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Centro de Referencia Perinatal Oriente

Facultad de Medicina, Universidad de Chile

NIPT: rendimiento en CNVs y enfermedades monogénicas

Joaquín López G.

Becado PTE Genética Clínica

Tutora: Dra. Catherine Diaz

Hoja de ruta

- NIPT: introducción y detalles técnicos generales
- Usos clásicos en clínica
- Técnica y rendimiento en variantes de número de copias (CNV)
- Técnica y rendimiento en enfermedades monogénicas

Introducción y detalles técnicos generales

Pruebas prenatales no invasivas (NIPT)

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- Técnicas moleculares orientadas a evaluar anomalías genéticas prenatales sin recurrir a procedimientos invasivos
- Fundamentado en presencia de ADN libre fetal en sangre materna (cffDNA) (1997)

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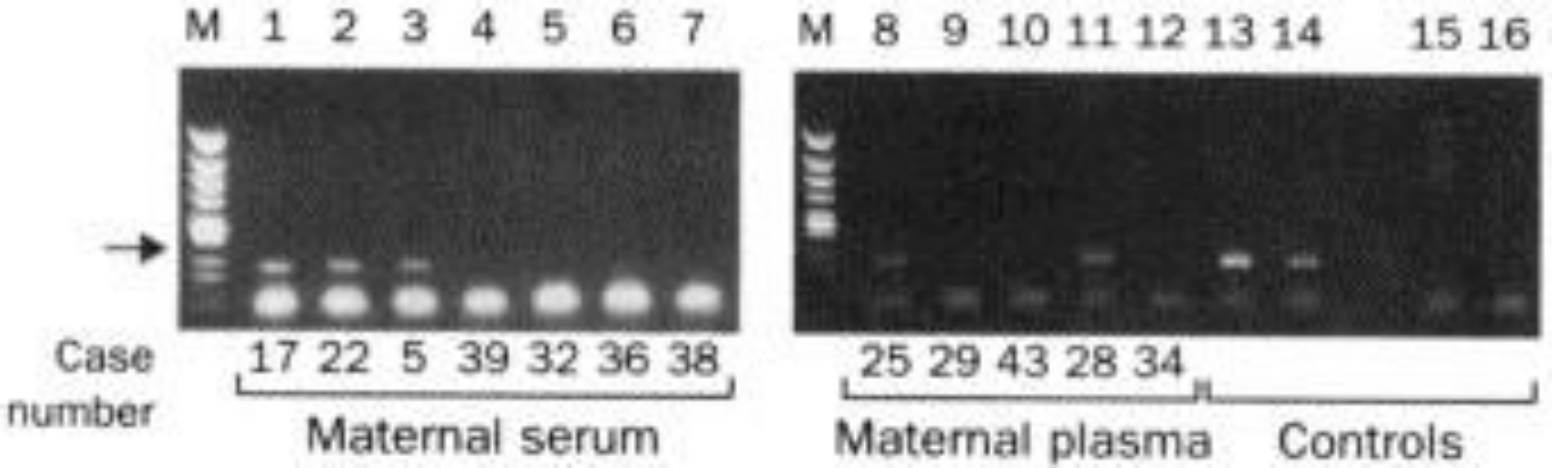
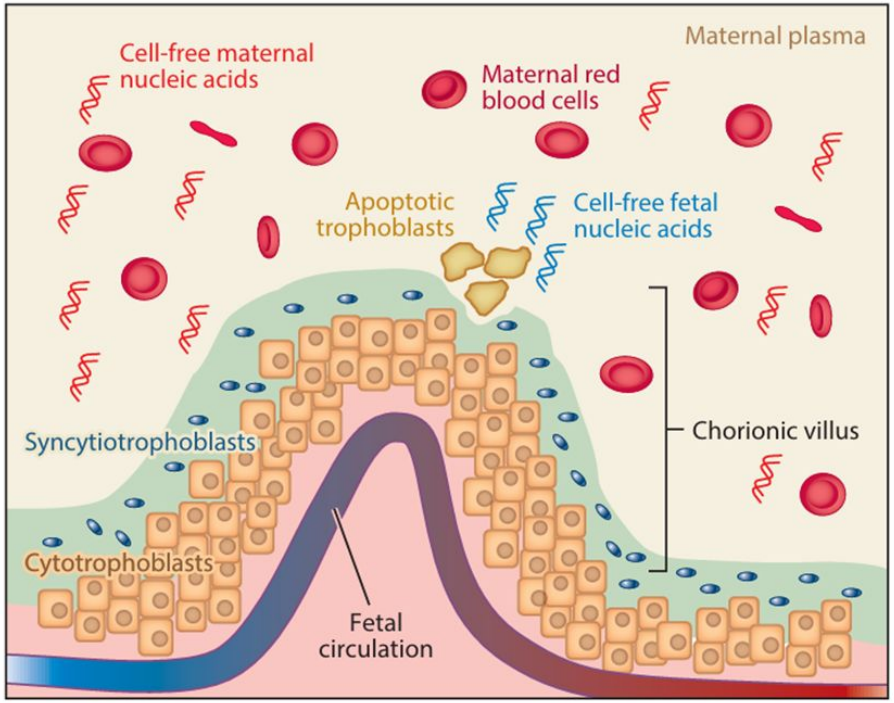


Detección de material de cromosoma Y en sangre materna (carriles 1 - 12)

Lo, Y. M. et al. (1997). Presence of fetal DNA in maternal plasma and serum. *Lancet (London, England)*, *350*(9076), 485–487.

ADN libre fetal en sangre materna

- cffDNA es representativo de genoma fetal
- Se origina de células trofoblásticas placentarias
- Detectable desde 4 semanas de gestación



Norton, M. Circulating cell-free DNA and screening for trisomies. NEJM 2022. 387(14), 1322-1324.

Fracción fetal

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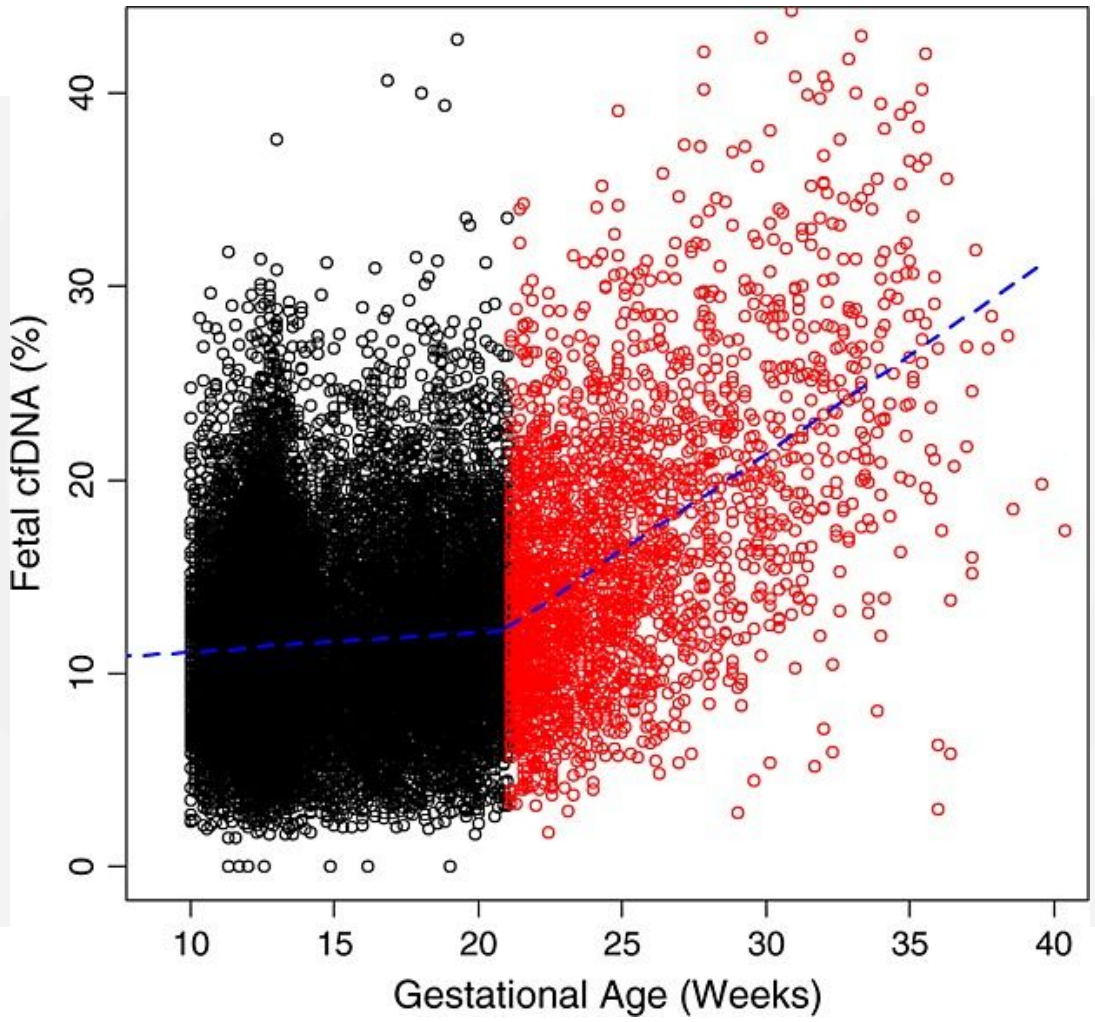
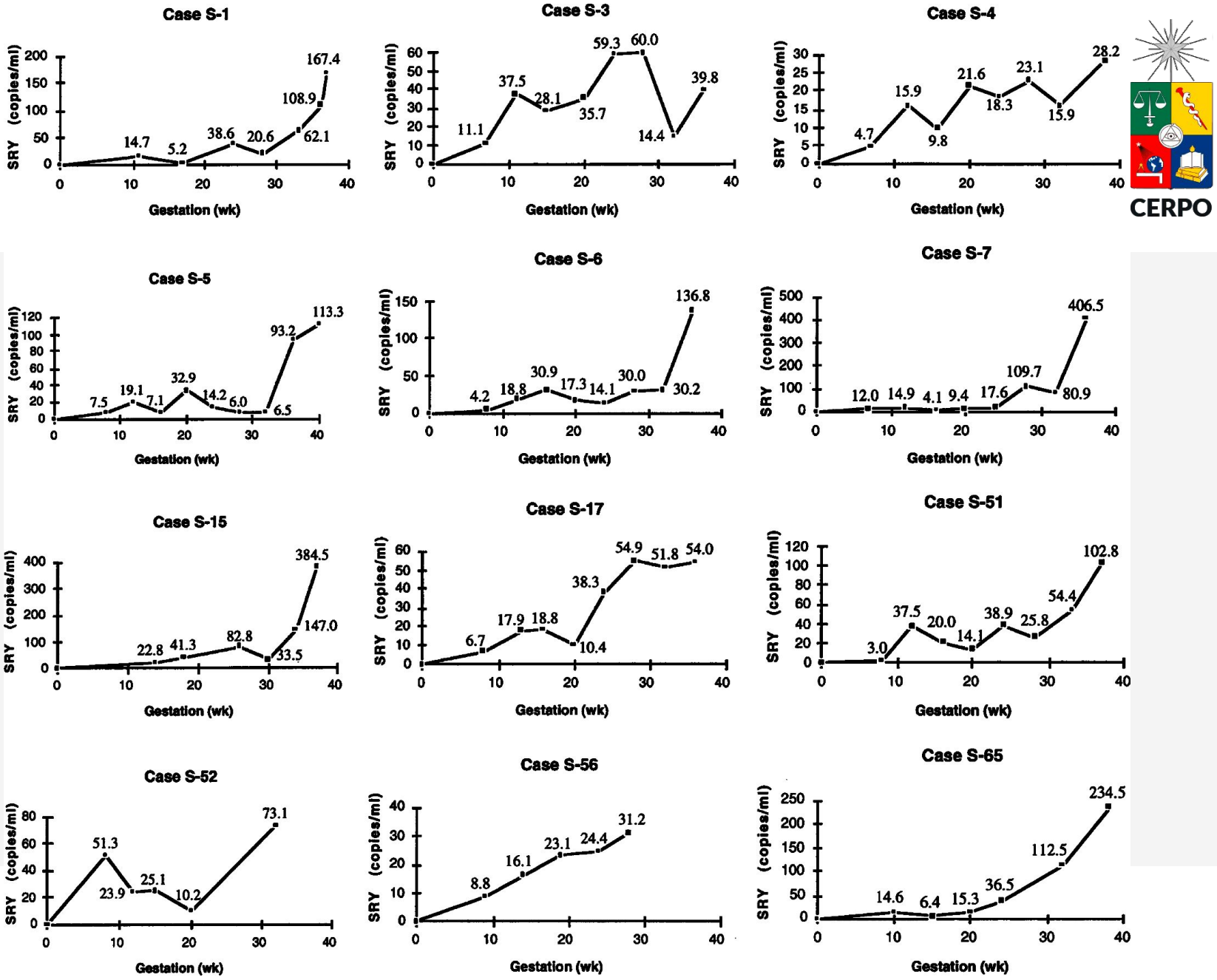
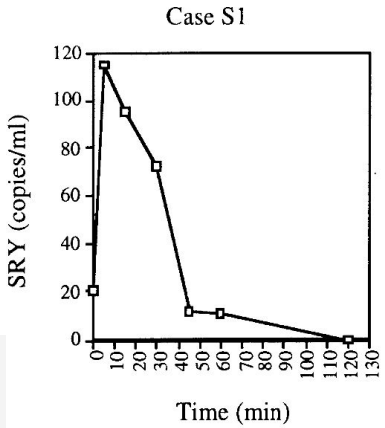
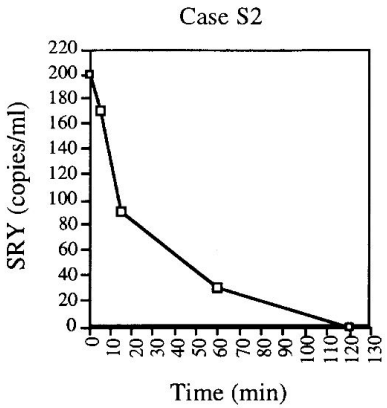


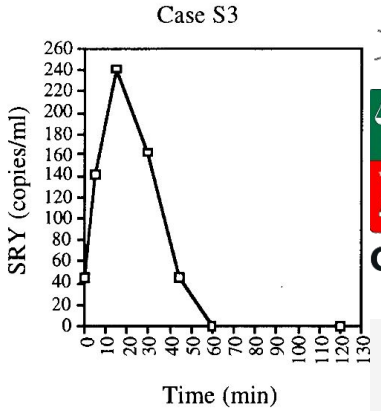
Wang, E. et al. (2013). Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasma. *Prenatal diagnosis*, *33*(7), 662–666.

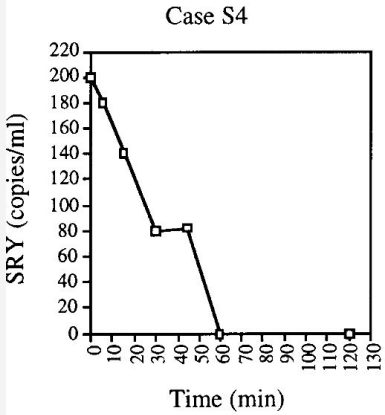


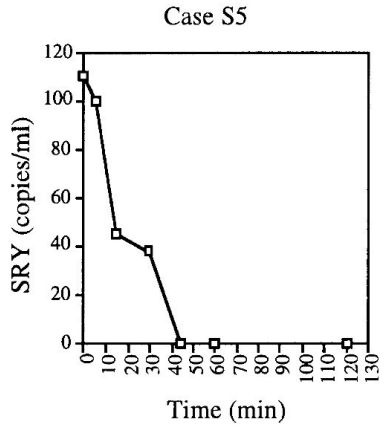
Lo, Y. M. et al. (1998). Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. *American journal of human genetics*, *62*(4), 768–775.

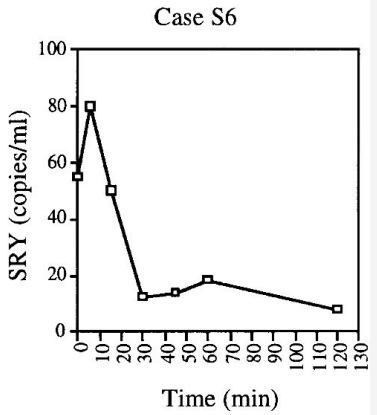


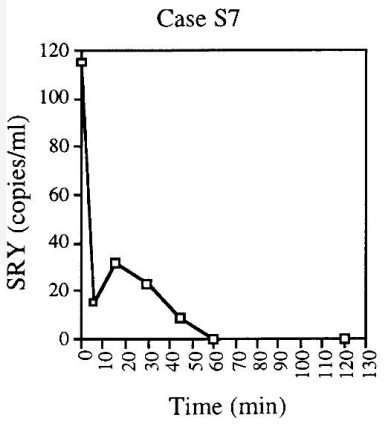


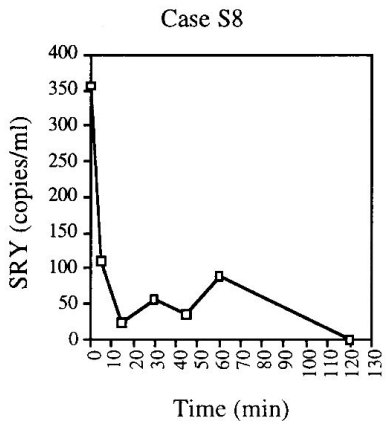












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Clearance de cffDNA ocurre en horas post parto

Lo, Y. M. et al. (1999). Rapid clearance of fetal DNA from maternal plasma. *American journal of human genetics*, *64*(1), 218–224.

Table 1. Proportion of pregnancies with ≥4% fetal cell-free DNA on first blood draw

Maternal weight bin (kg)	*n*	Pregnancies with ≥4% fetal cell-free DNA (%)
<50	809	99.8
≥50 < 60	4825	99.6
≥60 < 70	6224	99.2
≥70 < 80	4313	98.8
≥80 < 90	2574	98.2
≥90 < 100	1608	96.3
≥100 < 110	921	93.9
≥110 < 120	508	89.8
≥120 < 130	298	87.9
≥130 < 140	172	81.4
≥140	132	71.2

Mayor masa materna se correlaciona a menor porcentaje de cffDNA

Lo, Y. M. et al. (1999). Rapid clearance of fetal DNA from maternal plasma. *American journal of human genetics*, *64*(1), 218–224.

Positive correlation	Statistical values	Negative correlation	Statistical values	No correlation
PAPP-A	0.1493 (0.0921-0.2064), <0.001†; Increased by about 1% per 0.5 MoM increase in PAPP-A (24); 0.133 (0.119-0.146), <0.0001† (25)	Maternal body weight	−0.0093 (−0.0114 to −0.0071), <0.001†; Decreased by about 1% per 10 kg (24); $P <$ 0.001 (26); $P < 0.001$ (27)	Maternal age (26)
Free β-hCG	0.0706 (0.0434-0.0978), <0.001†; Increased by about 0.4% per 0.5 MoM increase in β-hCG (24); 0.140 (0.128-0.152), <0.0001 (25)†	BMI	−0.541 (−0.697 to −0.385), <0.0001† (28); −0.0022, <0.0001, 0.1241‡ (29); −0.295 (−0.329 to −0.26), <0.001† (30)	Assisted reproductive pregnancy (31)
		Maternal age	−0.202 (−0.316 to −0.089), 0.0005† (28); −0.081 (−0.103 to −0.059), < 0.001† (30); $P < 0.05$ (32)	Serological screening risk (33, 34)
		Racial origin	African American ($P = 0.007$) (35); Asian women ($P = 0.03$) (36); South Asian, −0.019 (−0.032 to −0.005), 0.008† (25)	Maternal smoking (37)
		Assisted reproductive pregnancy	−0.033 (−0.050 to −0.016), <0.001† (38)	Low molecular weight heparin (39)
		Low molecular weight heparin	37.5, 11.19–125.87, <0.0001§ (40)	Pre-existing diabetes (41)
		Drug use	−0.6 (−1.2 to 0.1), 0.02† (42)	Hyperthyroidism (41)
		Physical activity	$P < 0.01$, a decrease varying from 1–17 percentage points (43)	Gestational diabetes (44)
		Maternal diseases	−4.1 (−5.7 to −2.5), < 0.05† (45)	HBsAg (30, 41)
		Pre-existing hypertension	RMoM of 0.85 ($P = 0.02$) (41)	

PAPP-A, serum pregnancy-associated plasma protein; Free β-hCG, free β-subunit of human chorionic gonadotropin; BMI, body mass index; HBsAg, maternal carriers of the hepatitis B virus surface antigen; RMoM, the ratio between mean adjust multiples of the median value and theoretical "one"; aOR, the adjusted odds ratio adjusting for BMI, hypertension, anticoagulation use, and gestational age at circulating cell-free DNA blood draw.

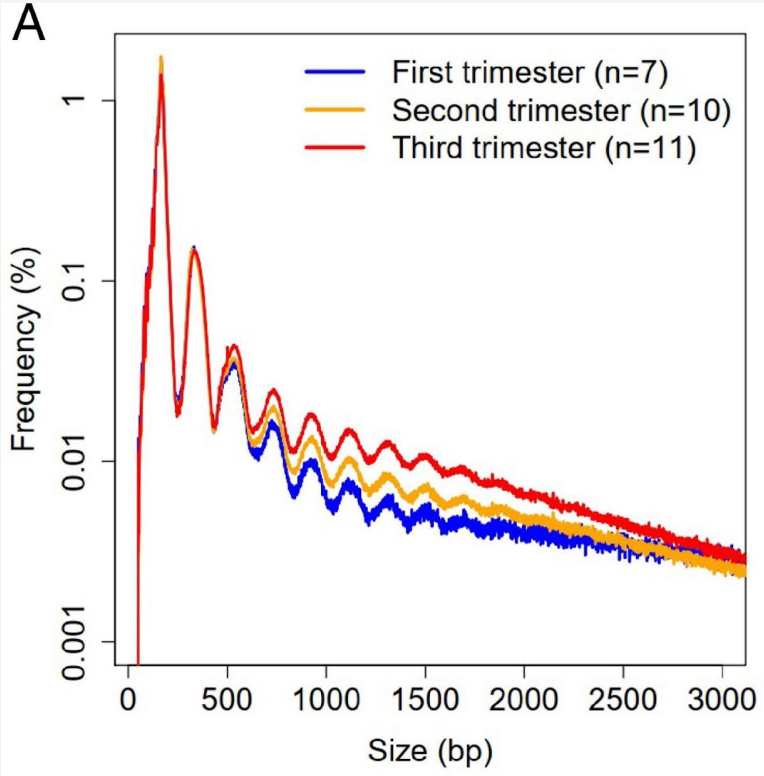
†Regression coefficient (95% confidence interval), P; ‡Regression coefficient, P; Intercept: §aOR, (95% confidence interval), P.

Significant at $P < 0.05$.

Deng, C., & Liu, S. (2022). Factors Affecting the Fetal Fraction in Noninvasive Prenatal Screening: A Review. *Frontiers in pediatrics*, *10*, 812781

ADN libre fetal en sangre materna

- Longitud promedio cffDNA ~143 bp
- Longitud promedio cfDNA materno ~166 bp
- Sugerido recientemente sesgo por uso inicial de tecnologías de secuenciación corta



Yu, S. et al. (2021). Single-molecule sequencing reveals a large population of long cell-free DNA molecules in maternal plasma. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(50), e2114937118.

NIPT en clínica

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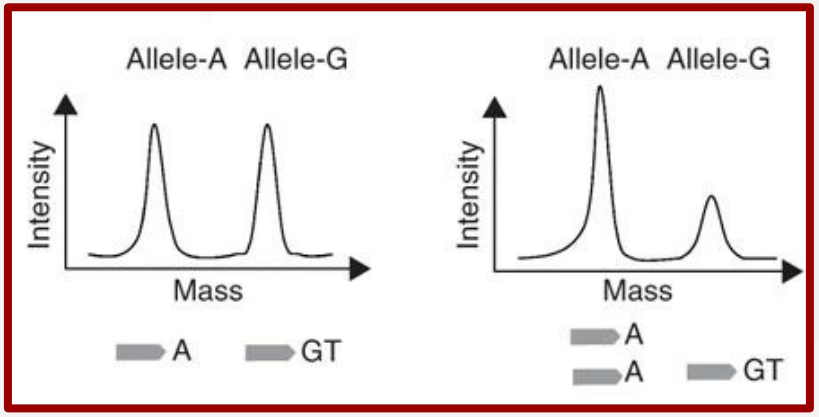
Technical Report | Published: 07 January 2007

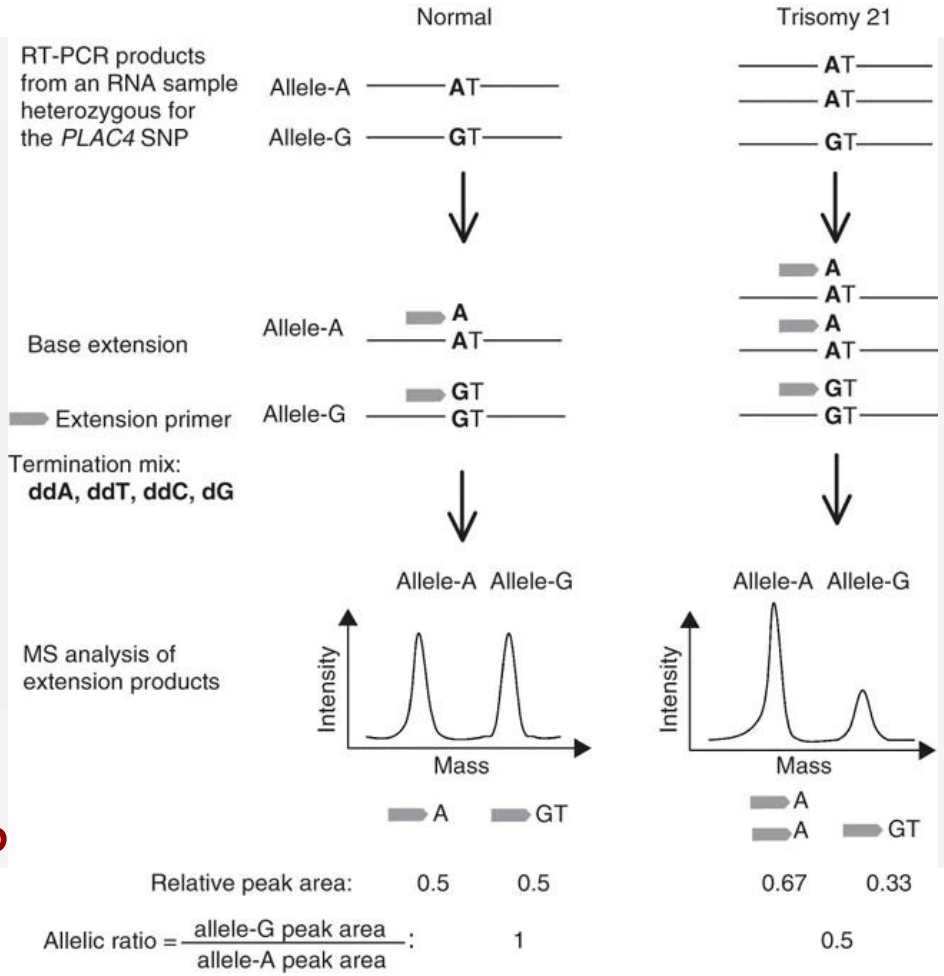
Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection

Y M Dennis Lo, Nancy B Y Tsui, Rossa W K Chiu, Tze K Lau, Tse N Leung, Macy M S Heung, Ageliki Gerovassili, Yongjie Jin, Kypros H Nicolaides, Charles R Cantor & Chunming Ding

Nature Medicine **13**, 218–223 (2007) | Cite this article



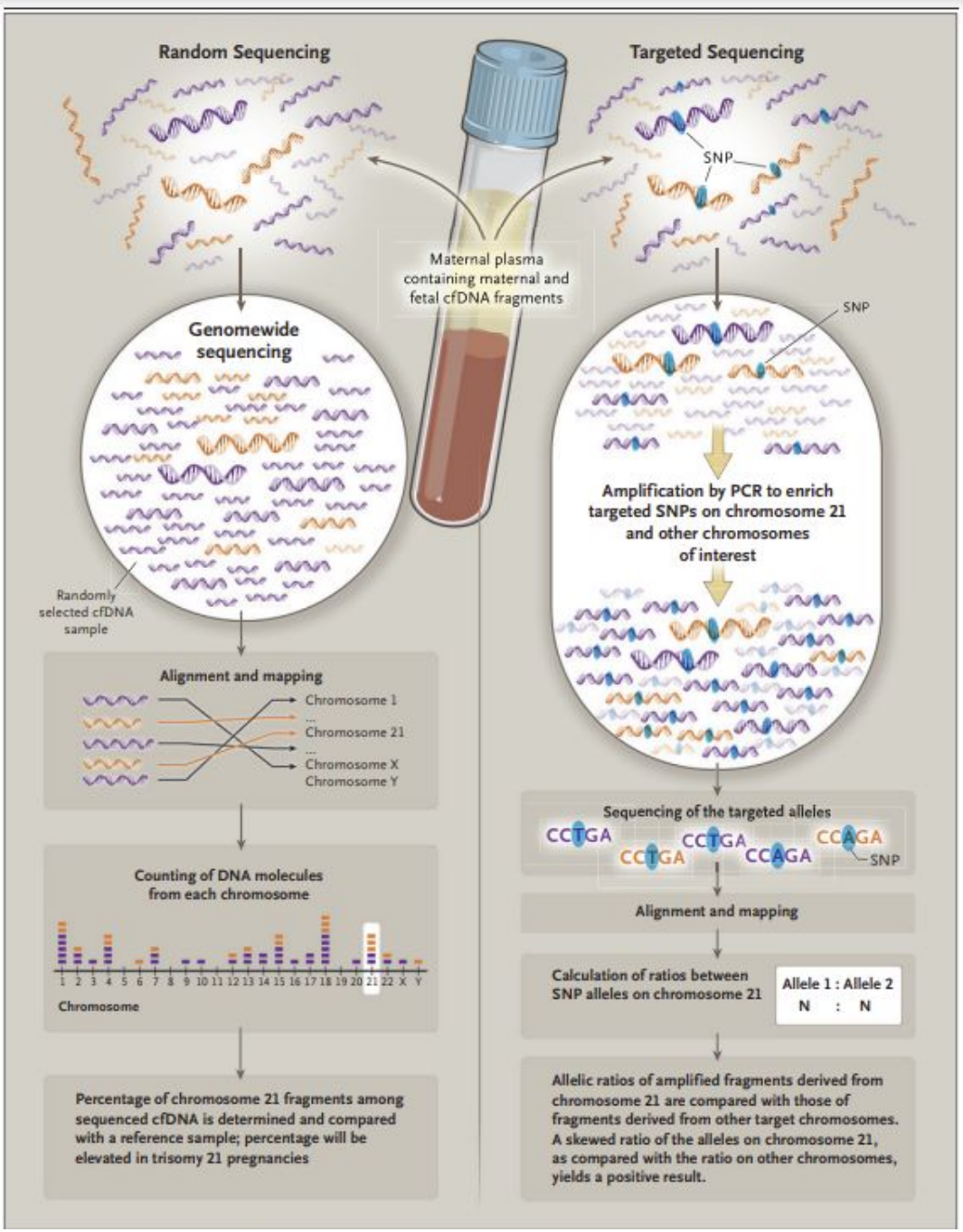
Cambio en señal relativa de SNP en cromosoma 21



Lo, Y. M. et al. (2007). Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection. *Nature medicine*, *13*(2), 218–223.

Genome-wide seq

Amplificación sitio-dirigida



Norton, M. Circulating cell-free DNA and screening for trisomies. NEJM 2022. 387(14), 1322-1324.

NIPT en clínica

- Aprobación uso clínico para estudio de aneuploidías (T21 y otras):
  - EEUU y Hong Kong: 2011
  - Países Bajos: 2014 (TRIDENT-1)
  - UK: 2015
  - China: 2016

Jayashankar, S. S. et al. (2023). Non-Invasive Prenatal Testing (NIPT): Reliability, Challenges, and Future Directions. *Diagnostics (Basel, Switzerland)*, *13*(15), 2570

Fetal Diagnosis and Therapy

Original Paper

Fetal Diagn Ther 2014;35:156–173
DOI: 10.1159/000358326

Received: November 13, 2013
Accepted after revision: December 26, 2013
Published online: February 8, 2014

Analysis of Cell-Free DNA in Maternal Blood in Screening for Aneuploidies: Meta-Analysis

M.M. Gil[a] R. Akolekar[a, b] M.S. Quezada[a] B. Bregant[a] K.H. Nicolaides[a]

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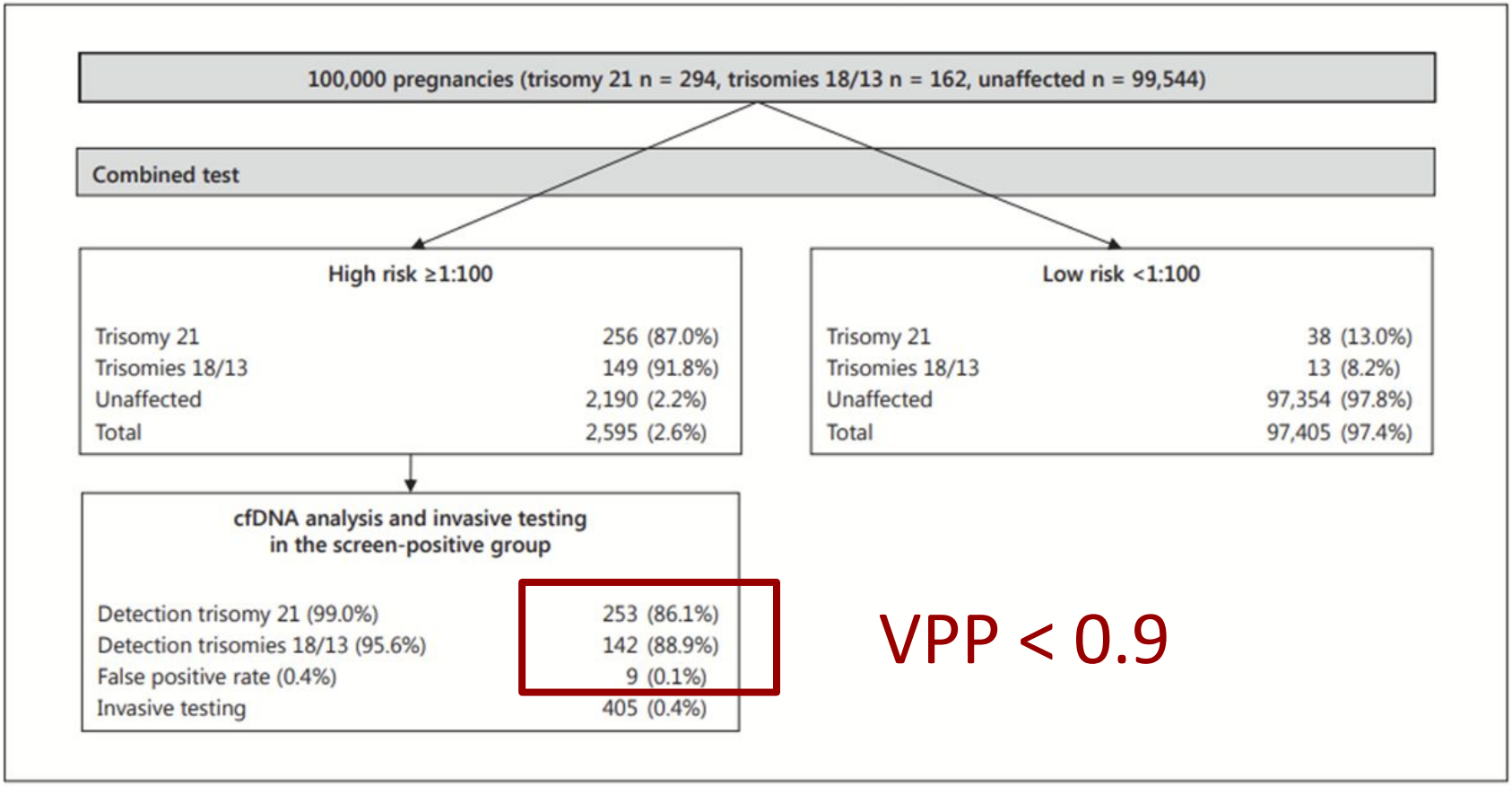

VPP < 0.9

Gil, M. et al.. (2017). Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *50*(3), 302–314.

Cases of TP FP distribution in different types of aneuploides

NIPT positive result	Cases n (%)	True positive	False positive	PPV (%)	FPR (%)
T21	144	124	20	86.1	13.9
T18	45	26	19	57.8	42.2
T13	28	7	21	25.0	75.0
SCAs	146	60	86	41.1	58.9
Monosomy X	40	8	32	20.0	80.0
XXX	17	4	13	23.5	76.5
XXY	16	11	5	68.8	25.0
XYY	8	5	3	62.5	37.5
RCAs	53	9	44	17.0	83.0
CNVs	52	21	31	40.4	59.6
Total	468	247	221	52.8	47.2

Liu, S. et al. (2022). Positive predictive value estimates for noninvasive prenatal testing from data of a prenatal diagnosis laboratory and literature review. *Molecular cytogenetics*, *15*(1), 29.

Table 4.

Cases of TP and FP distributions in different risk groups

Group	Total	True Positive	False Positive	PPV (%)	*P* value
All aneuploides					
Low risk	100	32	68	32.0	
Moderate risk	91	36	55	39.6	0.276
High risk-one factor	189	102	87	54.0	< 0.001
High risk-two factors or more	88	77	11	87.5	< 0.001
T21/T18/T13					
Low risk	33	12	21	36.4	
Moderate risk	35	20	15	57.1	0.086
High risk-one factor	80	58	22	72.5	< 0.001
High risk-two factors or more	69	67	2	97.1	< 0.001

Liu, S. et al. (2022). Positive predictive value estimates for noninvasive prenatal testing from data of a prenatal diagnosis laboratory and literature review. *Molecular cytogenetics*, *15*(1), 29.

Original Research ajog.org

OBSTETRICS

Clinical validation of a noninvasive prenatal test for genomewide detection of fetal copy number variants

Roy B. Lefkowitz, PhD; John A. Tynan, PhD; Tong Liu, PhD; Yijin Wu, PhD; Amin R. Mazloom, PhD; Eyad Almasri, MS; Grant Hogg, MS; Vach Angkachatchai, PhD; Chen Zhao, PhD; Daniel S. Grosu, MD; Graham McLennan, MS; Mathias Ehrich, MD

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TABLE 3
Clinical performance for indicated abnormalities and fetal sex

Abnormality	Concordant positive	Discordant positive	Concordant negative	Discordant negative	Sensitivity (95% CI)	Specificity (95% CI)
T21	85	0	1081	0	100% (94.6–100%)	100% (99.6–100%)
T18	27	0	1139	0	100% (84.4–100%)	100% (99.6–100%)
T13	15	0	1151	0	100% (74.7–100%)	100% (99.6–100%)
SCA	26	1	1117	0	100% (84.0–100%)	99.9% (99.4–100%)
CNVs[a]	42	1	1122	1	97.7% (86.2–99.9%)	99.9% (99.4–100%)

Analyte	Concordant male	Discordant male	Concordant female	Discordant female	Accuracy (95% CI)
Fetal sex	583	4	578	1	99.6% (98.9–99.8%)

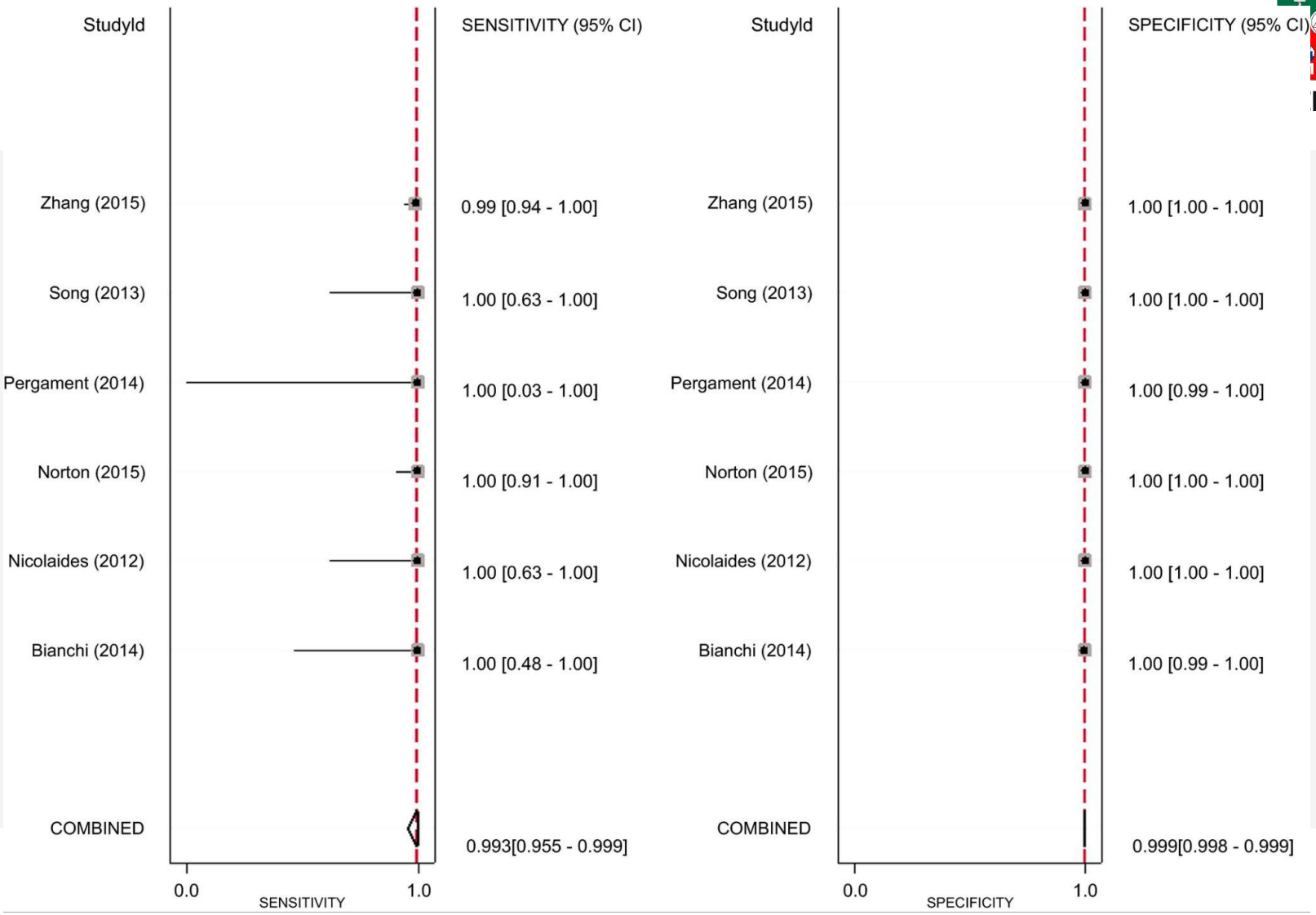
CI, confidence interval; *CNVs*, copy number variants; *SCA*, sex chromosome aneuploidy; *T13*, trisomy 13; *T18*, trisomy 18; *T21*, trisomy 21.

[a] Includes 8 samples with detected whole chromosome trisomies, and 35 samples with subchromosomal CNVs.

Lefkowitz et al. Clinical validation of genomewide cell-free DNA testing. Am J Obstet Gynecol 2016.

Lefkowitz, et al. (2016). Clinical validation of a non-invasive prenatal test for genome-wide detection of fetal copy number variants. AJOG

Población general bajo riesgo trisomía 21



Iwarsson, E. et al. (2017). Analysis of cell-free fetal DNA in maternal blood for detection of trisomy 21, 18 and 13 in a general pregnant population and in a high risk population - a systematic review and meta-analysis. *Acta obstetricia et gynecologica Scandinavica*, *96*(1), 7–18.

Población general bajo riesgo trisomía 13 (a) y 18 (b)

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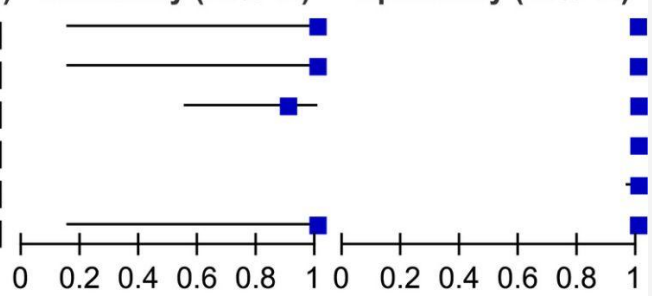
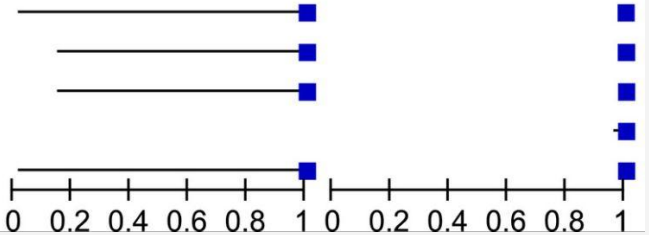
(a)

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Bianchi 2014	2	3	0	1902	1.00 [0.16, 1.00]	1.00 [1.00, 1.00]
Nicolaides 2012	2	2	0	1945	1.00 [0.16, 1.00]	1.00 [1.00, 1.00]
Norton 2015	9	1	1	15830	0.90 [0.55, 1.00]	1.00 [1.00, 1.00]
Pergament 2014	0	0	0	474	Not estimable	1.00 [0.99, 1.00]
Shaw 2014	0	0	0	100	Not estimable	1.00 [0.96, 1.00]
Song 2013	2	1	0	1738	1.00 [0.16, 1.00]	1.00 [1.00, 1.00]



(b)

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Bianchi 2014	1	1	0	891	1.00 [0.03, 1.00]	1.00 [0.99, 1.00]
Norton 2015	2	2	0	11181	1.00 [0.16, 1.00]	1.00 [1.00, 1.00]
Pergament 2014	2	0	0	472	1.00 [0.16, 1.00]	1.00 [0.99, 1.00]
Shaw 2014	0	0	0	100	Not estimable	1.00 [0.96, 1.00]
Song 2013	1	0	0	1740	1.00 [0.03, 1.00]	1.00 [1.00, 1.00]



Iwarsson, E. et al. (2017). Analysis of cell-free fetal DNA in maternal blood for detection of trisomy 21, 18 and 13 in a general pregnant population and in a high risk population - a systematic review and meta-analysis. *Acta obstetricia et gynecologica Scandinavica*, *96*(1), 7–18.

	False negative (FN)	True negative (TN)	Total negative (FN+TN)	Percentage (FN/(FN+TN)
T21 High	8	105 575	105 583	0.008
T21 Average	1	62 107	62 108	0.002
T18 High	15	146 129	146 144	0.010
T18 Average	1	21 989	21 990	0.005
T13 High	10	137 499	137 509	0.007
T13 Average	0	14 384	14 384	-

False negative (FN)

True Negative (TN)

Iwarsson, E. et al. (2017). Analysis of cell-free fetal DNA in maternal blood for detection of trisomy 21, 18 and 13 in a general pregnant population and in a high risk population - a systematic review and meta-analysis. *Acta obstetricia et gynecologica Scandinavica*, *96*(1), 7–18.

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Supporting Information Table S7. Proportion of false positives

	False positive (FP)	True positive (TP)	Total positive (FP+TP)	Percentage (FP/(FP+TP)
T21 High	52	1839	1891	2.7
T21 Average	37	156	193	20
T18 High	70	566	636	12
T18 Average	7	15	22	31
T13 High	56	134	190	30
T13 Average	3	6	9	33

False positive (FP)

True positive (TP)

Iwarsson, E. et al. (2017). Analysis of cell-free fetal DNA in maternal blood for detection of trisomy 21, 18 and 13 in a general pregnant population and in a high risk population - a systematic review and meta-analysis. *Acta obstetricia et gynecologica Scandinavica*, *96*(1), 7–18.

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Table 1
Cell free DNA performance for sex chromosome abnormalities.

Sex chromosomal aneuploidy	Sensitivity	Specificity	Positive predictive value
45,X	98.8%	99.4%	14.5–32.0%
47,XXY	100%	100%	67.6–97.7%
47,XXX	100%	99.6%	57.5–61.6%
47,XYY	100%	100%	70.9–100%

Based on *Shear* et al., *Prenat Diagn, 2023,* and *Bussolaro* et al., *Am J Obstet Gynecol MFM, 2023.*

Swanson, K. et al. Expanded applications of cell free fetal DNA screening, Best Practice & Research Clinical Obstetrics & Gynaecology, Volume 98,2025,102574,

NIPT en aneuploidías

- Muestra positiva requiere confirmación
  - Mosaicismo placentario
  - Rearreglos balanceados maternos
  - cfDNA por neoplasia materna
  - Gemelo evanescente

NIPT en variantes de número de copias (CNV)

NIPT en CNVs

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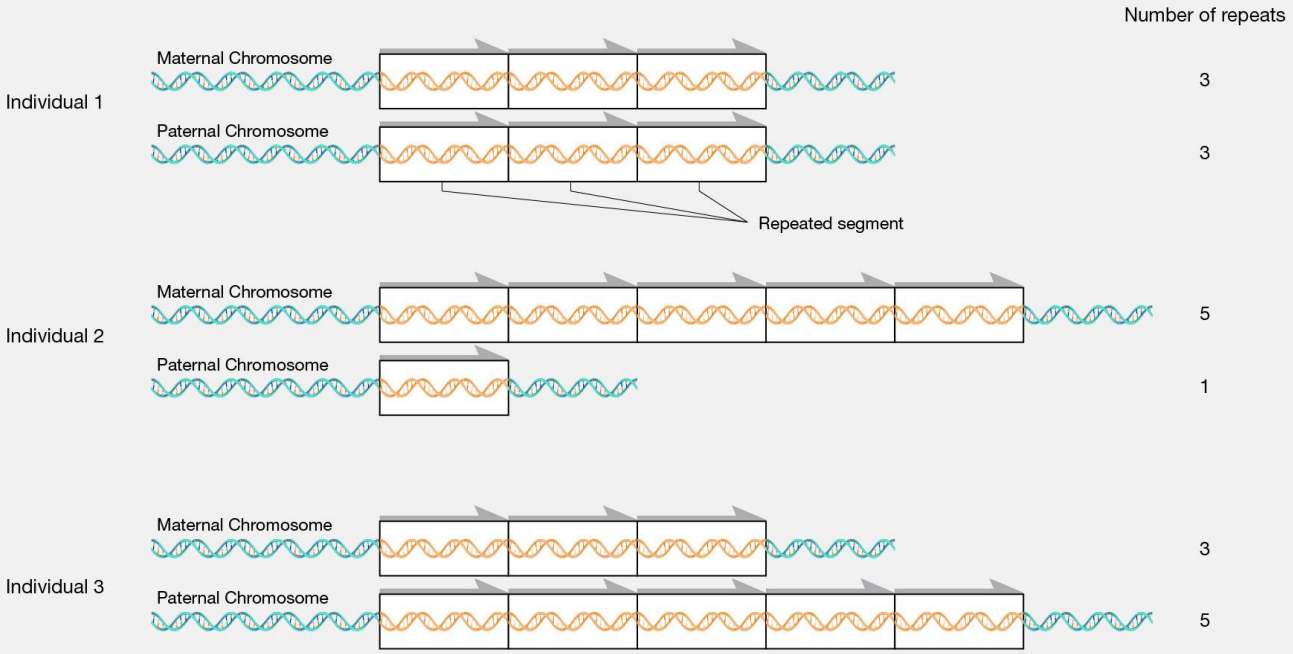
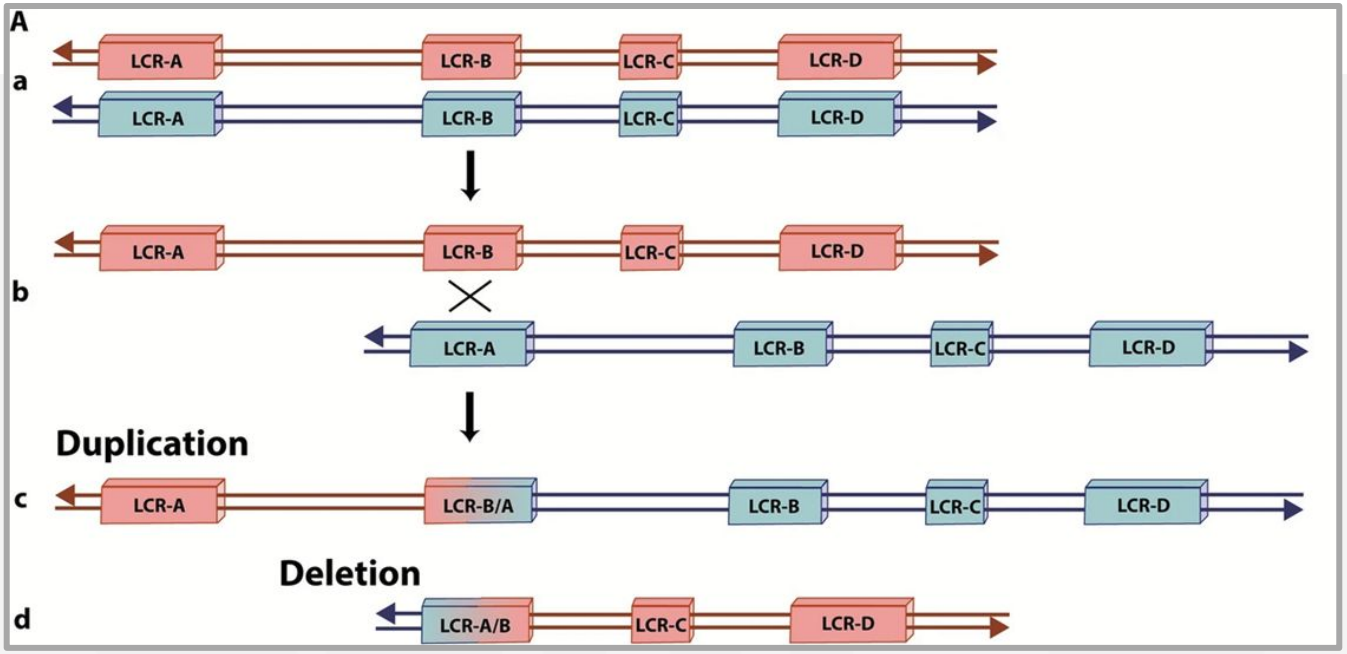
Deleciones o duplicaciones a lo largo del genoma





Mecanismo asociado a recombinación homóloga no alélica en regiones de alta homología

Burssed, B., Zamariolli, M., Bellucco, F. T., & Melaragno, M. I. (2022). Mechanisms of structural chromosomal rearrangement formation. *Molecular cytogenetics*, *15*(1), 23. https://doi.org/10.1186/s13039-022-00600-6

NIPT en CNVs

Raras por separado, pero son el 6% de fetos con anomalías anatómicas al US (Wapner, 2012)

- 90 - 95% del(22)(q11.2) son *de novo*

Sin factores de riesgo identificados

Consecuencia depende de localización y tamaño

Wapner, R. et al. (2012). Chromosomal microarray versus karyotyping for prenatal diagnosis. *The New England journal of medicine*, *367*(23), 2175–2184.

Síndrome de Microdeleción	Deleción Cromosómica	Características Principales
Síndrome de deleción 22q11.2	22q11.2	Defectos cardíacos, retraso en el desarrollo, paladar hendido, inmunodeficiencia, problemas psiquiátricos.
Síndrome de deleción 1p36	1p36	Discapacidad intelectual, hipotonía, problemas de audición y visión, convulsiones.
Síndrome de Williams-Beuren	7q11.23	Rasgos faciales característicos, problemas cardiovasculares, personalidad sociable, habilidades verbales fuertes.
Síndrome de Smith-Magenis	17p11.2	Discapacidad intelectual, problemas de sueño, comportamiento autolesivo, retraso en el habla.
Síndrome de Prader-Willi	15q11-q13 (deleción paterna)	Hipotonía, obesidad, hiperfagia, retraso en el desarrollo, hipogonadismo.
Síndrome de Angelman	15q11-q13 (deleción materna)	Discapacidad intelectual severa, problemas de equilibrio, comportamiento alegre, crisis epilépticas.
Síndrome de deleción 16p11.2	16p11.2	Retraso en el desarrollo, problemas de lenguaje, riesgo de autismo, bajo peso al nacer.
Síndrome de Wolf-Hirschhorn	4p16.3	Rasgos faciales distintivos ("casco de guerrero griego"), retraso en el desarrollo, convulsiones, anomalías esqueléticas.
Síndrome de Jacobsen	11q23	Retraso en el desarrollo, problemas de coagulación, anomalías cardíacas, características faciales distintivas.
Síndrome Cri-du-chat	5p15.2	Llanto característico (maullido de gato), retraso en el desarrollo, discapacidad intelectual, microcefalia.

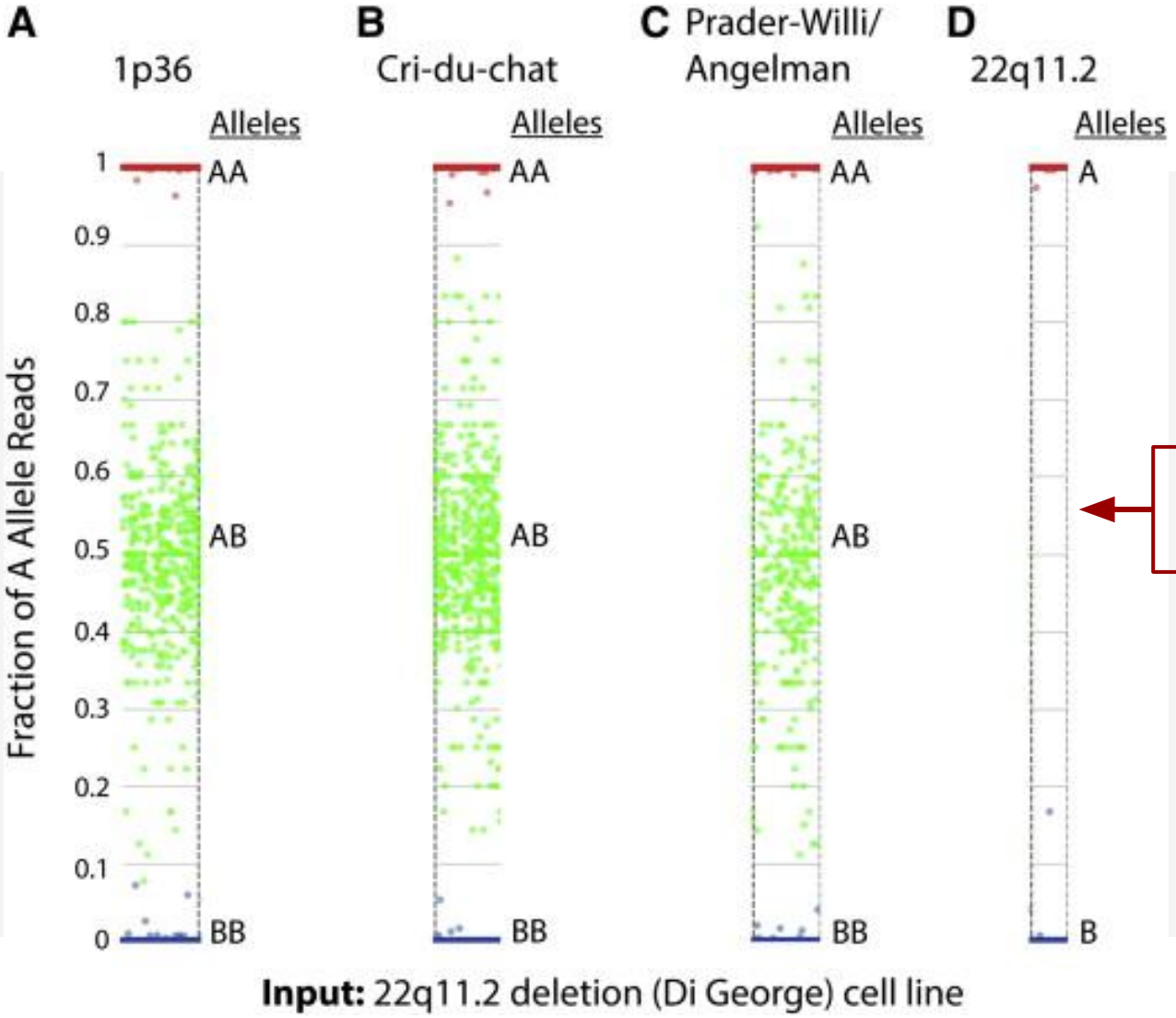
CERPO

NIPT en CNVs

Técnicas en uso para estudio prenatal de CNVs:

- SNP array / hibridación genómica comparativa
- NGS

SNP array

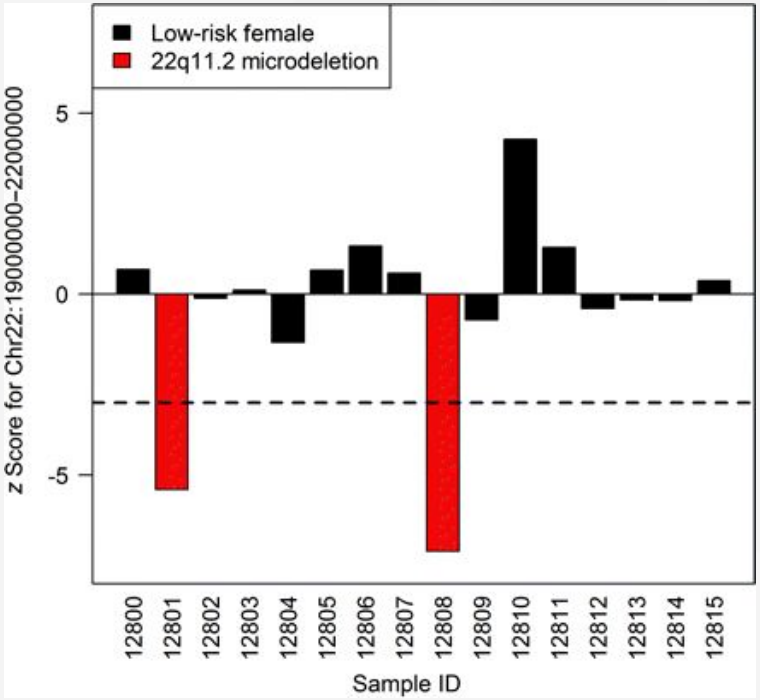


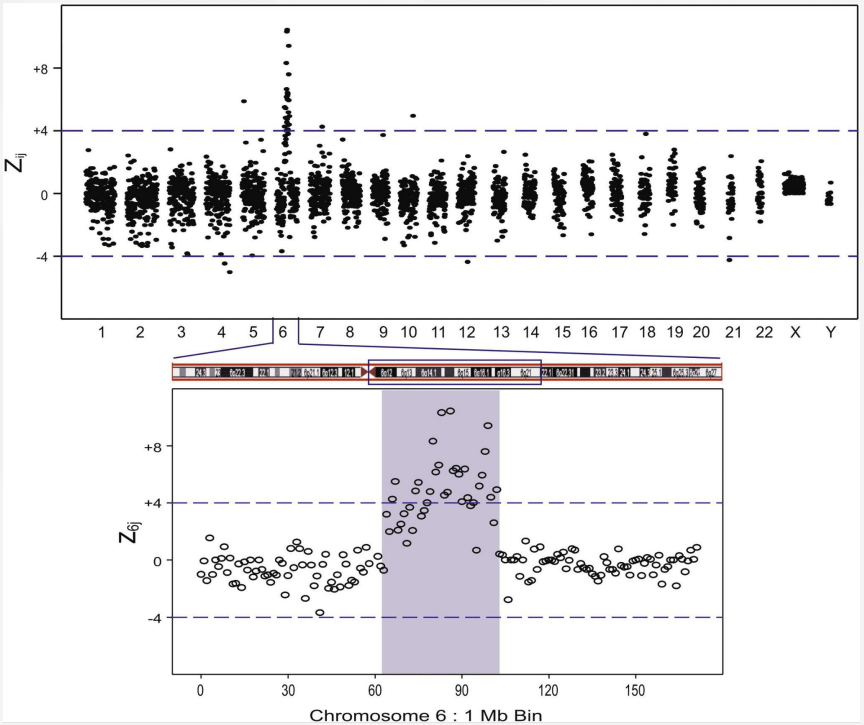
Pérdida de heterocigosidad

Wapner, R. et al. (2015). Expanding the scope of noninvasive prenatal testing: detection of fetal microdeletion syndromes. *American journal of obstetrics and gynecology*, *212*(3), 332.e1–332.e3329.

NGS

Evalúa genoma completo, determina regiones sobre/subrepresentadas





Jensen, T. J., Dzakula, Z., Deciu, C., van den Boom, D., & Ehrich, M. (2012). Detection of microdeletion 22q11.2 in a fetus by next-generation sequencing of maternal plasma. *Clinical chemistry*, *58*(7), 1148–1151.

Srinivasan, A. et al. (2013). Noninvasive detection of fetal subchromosome abnormalities via deep sequencing of maternal plasma. *American journal of human genetics*, *92*(2), 167–176.

Primary research | Open access | Published: 02 July 2021

Performances of NIPT for copy number variations at different sequencing depths using the semiconductor sequencing platform

Jiexia Yang, Jing Wu, Haishan Peng, Yaping Hou, Fangfang Guo, Dongmei Wang, Haoxin Ouyang, Yixia Wang & Aihua Yin

CERPO

Index	CNV size	Prenatal diagnostic validated by CMA			Total PPV
		Positive	Negative	Positive rate (%)	
NIPT	CNV (< 3Mb)	21	21	50.00	61/197(30.96%)
	CNV (3–5Mb)	8	17	32.00	
	CNV (5–10Mb)	12	19	38.71	
	CNV (> 10Mb)	20	79	20.20	
NIPT-PLUS	CNV (< 3Mb)	19	17	52.78	41/94(43.61%)*
	CNV (3–5Mb)	7	10	41.18	
	CNV (5–10Mb)	5	13	27.78	
	CNV (> 10Mb)	10	13	43.48*	

PPV positive predictive value

*Significant different between 0.15X and 0.4X sequencing depth

NGS: VPP podría depender de profundidad de lectura y tamaño de CNV

Yang, J. et al. (2021). Performances of NIPT for copy number variations at different sequencing depths using the semiconductor sequencing platform. *Human genomics*, *15*(1), 41.

Expanding the scope of noninvasive prenatal testing: detection of fetal microdeletion syndromes

Ronald J. Wapner, MD [a] · Joshua E. Babiarz, PhD [b] · Brynn Levy, MSc (Med), PhD [a] · ... · Susan J. Gross, MD [b] · Matthew Hill, PhD [b] · Peter Benn, DSc [c] ... Show more

CERPO

Table4. Estimated positive predictive value and negative predictive value

Disorder	Incidence (1:n)	Frequency of deletion evaluated	Positive predictive value,[a] %	Negative predictive value,[b] %
22q11.2 del	2000	0.87	5.3	>99.99
Prader-Willi	10,000	0.28	4.6	>99.99
Angelman	12,000	0.28	3.8	>99.99
1p36 del	5000	0.60	17.0	>99.99
Cri-du-chat	20,000	0.65	5.3	>99.99

Wapner. Noninvasive screening for fetal microdeletion syndromes. Am J Obstet Gynecol 2015.

* Basado en SNP array

Wapner, R. et al. (2015). Expanding the scope of noninvasive prenatal testing: detection of fetal microdeletion syndromes. *American journal of obstetrics and gynecology*, *212*(3), 332.e1–332.e3329.

SYSTEMATIC REVIEW

BJOG An International Journal of Obstetrics and Gynaecology

The accuracy of cell-free DNA screening for fetal segmental copy number variants: A systematic review and meta-analysis

Yvette C. Raymond[1] | Melissa L. Acreman[2] | Sofia Bussolaro[3] | Ben W. Mol[1,4] | Shavi Fernando[1,5] | Melody Menezes[6,7] | Fabricio Da Silva Costa[8,9] | Ilaria Fantasia[10] | Daniel Lorber Rolnik[1,5]

CERPO

- 63 artículos (2015 - 2022) (> 1.5 millón de mujeres sometidas a estudio de cfDNA)
- 5481 resultados de alto riesgo para CNV, 3737 confirmados

Raymond, Y. et al. (2023). The accuracy of cell-free DNA screening for fetal segmental copy number variants: A systematic review and meta-analysis. *BJOG : an international journal of obstetrics and gynaecology*, *130*(6), 549–559

SYSTEMATIC REVIEW

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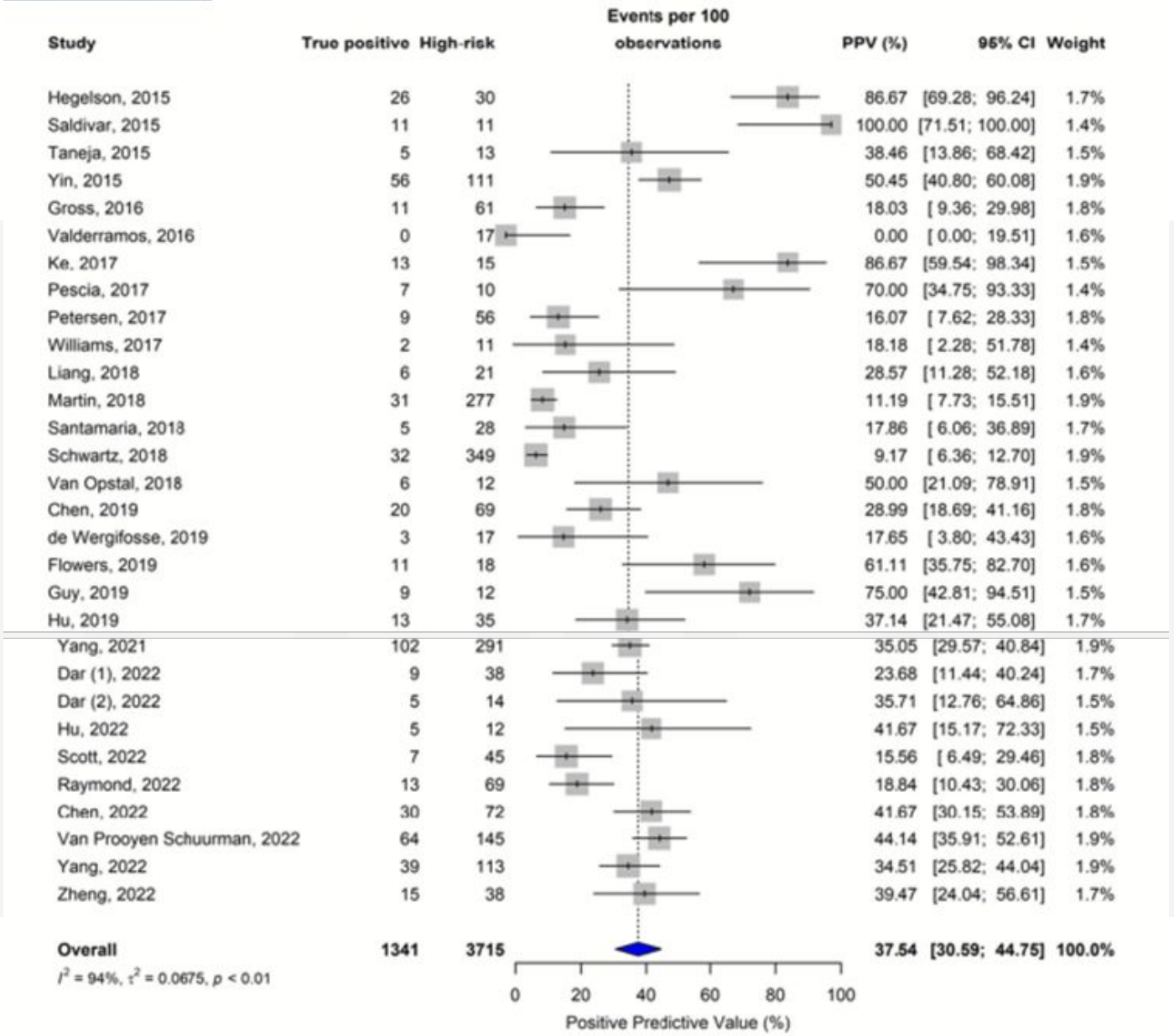
Metodología (n° de estudios):

- WGS: 44
- Array SNP: 6
- No reportado: 10
- Plataformas múltiples: 1

Raymond, Y. et al. (2023). The accuracy of cell-free DNA screening for fetal segmental copy number variants: A systematic review and meta-analysis. *BJOG : an international journal of obstetrics and gynaecology*, *130*(6), 549–559

An International Journal of Obstetrics and Gynaecology



Raymond, Y. et al. (2023). The accuracy of cell-free DNA screening for fetal segmental copy number variants: A systematic review and meta-analysis. *BJOG : an international journal of obstetrics and gynaecology*, *130*(6), 549–559

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CERPO

- VPP conjunto: 37.5% (1 de cada 3 resultados de alto riesgo confirmados)
  - Alto riesgo pre-test: 51.5% (95% CI 24.9–77.8)
  - Bajo riesgo pre-test: 36% (95% CI 28.9–43.3)
- S: 77.4% (95% CI 65.7 - 86.0)
- E: 99.4% (95% CI 98.0 - 9.8)

Raymond, Y. et al. (2023). The accuracy of cell-free DNA screening for fetal segmental copy number variants: A systematic review and meta-analysis. *BJOG : an international journal of obstetrics and gynaecology*, *130*(6), 549–559

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CERPO

- **VPP específico**:
  - Deleción 22q11.2: 49%
  - Microdeleción 15q: 26%
  - del(5p-) (Cri-du-Chat): 31%
- ¡Pero todas eran consideradas de alto riesgo pre test!
- Sin estudios de bajo riesgo de sesgo para patologías específicas

Raymond, Y. et al. (2023). The accuracy of cell-free DNA screening for fetal segmental copy number variants: A systematic review and meta-analysis. *BJOG : an international journal of obstetrics and gynaecology*, *130*(6), 549–559

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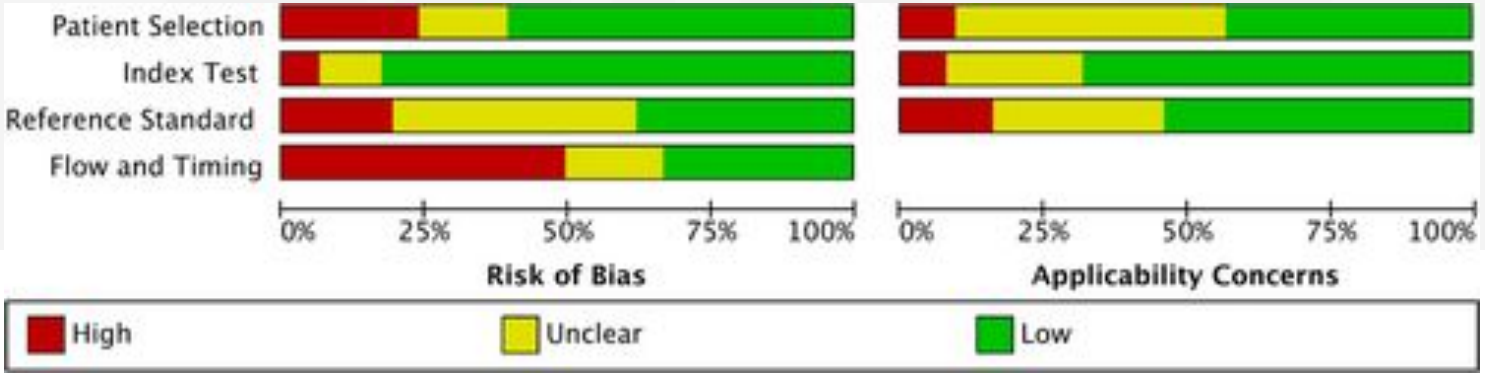
CERPO

- Heterogeneidad marcada entre estudios (I^2 > 75%)
- Alto riesgo de sesgo en publicaciones
  - Solo cuatro publicaciones con cuatro parámetros en “bajo riesgo”



Raymond, Y. et al. (2023). The accuracy of cell-free DNA screening for fetal segmental copy number variants: A systematic review and meta-analysis. *BJOG : an international journal of obstetrics and gynaecology*, *130*(6), 549–559

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CERPO

- Limitación además en estratificación artificial de riesgo (“alto” o “bajo” según 50% de riesgo pre test)

Raymond, Y. et al. (2023). The accuracy of cell-free DNA screening for fetal segmental copy number variants: A systematic review and meta-analysis. *BJOG : an international journal of obstetrics and gynaecology*, *130*(6), 549–559

En resumen:

- NIPT para CNVs tiene menor rendimiento que para trisomías comunes
- Consejería debe aclarar que estudio es no confirmatorio

CERPO

ACMG PRACTICE GUIDELINE

Noninvasive prenatal screening (NIPS) for fetal chromosome abnormalities in a general-risk population: An evidence-based clinical guideline of the American College of Medical Genetics and Genomics (ACMG)

Recommendation: ACMG SUGGESTS THAT NIPS FOR 22q11.2 DELETION SYNDROME BE OFFERED TO ALL PATIENTS (CONDITIONAL RECOMMENDATION, BASED ON MODERATE CERTAINTY OF THE EVIDENCE)

Recommendation: AT THIS TIME, THERE IS INSUFFICIENT EVIDENCE TO RECOMMEND OR NOT RECOMMEND NIPS FOR THE IDENTIFICATION OF RATs (NO RECOMMENDATION, OWING TO LACK OF CLINICALLY RELEVANT EVIDENCE)

Recommendation: AT THIS TIME, THERE IS INSUFFICIENT EVIDENCE TO RECOMMEND ROUTINE SCREENING FOR CNVs OTHER THAN 22q11.2 DELETIONS (NO RECOMMENDATION, OWING TO LACK OF CLINICALLY RELEVANT EVIDENCE AND VALIDATION)

Recommendation: ACMG RECOMMENDS THAT NIPS BE OFFERED TO PATIENTS WITH A SINGLETON GESTATION TO SCREEN FOR FETAL SCA (STRONG RECOMMENDATION, BASED ON HIGH CERTAINTY OF EVIDENCE)

Dungan, J. S. et al. (2023). Noninvasive prenatal screening (NIPS) for fetal chromosome abnormalities in a general-risk population: An evidence-based clinical guideline of the American College of Medical Genetics and Genomics (ACMG). *Genetics in medicine : official journal of the American College of Medical Genetics*, *25*(2), 100336.

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Table 2
Recommendations, opinions, and guidelines from governing bodies.

	Common aneuploidies	Sex chromosome aneuploidies	Rare autosomal trisomies	Microdeletions	Single gene disorders	Rh status
International society for prenatal diagnosis	Recommend	Insufficient data to recommend	Insufficient data to recommend	Insufficient data to recommend	Not included in statement	Not included
Royal college of obstetricians & gynaecologists	Recommend	Not included in statement	Not included in statement	Not included in statement	Not included in statement	Recommend
American college of obstetricians & gynecologists	Recommend	No recommendation provided	Not included in statement	Insufficient data to recommend	Insufficient data to recommend	Reasonable in setting of Rh immunoglobulin shortage
Society for maternal-fetal medicine	Recommend	No recommendation provided	Not included in statement	Insufficient data to recommend	Insufficient data to recommend	Not included

Swanson K., Norton M., Expanded applications of cell free fetal DNA screening, Best Practice & Research Clinical Obstetrics & Gynaecology, Volume 98, 2025, 102574, ISSN 1521-6934,

CERPO

Centro de Referencia Perinatal Oriente

Facultad de Medicina, Universidad de Chile

NIPD: enfermedades monogénicas

Primeras enfermedades

Aprobación para uso clínico

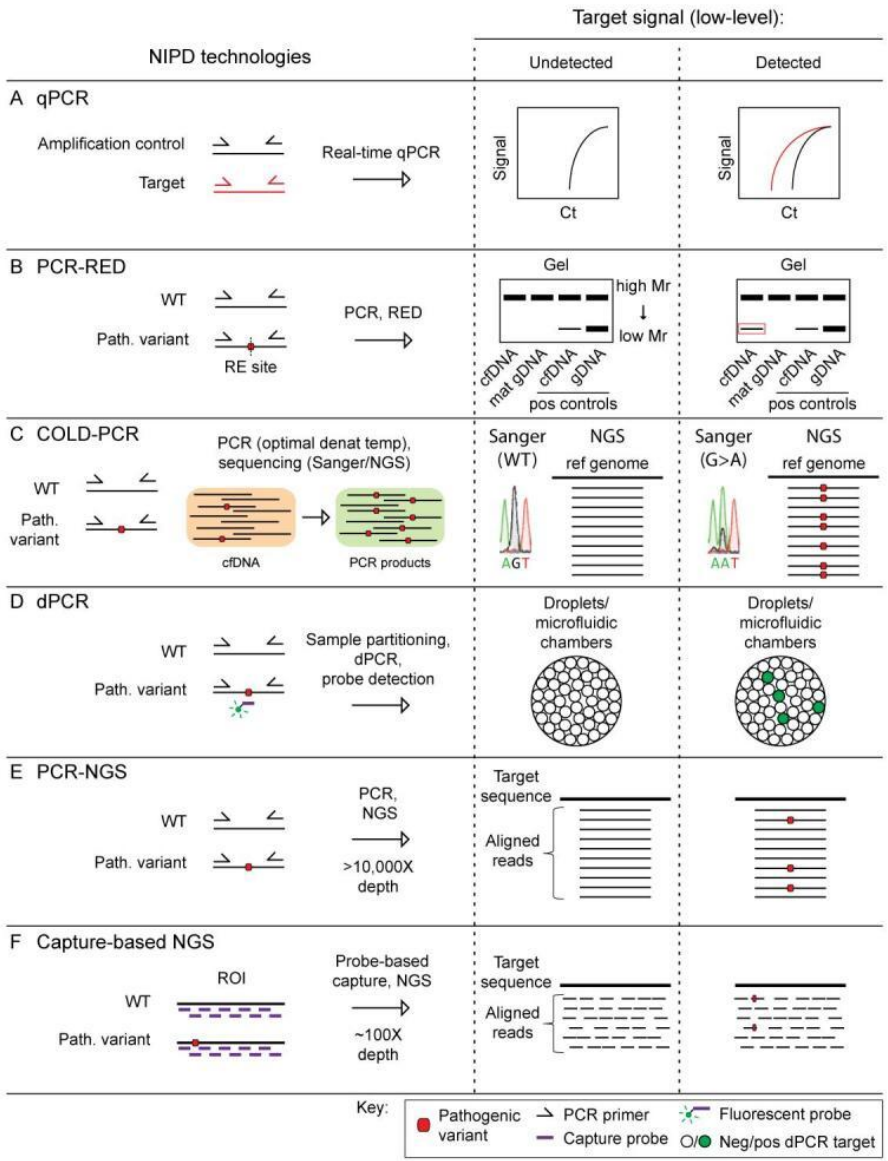
Timeline	Milestone for NIPD	Technology	Setting
1997	Discovery of cffDNA in maternal plasma	PCR	Research
2000	NIPD for SGDs (skeletal dysplasias)	PCR	Research
2002	Prenatal exclusion: AR conditions (CF, β-thalassaemia, CAH)	PCR	Research
2008	NIPD for SGDs, parents same mutation, male fetsuses	dPCR and RMD	Research
2010	Whole fetal genome mapping: parental haplotype analysis	NGS	Research
2011	NIPD for fetal sex determination approved	PCR	Public Health
	Routine 3rd trimester *RHD* genotyping: direct anti-RhD immunoglobulin administration	PCR	Public Health
2012	NIPD for SGDs, parents same mutation, male and female fetuses	NGS	Research
	NIPD for selected SGDs approved	PCR-RED	Public Health
2013	NIPD using gene panels approved	NGS	Public Health
	Molecular karyotyping of subchromosomal abnormalities	NGS	Research
2015	Molecular counting for NIPD of SGDs, cfDNA sample only	ddPCR and RMD	Research
2016	NIPD for recessive conditions approved	NGS and RHDO	Public Health
2017	Proband-free NIPD for recessive conditions	NGS and RHDO	Public Health
	Cell-based NIPD for SGDs	NGS	Research
2018	cfDNA screening for monogenic disorders	NGS	Commercial
	NIPD for paternal and *de novo* pathogenic variant detection, and fetal sexing	ddPCR	Public Health
2019	NIPD for low-risk pregnancies	NGS	Commercial
2021	Discovery of long cfDNA fragments by long-read sequencing	PacBio SMRT	Research

CERPO

Uso en enfs. monogénicas

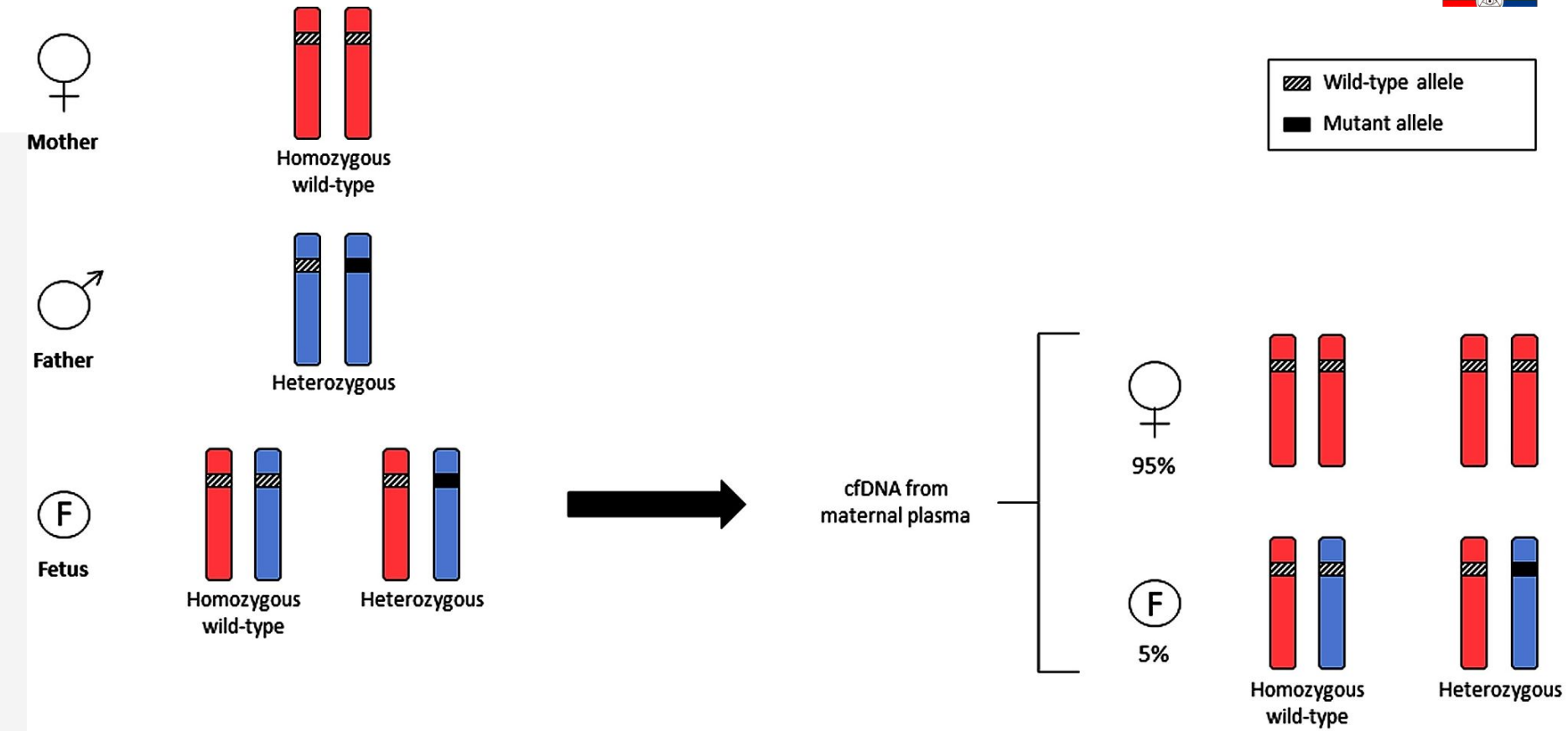
- **Primeras enfermedades**: acondroplasia y distrofia miotónica (2000)
- **Servicios clínicos**: inicialmente RhD y determinación de cromosomas sexuales
  - Luego fibrosis quística, variantes en FGFR2/3, atrofia muscular espinal, distrofia muscular de Duchenne
- Diferencia técnica en variantes paternas/de novo y maternas

Detección de variantes paternales/*de novo*



Múltiples métodos de detección basados en PCR/NGS

Detección de variantes paternales/*de novo*



Detectado/No detectado

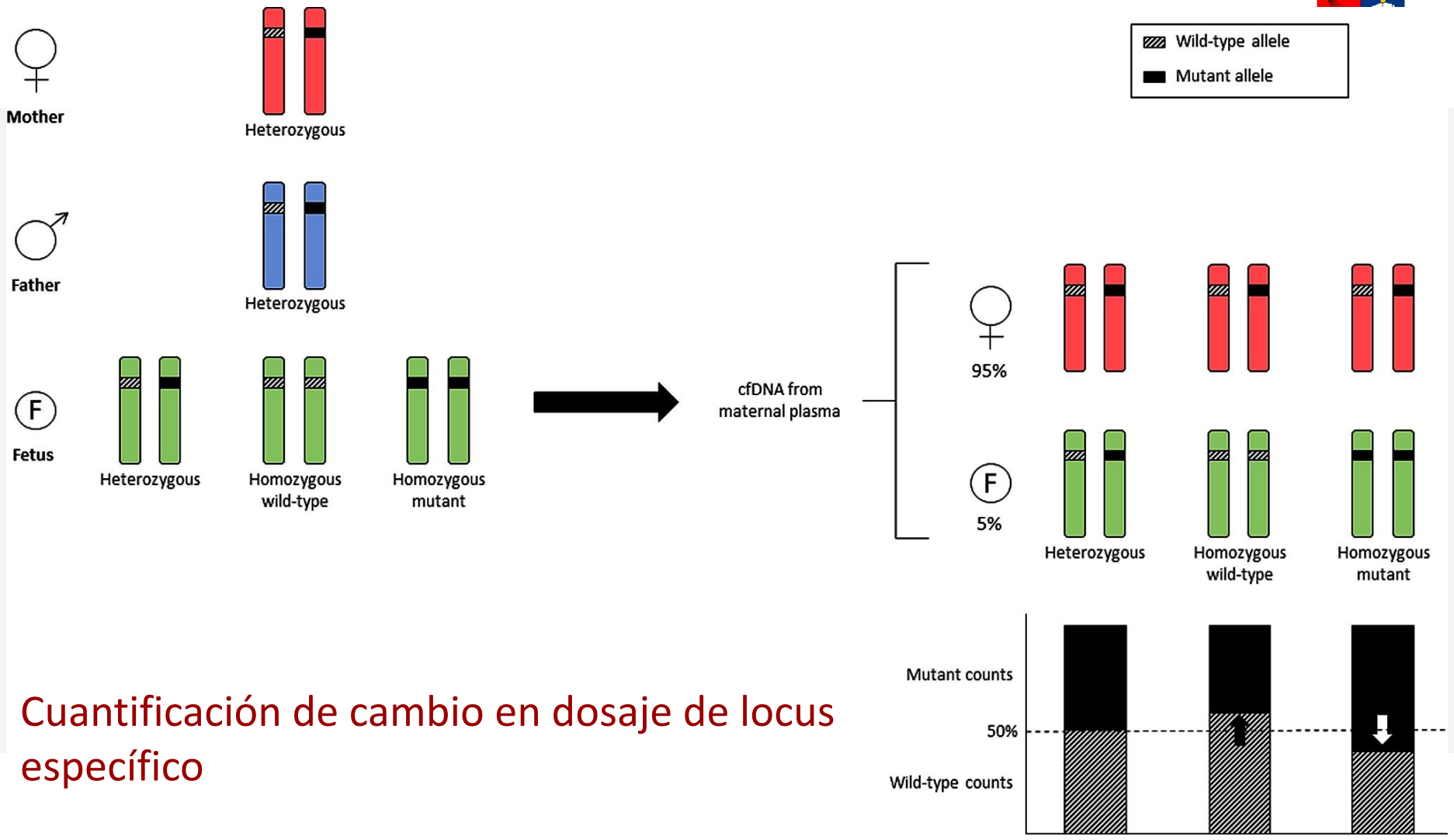
Scotchman, E. et al. (2020). Non-invasive prenatal diagnosis and screening for monogenic disorders. *European journal of obstetrics, gynecology, and reproductive biology*, *253*, 320–327.

Detección de variantes heredadas maternas

CERPO

- Alto fondo de material genético materno
- PCR-NGS no es cuantitativo fiable en este contexto por profundidad de lectura
- Requiere análisis por métodos altamente sensibles: dosis mutacional relativa (RMD), dosaje relativo de haplotipo (RHDO)

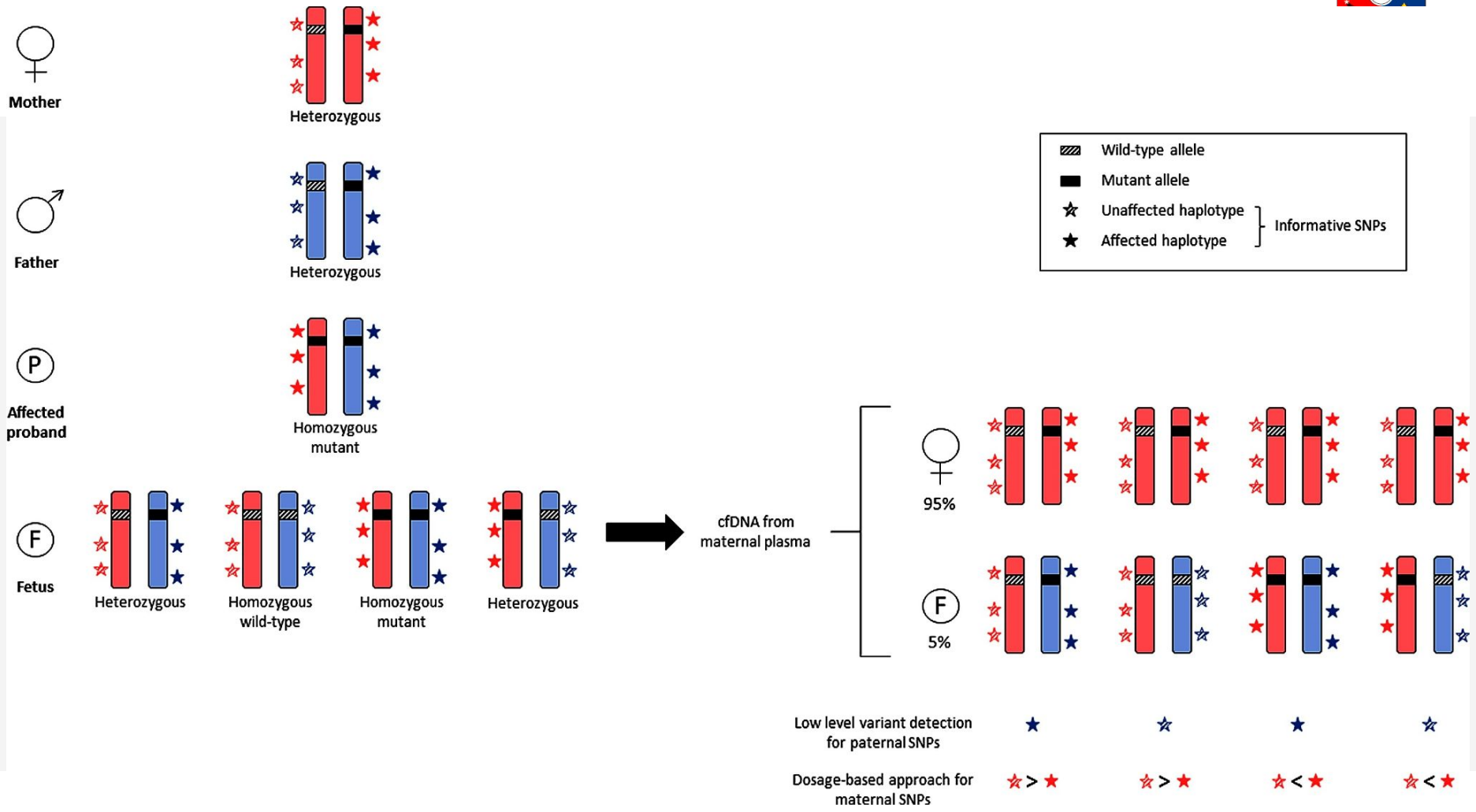
Dosis mutacional relativa (RMD)



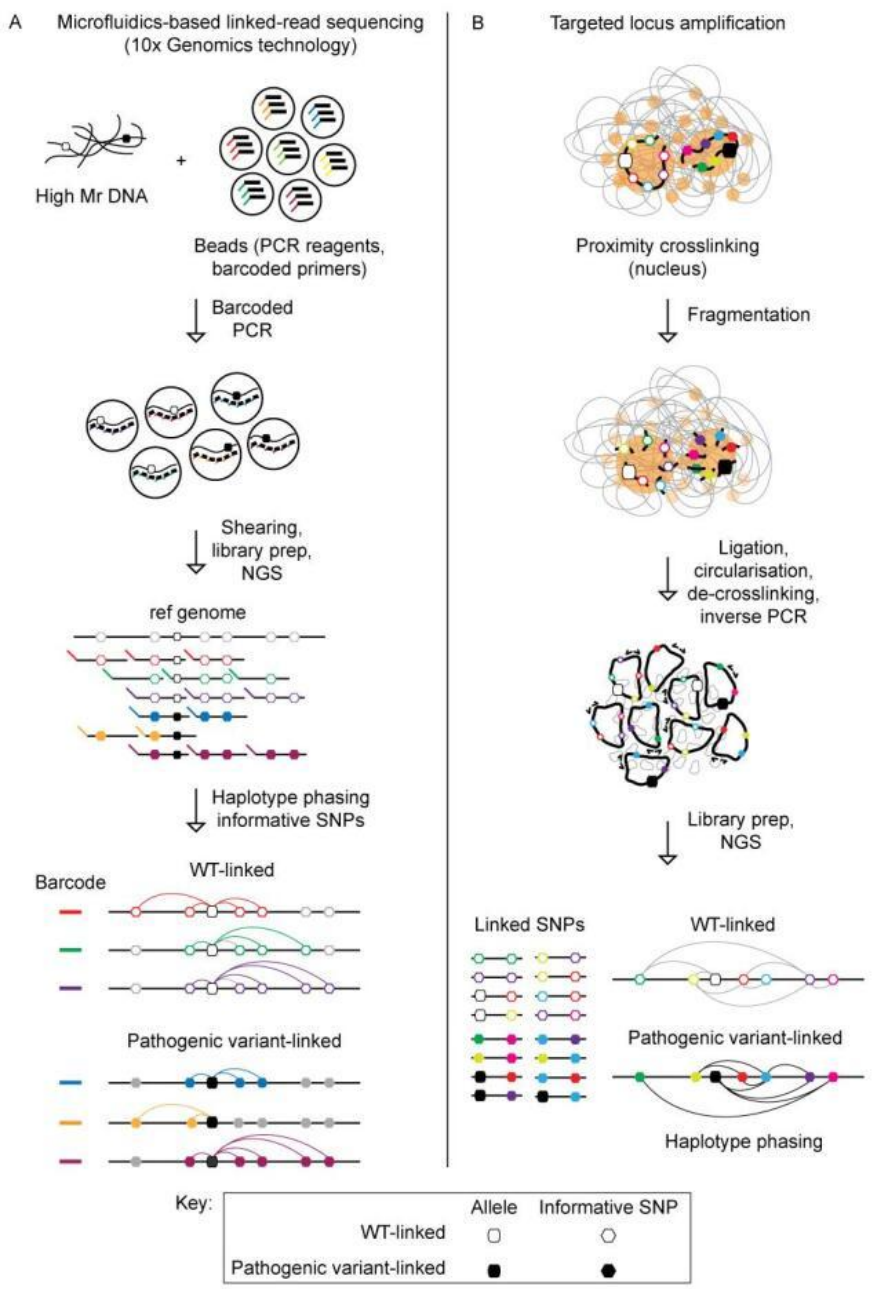
Cuantificación de cambio en dosaje de locus específico

Scotchman, E. et al. (2020). Non-invasive prenatal diagnosis and screening for monogenic disorders. *European journal of obstetrics, gynecology, and reproductive biology*, *253*, 320–327.

Análisis de dosaje relativo de haplotipo (RHDO)



Scotchman, E. et al. (2020). Non-invasive prenatal diagnosis and screening for monogenic disorders. *European journal of obstetrics, gynecology, and reproductive biology*, *253*, 320–327.

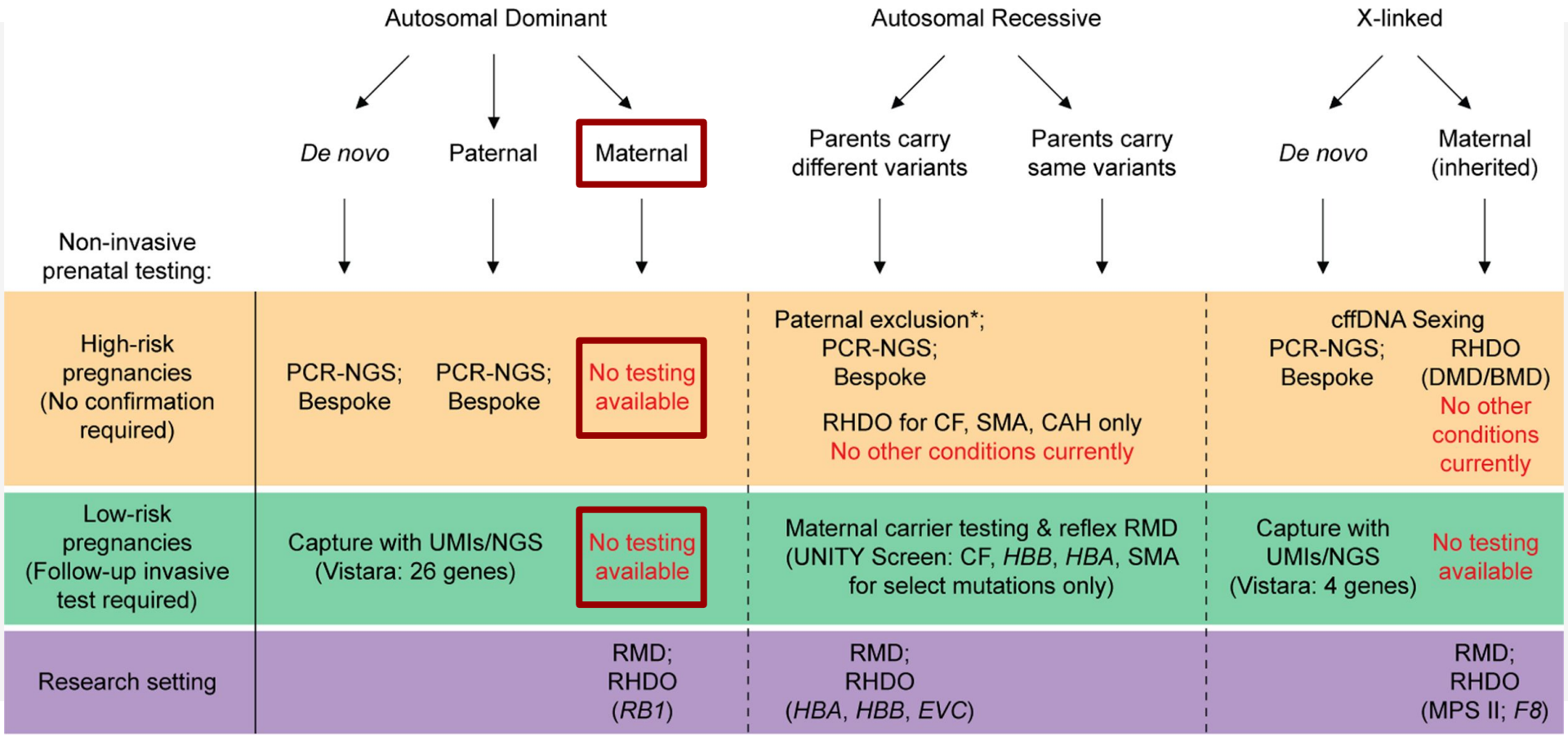


CERPO

Métodos en desarrollo para RHDO sin probando, aún no disponibles en clínica

Hanson, B. et al. (2023). Non-invasive prenatal diagnosis (NIPD): current and emerging technologies. *Extracellular vesicles and circulating nucleic acids*, *4*(1), 3–26

CERPO



Hanson, B. et al. (2022). Non-invasive prenatal diagnosis (NIPD): how analysis of cell-free DNA in maternal plasma has changed prenatal diagnosis for monogenic disorders. Clinical science (London, England : 1979), 136(22), 1615–1629.

PRENATAL DIAGNOSIS

ORIGINAL ARTICLE | Open Access

Is It Feasible to Screen for Fetal De Novo or Paternally Inherited Pathogenic Single Nucleotide Variants in Maternal Plasma Cell-Free DNA? A Systematic Literature Review

Kristína Valovičová, Karin E. M. Diderich, Wichor M. Bramer, Sander Lamballais, Malgorzata Ilona Srebniak

First published: 24 May 2025 | https://doi.org/10.1002/pd.6822

CERPO

- Revisión sistemática: documentos con estudio de cfDNA >= 2 genes hasta febrero 2024 + citaciones abril 2025
- Exclusión: un gen evaluado, solo aneuploidía, reportes de caso
- 16 estudios en total

Valovičová, K. et al. (2025). Is It Feasible to Screen for Fetal De Novo or Paternally Inherited Pathogenic Single Nucleotide Variants in Maternal Plasma Cell-Free DNA? A Systematic Literature Review. *Prenatal diagnosis*, *45*(9), 1139–1150

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CERPO

- Estrategias de secuenciación usadas: paneles NGS (14), WES (2)
- Aproximación (número de estudios):
  - Solo: 3
  - Duo: 4
  - Trío: 7

Valovičová, K. et al. (2025). Is It Feasible to Screen for Fetal De Novo or Paternally Inherited Pathogenic Single Nucleotide Variants in Maternal Plasma Cell-Free DNA? A Systematic Literature Review. *Prenatal diagnosis*, *45*(9), 1139–1150

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CERPO

- > 5000 muestras
- Todos >= 9 semanas EG
- Sensibilidad: 88.9 - 100%
- Especificidad: 98.1 - 100%
- VPP conjunto: 98.9% (66.7 - 100%)
- Raros falsos positivos por mosaicismo materno

Valovičová, K. et al. (2025). Is It Feasible to Screen for Fetal De Novo or Paternally Inherited Pathogenic Single Nucleotide Variants in Maternal Plasma Cell-Free DNA? A Systematic Literature Review. *Prenatal diagnosis*, *45*(9), 1139–1150

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First published: 24 May 2025 | https://doi.org/10.1002/pd.6822

CERPO

- NIPT sería técnicamente viable en estos escenarios
- VPP comparable a trisomías frecuentes

Limitaciones:

- Cohortes pequeñas de alto riesgo pre test
- Algunos estudios con mal registro de estudios confirmatorios
- Requiere en muchos casos estudio trio

Valovičová, K. et al. (2025). Is It Feasible to Screen for Fetal De Novo or Paternally Inherited Pathogenic Single Nucleotide Variants in Maternal Plasma Cell-Free DNA? A Systematic Literature Review. *Prenatal diagnosis*, *45*(9), 1139–1150

JOURNAL ARTICLE

Routine Prenatal cfDNA Screening for Autosomal Dominant Single-Gene Conditions

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Sophie Adams ✉ , Olivia Maher Trocki , Christina Miller , Courtney Studwell , Meghan Bombalicki , Lori Dobson , Sofia Horan , Jordan Sargent , Michael Duyzend , Kathryn J Gray ... Show more

Clinical Chemistry, Volume 71, Issue 1, January 2025, Pages 129–140, https://doi.org/10.1093/clinchem/hvae189

Published: 03 January 2025 **Article history** ▾

CERPO

3480 embarazos de bajo riesgo, 73.6% < 11 semanas

- 0.51% resultados de alto riesgo (variantes patogénicas/probablemente patogénicas)
  - 6 probablemente fetales
  - 8 probablemente maternas AD
  - Sin falsos positivos
- Tres abortos asociados a diagnóstico, nueve con fenotipo leve asociado

Valovičová, K. et al. (2025). Is It Feasible to Screen for Fetal De Novo or Paternally Inherited Pathogenic Single Nucleotide Variants in Maternal Plasma Cell-Free DNA? A Systematic Literature Review. *Prenatal diagnosis*, *45*(9), 1139–1150

Y las maternas?

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Comprehensive Noninvasive Fetal Screening by Deep Trio-Exome Sequencing

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Noninvasive prenatal screening using deep trio-exome sequencing analysis includes parental samples, which enables the option of carrier screening in both biologic parents to identify pregnancies that are at high risk for recessive disorders. Our study showed that this approach to testing can accurately determine the fetal inheritance of paternal variants in the event that the mother does not carry the same variant (Fig. S7). Confirmation of the presence of a maternally inherited variant in the fetus would necessitate invasive testing.

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Table 2
Recommendations, opinions, and guidelines from governing bodies.

	Common aneuploidies	Sex chromosome aneuploidies	Rare autosomal trisomies	Microdeletions	Single gene disorders	Rh status
International society for prenatal diagnosis	Recommend	Insufficient data to recommend	Insufficient data to recommend	Insufficient data to recommend	Not included in statement	Not included
Royal college of obstetricians & gynaecologists	Recommend	Not included in statement	Not included in statement	Not included in statement	Not included in statement	Recommend
American college of obstetricians & gynecologists	Recommend	No recommendation provided	Not included in statement	Insufficient data to recommend	Insufficient data to recommend	Reasonable in setting of Rh immunoglobulin shortage
Society for maternal-fetal medicine	Recommend	No recommendation provided	Not included in statement	Insufficient data to recommend	Insufficient data to recommend	Not included

Swanson K., Norton M., Expanded applications of cell free fetal DNA screening, Best Practice & Research Clinical Obstetrics & Gynaecology, Volume 98, 2025, 102574, ISSN 1521-6934,

En conclusión…

- NIPT para diagnóstico de enfermedades monogénicas es técnicamente posible
- VPP alto, pero se requiere de más experiencia antes de emplear como tamizaje
- Priorización de enfermedades para diagnóstico con conducta activa asociada
  - SESH (Severe outcome, Early onset, Prevalence, and High analytical performance)