NIPT in monogenic diseases: a current perspective

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Monogenic diseases

- **A group of human conditions caused by inherited or de novo pathogenic variants in a single gene**
- **Individially rare, but! in aggregate, they are more common**
- **1/9 women are carriers of a serious genetic condition**
- **80% of affected babies with AR conditions are born to couples with no family history**
- **traditional diagnosis – pregnancies at risk (family history, suspicious ultrasound finding) → invasive procedures (CVS, AMC) and DNA analysis**

Monogenic diseases

cffDNA in maternal plasma

→ non-invasive prenatal screening (NIPT) for common aneuploidies and others

- screening test
- requires confirmation of positive results with an invasive test
- discordant results (placental mosaicism, maternal rearrangements, vanishing twin and other problems

→ non-invasive prenatal diagnosis (NIPD) of monogenic diseases

- diagnostic test
- placental mosaicism has not been reported for single-gene diseases
- does not require an invasive test to confirm a positive result
- can be carried earlier than CVS or AMC (sufficient cffDNA levels are from around 6-7 weeks)

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Monogenic diseases

NIPD for monogenic diseases are technologically more challenging compared to NIPT

- fetal fraction is relatively low 5-20%
- therefore the need for highly sensitive detecting techniques
- precise confirmation of fetal fraction to avoid false negative results
- cffDNA fragments are short → problems detecting large deletions, duplications and rearrangements
- maternal somatic mosaicism can be a problem in some cases

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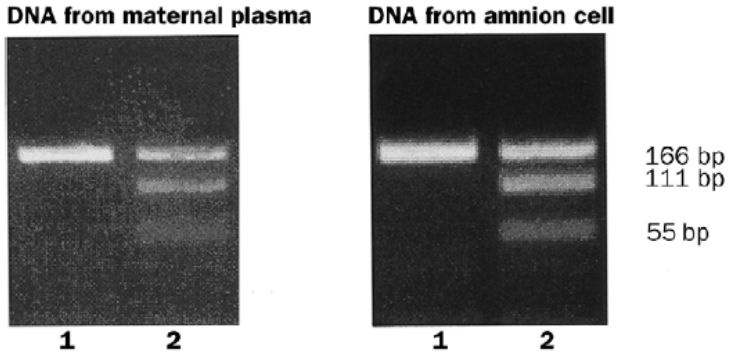
Monogenic diseases

2000 – non-invasive diagnosis of achondroplasia, published in Lancet

Prenatal DNA diagnosis of a single-gene disorder from maternal plasma

Hiroshi Saito, Akihiko Sekizawa, Taro Morimoto, Makoto Suzuki, Takumi Yanaihara

Achondroplasia is a short-limb disorder caused by a point mutation in a single gene. To diagnose such a disorder prenatally requires the use of invasive procedures such as amniocentesis. However, using PCR and restriction fragment length polymorphism analysis, we were able to detect the mutation in the plasma of a woman carrying a fetus suspected of having achondroplasia. The detection of a fetus-derived mutant gene from maternal plasma may therefore permit non-invasive prenatal diagnosis of single-gene disorders.



PCR amplification and restriction fragment length polymorphism analysis of genomic DNA from maternal plasma and amniotic fluid

Lane 1=before *Sfc*I digestion; lane 2=after *Sfc*I digestion.

2000 – non-invasive diagnosis of myotonic dystrophy, published in Clinical Chemistry

Prenatal Diagnosis of Myotonic Dystrophy Using Fetal DNA Obtained from Maternal Plasma

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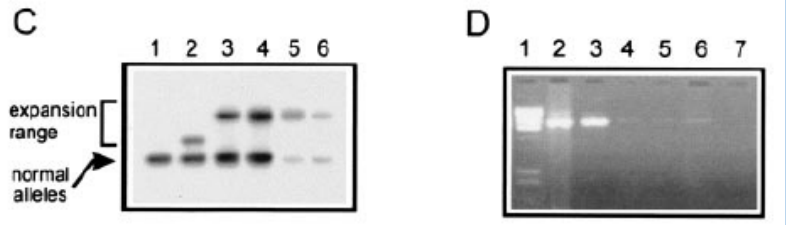


Fig. 1. Electropherograms of alleles at the *CFSPO* locus (*A*) and X-Y amelogenin PCR products (*B*), autoradiograph of CTG expansion at the *DMPK* locus (*C*), and gel showing amplification of *BPY2* (*D*).

Techniques for sgNIPT

Table 1 **Examples of NIPD applications with technologies used**

Inheritance	Methodology	Gene	Disease
Autosomal dominant/*de novo*	PCR-RED, dPCR, [a]Amplicon NGS [a]Bespoke testing for individual family	[a]*FGFR3* [a]*FGFR2*	Achondroplasia,[9,11] Thanatophoric dysplasia,[8] Apert syndrome[6]
	PCR	*DMPK*	Myotonic dystrophy[12]
	Semi-qPCR; PCR and automated fragment analysis	*HTT*	Huntington[13]
Autosomal recessive – paternal exclusion	[a]Amplicon NGS	[a]*CFTR*	Cystic fibrosis[14]
	dPCR	*PKHD1*	Autosomal recessive polycystic kidney disease[6]
	Polymorphic markers fluorescence PCR and fragment size analysis	*CYP21A2*	Congenital adrenal hyperplasia[15]
	qPCR	*HBB*	β-thalassaemia[16]
	[a]Amplicon NGS	*HBB*	β-thalassaemia[17]
Autosomal recessive – definitive diagnosis	RMD – dPCR	*HBB*	β-thalassaemia[18]
	dPCR	*HBB*	Sickle cell anaemia[19]
	ddPCR	*MUT*	Methylmalonic acidaemia[20]
	cSMART	*ATP7B*	Wilson disease[21]
	[a]NGS-RHDO	[a]*CYP21A2*	Congenital adrenal hyperplasia[22]
	[a]dPCR + NGS-RHDO	[a]*HBB*	β-thalassaemia[23]
	[a]NGS-RHDO	[a]*CFTR*	Cystic fibrosis[10]
	[a]NGS-RHDO	[a]*SMN1 & 2*	Spinal Muscular atrophy[24]
X-linked – definitive diagnosis	[a]NGS-RHDO	[a]*DMD*	Duchene muscular dystrophy/ Becker muscular dystrophy[25]
	dPCR	*F8, F9*	Haemophilia[26]

cSMART, circulating single-molecule amplification and resequencing technology; dPCR, digital PCR; ddPCR, droplet digital PCR; NGS, next-generation sequencing; PCR-RED, polymerase chain reaction–restriction enzyme digest; qPCR, quantative PCR; RHDO, relative haplotype dosage; RMD, relative mutation dosage; NIPD, non-invasive prenatal diagnosis.

[a]Those tests in current practice in accredited laboratories.

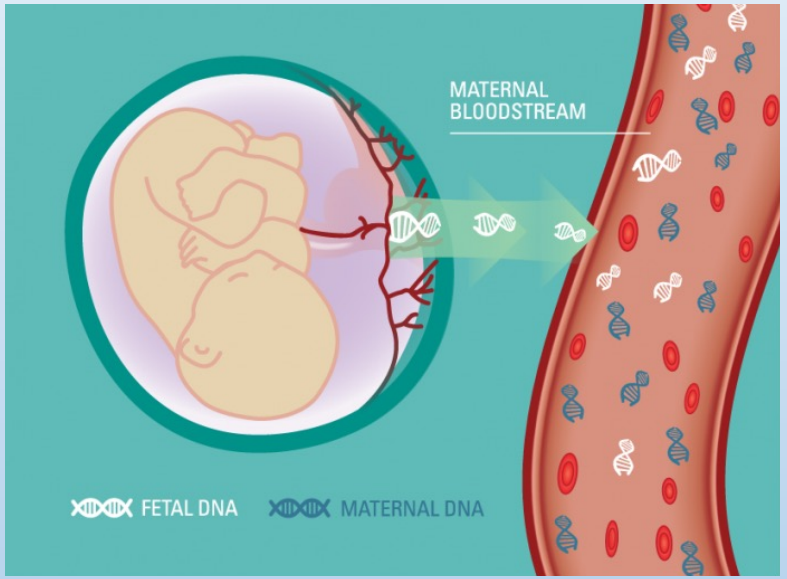
Jenkins LA et al. Prenat Diagn 2018

Techniques for single gene NIPD

Two main scenarios:

1. **De novo and paternally-inherited variants for dominant conditions or recessive conditions where the mother and father carry different mutations**
   - technically easier
   - low level variant detection
   - mutation is not present in the maternal cfDNA but only in the small fetal fraction

2. **Dosage-based techniques for the detection of maternally-inherited variants in autosomal recessive, X-linked or dominant disorders**
   - more challenging, requires a complex approach
   - we are trying to detect variants which is present in fetal as well as maternal cfDNA



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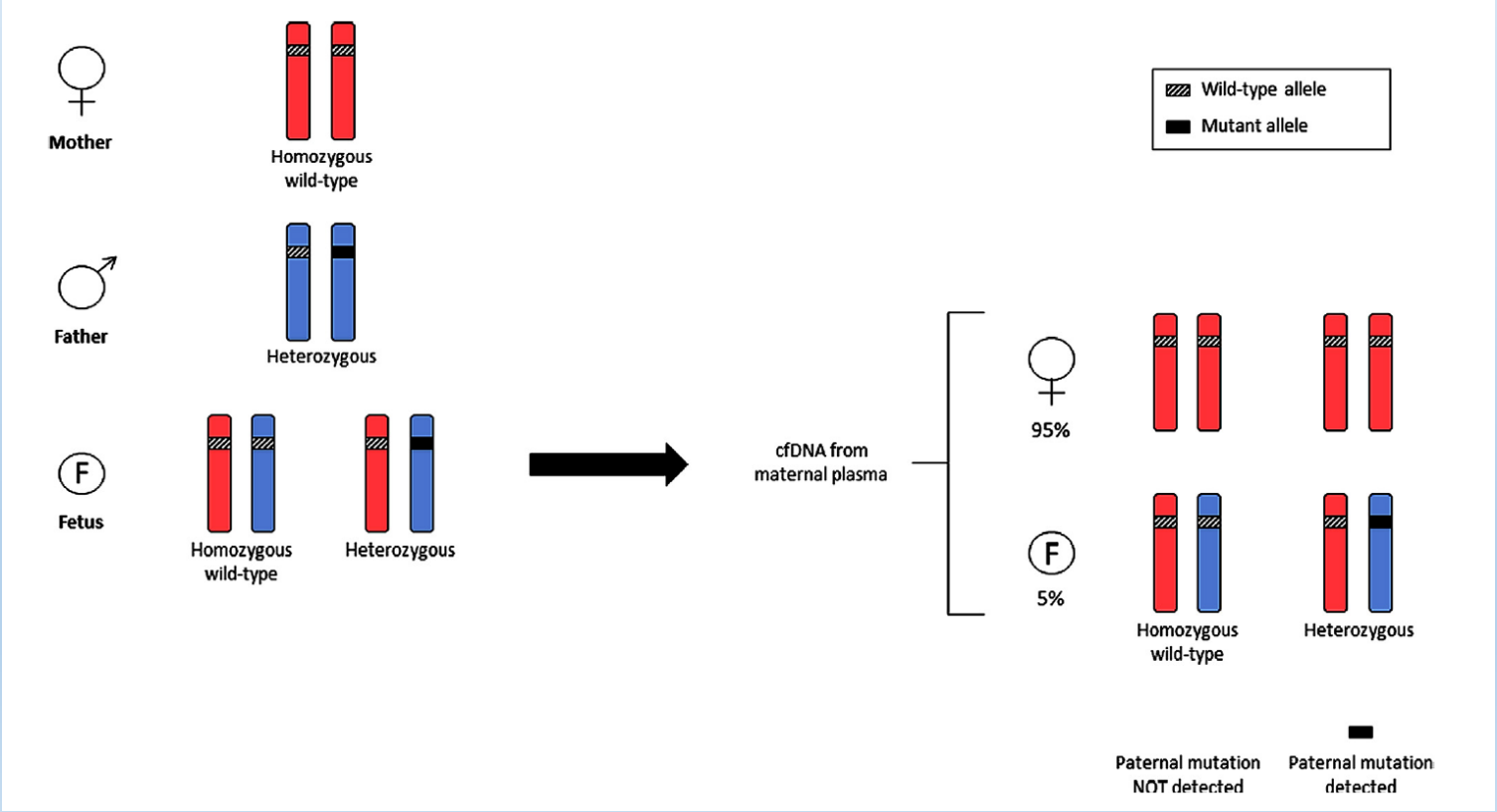
Techniques for single gene NIPD

1. **De novo and paternally-inherited variants for dominant conditions or recessive conditions where the mother and father carry different mutations**
   - PCR-based methods – for testing of individual mutation
   - NGS-based methods – allow to test of many causative mutations in a gene



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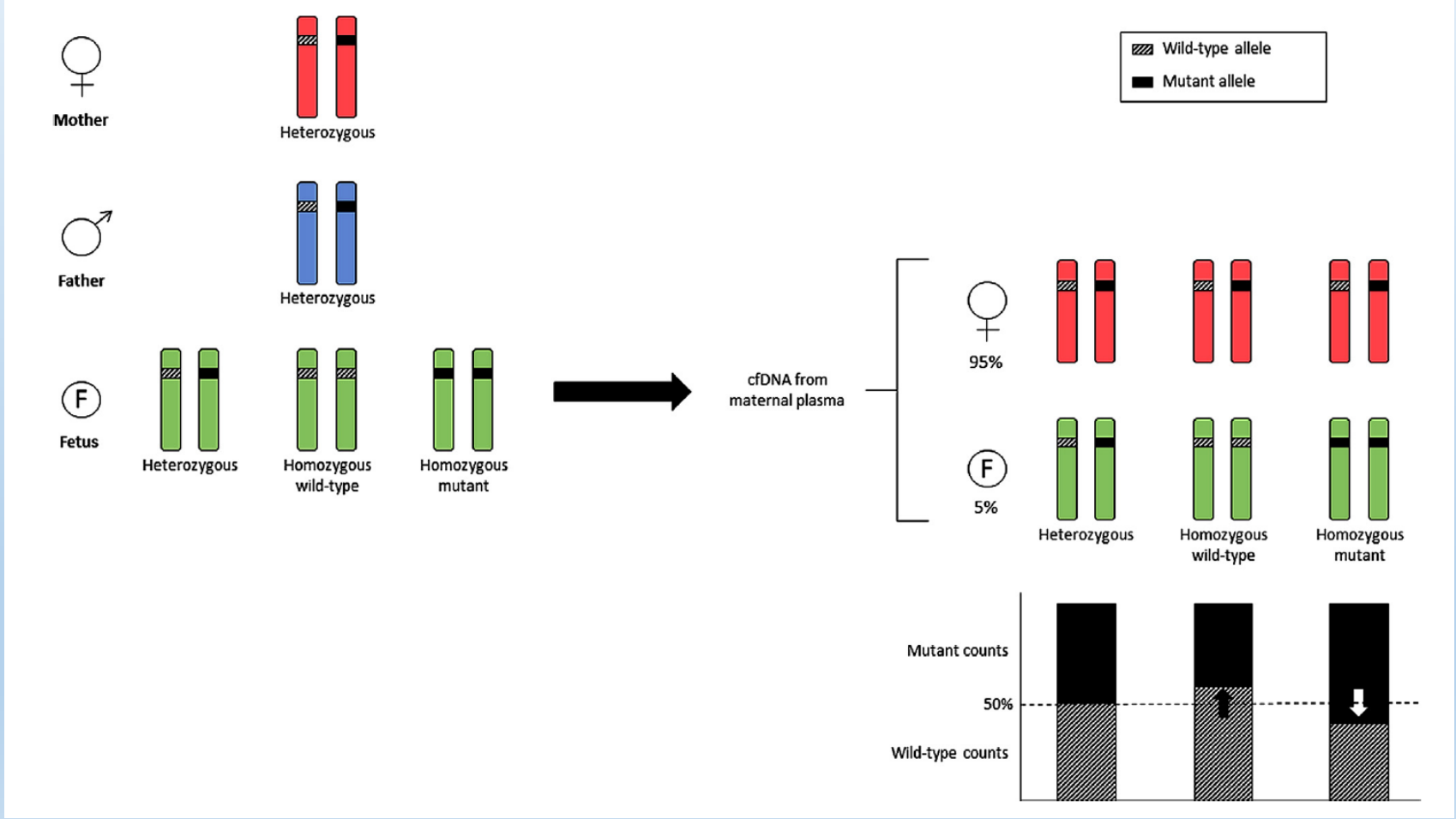
Techniques for single gene NIPD

2. Dosage-based techniques for the detection of maternally-inherited variants in autosomal recessive, X-linked or dominant disorders



- **Relative haplotype dosage analysis (RHDO)**
  - multiple SNPs surrounding mutation
  - we need both parents and affected proband or sibling
- **Relative mutation dosage (RDO)**
  - dPCR techniques
  - does not require family haplotype, only blood sample from the mother is sufficient

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Techniques for single gene NIPD

1. **De novo and paternally-inherited variants for dominant conditions or recessive conditions where the mother and father carry different mutations**
   - Fetal sex determination
   - RhD status
   - Achondroplasia, thanatophoric dysplasia, myotonic dystrophy
   - AB0 group prediction
   - CF, beta-thalassemia

2. **Dosage-based techniques for the detection of maternally-inherited variants in autosomal recessive, X-linked or dominant disorders**
   - CF, DMD, SMA
   - haemophilia, CAH, beta-thalassemia, maple syrup urine disease, cobalamin C deficiency, sickle-cell disease

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GeneSAFE™ Inherited screens for **5 common inherited recessive genetic disorders**, such as **Cystic Fibrosis, β-Thalassemia, Sickle cell anemia, Deafness autosomal recessive type 1A, Deafness autosomal recessive type 1B**

GENE	SYNDROMIC DISORDERS
JAG1	Alagille syndrome
CHD7	CHARGE syndrome
HDAC8	Cornelia de Lange syndrome 5
NIPBL	Cornelia de Lange syndrome 1
MECP2	Rett syndrome
NSD1	Sotos syndrome 1
ASXL1	Bohring-Opitz syndrome
SETBP1	Schinzel-Giedion syndrome
SIX3	Holoprosencephaly
	SINDROME DI NOONAN
BRAF	Cardiofaciocutaneous syndrome 1
CBL	Noonan syndrome-like disorder with or without juvenile myelomonocytic leukemia (NSLL)
KRAS	Noonan syndrome/cancers
MAP2K1	Cardiofaciocutaneous syndrome 3
MAP2K2	Cardiofaciocutaneous syndrome 4
NRAS	Noonan syndrome 6/cancers
PTPN11	Noonan syndrome 1/ LEOPARD syndrome/cancers
PTPN11	Juvenile myelomonocytic leukemia (JMML)
RAF1	Noonan syndrome 5/LEOPARD syndrome 2
RIT1	Noonan syndrome 8
SHOC2	Noonan syndrome-like disorder with loose anagen hair
SOS1	Noonan syndrome 4

GENE	SKELETAL DISORDERS
COL2A1	Achondrogenesis, type II or hypochondrogenesis
FGFR3	Achondroplasia
	CATSHL syndrome
	Crouzon syndrome with acanthosis nigricans
	Hypochondroplasia
	Muenke syndrome
	Thanatophoric dysplasia, type I
	Thanatophoric dysplasia, type II
COL1A1	Ehlers-Danlos syndrome, classic
	Ehlers-Danlos syndrome, type VIIA
	Osteogenesis imperfecta, type I
	Osteogenesis imperfecta, type II
	Osteogenesis imperfecta, type III
	Osteogenesis imperfecta, type IV
COL1A2	Ehlers-Danlos syndrome, cardiac valvular form
	Ehlers-Danlos syndrome, type VIIB
	Osteogenesis imperfecta, type II
	Osteogenesis imperfecta, type III
	Osteogenesis imperfecta, type IV
	CRANIOSYNOSTOSIS SYNDROMES
FGFR2	Antley-Bixler syndrome without genital anomalies or disordered steroidogenesis
	Apert syndrome
	Crouzon syndrome
	Jackson-Weiss syndrome
	Pfeiffer syndrome type 1
	Pfeiffer syndrome type 2
	Pfeiffer syndrome type 3

GeneSafe™ INHERITED

GeneSafe Inherited

screens for 5 common inherited recessive genetic disorders

Learn More

GeneSafe DeNovo

screens for 44 severe genetic disorders due to de novo mutations (a gene mutation that is not inherited)

Learn More

GeneSafe™ DE NOVO

Routine testing

NIPD for monogenic conditions is technically possible and available

Its usage is still largely limited to pregnancies at increased risk

?? the use of cfDNA to screen for common monogenic conditions

Is it ready for routine clinical use in low-risk pregnancies ??

Vora NL, Langlois S, Chitty LS. The debate during ISPD conference in Edinburgh, UK, 2023

Routine testing

Arguments for (dr. Neeta Vora):

- **AR conditions (clinically available for CF, SMA, hemoglobinopathies)**
  - **it allows the identification of affected pregnancies without the need for involving the partner in testing (e.g. in the USA less than 50% of partners of pregnant women who are carriers complete the screening and also 50% pregnancies are unplanned, therefore the current conception of preconceptual carrier screening or early in pregnancy may be insufficient**
  - **Single blood draw is needed from the pregnant women**
  - **Both PPV and NPV of sgNIPT is superior to traditional carrier screening**
  - **The time to identify high-risk fetus is shorter than with the traditional screening**
  - **Cost-effectiveness**
  - **Better management of affected pregnancies (new therapy possibilities)**

- **AD conditions (clinically available for up to 25 AD de novo conditions)**
  - **Identification of affected fetuses that would have either presented later in pregnancy with fetal anomalies or not been detected prenatally given normal ultrasound**

Vora NL, Langlois S, Chitty LS. The debate during ISPD conference in Edinburgh, UK, 2023

Routine testing

Arguments against (dr. Lyn Chitty):

- **AR conditions**
  - **Traditional carrier screening can be carried out in many jurisdictions and added value of routine sgNIPT has not been clearly demonstrated**
  - **The limited number of variants in the screened genes**
  - **The available studies have suboptimal follow-up and lack of clinical validation**

- **AD conditions**
  - **Total lack of validation studies, suboptimal follow-up in the available studies**
  - **Risk of false reassurance in case of negative results and unnecessary invasive procedure in case of false positive results**

Vora NL, Langlois S, Chitty LS. The debate during ISPD conference in Edinburgh, UK, 2023

Routine testing

Arguments against:

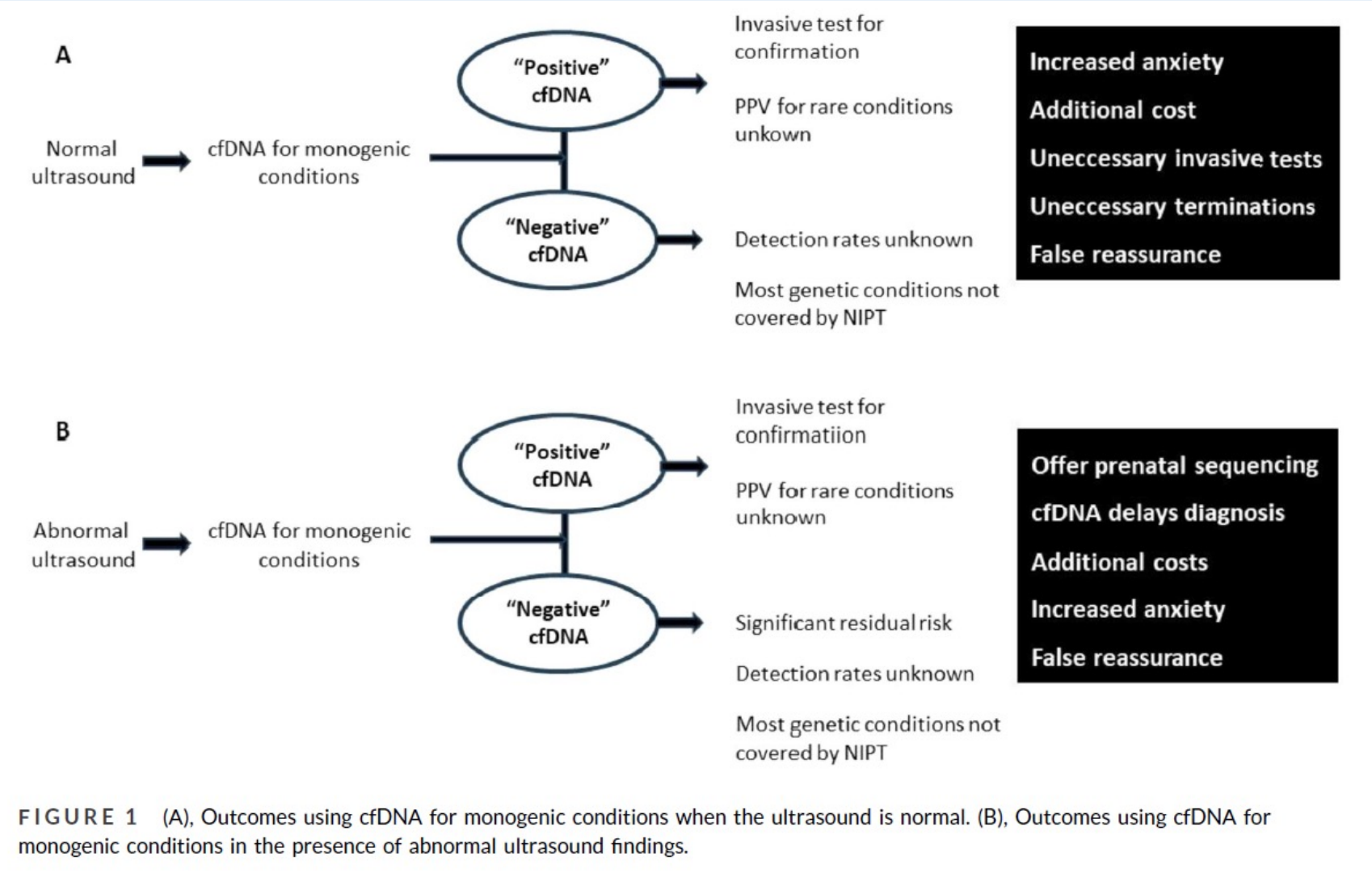

FIGURE 1 (A), Outcomes using cfDNA for monogenic conditions when the ultrasound is normal. (B), Outcomes using cfDNA for monogenic conditions in the presence of abnormal ultrasound findings.

Vora NL, Langlois S, Chitty LS. The debate during ISPD conference in Edinburgh, UK, 2023

Summary

- **NIPD for monogenic conditions is a rapidly developing field**
- **It can offer safe and accurate testing to couple with a relevant family history or following an abnormal ultrasound findings**
- **Techniques for paternally-inherited and de novo variants**
- **Techniques for more challenging maternally-inherited variants**
- **?? time to offer to low-risk pregnant women ??**
- **Further clinical validation studies are needed before broad implementation**

Thank you for your attention!

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