NIPD per Malattie Monogeniche ed Rh Fetale

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Non Invasive Prenatal Diagnosis (NIPD)

THE LANCET

Early report

1997

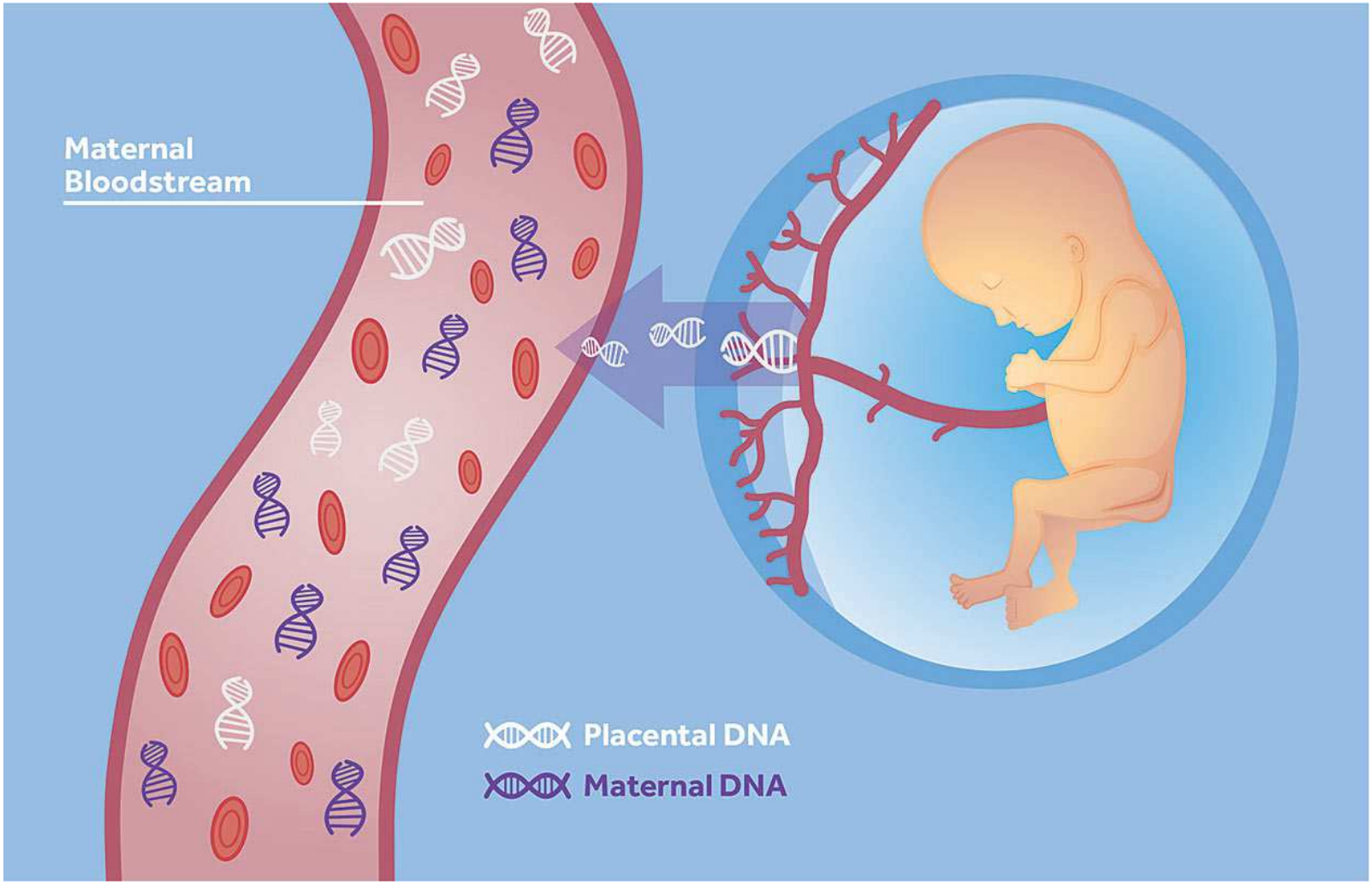
Presence of fetal DNA in maternal plasma and serum

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

Findings Fetus-derived Y sequences were detected in 24 (80%) of the 30 maternal plasma samples, and in 21 (70%) of the 30 maternal serum samples, from women bearing male fetuses. These results were obtained with only 10 µL of the samples. When DNA from nucleated blood cells extracted from a similar volume of blood was used, only five (17%) of the 30 samples gave a positive Y signal. None of the 13 women bearing female fetuses, and none of the ten non-pregnant control women, had positive results for plasma, serum or nucleated blood cells.

Interpretation Our finding of circulating fetal DNA in maternal plasma may have implications for non-invasive prenatal diagnosis, and for improving our understanding of the fetomaternal relationship.

cffDNA orignates from trophoblast



The cell-free fetal DNA

Early Human Development (2007) 83, 563–566

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/earlhumdev

Early detection of cell-free fetal DNA in maternal plasma

S. Illanes [a,*], M. Denbow [a], C. Kailasam [a], K. Finning [b], P.W. Soothill [a]

[a] Fetal Medicine Research Unit, Division of Obstetrics and Gynaecology, University of Bristol, Bristol, UK
[b] National Blood Service, Southmead Road, Bristol, UK

Table 2 The Y signal *DYS14* amplifications in maternal blood in the IVF pregnancies (I to VI) at 2, 4, 5 and 6 weeks' gestation

IVF study		No. Ct<45	No. Ct<40	Predicted sex	Sex at birth*
I	2 weeks	0	0		
	4 weeks	8	7		
	5 weeks	5	5		
	6 weeks	8	8	M	M
II	2 weeks	0	0		
	4 weeks	0	0		
	5 weeks	4	2		
	6 weeks	5	5	M	M
III	2 weeks	0	0		
	4 weeks	0	0		
	5 weeks	0	0		
	6 weeks	8	8	M	M
IV	2 weeks	0	0		
	4 weeks	0	0		
	5 weeks	0	0		
	6 weeks	4	2	E	M
V	2 weeks	0	0		
	4 weeks	0	0		
	5 weeks	5	0	E	M
VI	2 weeks	3	1		
	4 weeks	0	0		
	5 weeks	8	4	E	M

No sample was obtained in two cases at 6 weeks' gestation. The data are the number of replicates out of eight with a Ct number at the PCR cycle cut-offs of <45 and <40 (M: male, E: equivocal).
*Of at least one male fetus.

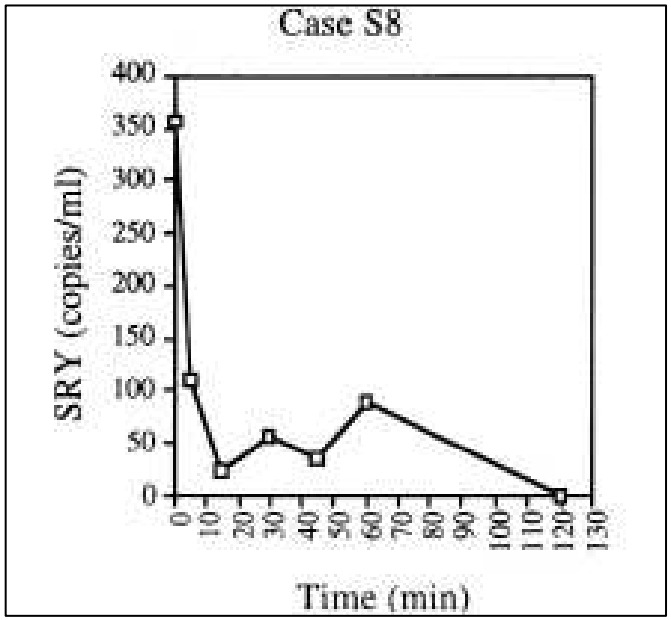
The cell-free fetal DNA

Am. J. Hum. Genet. 64:218–224, 1999

Rapid Clearance of Fetal DNA from Maternal Plasma

Y. M. Dennis Lo,[1] Jun Zhang,[1] Tse N. Leung,[2] Tze K. Lau,[2] Allan M. Z. Chang,[2] and N. Magnus Hjelm[1]

Departments of [1]Chemical Pathology and [2]Obstetrics and Gynecology, Chinese University of Hong Kong, Prince of Wales Hos New Territories, Hong Kong



Summary

Fetal DNA has been detected in maternal plasma during pregnancy. We investigated the clearance of circulating fetal DNA after delivery, using quantitative PCR analysis of the sex-determining region Y gene as a marker for male fetuses. We analyzed plasma samples from 12 women 1–42 d after delivery of male babies and found that circulating fetal DNA was undetectable by day 1 after delivery. To obtain a higher time-resolution picture of fetal DNA clearance, we performed serial sampling of eight women, which indicated that most women (seven) had undetectable levels of circulating fetal DNA by 2 h postpartum. The mean half-life for circulating fetal DNA was 16.3 min (range 4–30 min). Plasma nucleases were found to account for only part of the clearance of plasma fetal DNA. The rapid turnover of circulating DNA suggests that plasma DNA analysis may be less susceptible to false-positive results, which result from carryover from previous pregnancies, than is the detection of fetal cells in maternal blood; also, rapid turnover may be useful for the monitoring of feto-maternal events with rapid dynamics. These results also may have implications for the study of other types of nonhost DNA in plasma, such as circulating tumor-derived and graft-derived DNA in oncology and transplant patients, respectively.

Applicazioni (pre NGS)

- **Sesso Fetale**
- **Rh**
- **Malattie Monogeniche (Alleli Paterni)**

Già da 8°-9° s.g. (Frazione Fetale non deve essere molto elevata)

Ricerca Aneuploidie

Dato QUANTITATIVO

(conto quanto DNA c'è per ogni cromosoma)

Necessario NGS

Sesso

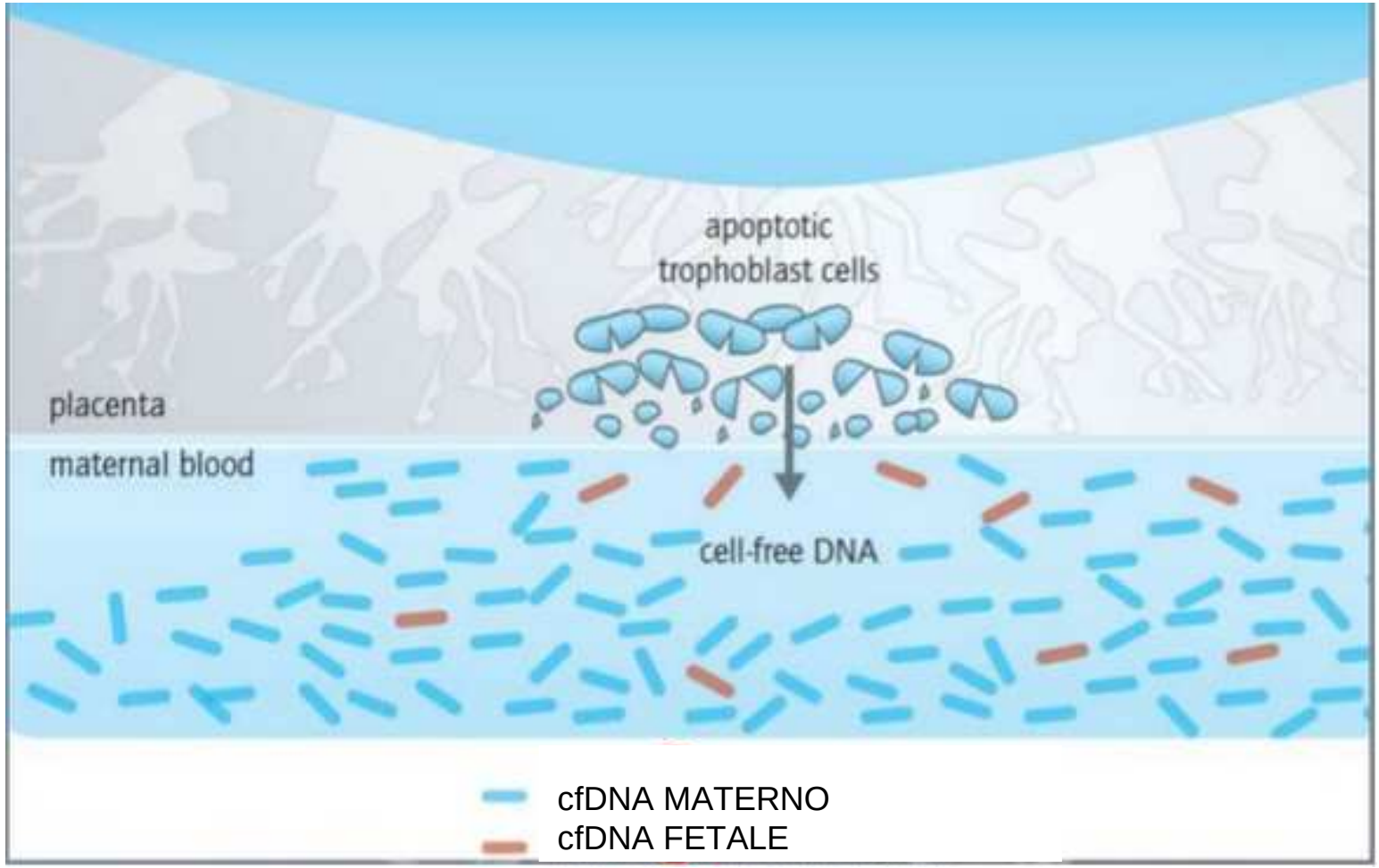
Rh

Mutazioni puntiformi

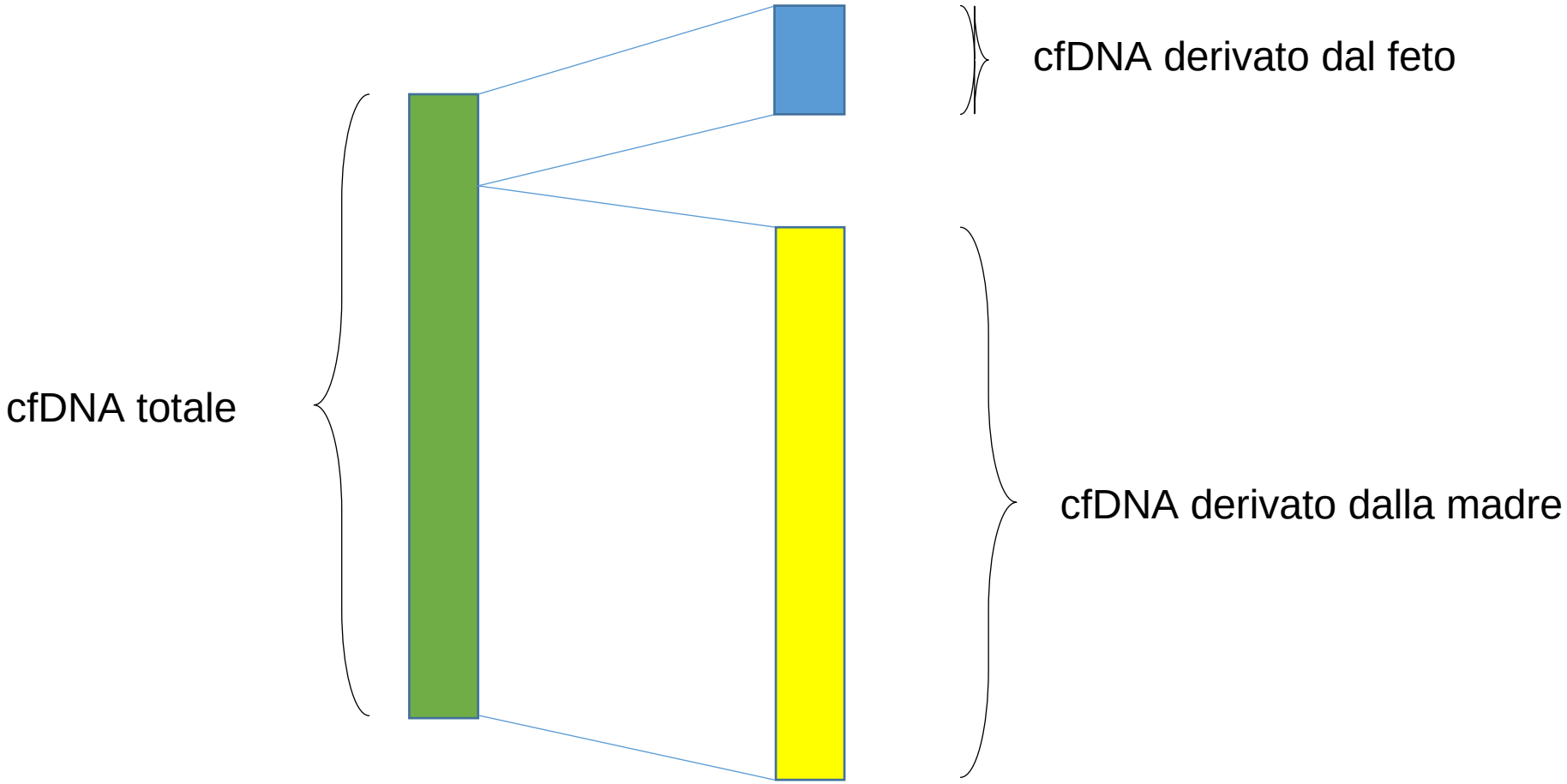
Dato QUALITATIVO

(C'è o non c'è)

Basta BIO MOLECOLARE CLASSICA



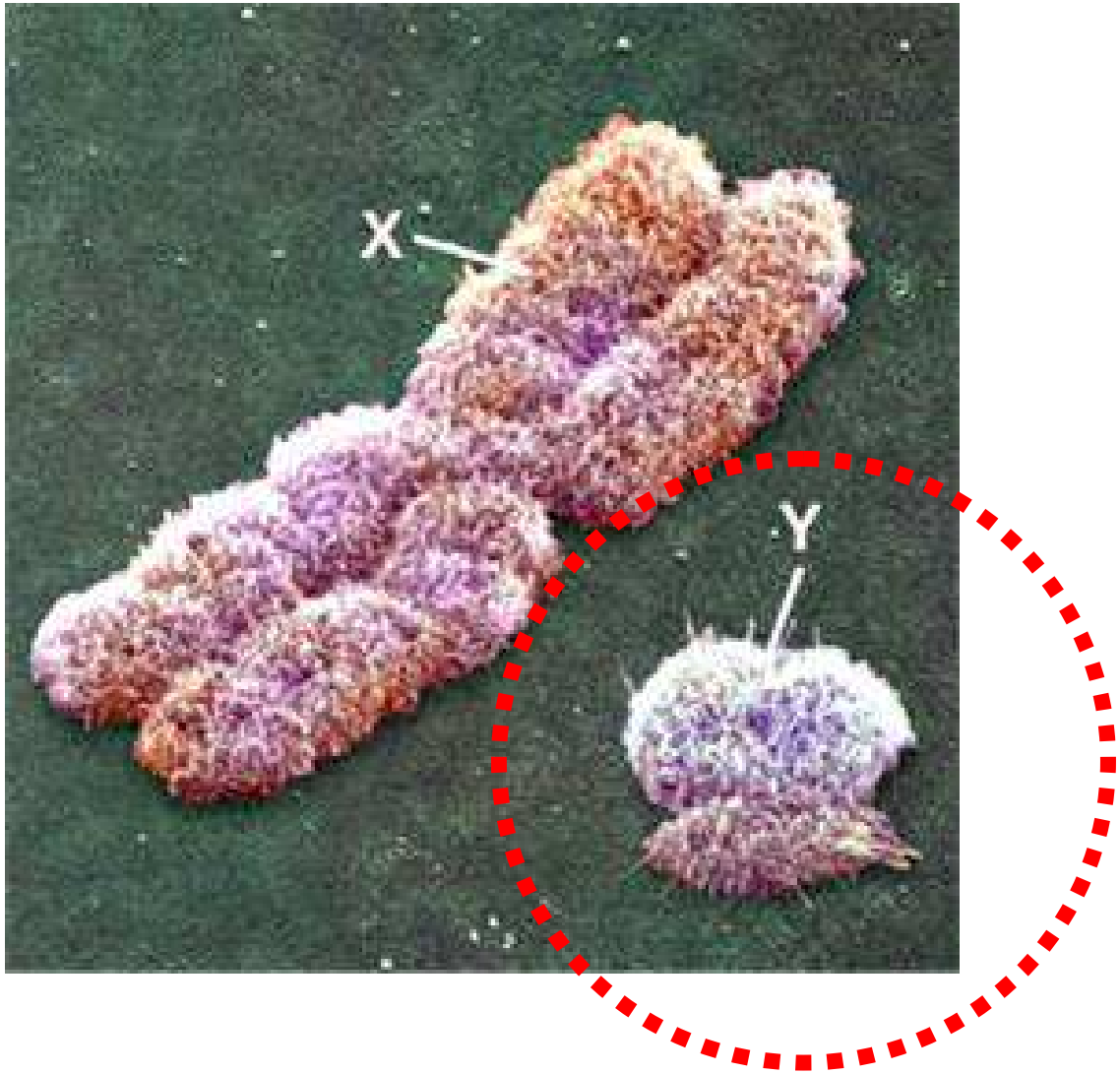
Cosa è di origine fetale ma non materna?



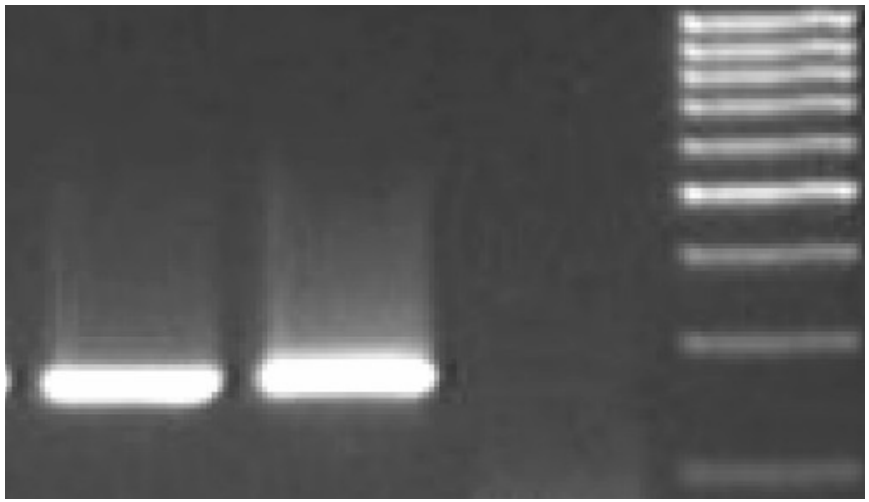
$$\text{Fetal fraction} = \frac{\text{cfDNA derivato dal feto}}{\text{cfDNA totale}}$$

Per queste applicazioni la Fetal Fraction è un po’ meno critica (rispetto alla ricerca per aneuploidie)

Possibile analisi più precoce


X
Y

Per determinare il sesso fetale basta amplificare con PCR «standard» sequenze specifiche del cromosoma Y



Se amplificazione positiva il feto sarà maschio
altrimenti sarà femmina

(protocolli «moderni usano RQ-PCR)

Nel caso di malattie XL (DMD) sapere che il feto è femmina potrebbe evitare il ricorso alla Diagnosi invasiva

Rh pos

Rh neg

Nei feti maschi analisi chr Y fa da controllo Positivo

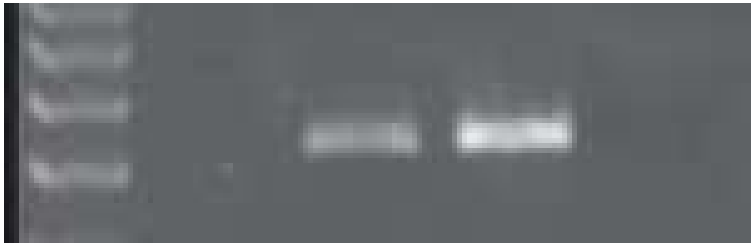
Problema nei feti femmina

possibile analisi di altri marcatori polimorfici che permettono di identificare alleli fetali in oltre 95-99% dei casi (e determinazione della frazione fetale)

Madre	Rh neg	
Padre	Rh pos	rischio immunizzazione materna contro antigene Rh m.emolitica del neonato in gravidanze successive

Determinazione precoce permette di evitare profilassi o di programmare opportuni monitoraggi

1 2



Oggi Tecniche più «Raffinate»

Clin Chem. 2015 Nov;61(11):1399-407. doi: 10.1373/clinchem.2015.239137. Epub 2015 Sep 9.

Fetal Sex and RHD Genotyping with Digital PCR Demonstrates Greater Sensitivity than Real-time PCR.

Sillence KA[1], Roberts LA[2], Hollands HJ[2], Thompson HP[1], Kiernan M[1], Madgett TE[1], Ross Welch C[2], Avent ND[3].

Author information

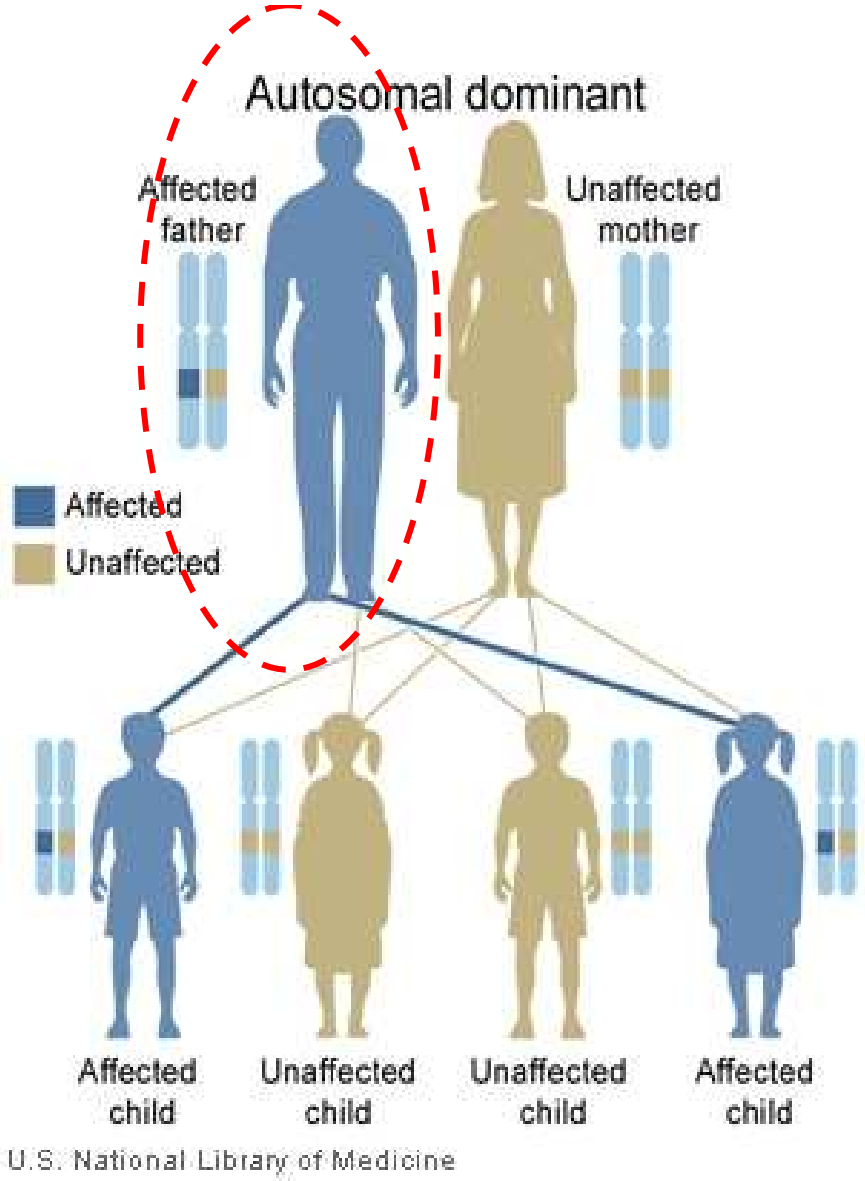
Analisi possibili anche con Fetal Fraction molto bassa

Malattie monogeniche

AD ricerca mutazioni Paterne nel feto

AR esclusione trasmissione

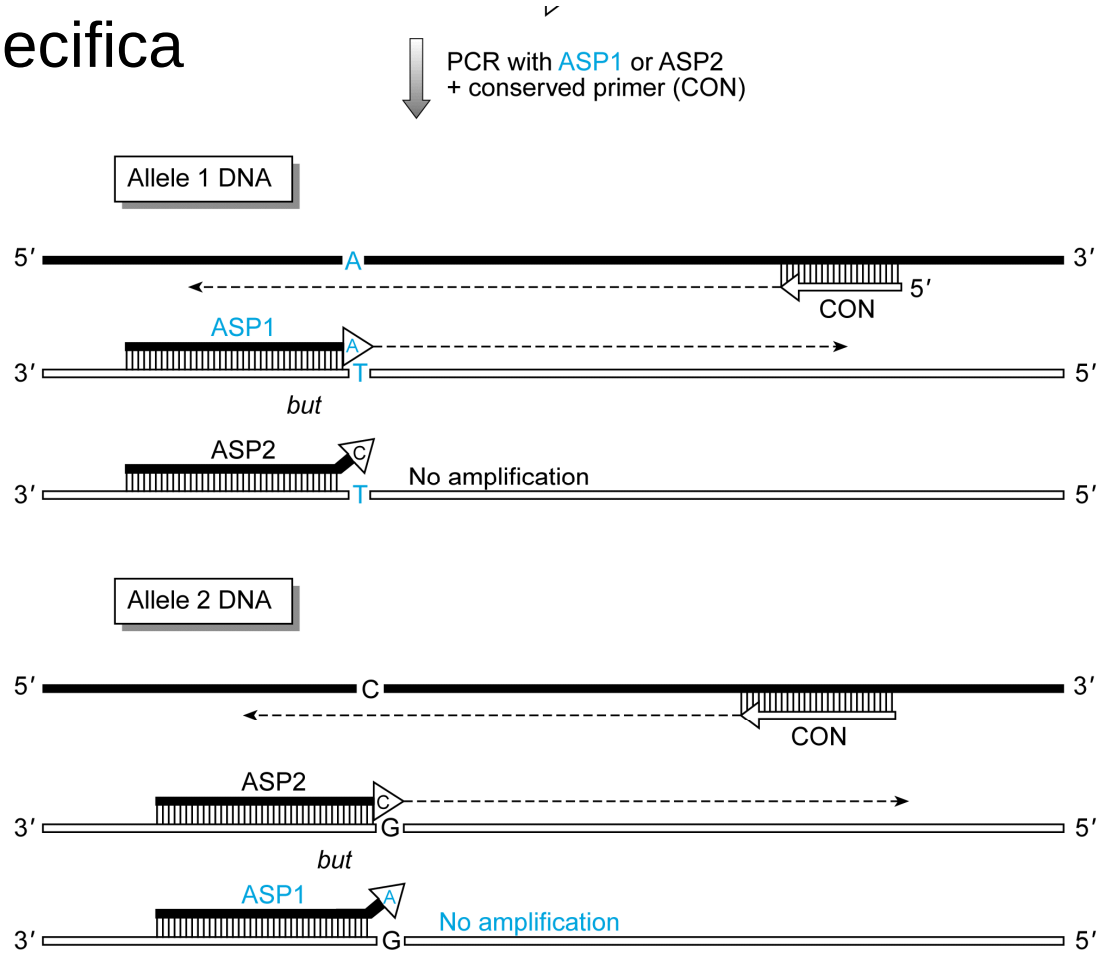
XL (al momento non applicazioni)



CERCO MUTAZIONE PATERNA SU cfDNA MATERNO

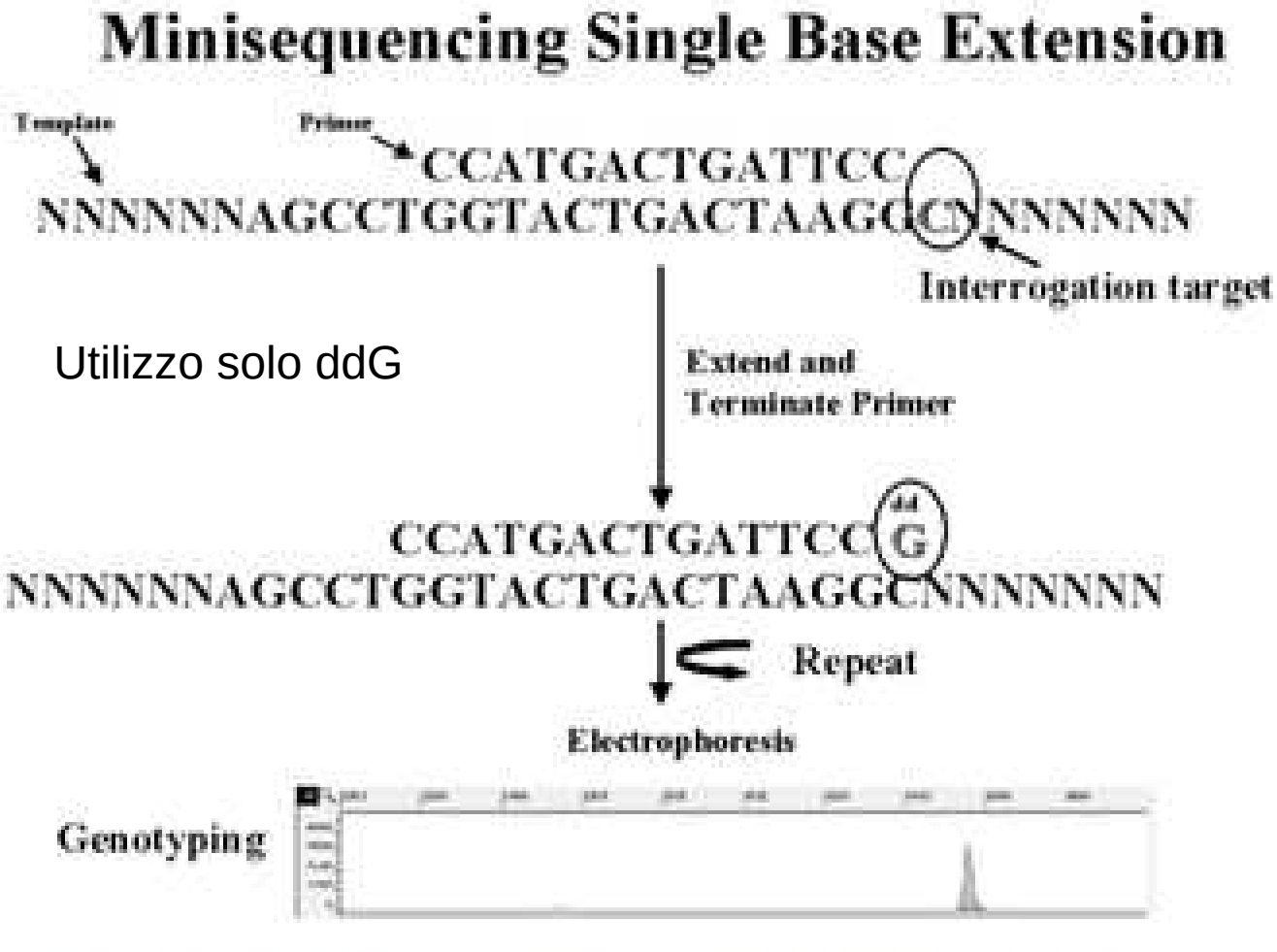
Possibili vari approcci

PCR allele specifica



Possibili vari approcci

Minisequencing



Paternal NF1 point mutation

CVS confirmed finding

Possibili anche altre metodiche.
Queste sono relativamente economiche e semplici da implementare (spt. Minisequencing)

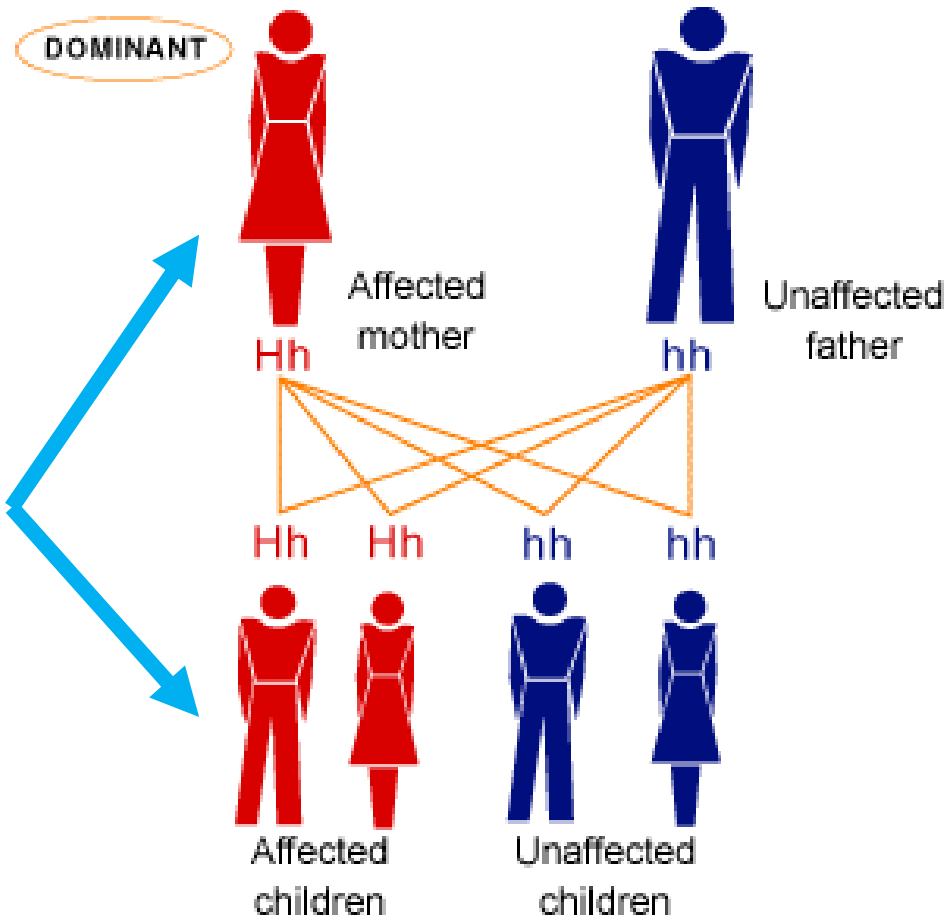
In questo caso è una tecnica potenzialmente «diagnostica»

(se trovo la mutazione paterna sul cfDNA materno il feto sarà *sicuramente* affetto)

Teoricamente meno problemi rispetto ad analisi per aneuploidie (come diagnosi di mutazionie su villi coriali)

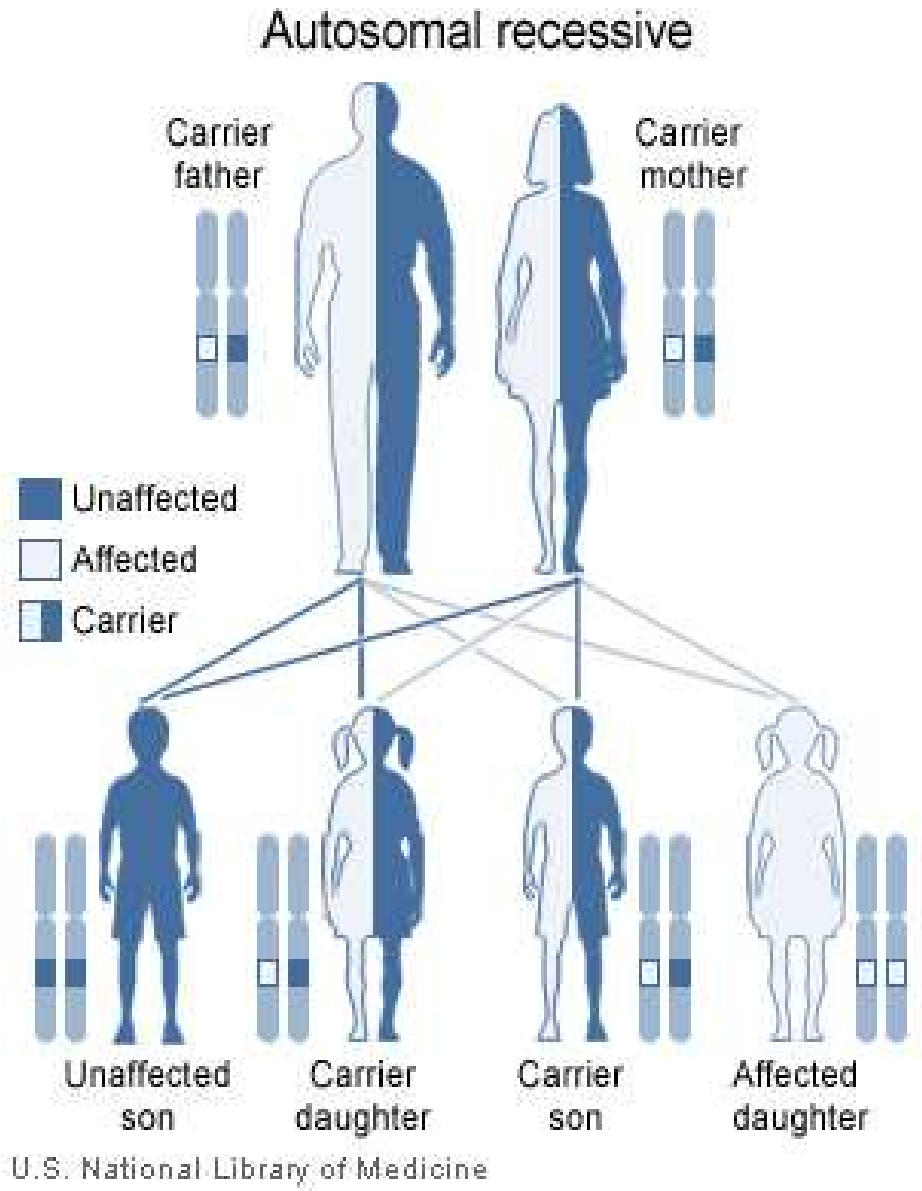
(in pratica esperienza relativamente poca)

Stessa mutazione



NIPD con metodiche «classiche» non possibile

Non riesco a discriminare quali molecolde del cfDNA derivano dal feto e quali dalla madre



Possibile se mutazione Materna è DIVERSA da quella Paterna

Ricerco mutazione PATERNA come per le AD

In questo caso identifico solo feti «a rischio»

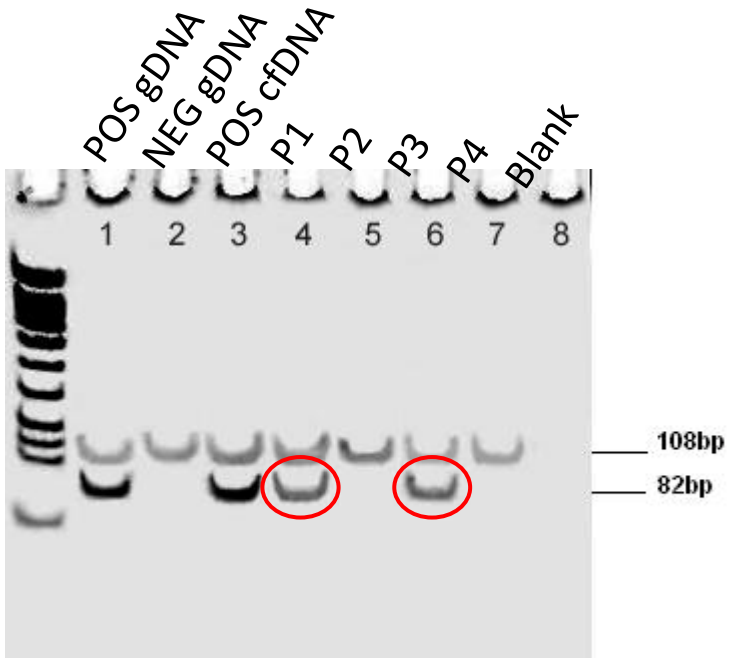
Se ricerca negativa escludo patologia
(feto al max portatore)

Se ricerca positiva feto 50% Portatore
50% Affetto

Dimezzo le dgn. Invasive

Analysis of paternal *CFTR* F508del mutation in maternal plasma

FATHER F508del
MOTHER other *CFTR* mutation (non-F508del)



«Positive» women underwent CVS

1 fetus had not inherited maternal mutation

1 fetus had both mutations > TOP

If both parents carry the same mutant allele

NIPD not possibile

(Will not work for SMA)

In passato

Tentativi di arricchire cffDNA in base alle dimensioni (frammenti più piccoli rispetto a frammenti di derivaione materna)

Profili di metilazione

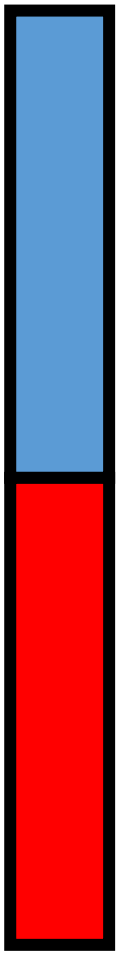
NGS (e digital PCR)

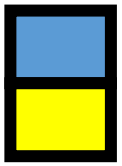
In teoria permette analisi di mutazioni AD Materne

E ricerca di ambedue gli alleli nelle AR

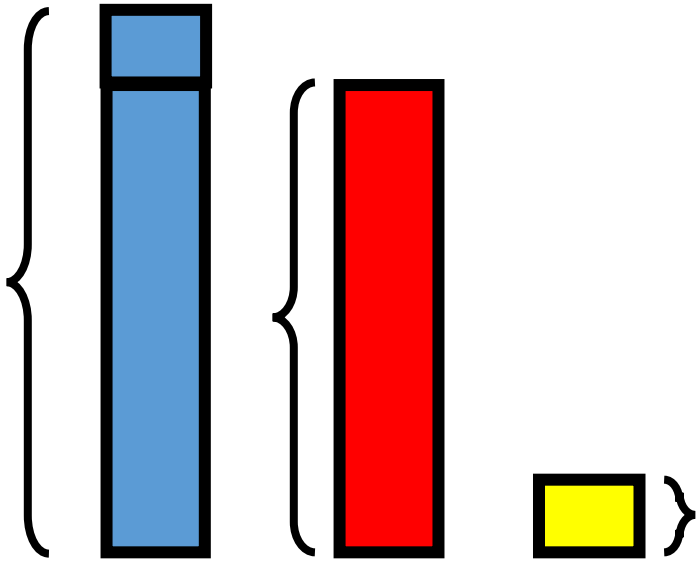
DNA di derivazione
MATERNA

DNA di derivazione
FETALE





NGS permette di quantificare con precisione l’abbondanza relativa dei vari alleli.



Necessario «very deep» sequencing per avere differenze statisticamente significative

Messa a punto complessa (cinetiche di amplificazione dei vari frammenti non omogenee)

Molte mutazioni diverse

Costo elevato per messa a punto della singola analisi

numerosità pazienti scarsa (eccetto forse CF e emoglobinopatie)

Clinical Chemistry 58:6
1026–1032 (2012)

Digital PCR Analysis of Maternal Plasma for Noninvasive Detection of Sickle Cell Anemia

Angela N. Barrett,[1,2] Thomas C.R. McDonnell,[1] K.C. Allen Chan,[3] and Lyn S. Chitty[2,4*]

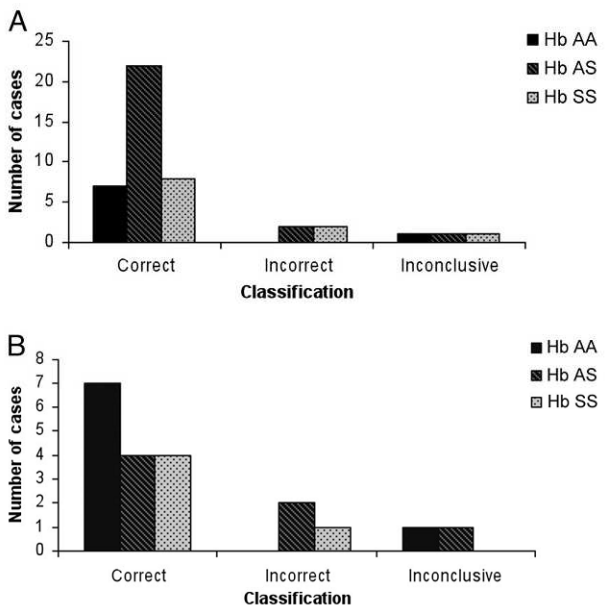


Fig. 1. Genotype for each classification for male-bearing (A) and female-bearing (B) pregnancies.

The number of samples with a particular genotype is shown for correct, incorrect, and inconclusive classifications.

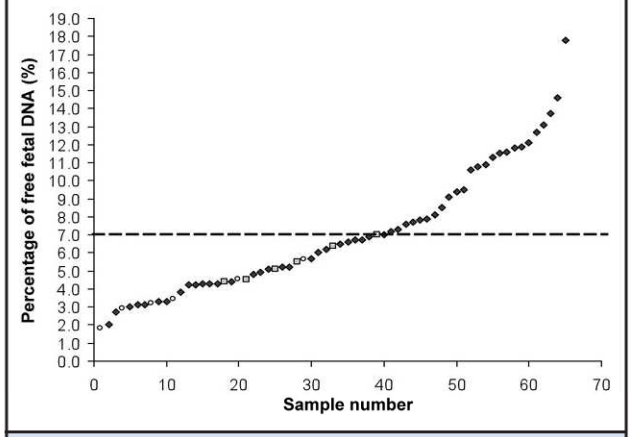


Fig. 2. Classification for male- and female-bearing pregnancies with an increasing cffDNA percentage.

At cffDNA percentages >7% (dashed line), all samples are classified correctly: ◆, correct result; ■, incorrect result; ○, unclassified.

Altre applicazioni possibili

Paternità

Ricerca di mutazioni de novo (NGS)

es pannello RAS ad alto covrage

in feti con NT aumentata

…