CERPO

Centro de Referencia Perinatal Oriente

Facultad de Medicina, Universidad de Chile

Exámenes genéticos IV: Next generation sequencing (NGS). Técnica y rendimiento prenatal.

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Obstetricia y Ginecología - Medicina Materno Fetal - Genética Clínica.

Relevancia test genéticos

Alrededor de **3 a 5 %** de los embarazos presentan alteraciones fetales estructurales en el screening ecográfico.

Causas: infecciosas, teratogénicas, genéticas.
Genéticas → 40% de las malformaciones congénitas.

La evolución de los test genéticos ha generado un aumento progresivo de la tasa de diagnóstico prenatal de trastornos genéticos en los fetos portadores de alteraciones fenotípicas detectadas en el tamizaje.

Test genéticos

CERPO

1980- 1990 → 2000 → actualidad

Citogenética clásica:

- Variaciones numéricas y estructurales
- variaciones desde 5-10 MB
- **Cariograma, FISH**

Citogenética molecular:

- Microdeleciones, duplicaciones (CNV)
- resolución entre 50-100 kB
- **QF PCR, Array (SNP y CGH)**

Genética molecular:

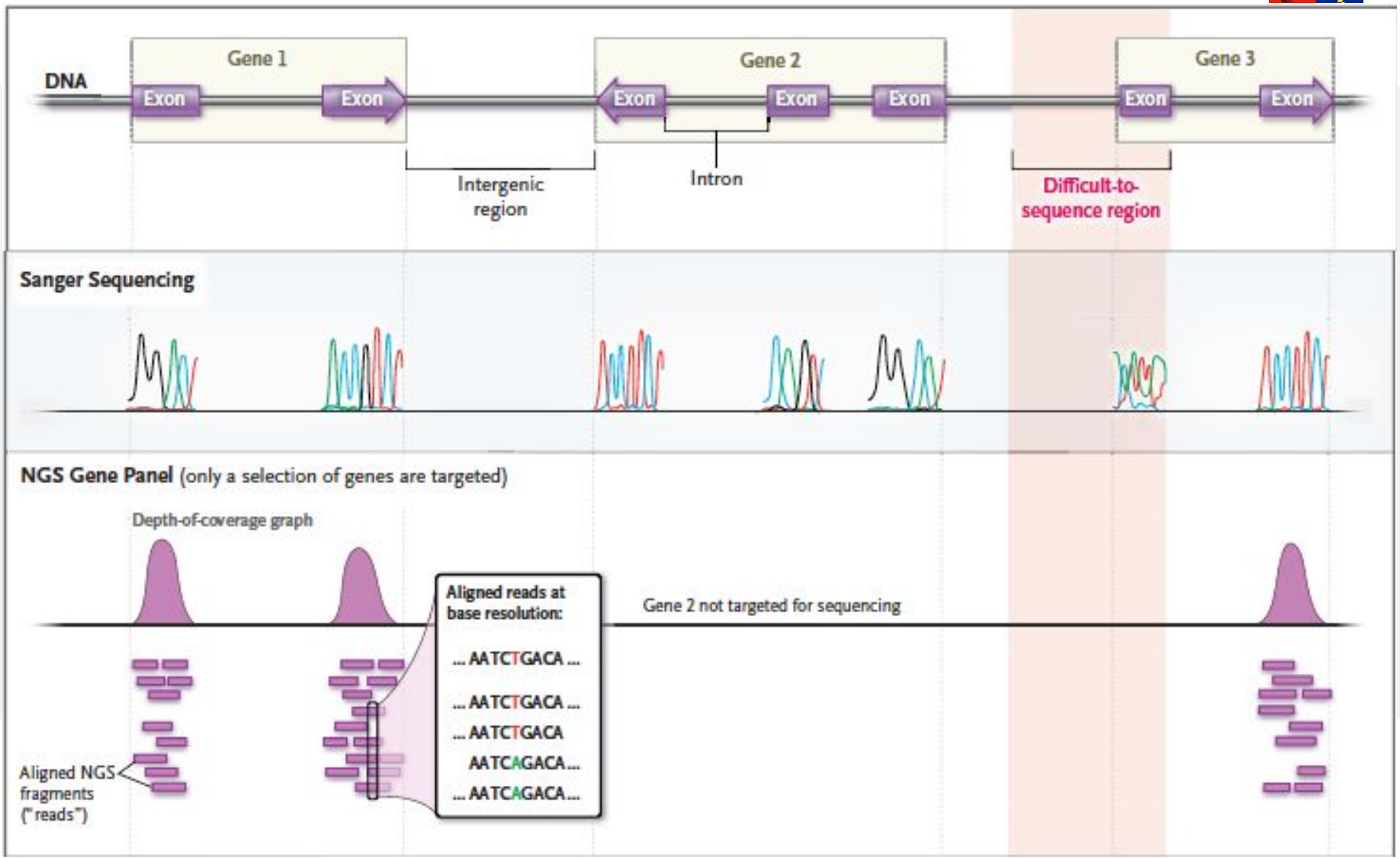
- Secuenciación de primera generación (Sanger) y Next generation sequencing (NGS), detecta variaciones de secuencias únicas (uno o más nucleótidos)
- **Panel NGS (2005): Whole exome sequence (WES),** resolución de 1 par de bases, detectando, +/- 2000 variantes por persona. **Whole genome sequence (WGS),** resolución 1 par de bases, detectando +/- 4 a 5 millones de variantes por persona.

a	Light microscope	G-banded karyotype	Microarray	Whole-exome sequence	Whole-genome sequence
Appearance				CGGATGATTACCCGTT G.....GCTC TAGCTAGCTATA....	CGGATGATTACCCGTT GATATAGCTCTCGCTC GCTCTAGCTAGCTATA GGCTATGGGTGGGGGC
Resolution	Entire chromosome	5–10 Mb	50–100 kb	1 bp	1 bp
Number of loci probed	N/A	~500	~0.05–2 million	~50 million	3 billion
Variants detected	Aneuploidy, polyploidy	Variants >5 Mb	Copy number variants	Coding regions	Majority of variants
Variants per person	0 or 1	0 or 1	10–100s	~20,000	4–5 million
Diagnostic yield	Low →				High
Incidental findings	Low →				High

Genoma completo: cariotipo, Array CGH, Array SNP, NGS WGS (genoma)

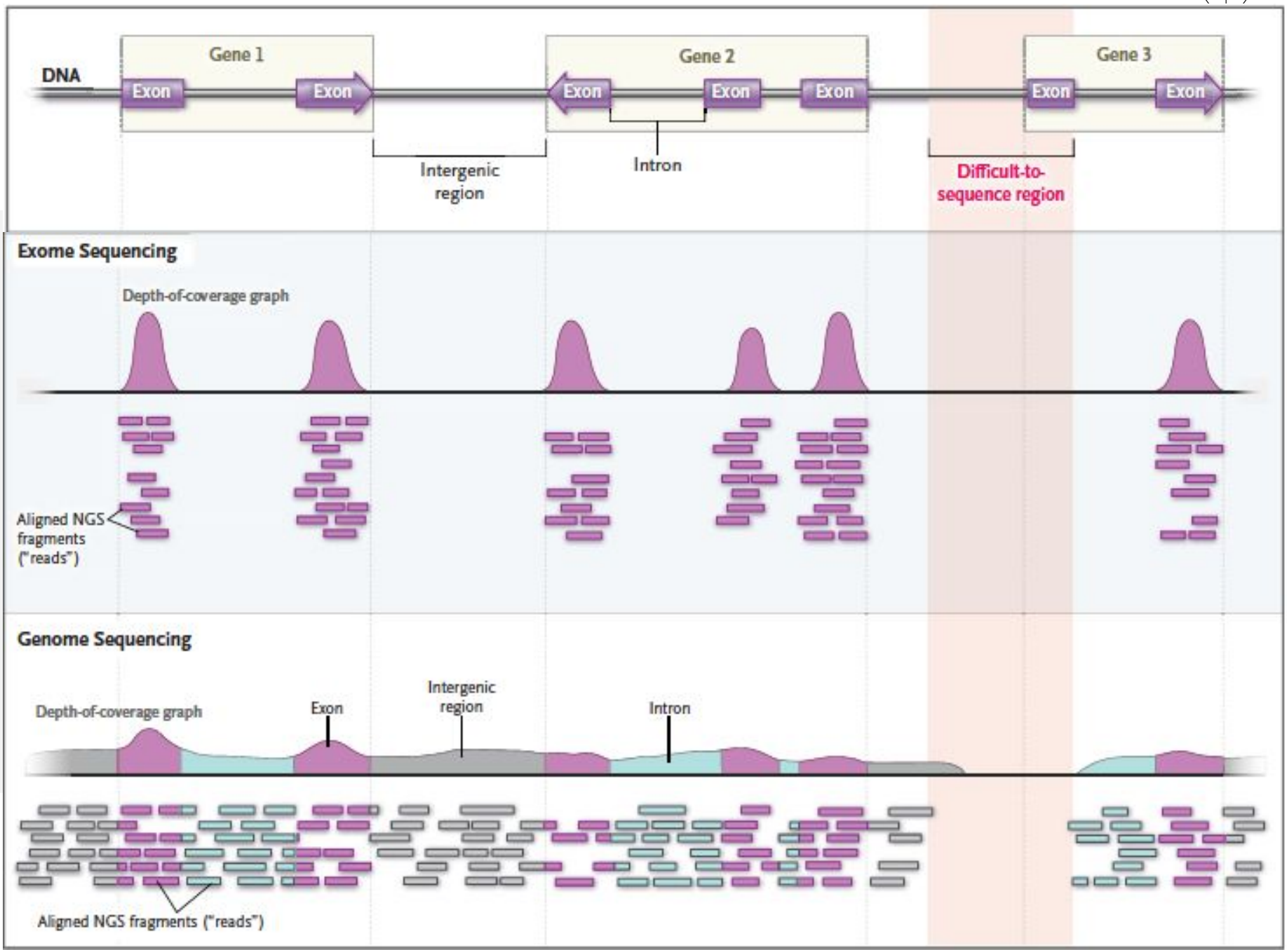
Regiones específicas del genoma: QF-PCR, FISH, MLPA, Sanger, NGS: WES (exoma) y panel genético.

Secuenciación DNA

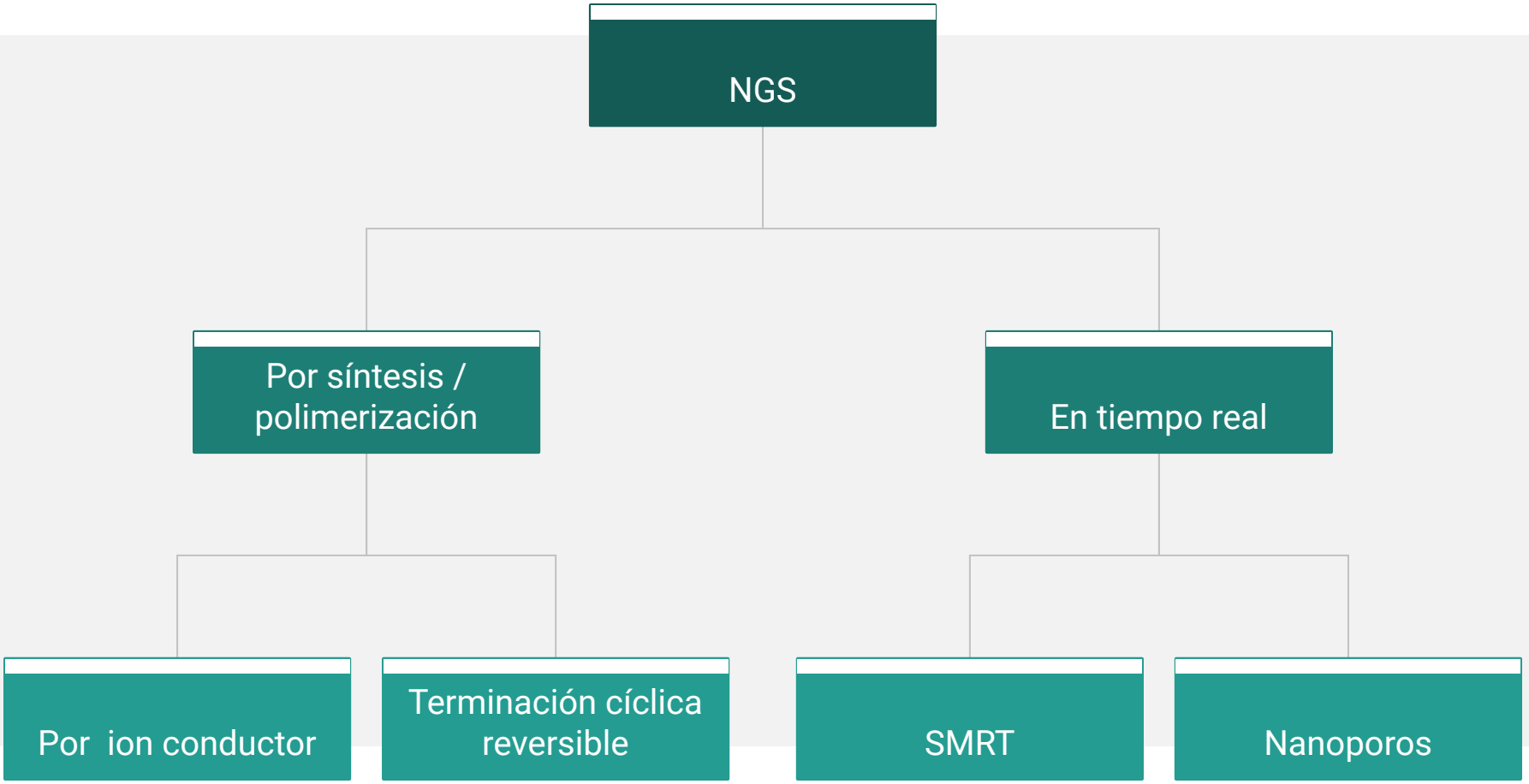


N Engl J Med 2018;379:1353-62. DOI: 10.1056/NEJMra1711801

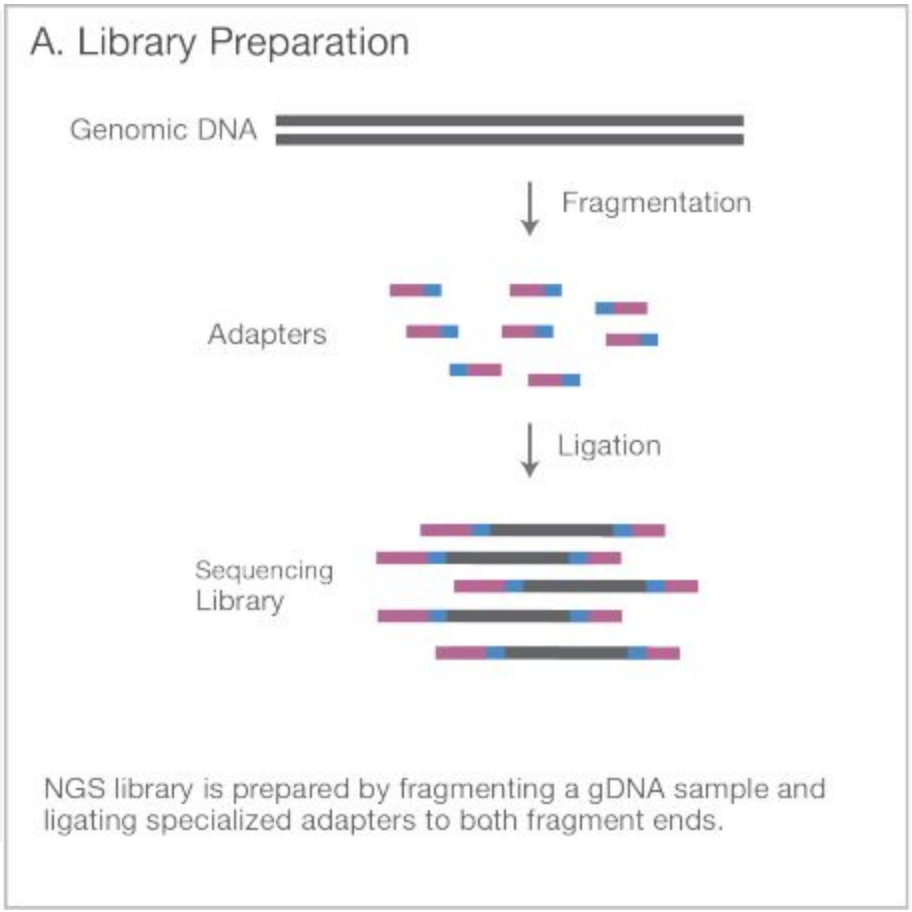
N Engl J Med 2018;379:1353-62. DOI: 10.1056/NEJMra1711801

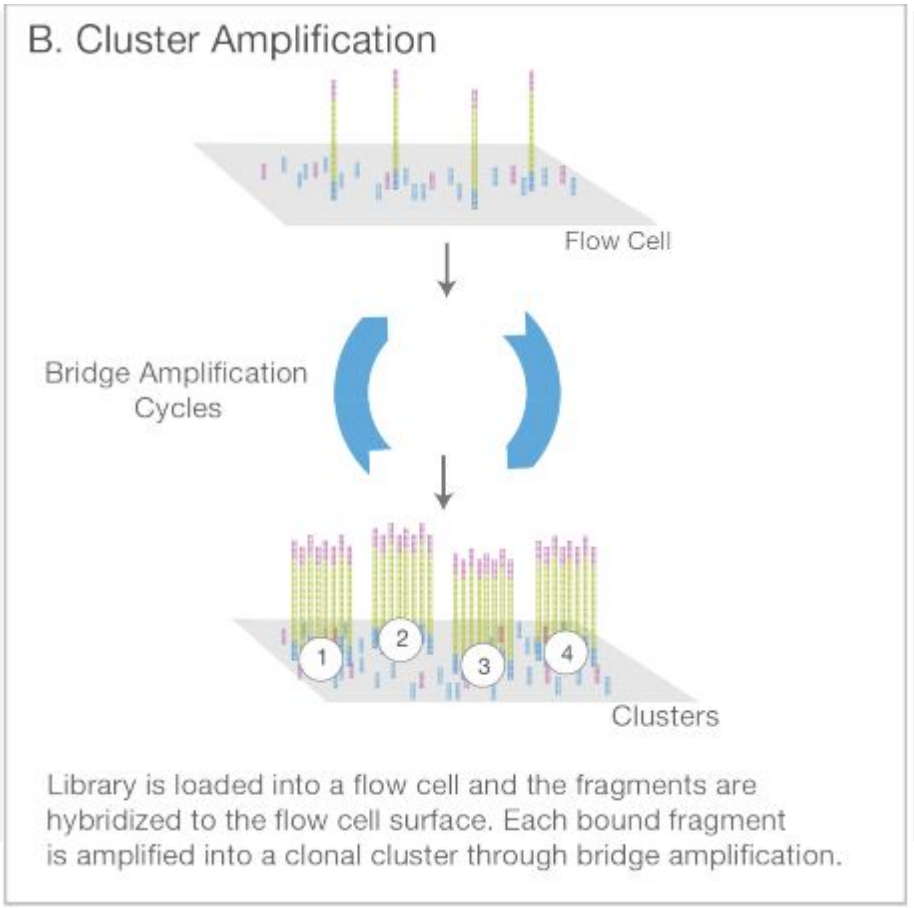


Tipos de secuenciación



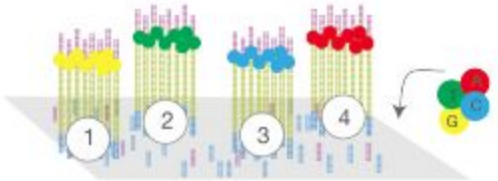
Secuenciación por síntesis





https://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf

C. Sequencing



Sequencing Cycles

Digital Image

Data is exported to an output file

```
Cluster 1 > Read 1: GAGT...
Cluster 2 > Read 2: TTGA...
Cluster 3 > Read 3: CTAG...
Cluster 4 > Read 4: ATAC...
```

Text File

Sequencing reagents, including fluorescently labeled nucleotides, are added and the first base is incorporated. The flow cell is imaged and the emission from each cluster is recorded. The emission wavelength and intensity are used to identify the base. This cycle is repeated "n" times to create a read length of "n" bases.

D. Alignment and Data Anaylsis

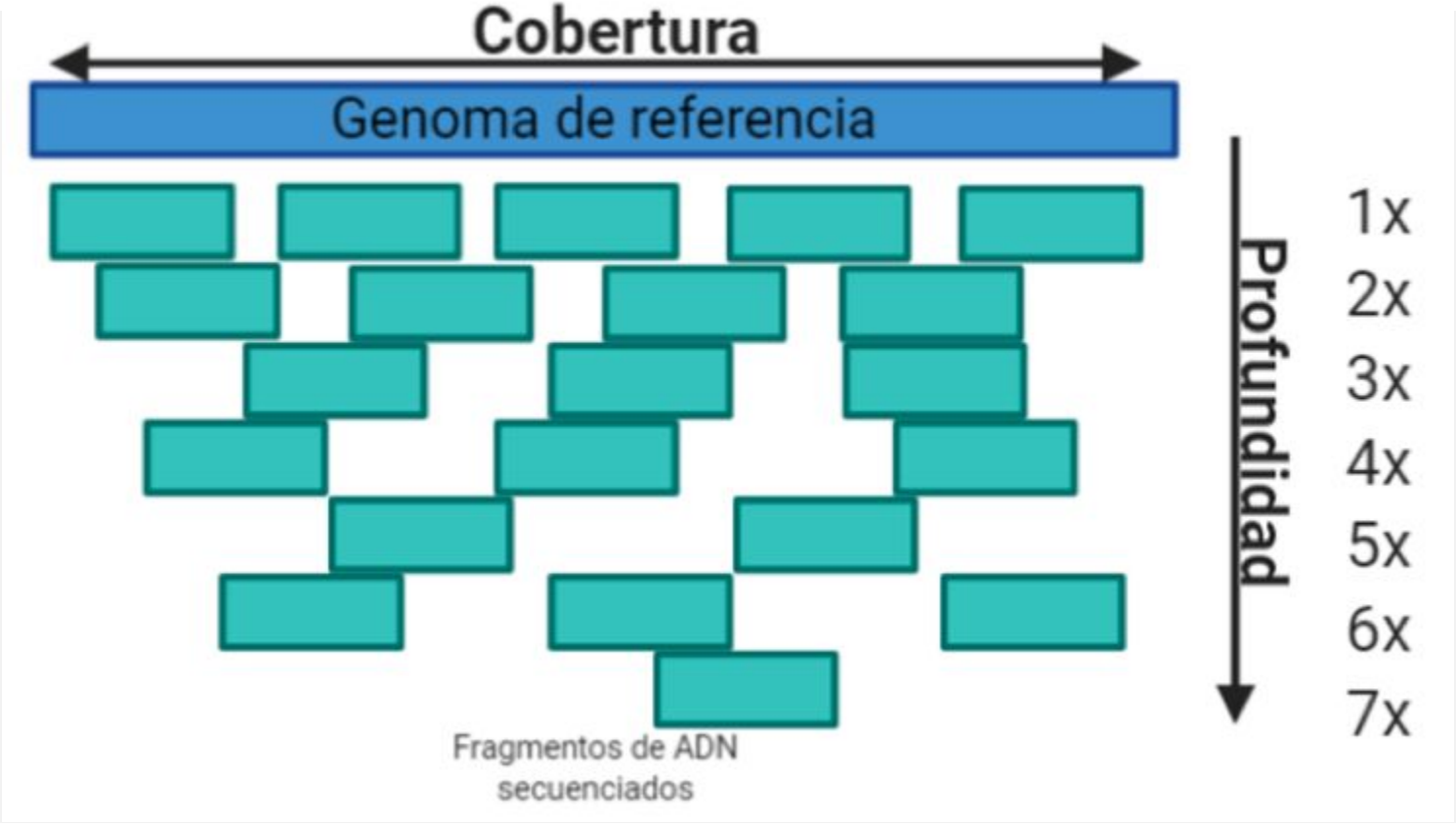
Reads

```
    ATGGCATTGCAATTTGACAT
     TGGCATTGCAATTTG
AGATGGTATTG
 GATGGCATTGCAA
      GCATTGCAATTTGAC
    ATGGCATTGCAATT
AGATGGCATTGCAATTTG
```

Reference Genome

```
AGATGGTATTGCAATTTGACAT
```

Reads are aligned to a reference sequence with bioinformatics software. After alignment, differences between the reference genome and the newly sequenced reads can be identified.


Cobertura
Genoma de referencia
Profundidad
1x
2x
3x
4x
5x
6x
7x
Fragmentos de ADN
secuenciados

Genet Med. 2015 May ; 17(5): 405–424. doi:10.1038/gim.2015.30.

Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

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Clasificación de variantes

Patogénica	variantes notificadas previamente en pacientes con enfermedad y/o sobre las que se sospecha firmemente que son patógenas según estudios preclínicos
Probablemente patogénica	características de secuencia que probablemente estén implicadas en la patogénesis de la enfermedad pero para las cuales no se dispone de pruebas concluyentes de patogenicidad
Incierta (VUS)	algunas características que sugieren posibles consecuencias funcionales, pero para las cuales no hay evidencia suficiente. ya sea para un papel patógeno o benigno
Probablemente no patogénica	aquellas para las cuales puede haber datos débiles en la literatura médica que respalden la patogenicidad, pero para las cuales la mayoría de la evidencia sugiere que el efecto de la variante es benigno
No patogénica	variantes genéticas que no se prevé que alteren la expresión o función genética

Very strong evidence of pathogenicity

PVS1 Null variant (nonsense, frameshift, canonical +/–1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease

Caveats:

- Beware of genes where LOF is not a known disease mechanism (e.g. *GFAP*, *MYH7*)
- Use caution interpreting LOF variants at the extreme 3' end of a gene
- Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact
- Use caution in the presence of multiple transcripts

Strong evidence of pathogenicity

PS1 Same amino acid change as a previously established pathogenic variant regardless of nucleotide change

Example: Val->Leu caused by either G>C or G>T in the same codon

Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level

PS2 *De novo* (both maternity and paternity confirmed) in a patient with the disease and no family history

Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, *etc*. can contribute to non-maternity

PS3 Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect on the gene or gene product

Note: Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well-established

PS4 The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls

Note 1: Relative risk (RR) or odds ratio (OR), as obtained from case-control studies, is >5.0 and the confidence interval around the estimate of RR or OR does not include 1.0. See manuscript for detailed guidance.

Note 2: In instances of very rare variants where case-control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence.

Moderate evidence of pathogenicity

PM1 Located in a mutational hot spot and/or critical and well-established functional domain (*e.g.* active site of an enzyme) without benign variation

PM2 Absent from controls (or at extremely low frequency if recessive) (see Table 6) in Exome Sequencing Project, 1000 Genomes or ExAC

Caveat: Population data for indels may be poorly called by next generation sequencing

PM3 For recessive disorders, detected in *trans* with a pathogenic variant

Note: This requires testing of parents (or offspring) to determine phase

PM4 Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants

PM5 Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before

Example: Arg156His is pathogenic; now you observe Arg156Cys

Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level

PM6 Assumed *de novo*, but without confirmation of paternity and maternity

Supporting evidence of pathogenicity

PP1 Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease

Note: May be used as stronger evidence with increasing segregation data

PP2 Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease

PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc)

Caveat: As many *in silico* algorithms use the same or very similar input for their predictions, each algorithm should not be counted as an independent criterion. PP3 can be used only once in any evaluation of a variant.

PP4 Patient's phenotype or family history is highly specific for a disease with a single genetic etiology

PP5 Reputable source recently reports variant as pathogenic but the evidence is not available to the laboratory to perform an independent evaluation

Stand-Alone evidence of benign impact

BA1 Allele frequency is above 5% in Exome Sequencing Project, 1000 Genomes, or ExAC

Strong evidence of benign impact

BS1 Allele frequency is greater than expected for disorder (see table 6)

BS2 Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder with full penetrance expected at an early age

BS3 Well-established *in vitro* or *in vivo* functional studies shows no damaging effect on protein function or splicing

BS4 Lack of segregation in affected members of a family

Caveat: The presence of phenocopies for common phenotypes (*i.e.* cancer, epilepsy) can mimic lack of segregation among affected individuals. Also, families may have more than one pathogenic variant contributing to an autosomal dominant disorder, further confounding an apparent lack of segregation.

Supporting evidence of benign impact

BP1 Missense variant in a gene for which primarily truncating variants are known to cause disease

BP2 Observed in *trans* with a pathogenic variant for a fully penetrant dominant gene/disorder; or observed in *cis* with a pathogenic variant in any inheritance pattern

BP3 In-frame deletions/insertions in a repetitive region without a known function

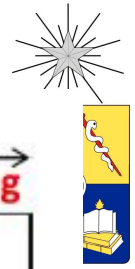
BP4 Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc)

Caveat: As many *in silico* algorithms use the same or very similar input for their predictions, each algorithm cannot be counted as an independent criterion. BP4 can be used only once in any evaluation of a variant.

BP5 Variant found in a case with an alternate molecular basis for disease

BP6 Reputable source recently reports variant as benign but the evidence is not available to the laboratory to perform an independent evaluation

BP7 A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved



	Benign		Pathogenic			
	Strong	**Supporting**	**Supporting**	**Moderate**	**Strong**	**Very Strong**
Population Data	MAF is too high for disorder *BA1/BS1* **OR** observation in controls inconsistent with disease penetrance *BS2*			Absent in population databases *PM2*	Prevalence in affecteds statistically increased over controls *PS4*	
Computational And Predictive Data		Multiple lines of computational evidence suggest no impact on gene /gene product *BP4* Missense in gene where only truncating cause disease *BP1* Silent variant with non predicted splice impact *BP7*	Multiple lines of computational evidence support a deleterious effect on the gene /gene product *PP3*	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before *PM5* Protein length changing variant *PM4*	Same amino acid change as an established pathogenic variant *PS1*	Predicted null variant in a gene where LOF is a known mechanism of disease *PVS1*
Functional Data	Well-established functional studies show no deleterious effect *BS3*		Missense in gene with low rate of benign missense variants and path. missenses common *PP2*	Mutational hot spot or well-studied functional domain without benign variation *PM1*	Well-established functional studies show a deleterious effect *PS3*	
Segregation Data	Non-segregation with disease *BS4*		Co-segregation with disease in multiple affected family members *PP1*	Increased segregation data →		
De novo Data				*De novo* (without paternity & maternity confirmed) *PM6*	*De novo* (paternity & maternity confirmed) *PS2*	
Allelic Data		Observed in *trans* with a dominant variant *BP2* Observed in *cis* with a pathogenic variant *BP2*		For recessive disorders, detected in *trans* with a pathogenic variant *PM3*		
Other Database		Reputable source w/out shared data = benign *BP6*	Reputable source = pathogenic *PP5*			
Other Data		Found in case with an alternate cause *BP5*	Patient's phenotype or FH highly specific for gene *PP4*			

Pathogenic

1. 1 Very Strong (PVS1) *AND*
   a. ≥1 Strong (PS1–PS4) *OR*
   b. ≥2 Moderate (PM1–PM6) *OR*
   c. 1 Moderate (PM1–PM6) and 1 Supporting (PP1–PP5) *OR*
   d. ≥2 Supporting (PP1–PP5)
2. ≥2 Strong (PS1–PS4) *OR*
3. 1 Strong (PS1–PS4) *AND*
   a. ≥3 Moderate (PM1–PM6) *OR*
   b. 2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR*
   c. 1 Moderate (PM1–PM6) *AND* ≥4 Supporting (PP1–PP5)

Likely Pathogenic

1. 1 Very Strong (PVS1) *AND* 1 Moderate (PM1–PM6) *OR*
2. 1 Strong (PS1–PS4) *AND* 1–2 Moderate (PM1–PM6) *OR*
3. 1 Strong (PS1–PS4) *AND* ≥2 Supporting (PP1–PP5) *OR*
4. ≥3 Moderate (PM1–PM6) *OR*
5. 2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR*
6. 1 Moderate (PM1–PM6) *AND* ≥4 Supporting (PP1–PP5)

Benign

1. 1 Stand-Alone (BA1) *OR*
2. ≥2 Strong (BS1–BS4)

Likely Benign

1. 1 Strong (BS1–BS4) and 1 Supporting (BP1–BP7) *OR*
2. ≥2 Supporting (BP1–BP7)

*Variants should be classified as Uncertain Significance if other criteria are unmet or the criteria for benign and pathogenic are contradictory.

Rendimiento Exoma

En población adulta y pediátrica es diagnóstico en un 25-29% de las veces.

Estudios iniciales informaron un rendimiento prenatal entre un 50-80% → sesgo por cohortes pequeñas y casos seleccionados

Dos estudios prospectivos a larga escala reportan un **rendimiento prenatal entre un 8 a 10% en fetos con anomalías fenotípicas** con **estudio previo con cariotipo y array normal.**

Emms, A.; Castleman, J.; Allen, S.;Williams, D.; Kinning, E.; Kilby, M. Next Generation Sequencing after Invasive Prenatal Testing in Fetuses with Congenital Malformations: Prenatal or Neonatal Investigation. Genes 2022, 13, 1517. https://doi.org

Petrovski S, Aggarwal V, Giordano JL, et al. Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. Lancet. 2019;393:758–767.

Lord J, McMullan DJ, Eberhardt RY, et al. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. Lancet. 2019;393:747–757.

Monaghan KG, Leach NT, Pekarek D, Prasad P, Rose NC; ACMG Professional Practice and Guidelines Committee. The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2020 Apr;22(4):675-680. doi: 10.1038/s41436-019-0731-7. Epub 2020 Jan 8. PMID: 31911674.

› Lancet. 2019 Feb 23;393(10173):758-767. doi: 10.1016/S0140-6736(18)32042-7.
Epub 2019 Jan 31.

Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study

Slavé Petrovski [1], Vimla Aggarwal [2], Jessica L Giordano [3], Melissa Stosic [3], Karen Wou [4], Louise Bier [5], Erica Spiegel [6], Kelly Brennan [6], Nicholas Stong [5], Vaidehi Jobanputra [7], Zhong Ren [5], Xiaolin Zhu [5], Caroline Mebane [5], Odelia Nahum [7], Quanli Wang [5], Sitharthan Kamalakaran [5], Colin Malone [5], Kwame Anyane-Yeboa [4], Russell Miller [6], Brynn Levy [7], David B (

Affiliations + expand

PMID: 30712878 DOI:

Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study

Jenny Lord*, Dominic J McMullan*, Ruth Y Eberhardt*, Gabriele Rinck, Susan J Hamilton, Elizabeth Quinlan-Jones, Elena Prigmore, Rebecca Keelagher, Sunayna K Best, Georgina K Carey, Rhiannon Mellis, Sarah Robart, Ian R Berry, Kate E Chandler, Deirdre Gilliers, Lara Cresswell, Sandra L Edwards, Carol Gardiner, Alex Henderson, Simon T Holden, Tessa Homfray, Tracy Lester, Rebecca A Lewis, Ruth Newbury-Ecob, Katrina Prescott, Oliver W Quarrell, Simon C Ramsden, Eileen Roberts, Dagmar Tapon, Madeleine J Tooley, Pradeep C Vasudevan, Astrid P Weber, Diana G Wellesley, Paul Westwood, Helen White, Michael Parker, Denise Williams, Lucy Jenkins, Richard H Scott, Mark D Kilby†, Lyn S Chitty†, Matthew E Hurles†, Eamonn R Maher†, for the Prenatal Assessment of Genomes and Exomes Consortium‡

Summary

Background Fetal structural anomalies, which are detected by ultrasonography, have a range of genetic causes, including chromosomal aneuploidy, copy number variations (CNVs; which are detectable by chromosomal microarrays), and pathogenic sequence variants in developmental genes. Testing for aneuploidy and CNVs is routine during the investigation of fetal structural anomalies, but there is little information on the clinical usefulness of genome-wide next-generation sequencing in the prenatal setting. We therefore aimed to evaluate the proportion of fetuses with structural abnormalities that had identifiable variants in genes associated with developmental disorders when assessed with whole-exome sequencing (WES).

Lancet 2019; 393: 747–57
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See Editorial page 717
See Comment page 719
*Contributed equally as first

Emms, A.; Castleman, J.; Allen, S.;Williams, D.; Kinning, E.; Kilby, M. Next Generation Sequencing after Invasive Prenatal Testing in Fetuses with Congenital Malformations: Prenatal or Neonatal Investigation. Genes 2022, 13, 1517. https://doi.org

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DOI: 10.1002/pd.6115

ORIGINAL ARTICLE

PRENATAL DIAGNOSIS WILEY

Diagnostic yield of exome sequencing for prenatal diagnosis of fetal structural anomalies: A systematic review and meta-analysis

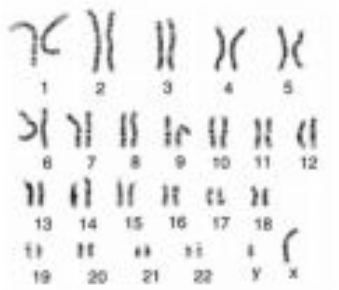
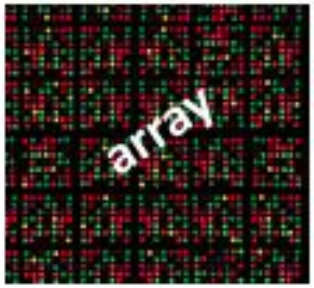
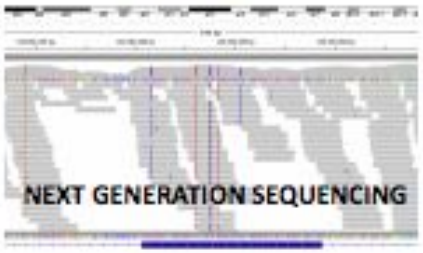
Rhiannon Mellis[1,2] | Kathryn Oprych[3] | Elizabeth Scotchman[1] | Melissa Hill[1,2] | Lyn S Chitty[1,2]

Revisión sistemática y metaanálisis: 66 estudios, 4350 fetos.
Rendimiento para todas las anomalías congénitas 31%, con variaciones significativas según **fenotipo** fetal.

Esquelético: 53% (IC 95% 42-63%) p<0.0001
Neuromuscular- Fetal akinesia deformation sequence 37% (IC 95% 20-54%) p<0.0001
Multisistema 29% (IC 95% 22-35%) p<0.0001
Hidrops/edema 22% (IC 95% 14-31%) p<0.0001
SNC 17% (IC 95% 12-22%) p<0.0001
Cardiológico 11% (IC 95% 7-16%) p<0.0001
Nefrourológico 9% (IC 95% 5-12%) p<0.0001

Craneofacial 9% (IC 95% 1-17%) p 0.02
RCF 4% (IC 95% -9 - 17%) p 0.59
TN aumentada aislada 2% (IC 95% 0-5%) p 0.04
Gastrointestinal 2% (IC 95% -4 - 8%) p 0.5
Respiratorio/Torácico 0% (IC 95% -7 - 7%) p 1
Pared abdominal 0% (IC 95% -31 - 31%) p 1

Mellis, R.; Oprych, K.; Scotchman, E.; Hill, M.; Chitty, L.S. Diagnostic yield of exome sequencing for prenatal diagnosis of fetal structural anomalies: A systematic review and meta-analysis. Prenat. Diagn. 2022, 42, 662–685.

2012-2014	2014-2020	2020	Futuro
Cariotipo	**Cariotipo molecular**	**NGS**	**NGS**
			**Exoma** ↓ **Genoma** ↓ **cfDNA**
30% detección de alteraciones numéricas y estructurales microscópicas	**4-6% detección de alteraciones** con cariotipo normal	**>10% detección de alteraciones** en malformaciones estructurales (cariotipo y arrayCGH normal)	

Tasa diagnóstica

Curso avanzado de diangósticio prenatal genético. Secuenciación prenatal de nueva generación (NGS). PhD. Ivon Cuscó. Hospital de la Santa Creu i Sant Pau 2024. Curso genética prenatal Clinic Barcelona.
Monaghan KG, Leach NT, Pekarek D, Prasad P, Rose NC; ACMG Professional Practice and Guidelines Committee. The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2020 Apr;22(4):675-680. doi: 10.1038/s41436-019-0731-7. Epub 2020 Jan 8. PMID: 31911674.

Recomendaciones ACMG 2020 en el uso de secuenciación de exoma en el diagnóstico prenatal

1. Considerar WES en gestaciones con anomalía fenotípica detectada ecográficamente y con **cariotipo y array CGH normal.**
2. Utilizar estrategia **Exoma TRIO** (mayor rendimiento diagnóstico, menor tiempo)
3. **Consejería pre-test**: informar sobre hallazgos secundarios o incidentales, paternidad.
4. No recomienda:
   a. Reportar **VUS** en genes no relacionadas con fenotipo fetal
   b. Reportar **portación** de enfermedades recesivas.

Monaghan KG, Leach NT, Pekarek D, Prasad P, Rose NC; ACMG Professional Practice and Guidelines Committee. The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2020 Apr;22(4):675-680. doi: 10.1038/s41436-019-0731-7. Epub 2020 Jan 8. PMID: 31911674.

Limitaciones Exoma

- Dificultad en detectar grandes ganancias, deleciones o translocaciones debido a las **cortas longitudes de lectura,** a excepción de realizar exoma con análisis de CNV.
- **Costo y disponibilidad**
- Detección de variantes como **hallazgo secundario o incidental**
- **Fenotipo fetal dinámico** → alteraciones no percibidas ecográficamente pueden cambiar la interpretación del examen en el periodo postnatal.

Mone, F.; Abu Subieh, H.; Doyle, S.; Hamilton, S.; Mcmullan, D.J.; Allen, S.; Marton, T.; Williams, D.; Kilby, M.D. Evolving fetal phenotypes and clinical impact of progressive prenatal exome sequencing pathways: Cohort study. Ultrasound Obs. Gynecol. 2022, 59, 723–730.
Chandler, N.J.; Scotchman, E.; Mellis, R.; Ramachandran, V.; Roberts, R.; Chitty, L.S. Lessons learnt from prenatal exome sequencing. Prenat. Diagn. 2022, 42, 831–844.

Exoma para CNV

Published online 4 June 2010

Nucleic Acids Research, 2010, Vol. 38, No. 14 e151
doi:10.1093/nar/gkq510

Using next-generation sequencing for high resolution multiplex analysis of copy number variation from nanogram quantities of DNA from formalin-fixed paraffin-embedded specimens

Henry M. Wood[1,*], Ornella Belvedere[1], Caroline Conway[1], Catherine Daly[1], Rebecca Chalkley[1], Melissa Bickerdike[1], Claire McKinley[1], Phil Egan[1], Lisa Ross[1], Bruce Hayward[2], Joanne Morgan[1], Leslie Davidson[3], Ken MacLennan[4], Thian K. Ong[5], Kostas Papagiannopoulos[6], Ian Cook[7], David J. Adams[8], Graham R. Taylor[1] and Pamela Rabbitts[1]

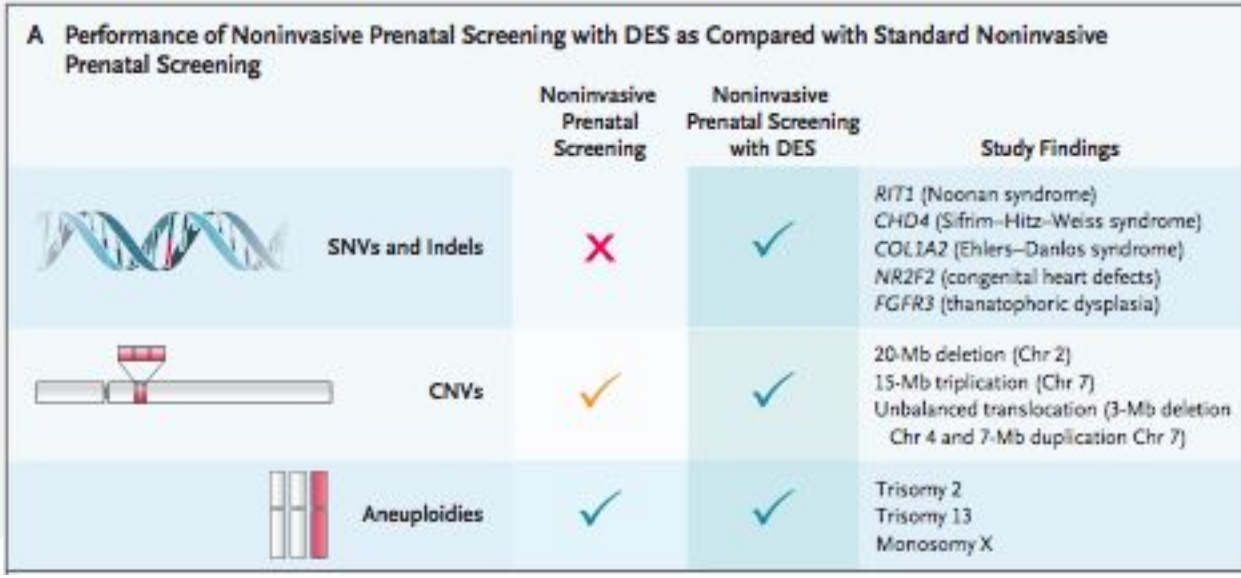
NGS puede utilizarse para evaluar la presencia de CNV con un alto rendimiento en muestras celulares, tumores congelados y muestras fijadas en parafina, mediante multiplexación de hasta 10 muestras en un carril de Illumina, logrando una fuerte correlación con los resultados obtenidos mediante array CGH.

Exoma en DNA libre fetal en sangre materna

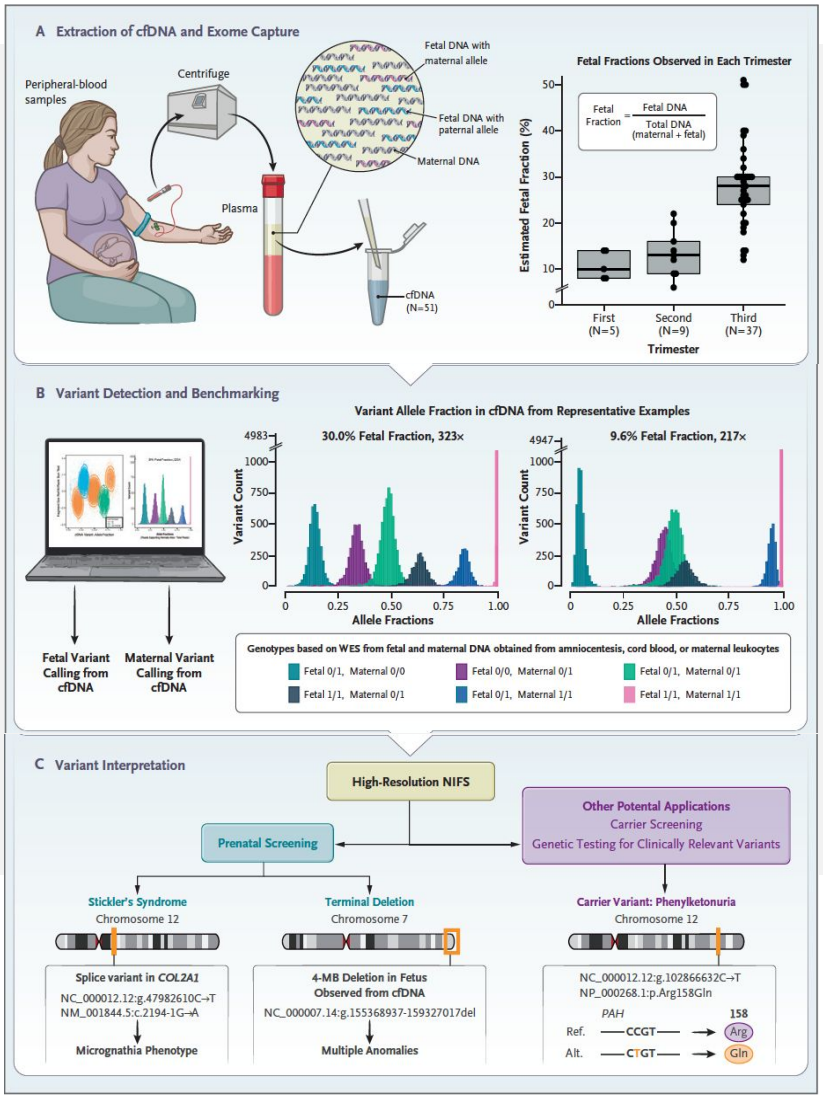
36 gestantes + parejas, con TN ≥5mm + anomalía fenotípica detectada en ecografía de 1º-2ºT.
Se realizó ES trio en DNA fetal libre en sangre materna.
Se comparó con resultados obtenidos en WES trio y WGS + CMA de los mismos casos.

100% detección de variantes patogénicas de novo.

Sensibilidad estimada en un 95,2% dependiente de la fracción de DNA fetal obtenida en la muestra y la cobertura de secuenciación.



Miceikaitė, I. , Hao, Q. , Brasch-Andersen, C. , Fagerberg, C. R. , Torring, P. M. , Kristiansen, B. S. , Ousager, L. B. , Sperling, L. , Ibsen, M. H. , Löser, K. & Larsen, M. J. (2023). *The New England Journal of Medicine, 389* (21), 2017-2019. doi: 10.1056/NEJMc2307918.


A Extraction of cfDNA and Exome Capture
Peripheral-blood samples
Centrifuge
Plasma
Fetal DNA with maternal allele
Fetal DNA with paternal allele
Maternal DNA
cfDNA (N=51)
Fetal Fractions Observed in Each Trimester
Fetal Fraction = Fetal DNA / Total DNA (maternal + fetal)
Estimated Fetal Fraction (%)
First (N=5)
Second (N=9)
Third (N=37)
Trimester
B Variant Detection and Benchmarking
Variant Allele Fraction in cfDNA from Representative Examples
30.0% Fetal Fraction, 323×
9.6% Fetal Fraction, 217×
Variant Count
Allele Fractions
Fetal Variant Calling from cfDNA
Maternal Variant Calling from cfDNA
Genotypes based on WES from fetal and maternal DNA obtained from amniocentesis, cord blood, or maternal leukocytes
Fetal 0/1, Maternal 0/0
Fetal 0/0, Maternal 0/1
Fetal 0/1, Maternal 0/1
Fetal 1/1, Maternal 0/1
Fetal 0/1, Maternal 1/1
Fetal 1/1, Maternal 1/1
C Variant Interpretation
High-Resolution NIFS
Prenatal Screening
Other Potental Applications
Carrier Screening
Genetic Testing for Clinically Relevant Variants
Stickler's Syndrome
Chromosome 12
Splice variant in COL2A1
NC_000012.12:g.47982610C→T
NM_001844.5:c.2194-1G→A
Micrognathia Phenotype
Terminal Deletion
Chromosome 7
4-MB Deletion in Fetus Observed from cfDNA
NC_000007.14:g.155368937-159327017del
Multiple Anomalies
Carrier Variant: Phenylketonuria
Chromosome 12
NC_000012.12:g.102866632C→T
NP_000268.1:p.Arg158Gln
PAH
158
Ref. CCGT Arg
Alt. CTGT Gln

Exoma post mortem

- Test de **segunda línea** posterior a cariotipo y array negativo.
- En fetos de embarazos interrumpidos, óbito fetal, muerte neonatal.
- Estudios informan rendimiento entre **20 a 37%** en muestras fetales post mortem.
- RS concluye que ¼ a ⅓ de embarazos interrumpidos o muertes perinatales se asocian a una causa identificable en WES/WGS.

Review > Genet Med. 2024 May 2:101159. doi: 10.1016/j.gim.2024.101159. Online ahead of print.

A systematic review to assess the utility of genomic autopsy using exome or genome sequencing in cases of congenital anomalies and perinatal death

Camille Schubert [1], Joanne Milverton [2], Stephen Goodall [3], Tracy Merlin [2]

Affiliations + expand

PMID: 38704678 DOI: 10.1016/j.gim.2024.101159

Emms, A.; Castleman, J.; Allen, S.;Williams, D.; Kinning, E.; Kilby, M. Next Generation Sequencing after Invasive Prenatal Testing in Fetuses with Congenital Malformations: Prenatal or Neonatal Investigation. Genes 2022, 13, 1517. https://doi.org

Quinlan-Jones, E.; Lord, J.; Williams, D.; Hamilton, S.; Marton, T.; Eberhardt, R.Y.; Rinck, G.; Prigmore, E.; Keelagher, R.; McMullan, D.J.; et al. Molecular autopsy by trio exome sequencing (ES) and postmortem examination in fetuses and neonates with prenatally identified structural anomalies. Genet. Med. 2019, 21, 1065–1073.

Yates, C.L.; Monaghan, K.G.; Copenheaver, D.; Retterer, K.; Scuffins, J.; Kucera, C.R.; Friedman, B.; Richard, G.; Juusola, J. Whole-exome sequencing on deceased fetuses with ultrasound anomalies: Expanding our knowledge of genetic disease during fetal development. Genet. Med. 2017, 19, 1171–1178.

Secuenciación genoma: WGS

- Secuenciación de 3 billones de pares de bases: exones e intrones (genes no codificantes).
- Rendimiento diagnóstico en evaluación, se propone igual o mayor al WES, con mayor incidencia de VUS, hallazgos incidentales y secundarios.
- Interpretación dificultosa por limitación de conocimiento en implicancias de variantes de zonas no codificantes.
- Detecta rearreglos estructurales (translocaciones, inversiones, inserciones), CNVs largas (eliminaciones y duplicaciones) que actualmente sólo son detectadas por Array.

Van den Veyver IB, Chandler N, Wilkins-Haug LE, Wapner RJ, Chitty LS; ISPD Board of Directors. International Society for Prenatal Diagnosis Updated Position Statement on the use of genome-wide sequencing for prenatal diagnosis. Prenat Diagn. 2022 May;42(6):796-803. doi: 10.1002/pd.6157. PMID: 35583085.

Secuenciación genoma: WGS

› Ultrasound Obstet Gynecol. 2024 May;63(5):658-663. doi: 10.1002/uog.27592.
Epub 2024 Apr 14.

Whole-genome sequencing in prenatally detected congenital malformations: prospective cohort study in clinical setting

E Westenius [1,2], P Conner [3,4], M Pettersson [1,2], E Sahlin [1,2], N Papadogiannakis [5,6], A Lindstrand [1,2], E Iwarsson [1,2]

Affiliations + expand

PMID: 38268232 DOI: 10.1002/uog.27592

Estudio prospectivo 50 casos con malformaciones congénitas.
Se realizó WGS TRIO logrando diagnóstico molecular en un 26% de los casos e identificando una variante clinicamente significativa en un 30% de los casos.

› Ultrasound Obstet Gynecol. 2024 May;63(5):664-671. doi: 10.1002/uog.27517.
Epub 2024 Apr 15.

Whole-genome sequencing analysis in fetal structural anomalies: novel phenotype-genotype discoveries

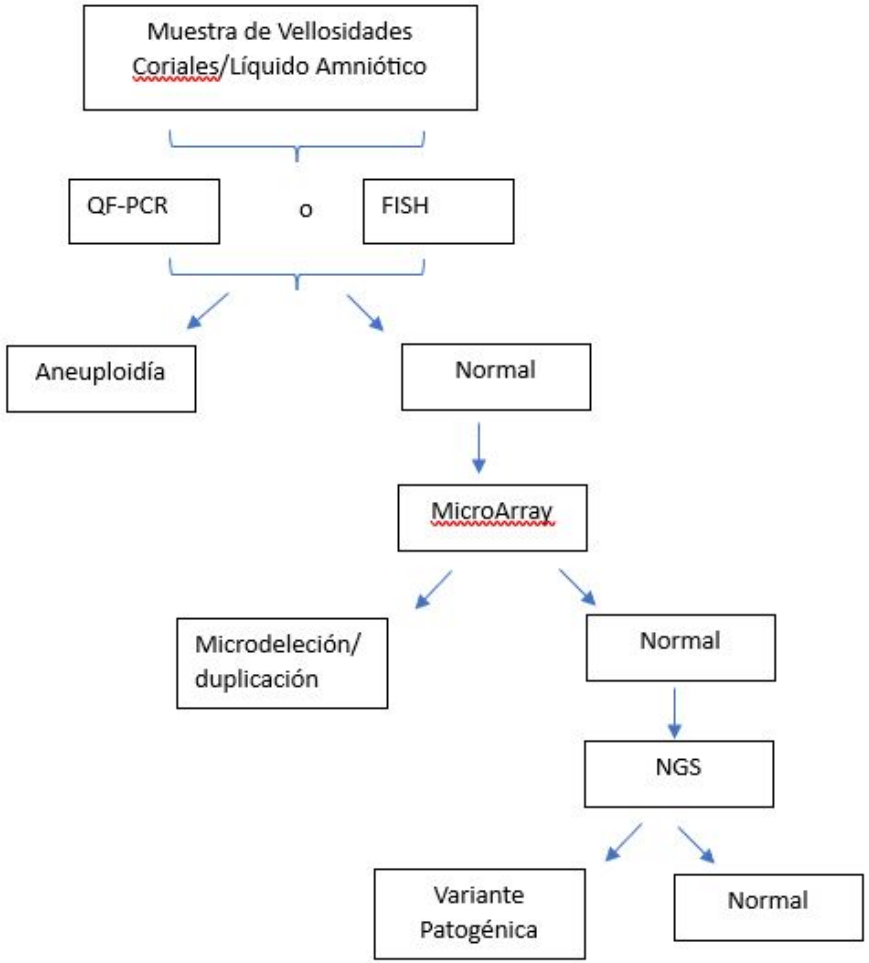
Q Qi [1], Y Jiang [1], X Zhou [1], Y Lü [1], R Xiao [2], J Bai [3], H Lou [3], W Sun [4], Y Lian [4], N Hao [1], M Li [1], J Chang [5]

Affiliations + expand

PMID: 37842862 DOI: 10.1002/uog.27517

WGS TRIO en 17 fetos con anomalías fenotípicas con resultado negativo de ES y CMA. Identificó variantes clinicamente significativas en un 11,8%.

Flujograma estudio genético



Extraído de Protocols Medicina Materno Fetal Hospital Clínic - Hospital Sant Joan De Déu Universitat de Barcelona www.medicinafetalbarcelona.org y modificado por Centro de Referencia Perinatal Oriente (CERPO).

CERPO

Centro de Referencia Perinatal Oriente

Facultad de Medicina, Universidad de Chile

Exámenes genéticos IV: Next generation sequencing (NGS). Técnica y rendimiento prenatal.

Autora: Dra. Clara Rioseco R.
Tutora: Dra. Catherine Díaz Sanhueza.
Obstetricia y Ginecología - Medicina Materno Fetal - Genética Clínica.