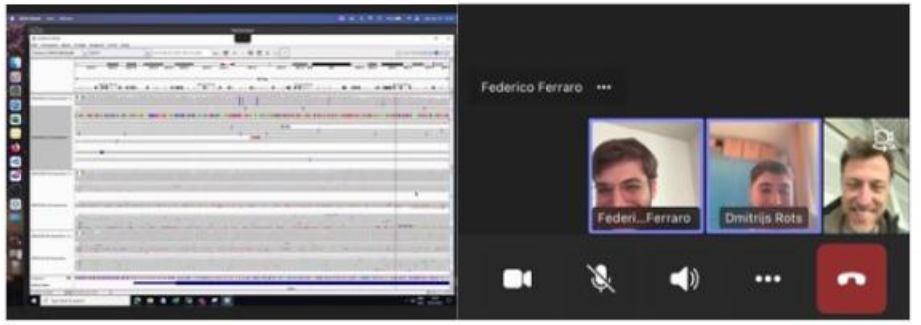
OXFORD NANOPORE TECHNOLOGY (ONT) ULTRA RAPID ANALYSIS





UR request	Sample prep	Sequencing & basecalling	Variant calling	Variant annotation & prioritization	Analysis & Interpretation	Result
	Trio	3x2 flowcells On the fly	On tower		2 laboratory specialists in parallel	



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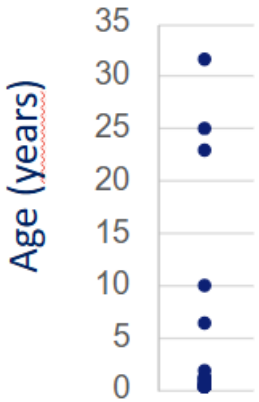
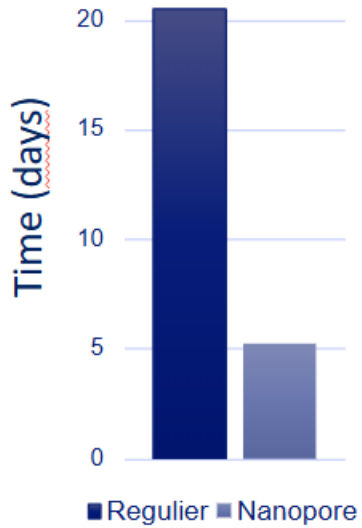
Results (one-year summary)



n=26 critically ill pt
median: 2 months

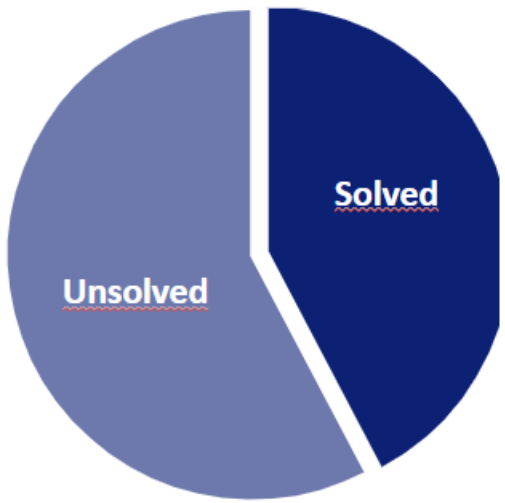
Turnaround time



20.5 days (Regular, range 6.1–42)
5.2 days (Nanopore, range 2–9.3)

Including weekend
In time for decision making?

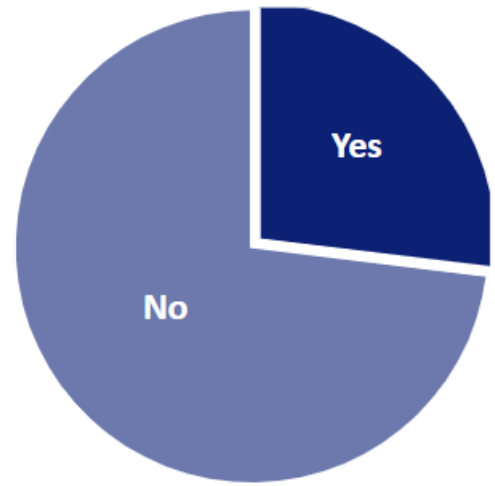
Yield



11/26 solved (43%)

Regular = Nanopore
But less tests
1 vs 2 (range 1-4)

Impact



7/26 (27%);
7/11 solved (64%)

i.e. medication switch,
termination of treatment

Smits, Ferraro, Drost et al, *under revision*

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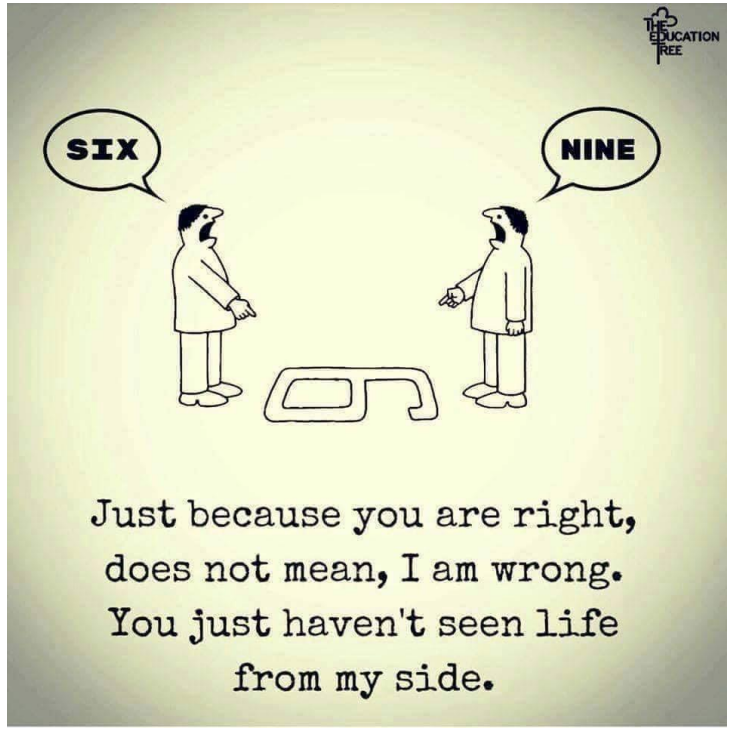
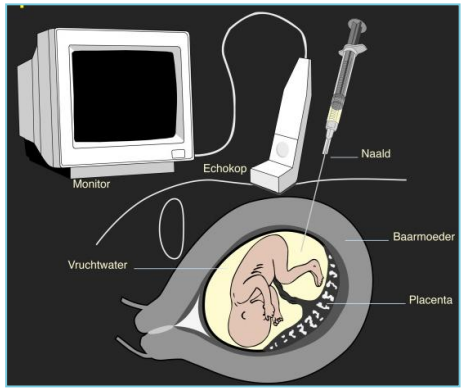
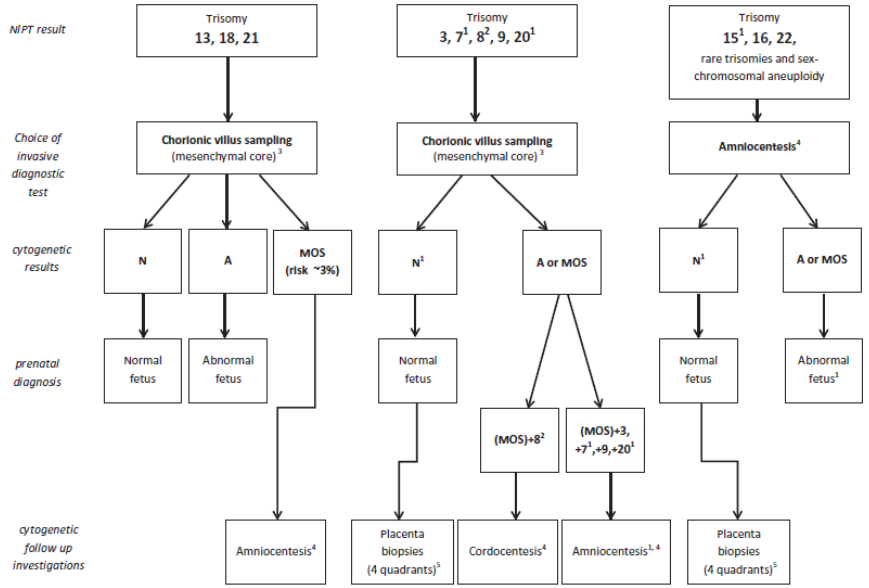
TAKE HOME MESSAGE

PRENATAL SETTING - ALLOW INDIVIDUAL CHOICES

- Facilitate reproductive autonomy
- Inform on the possibilities and limitations of available tests
- Let pregnant women choose the scope of screening and diagnosis
- Let pregnant women choose between chorionic villi sampling and amniocentesis (where possible)







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CURRENT RECOMMENDATION

NIPT + first and second trimester ultrasound in general population

Ultrasound anomalies:

High resolution CNV analysis

SNV analysis (WES/WGS) (in selected cases)

Normal NIPT, but abnormal ultrasound:

RAD if strong suggestion for T21, T18, T13

High resolution CNV analysis

SNV analysis (WES/WGS) (in most cases)

Other indications without ultrasound anomalies:

0.5 Mb microarray analysis as additional test + test according to the indication for invasive sampling

Negative results also have major impact on pregnancy management and well-being of patients

Avoid targeted testing in prenatal settings due to incomplete fetal phenotype and background risk for chromosomal aberrations

Avoid diagnostic delay – it is not only about finding a diagnosis, but also about rapid diagnosis

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Bioinformaticians
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