

Curso Biología Molecular CMR

Módulo 5

Diagnóstico Molecular en Medicina Reproductiva



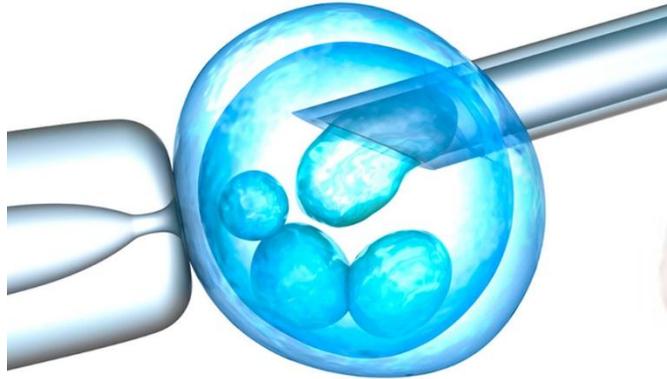
Dra. Ivana Canonero
Lic. Guadalupe Méjico

DIAGNÓSTICO GENÉTICO: ETAPAS



PRECONCEPCIONAL

PREIMPLANTACIONAL



PRENATAL

POSTNATAL

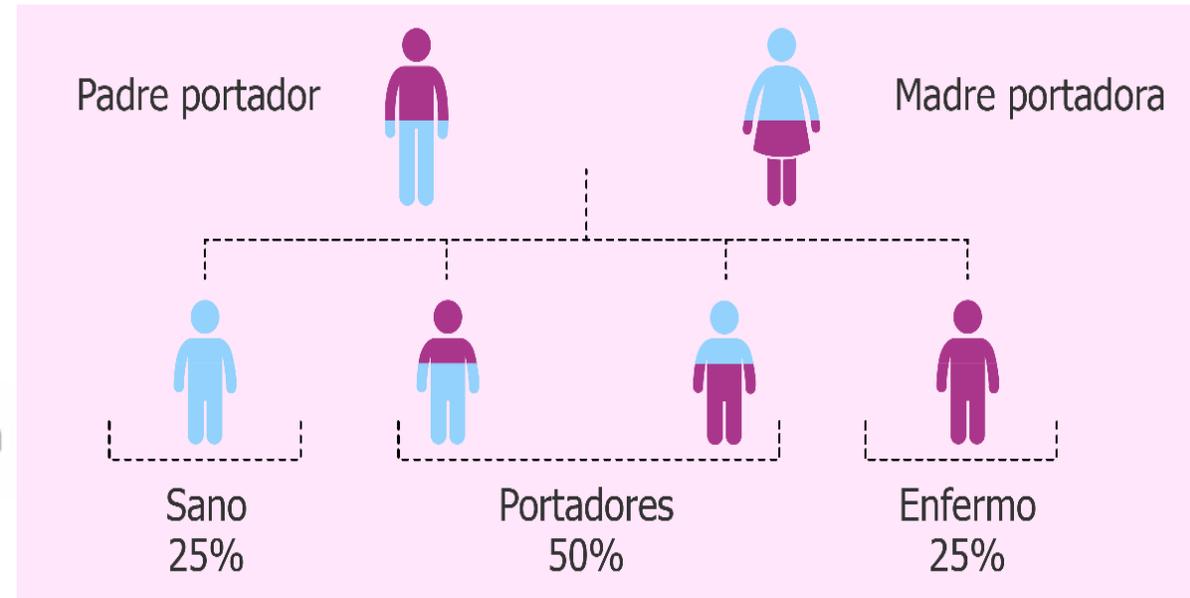


PRECONCEPCIONAL

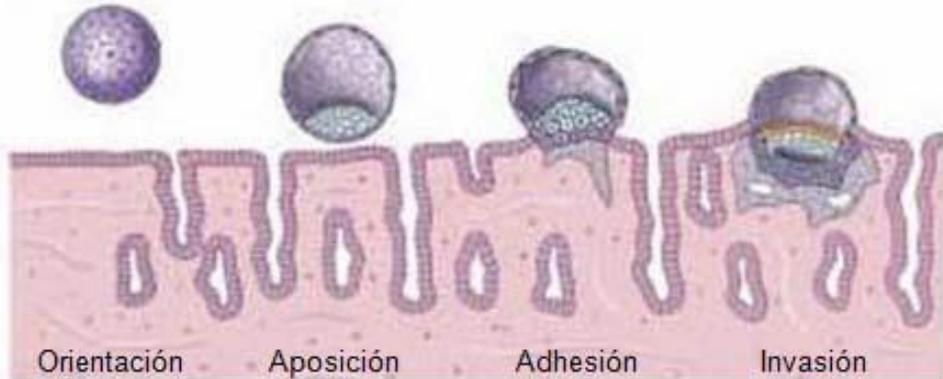


Brindar información a los progenitores de la posibilidad de transmisión de enfermedades hereditarias. Principalmente de aquellas de herencia recesiva.

- Enfermedades con frecuencia de portador alto: FQ (1/25), AME (1/40), FRAXA (1/125)
- Que se evalúa el mecanismo molecular involucrado
- Resultado: Riesgo de tener un hijo afectado, en función de la compatibilidad de los progenitores para las enfermedades testeadas
- También debería ser usado en dadores de gametos



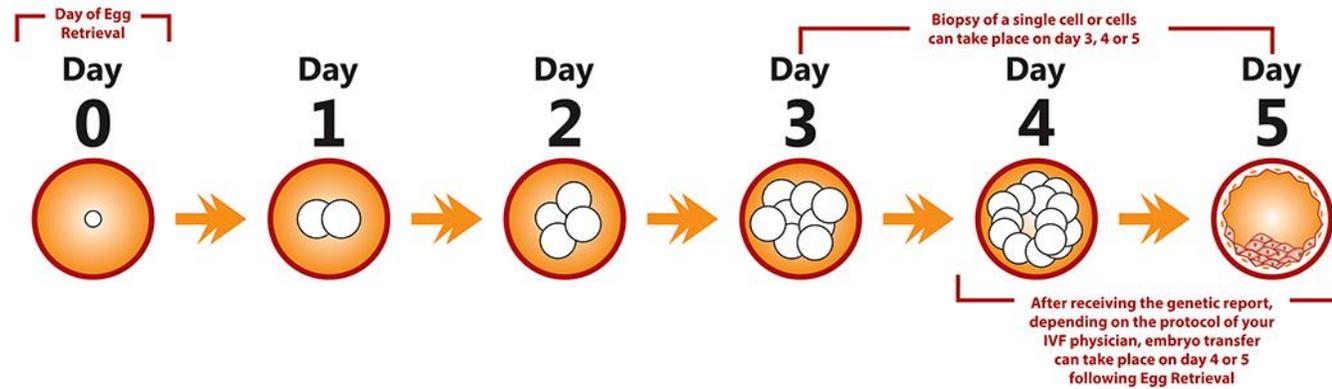
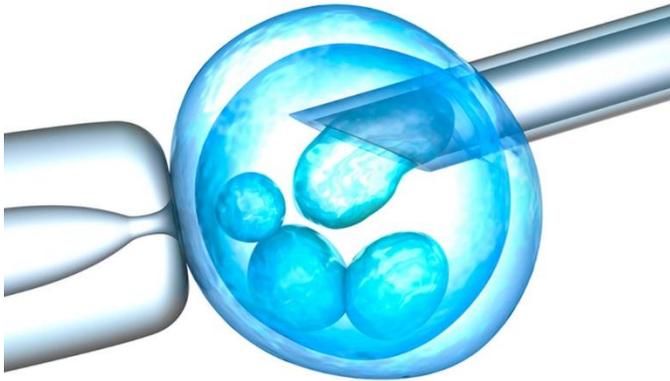
Test de receptividad endometrial



El endometrio varía a lo largo del ciclo menstrual y su principal función es la de estar preparado para la implantación embrionaria y la gestación.

- **Para quién?** Mujeres en las que se ha producido falla con embriones euploides y con útero morfológicamente y anatómicamente normal
- **Para qué?** Permite determinar la ventana de implantación personalizada y establecer en función del resultado una transferencia de embriones personalizada
- **Cómo y qué?**
 - Biopsia endometrial en día LH+7 (ciclo natural) o día P+5 (ciclo sustituido).
 - Se analiza la expresión de 238 genes implicados en la receptividad endometrial.
 - Los datos obtenidos clasifican al endometrio como receptivo o no receptivo.

PREIMPLANTACIONAL



PGT-A → Aneuploidías

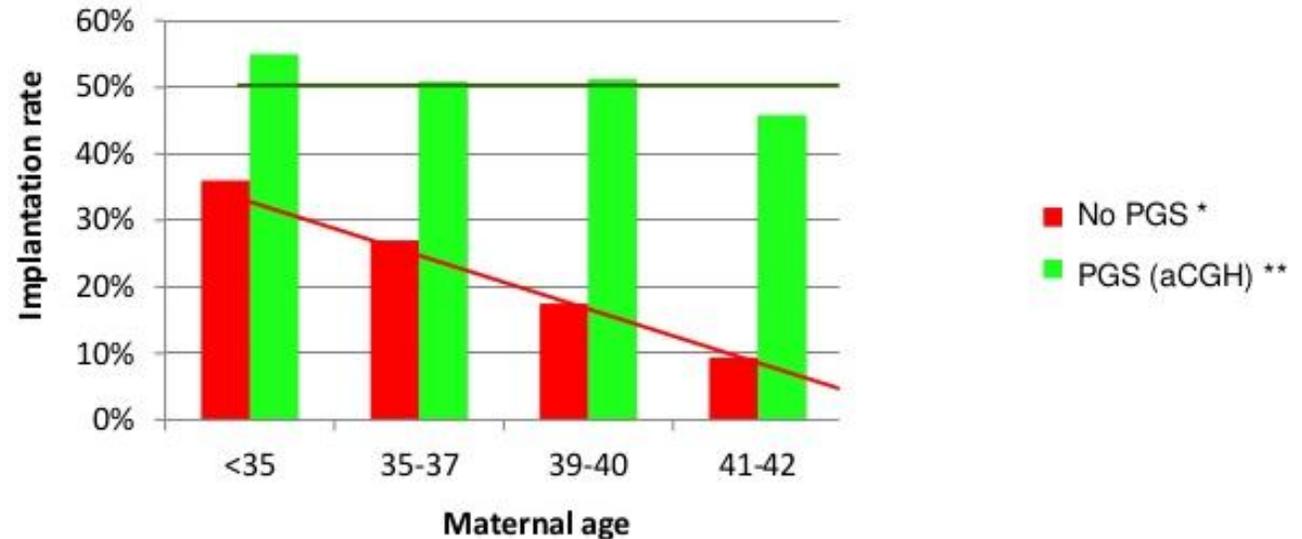
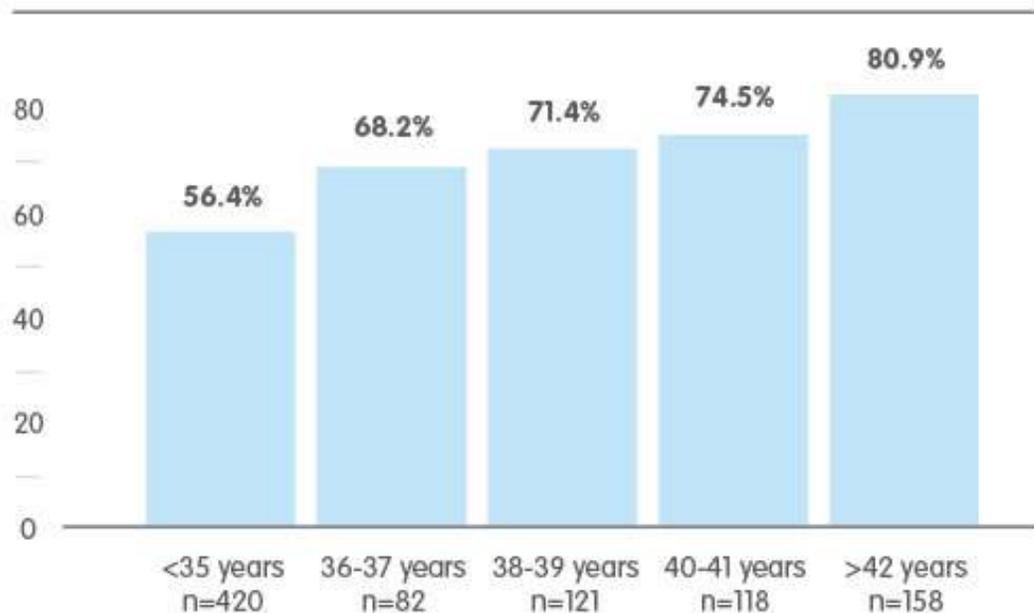
PGT-SR → Traslocaciones

PGT-M → Mendelianas

PGT-A

- Selección de embriones euploides en FIV
- El aumento de la edad materna conlleva un aumento en la falla de implantación.
- *PGT: Test Genético Preimplantacional* - A partir de biopsia embrionaria (D5) se realiza el análisis de aneuploidías en células del embrión
- Es un tamizaje que mejora la tasa de implantación.

Abnormal embryos per age



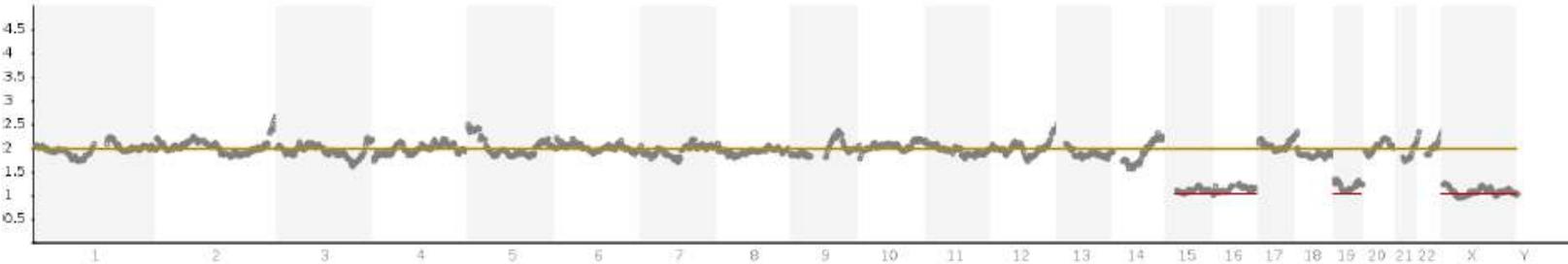
* SART 2011

** Harton, Munné et al. (2013) Fertil Steril. And unpublished data to 8/2013. N >800 blast biopsies

Resultados PGT-A

Resultado: 42, X0, -15, -16, -19

Interpretación: Con la metodología utilizada, se detectan múltiples monosomías cromosómicas, es decir, la presencia de una sola copia de los cromosomas X, 15, 16 y 19 en la muestra analizada.

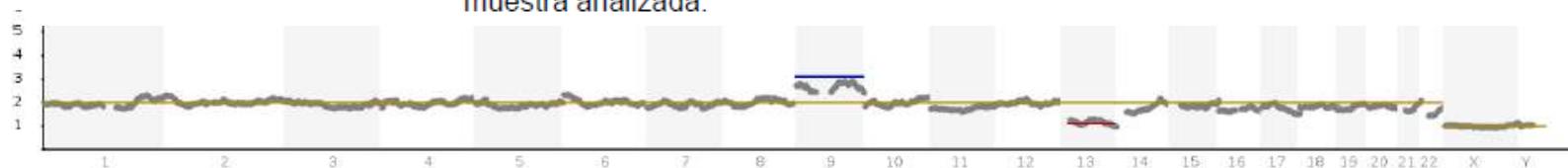


Para quién??

Edad Reproductiva avanzada
Fallo de implantación (+ 2)
Abortos recurrentes (+ 2)

Resultado: 46, XY, +9, -13

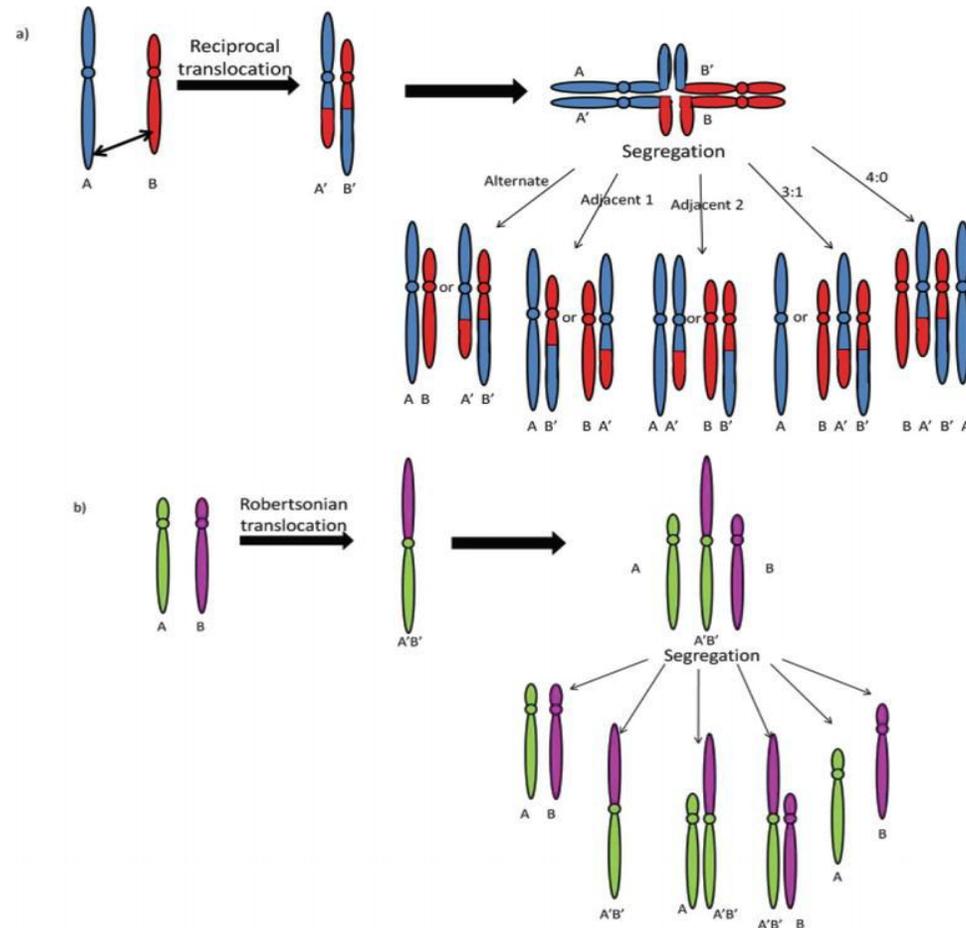
Interpretación: Con la metodología utilizada, se detecta trisomía del cromosoma 9, es decir, la presencia de tres copias del cromosoma 9, además se detecta monosomía del cromosoma 13, es decir, la presencia de una sola copia del cromosoma 13 en la muestra analizada.



PGT-SR

Para quién? Progenitores que presenten anomalías cromosómicas balanceadas (traslocaciones/inversiones).

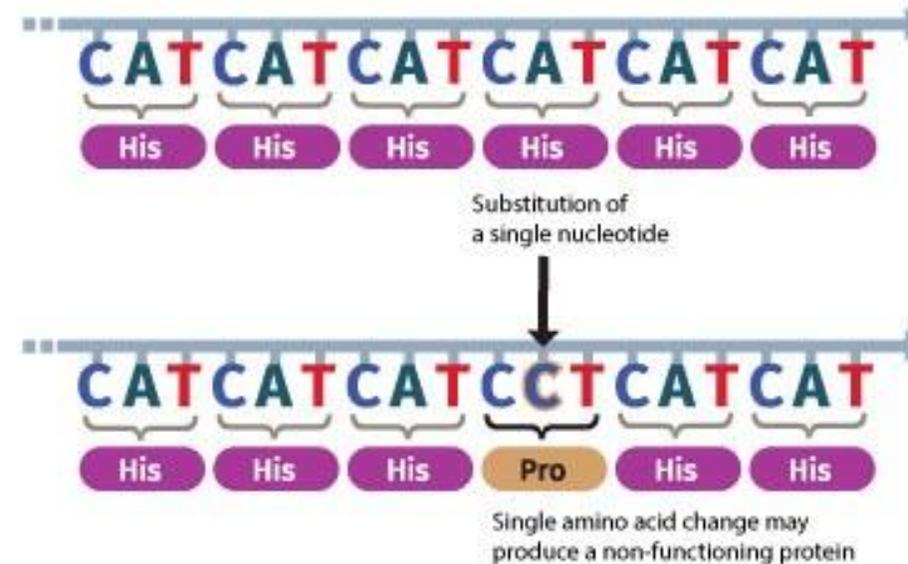
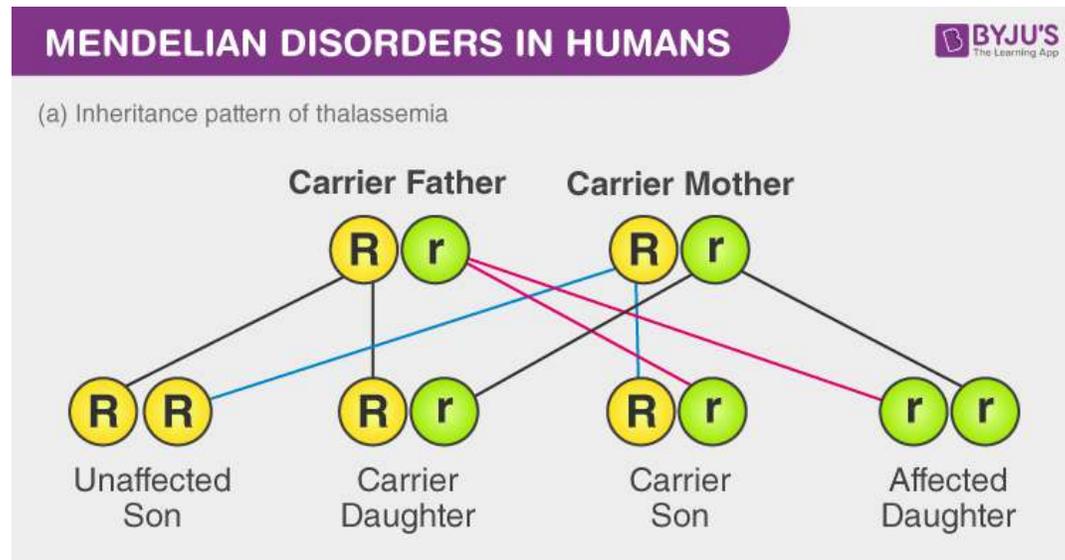
Para qué? Determinar embriones sin desequilibrios cromosómicos en parejas portadoras de anomalías cromosómicas estructurales (p.ej. translocaciones Robertsonianas, translocaciones recíprocas).



PGT-M

Para quién? Parejas con riesgo de tener descendencia con un desorden causado por una variante puntual conocida (monogénico)

Para qué? Selección de embriones que no sean portadores de la variante en cuestión a buscar.





NICS: Noninvasive Chromosome Screening

Método basado en WGS para la detección de aneuploidías o traslocaciones desbalanceadas, a partir del ADN secretado por los blastocistos al medio de cultivo.

Ventajas:

Evitar la biopsia del embrión
Metodología *a priori* menos compleja y menos costosa

Limitaciones:

FN → presencia de células del *cumulus* (origen materno)
FP → se piensa que pueden ser mosaico, células que el embrión elimina (rescate de trisomía)

- Serviría principalmente para la selección de embriones euploides, y cuando fuese aneuploide se debería confirmar por método invasivo.
- **Falsos negativos es un gran problema si se logra el embarazo y no se aborta espontáneamente!**

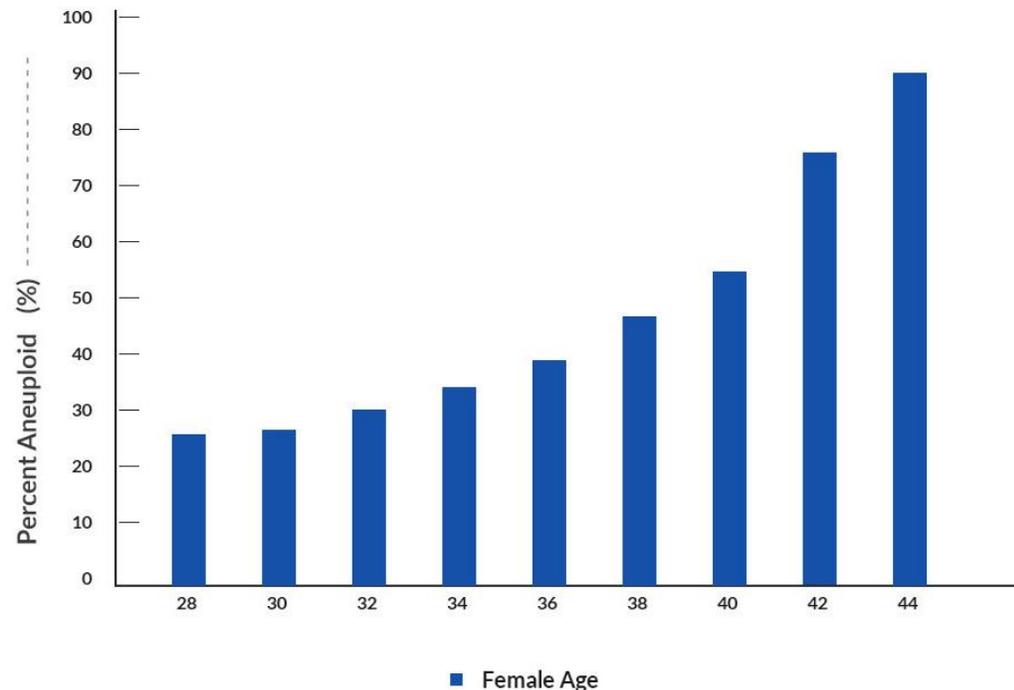
PRENATAL



Detectar precozmente anomalías congénitas con el fin de:

- Brindar **tratamiento** prenatal o perinatal
- **Tomar acciones** al momento del parto para controlar la afección
- **Interrupción voluntaria** del embarazo
- **Preparación emocional** para los progenitores

Percent of embryos with aneuploidy by female age



- ✓ Edad materna avanzada
- ✓ Abortadoras recurrentes
- ✓ Antecedentes familiares
- ✓ Voluntad o ansiedad materna

DIAGNÓSTICO GENÉTICO PRENATAL

Técnicas invasivas

- ✓ Riesgo genético mayor al riesgo de la prueba invasiva
- ✓ Cribado combinado de alto riesgo
- ✓ Malformación ecográfica
- ✓ Antecedentes familiares fuertes
- ✓ Ansiedad o angustia materna



BIOPSIA CORIAL

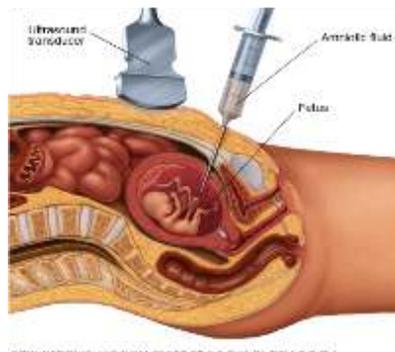
Semana 10-13

Más precoz

Técnica agresiva

Contaminación materna

Células del trofoctodermo



AMNIOCENTESIS

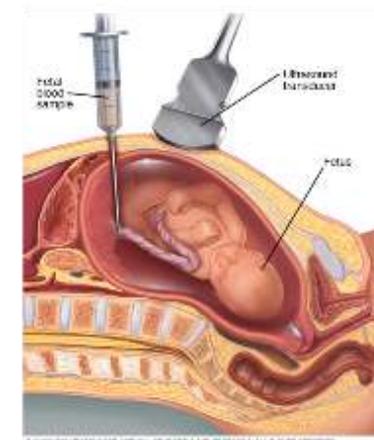
Semana 15-20

Técnica más habitual

Menor riesgo (expertiz)

Más fiabilidad diagnóstica

Células epiteliales (piel y sistema urinario)



CORDOCENTESIS

Semana 18-20

Dificultad técnica

Riesgo fetal alto

Sólo para técnicas diagnósticas específicas

Non-Invasive Prenatal Screening (NIPS)

BMJ

RESEARCH

Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study

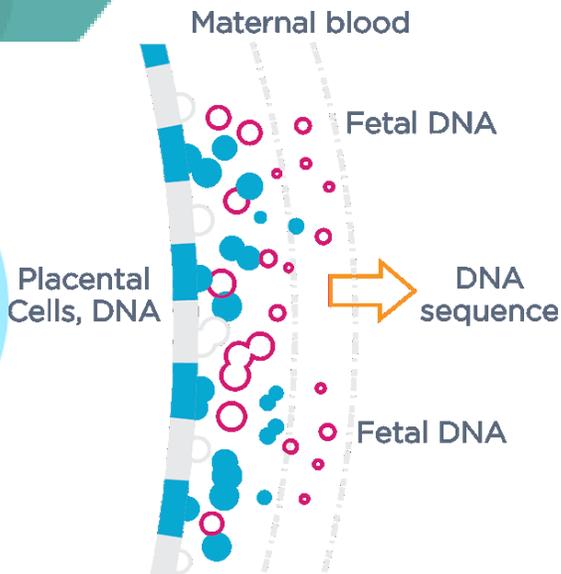
Rossa W K Chiu, professor,¹ Ranjit Akolekar, clinical research fellow,¹ Yama W L Zheng, student,¹ Tak Y Leung, professor,² Hao Sun, assistant professor,¹ K C Ai fellow,¹ Attie T J I Go, professor,³ Elizabeth T Lai William W K Lo, consultant,⁴ Wing C Leung, consultant,⁵ Helena Lam, consultant,⁶ Yu Y Kun Yugt, professor,⁴ Ryoko Minekawa, postdoctoral associate professor,⁷ Jun Wang, professor,⁷ Tze K Lau, professor,⁸ Kypros H Nicolaides, professor,⁹ Y M Dennis Lo, professor¹⁰

Análisis Trisomía 21: 753 embarazadas
Sensibilidad: 100%
Especificidad: 97.9%

¹Centre for Research into Circulating Fetal Nucleic Acids, Li Ka Shing Institute of Health Sciences, Department of Clinical Pathology, The Chinese University of Hong Kong, Hong Kong SAR, China
²Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, Hong Kong SAR, China

ABSTRACT
Objectives To validate the clinical efficacy and practical feasibility of massively parallel maternal plasma DNA sequencing to screen for fetal trisomy 21 among high risk pregnancies clinically indicated for amniocentesis or chorionic villus sampling.

Conclusion Multiplexed maternal plasma DNA sequencing analysis could be used to rule out fetal trisomy 21 among high risk pregnancies. If referrals for amniocentesis or chorionic villus sampling were based on the sequencing test results, about 98% of the invasive diagnostic procedures could be avoided.



Ácidos nucleicos circulantes en plasma (CNAs)

Descubiertos en 1948

1989 se asocia a cáncer (Biopsia líquida)

1997 Lo et al. ,presencia de CNAs fetales en plasma materno

Proviene de la apoptosis del trofoblasto (origen placentario)

Supone entre un 5-8% del ADN total materno

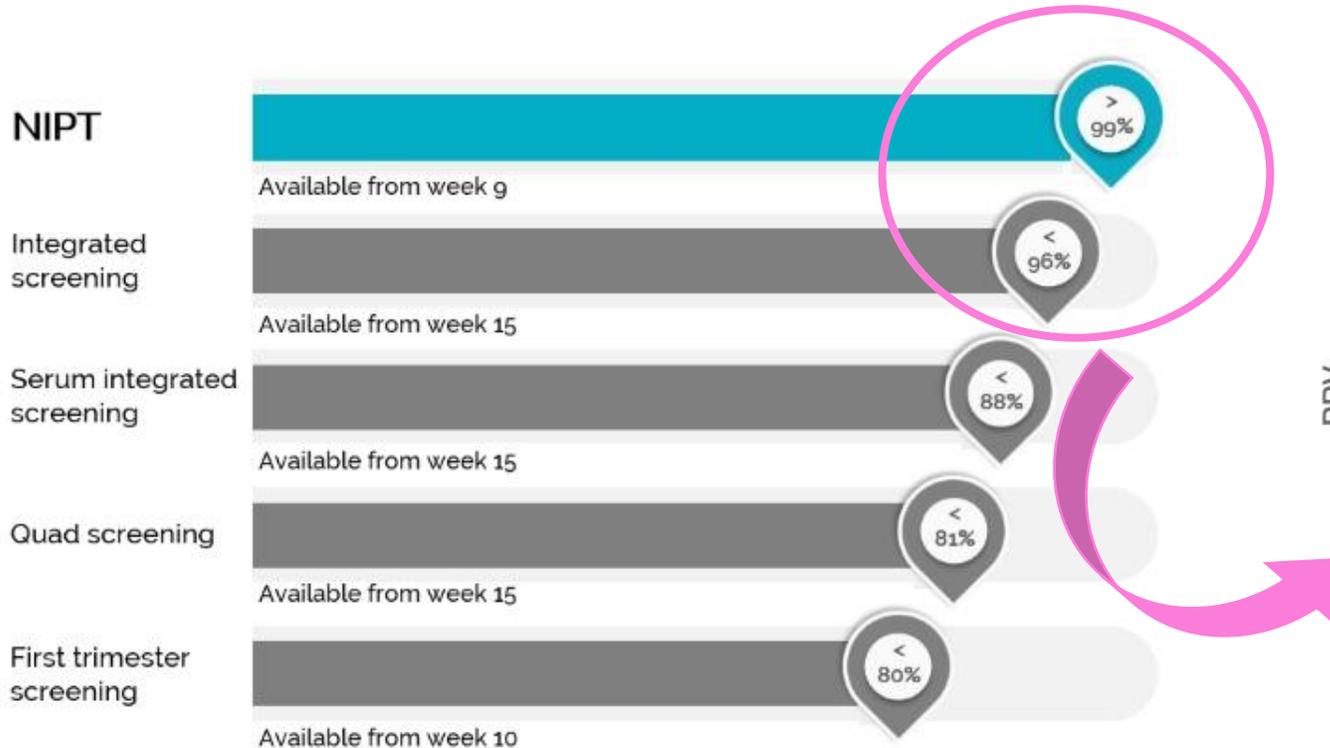
Crece durante el embarazo

Puede detectarse precozmente (semana 9)

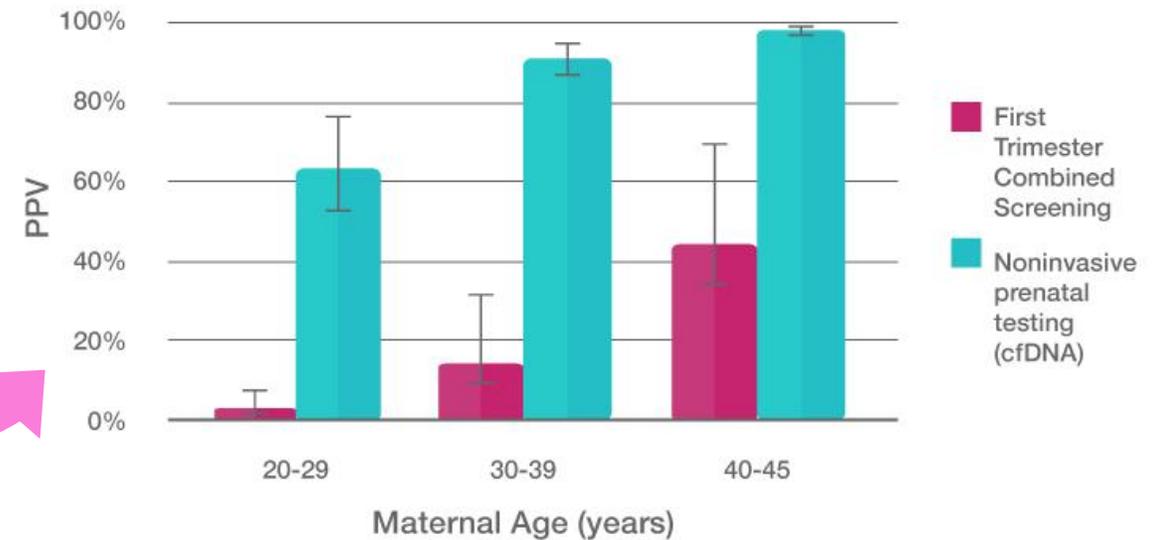
Desaparece luego del parto

Contenido de ADN mayor que en células fetales

Non-Invasive Prenatal Screening (NIPS)



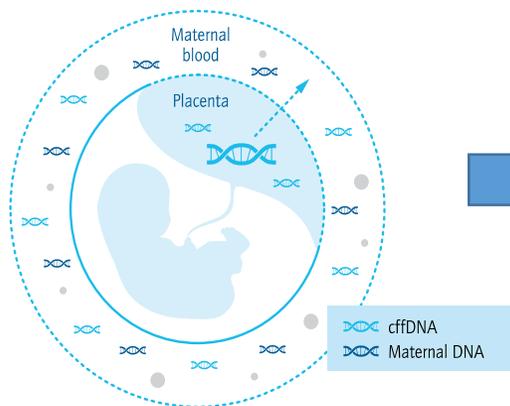
Trisomy 21 PPVs: NIPT vs. First Trimester Combined Screening



$$\text{PPV} = \frac{\text{VP}}{\text{VP} + \text{FP}}$$

Tasa de Falsos Positivos:
 Screening combinado: 5%
 NIPS: 0.1%

Flujo de trabajo NIPS



```
ATCTCTTGGCTCCAGCATCGATGAA
TCATTTAGAGGAAGTAAAAGTCGT
GAACTGTCAAAACTTTTAACACCG
TGTTGCTTCGGCGGCCCGCAAGC
GGCCTGCCGTGGCAGATCCCCAAC
TCTCTTGGCTCCAGCATCGATGAA
CAGCATCGATGAAGAACGCAGCGA
CGATACTTCTGAGTGTTCTTAGCG
CGGATCTCTTGGCTCCAGCATCGA
ACAACGGATCTCTTGGCTCCAGCA
CGGATCTCTTGGCTCCAGCATCGA
GATGAAGAACGCAGCGAAACGCGA
```



Extracción cfADN

- Sangre entera colectada en tubos especiales para evitar liberación del ADN genómico materno
- Se separa el plasma y se extrae ADN libre

Biblioteca y Secuenciación

- Preparación de bibliotecas Truseq Nano y enriquecimiento de regiones de interés
- Secuenciación con secuenciador Illumina® NextSeq500, HO 1x75pb

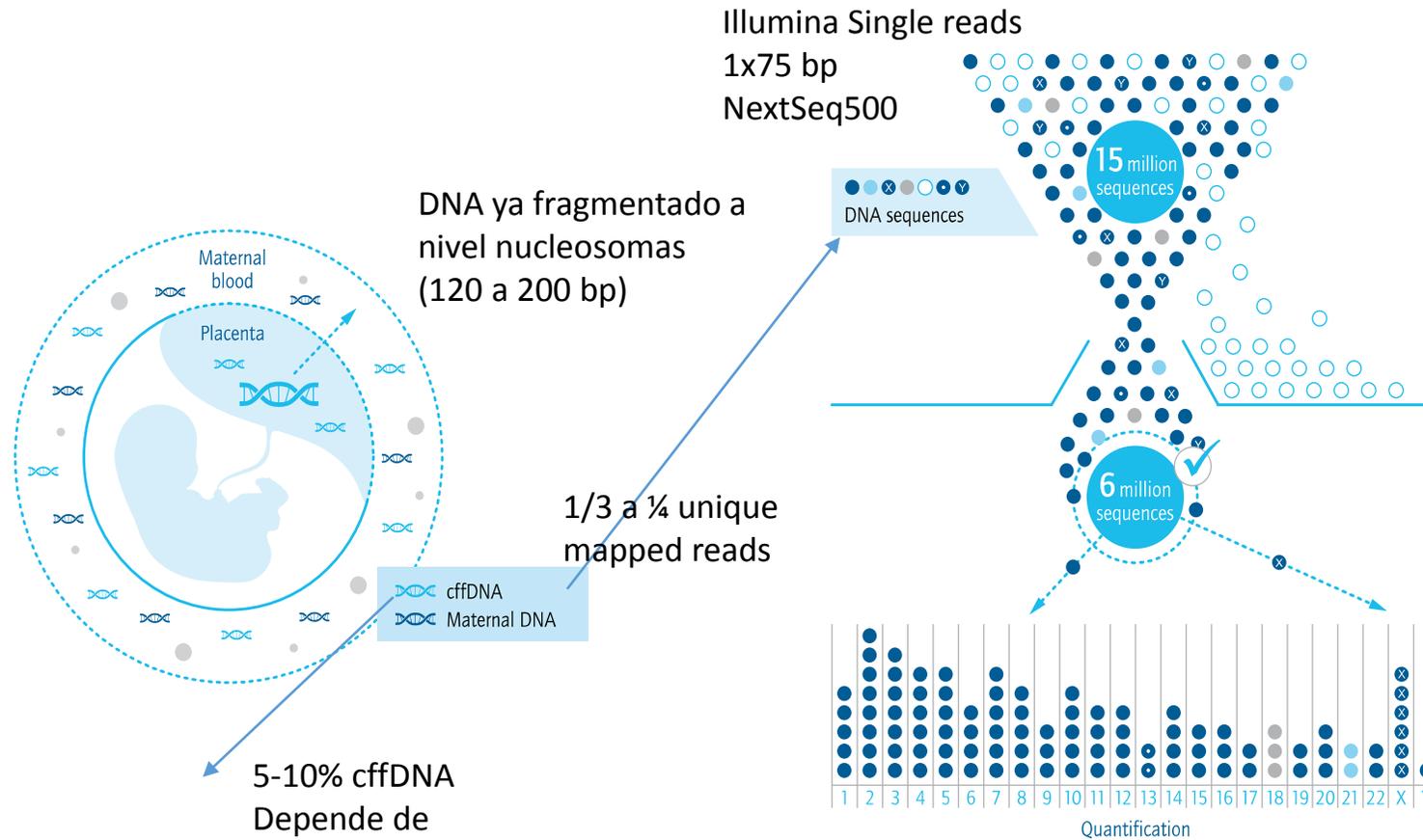
Análisis Bioinformático

- Conteo estadístico de secuencias para cada cromosoma y comparación contra la DB euploide
- Sistema de cuádruple puntaje e inspección visual de los plots
- Determinación de %FF y sexo fetal por machine learning

Análisis Genético

- Análisis de resultados y confección de reporte en contexto clínico
- Asesoramiento genético a cargo de médico genetista especialista

Non-Invasive Prenatal Screening (NIPS)

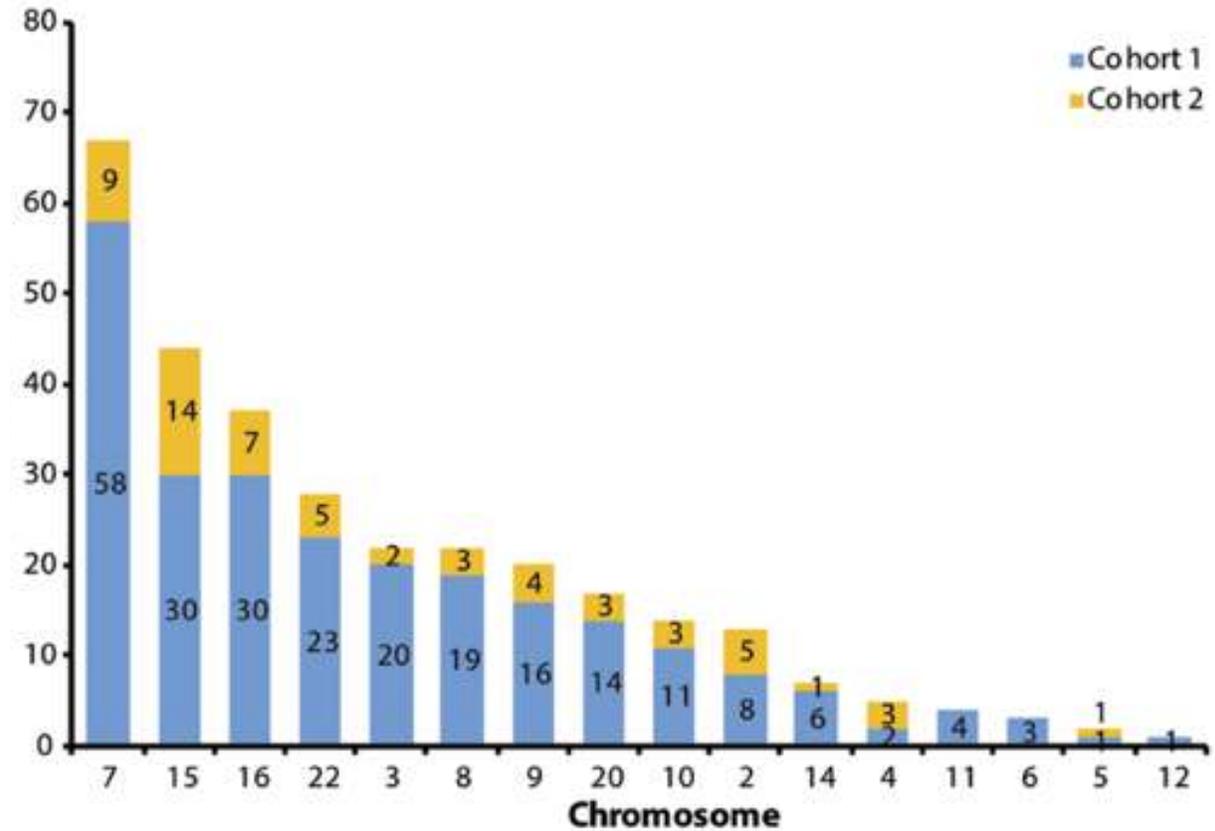
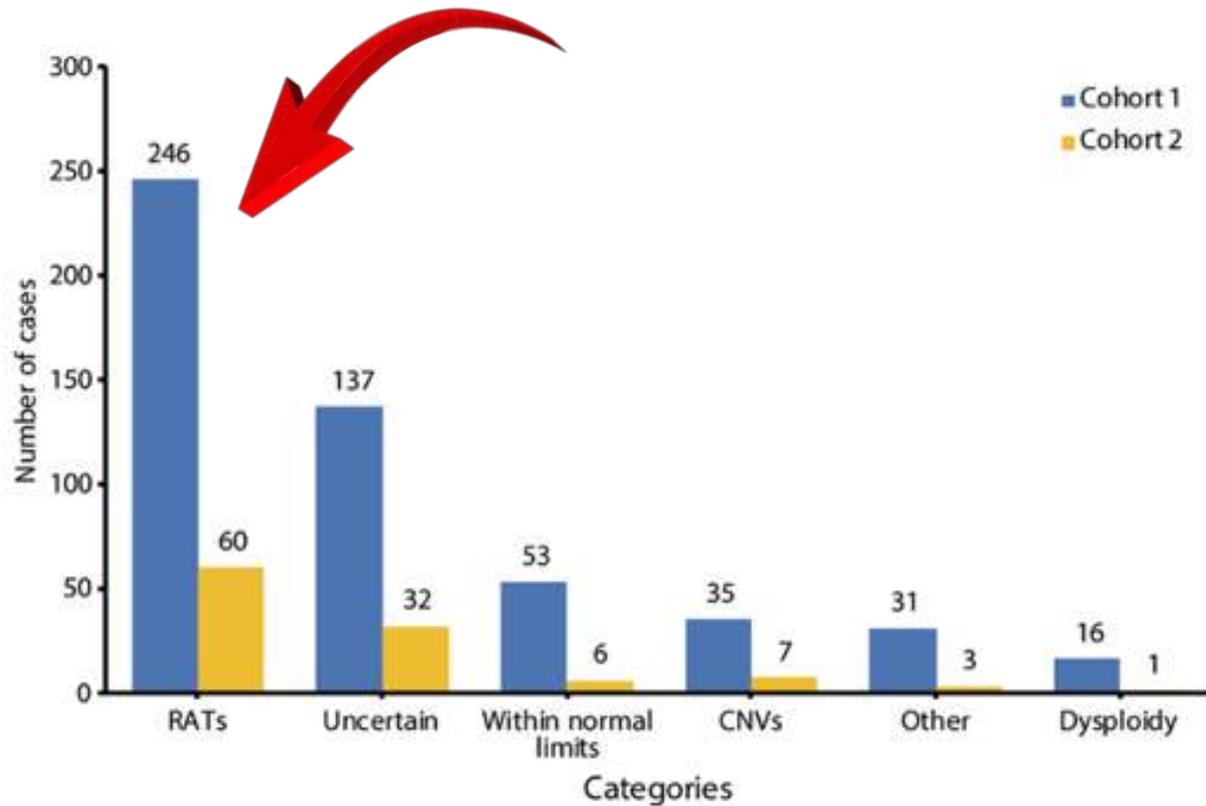


PREGNANCY

Rare autosomal trisomies, revealed by maternal plasma DNA sequencing, suggest increased risk of fetoplacental disease

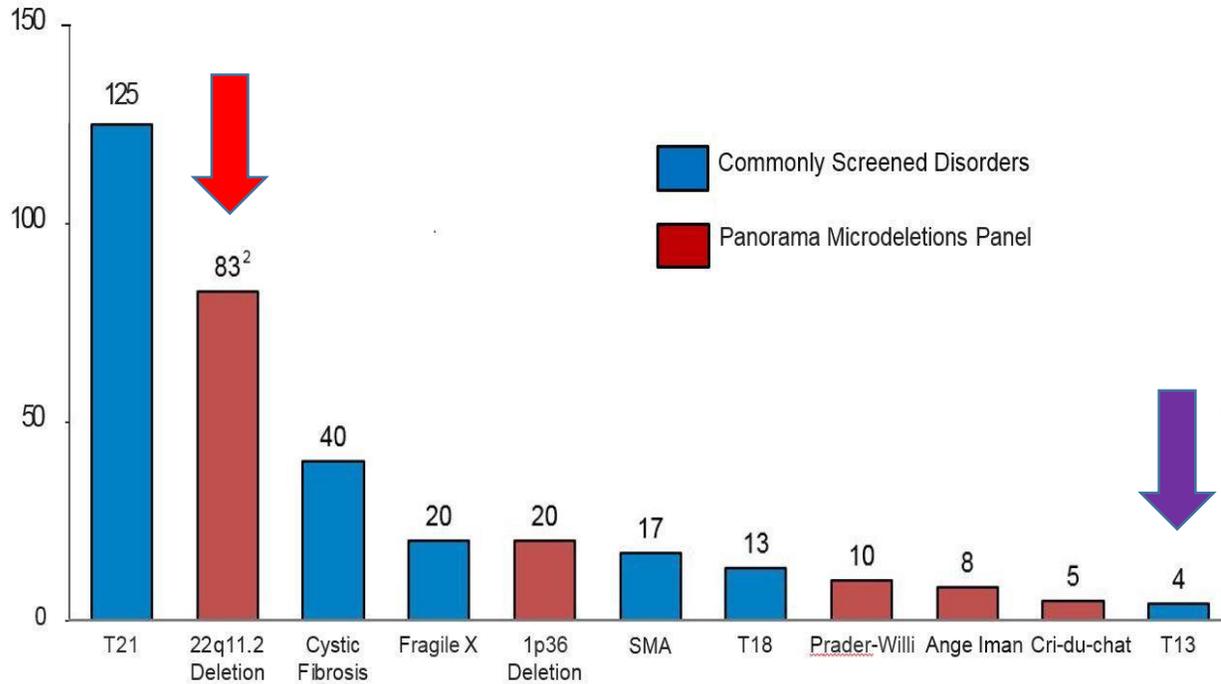
Mark D. Pertile,^{1,2*} Meredith Halks-Miller,^{3,4*} Nicola Flowers,¹ Catalin Barbacioru,⁴ Sarah L. Kinnings,³ Darcy Vavrek,³ William K. Seltzer,³ Diana W. Bianchi^{5,6†}

N=89,817



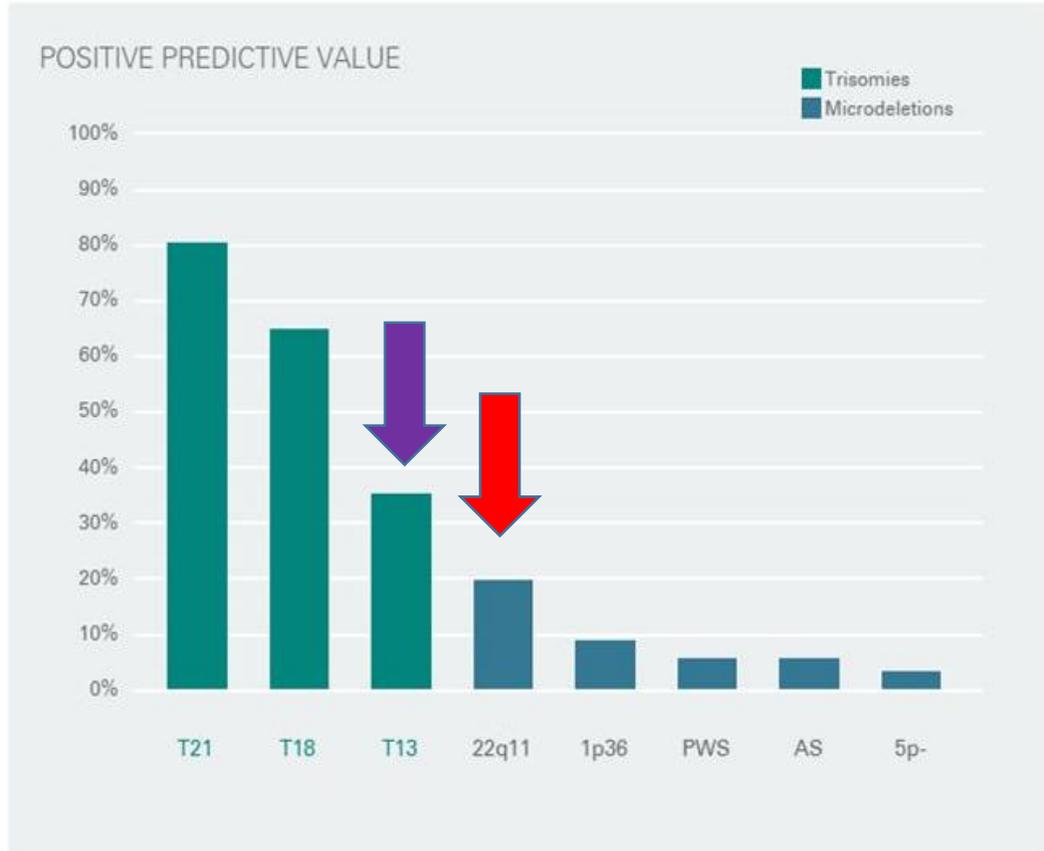
Microdeleciones???

Incidence out of 100,000 Births¹



1. Hall. Panorama™ Non-invasive Prenatal Screening for Microdeletion Syndromes. 2013.
2. Gross, et al. Clinical experience with single-nucleotide polymorphism-based non-invasive prenatal screening for 22q11.2 deletion syndrome. Ultrasound Ob Gyn, 2016.

POSITIVE PREDICTIVE VALUE (PPV) OF THEORETICAL GENETIC TEST WITH FALSE-POSITIVE RATE (FPR) OF 0.1%*



Modelos de reporte



Información de la Paciente

Apellido Paciente: Fecha de toma de muestra: 20.07.18
 Nombre Paciente: Edad materna: 31
 ID acceso: Médico referente:
 Gestación: Única Día de reporte: 6.08.18
 IMC (kg/m2): 19,34

RESULTADOS DEL ANÁLISIS

EDAD GESTACIONAL
14 sem

FRACCIÓN ADN FETAL
8.47 %

**ANEUPLOIDÍAS
NO
DETECTADAS**

HALLAZGOS INCIDENTALES

NO DETECTADOS

CRÓMOSOMA Y

DETECTADO

CRÓMOSOMAS ANALIZADOS SIN HALLAZGOS RELEVANTES

21, 18, 13, X, 7, 9, 14, 16, 16, 22

Resultados detallados

Condición testeada	% Riesgo a priori *	% Riesgo personalizado **	Resultado
Trisomía 21	0.17%	< 0.01%	NO DETECTADA
Trisomía 18	0.08%	< 0.01%	NO DETECTADA
Trisomía 13	0.08%	< 0.01%	NO DETECTADA
Monosomía X			NO DETECTADA
Otras Trisomías			NO DETECTADAS

(*) Riesgo a priori calculado según: Snijders RJL, Sundberg K, Holzgräbe W, Henry G, Nicolaides KH. Maternal age and gestation-specific risk for trisomy 21. Ultrasound Obstet Gynecol. 1999;13(3):167-70. APA Nicolaides, K. H. (2003). Screening for chromosomal defects. Ultrasound in Obstetrics & Gynecology, 21(6), 313-321. (**) Riesgo personalizado calculado según: Skkama-estdatz B, Johansson LF, De Boer EN, et al. NIFTYC: an online tool for clinical interpretation of non-invasive prenatal testing (NPT) results. Sci Rep. 2016;6:30059.



Información de la Paciente

Apellido Paciente: Fecha de toma de muestra: 14.07.18
 Nombre Paciente: Edad materna: 32
 ID acceso: Médico referente:
 Gestación: Única Día de reporte: 6.08.18
 IMC (kg/m2): 25

RESULTADOS DEL ANÁLISIS

EDAD GESTACIONAL
17 sem

FRACCIÓN ADN FETAL
8.32 %

**ANEUPLOIDÍAS
DETECTADA**

Trisomía 18

HALLAZGOS INCIDENTALES

NO DETECTADOS

CRÓMOSOMA Y

NO DETECTADO

CRÓMOSOMAS ANALIZADOS SIN HALLAZGOS RELEVANTES

21, 13, X, 7, 9, 14, 16, 16, 22

Resultados detallados

Condición testeada	% Riesgo a priori *	% Riesgo personalizado **	Resultado
Trisomía 21	< 0.01%	< 0.01%	NO DETECTADA
Trisomía 18	0.04%	> 99.99%	DETECTADA
Trisomía 13	0.04%	< 0.01%	NO DETECTADA
Monosomía X			NO DETECTADA
Otras Trisomías			NO DETECTADAS

(*) Riesgo a priori calculado según: Snijders RJL, Sundberg K, Holzgräbe W, Henry G, Nicolaides KH. Maternal age and gestation-specific risk for trisomy 21. Ultrasound Obstet Gynecol. 1999;13(3):167-70. APA Nicolaides, K. H. (2003). Screening for chromosomal defects. Ultrasound in Obstetrics & Gynecology, 21(6), 313-321. (**) Riesgo personalizado calculado según: Skkama-estdatz B, Johansson LF, De Boer EN, et al. NIFTYC: an online tool for clinical interpretation of non-invasive prenatal testing (NPT) results. Sci Rep. 2016;6:30059.



POSTNATAL



Objetivo

- Establecer las bases moleculares de la patología del paciente
- Brindar herramientas que puedan utilizarse para la prognosis, tratamiento y asesoramiento familiar
- Diferentes técnicas moleculares en función de la anomalía que se quiera identificar



Cariotipo
FISH



Array (BACs, oligos,
SNPs, de expresión,
etc)



PCR (distintos
tipos)



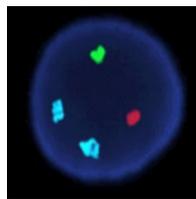
Secuenciador
Sanger



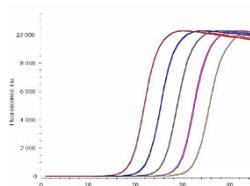
Next Generation
Sequencing



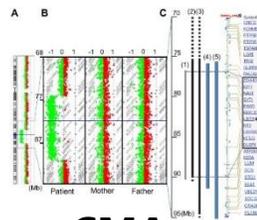
¿Cuál estudio es el mejor?



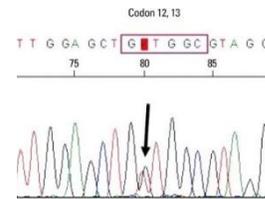
FISH



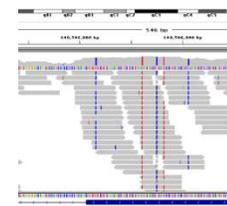
PCR



CMA

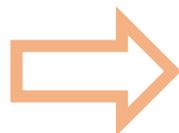


SANGER

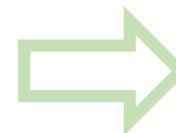


NGS

Evaluación clínica detallada:
Antecedentes familiares
Estudios previos
Sospecha clínica



Etiología del mecanismo molecular de sospecha



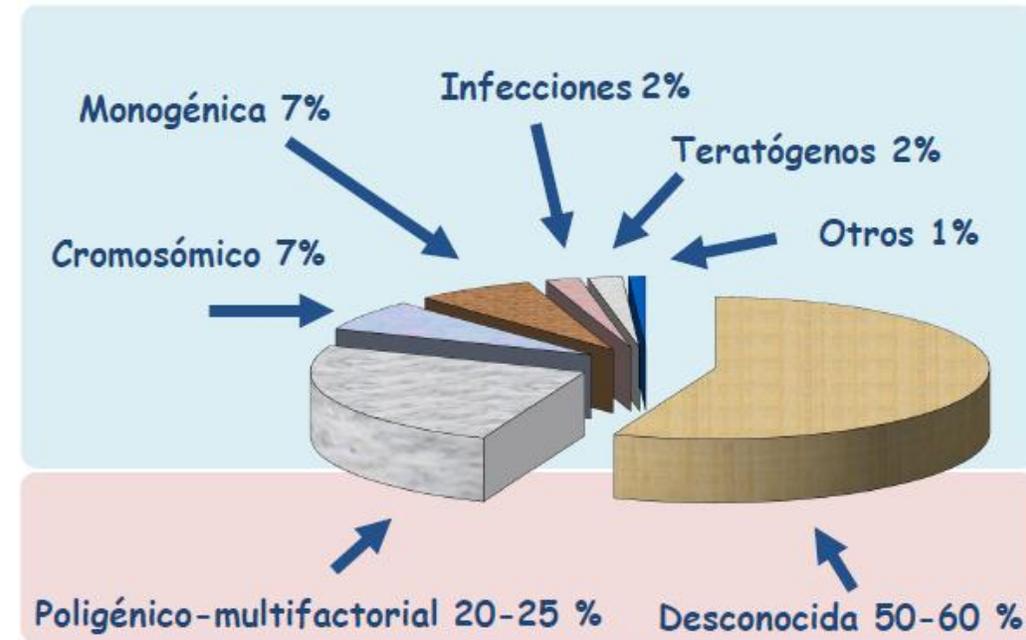
Técnica molecular más adecuada



Anomalías Congénitas

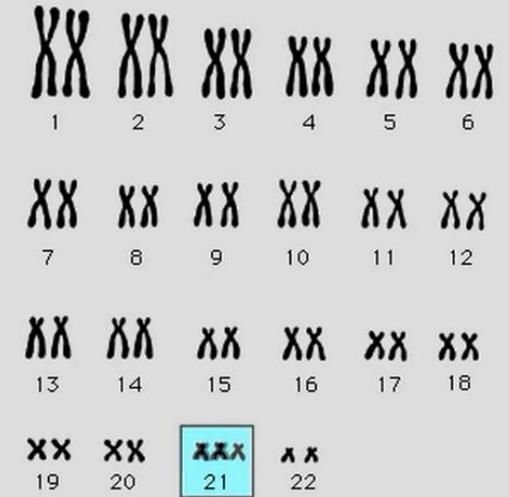
“Las anomalías congénitas se denominan también defectos de nacimiento, trastornos congénitos o malformaciones congénitas. Se trata de anomalías estructurales o funcionales, como los trastornos metabólicos, que ocurren durante la vida intrauterina y se detectan durante el embarazo, en el parto o en un momento posterior de la vida”

- Se manifiestan de forma prenatal (ej.: polidactilia) o al nacimiento (ej.: sordera congénita)
- Afectan un 2-3% de los RNV
- La etiología de las anomalías congénitas es en gran parte desconocida, siendo algunas de ellas de origen genético.

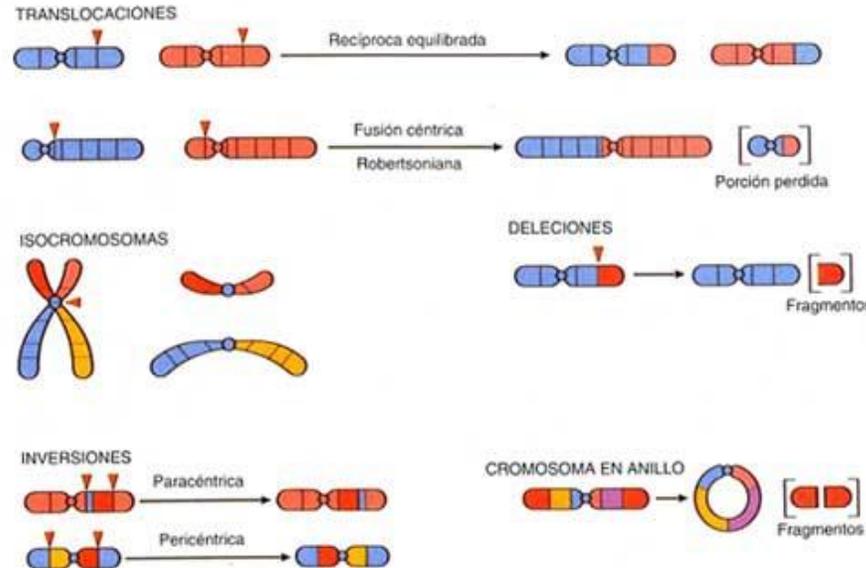
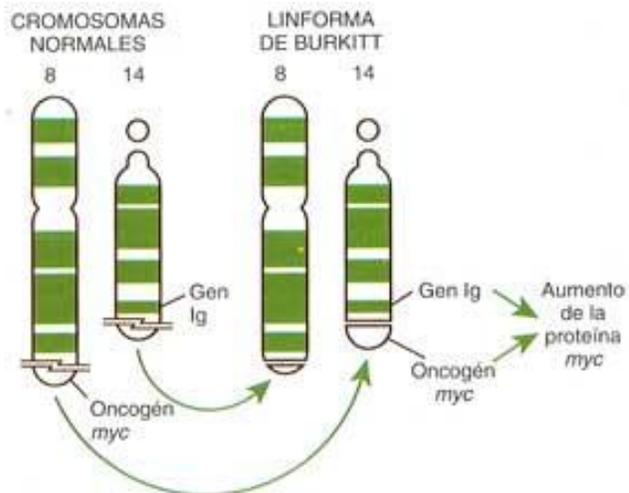


Anomalías Cromosómicas

NUMÉRICAS



ESTRUCTURALES



Síndromes de microdelección y microduplicación


Genetics and Molecular Biology, 37, 1 (suppl), 210-219 (2014)
 Copyright © 2014, Sociedade Brasileira de Genética. Printed in Brazil
 www.sbg.org.br

Review Article

New microdeletion and microduplication syndromes: A comprehensive review

Julián Nevado^{1,2*}, Raffaella Mergener^{3*}, María Palomares-Bralo^{1,2}, Karen Regina Souza³, Elena Vallespín^{1,2},
 Rocío Mena^{1,2}, Víctor Martínez-Glez^{1,2}, María Ángeles Mori^{1,2}, Fernando Santos^{1,4}, Sixto García-Miñaur^{1,4},
 Fé García-Santiago^{1,5}, Elena Mansilla^{1,5}, Luis Fernández^{1,6}, María Luisa de Torres^{1,5}, Mariluce Riegel^{3,7,8}
 and Pablo Laranzina^{1,4,8S}

+ de 300 síndromes!!!

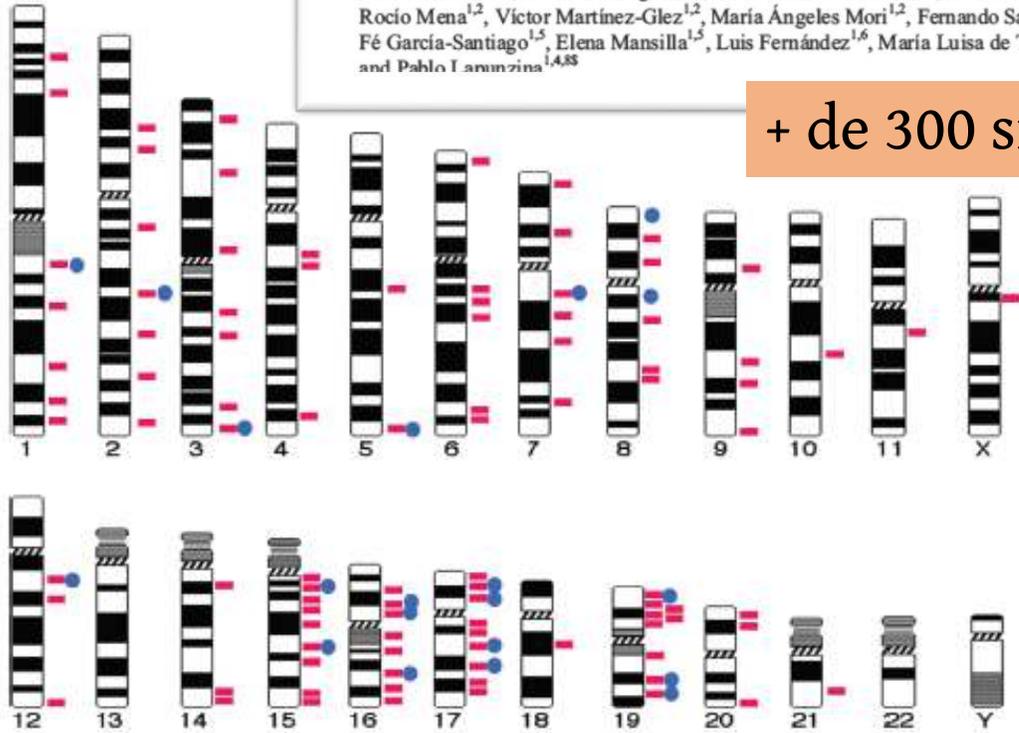
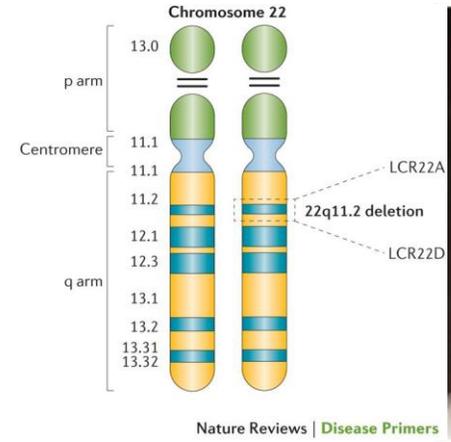
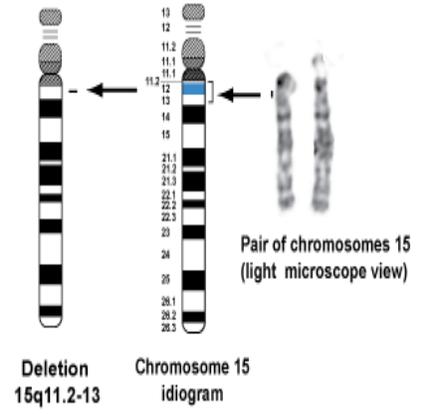


Figure 3 - New microdeletion and microduplication syndromes discovered over the last three to five years. Red squares indicate reported microdeletions and blue circles indicate reported microduplications.



Síndrome DiGeorge

Síndrome Angelman

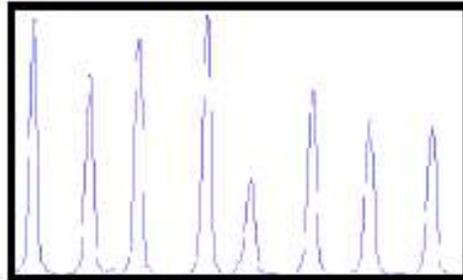


Deletion 15q11.2-13
Chromosome 15 idiogram

Métodos de detección de CNVs

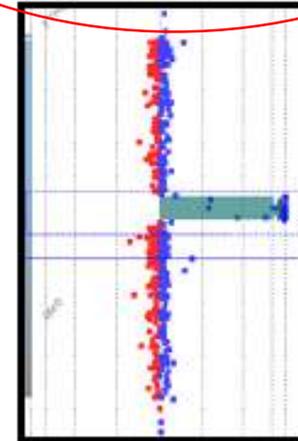
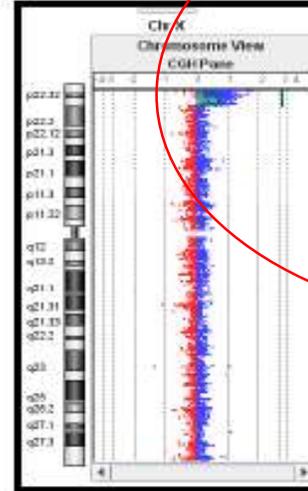
MLPA:

Resolución: 130-1 pb
Región investigada: 100 locus
(dos kits)
Tasa diagnóstica: 9-12%



Array:

Resolución: >200 Kb
Región investigada: todo el genoma
Tasa diagnóstica: 15-20%



Citogenética:

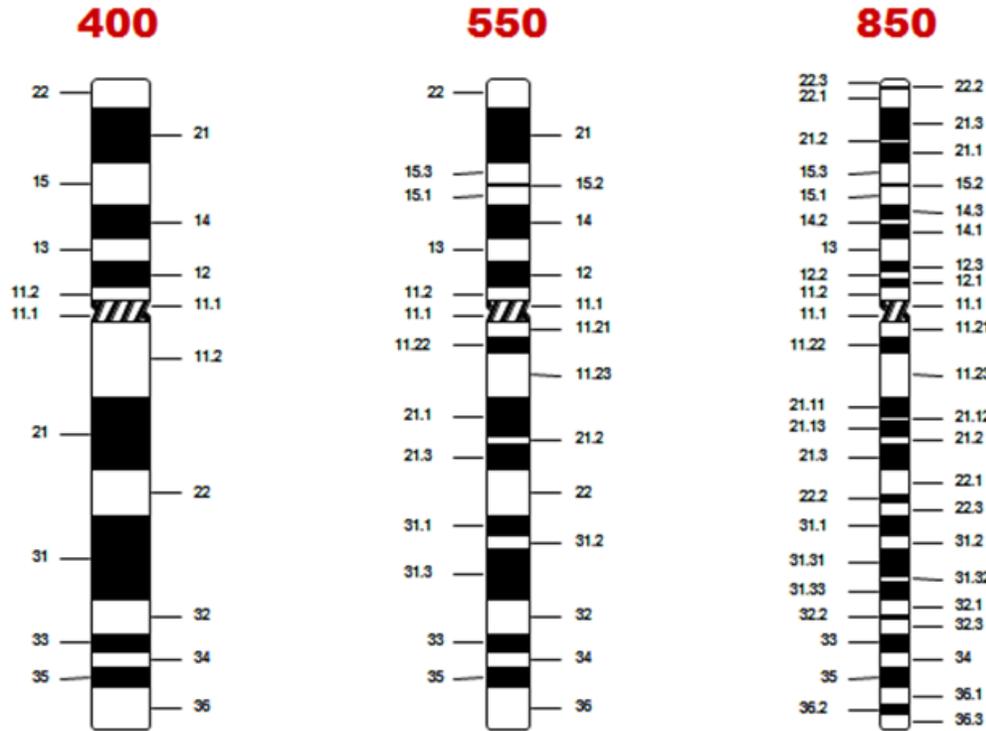
Resolución: >6-10 Mb
Región investigada: todo el genoma
Tasa diagnóstica: 3%

Array con cobertura exónica 500 genes:

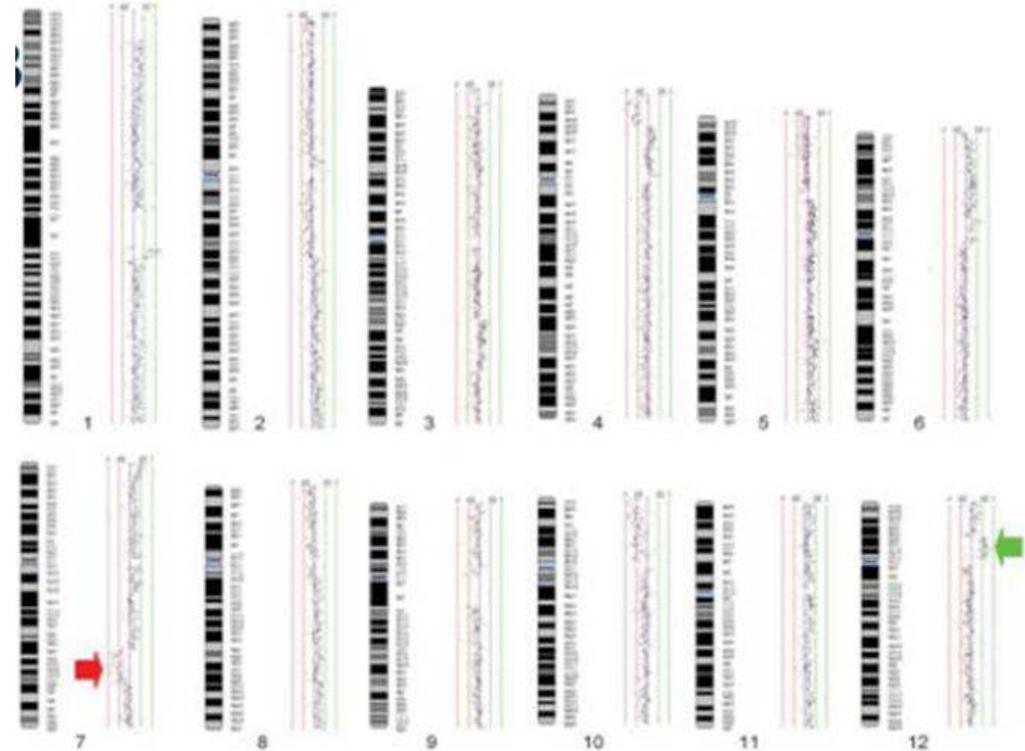
Resolución: >200 Kb- exónica
Región investigada: todo el genoma
Tasa diagnóstica: >20%?



Cariotipo vs CMA



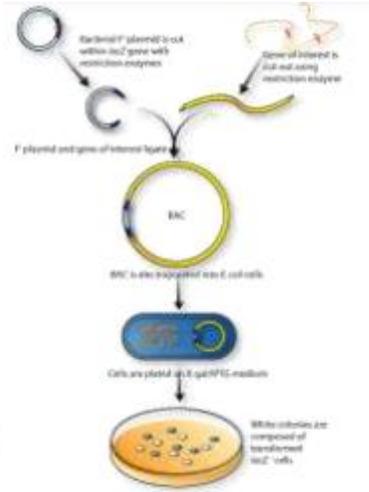
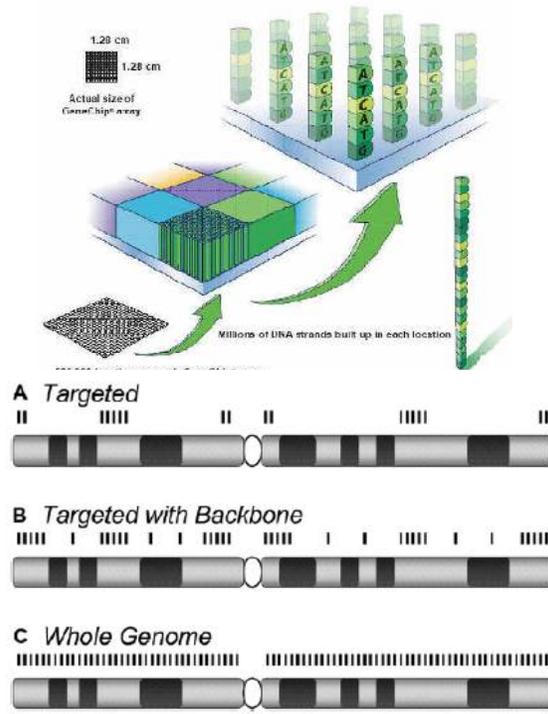
Resolución 3-10Mb



Resolución 150Kb en backbone
25Kb en ROI

Características de los arrays

TIPOS



UTILIZADOS EN



ORIGINAL

Array CGH como primera opción en el diagnóstico genético: 1.000 casos y análisis de coste-beneficio[☆]



Neus Castells-Sarret^{a,b,*}, Anna M. Cueto-González^{a,c}, Mar Borregan^c, Fermina López-Grondona^a, Rosa Miró^b, Eduardo Tizzano^{a,d} y Alberto Plaja^{a,b}

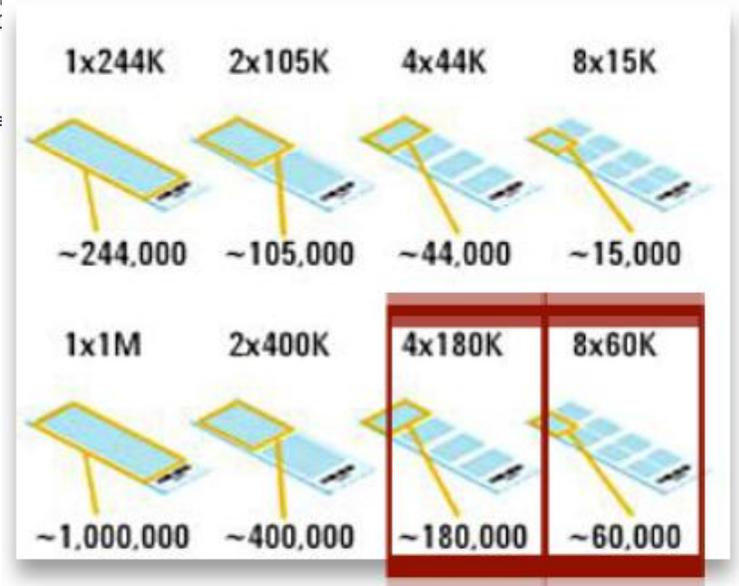
^a Área de Genética Clínica i Molecular, Hospital Vall d'Hebron, Barcelona, España

^b Departament de Biologia Cel·lular, Fisiologia i Bioquímica, Universitat de Barcelona, Barcelona, España

^c Facultat de Medicina, Departament de Ciències Bàsiques, Universitat de Barcelona, Barcelona, España

^d CIBERER, Barcelona, España

Recibido el 5 de abril de 2017; aceptado el 12 de mayo de 2017



FORMATO Y RESOLUCIÓN

CMA...En postnatal

ARTICLE

Consensus Statement: Chromosomal Microarray
Is a First-Tier Clinical Diagnostic Test for Individuals
with Developmental Disabilities or Congenital Anomalies

David T. Miller,^{1,*} Margaret P. Adam,^{2,3} Swaroop Aradhya,⁴ Leslie G. Biesecker,⁵ Arthur R. Brothman,⁶
Nigel P. Carter,⁷ Deanna M. Church,⁸ John A. Crolla,⁹ Evan E. Eichler,¹⁰ Charles J. Epstein,¹¹

The American Journal of Human Genetics 86, 749–764, May 14, 2010

33 estudios
N=21,698 pacientes

RENDIMIENTO DIAGNÓSTICO: 15-20%



FIRST-TIER

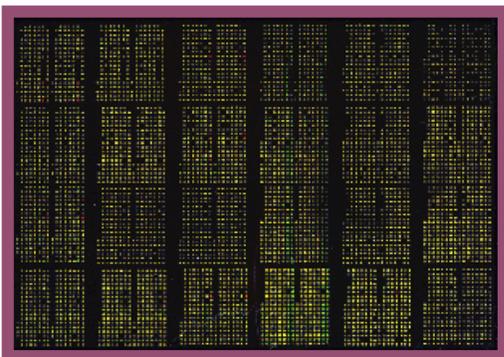
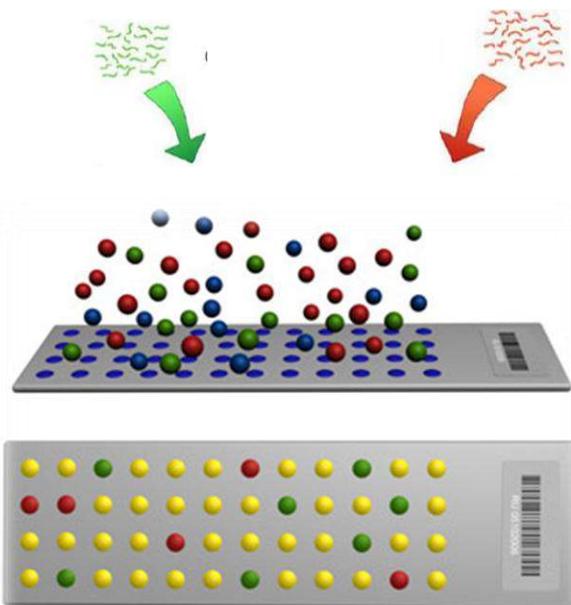
- Discapacidad intelectual/Retraso global del desarrollo
- Trastorno espectro autista
- Malformaciones congénitas
- Talla baja
- Epilepsia

REFLEX

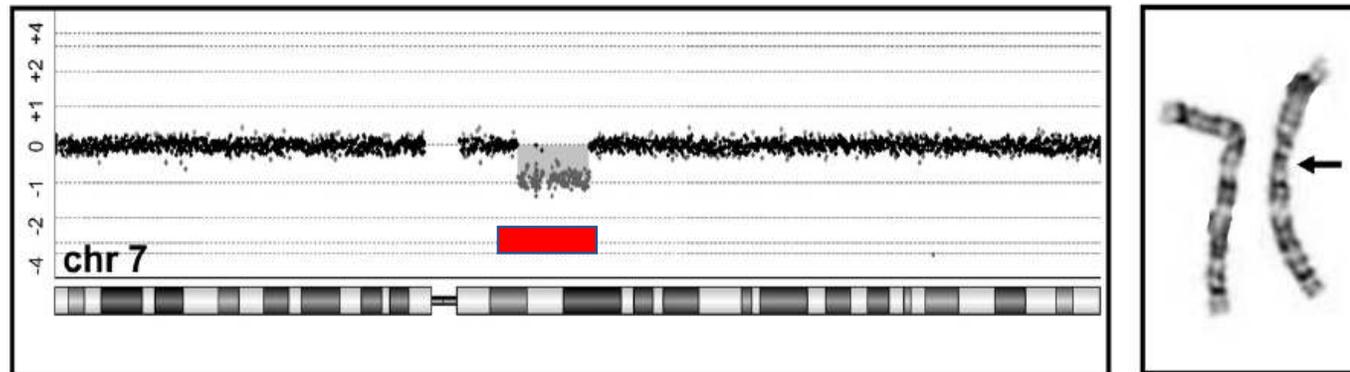
- Cromosoma marcador
- Traslocación balanceada
- Cariotipo alterado para definir región involucrada

chromo

- HÉRITAS MICROARRAY CROMOSÓMICO -



CGH array: Hibridación Genómica Comparativa



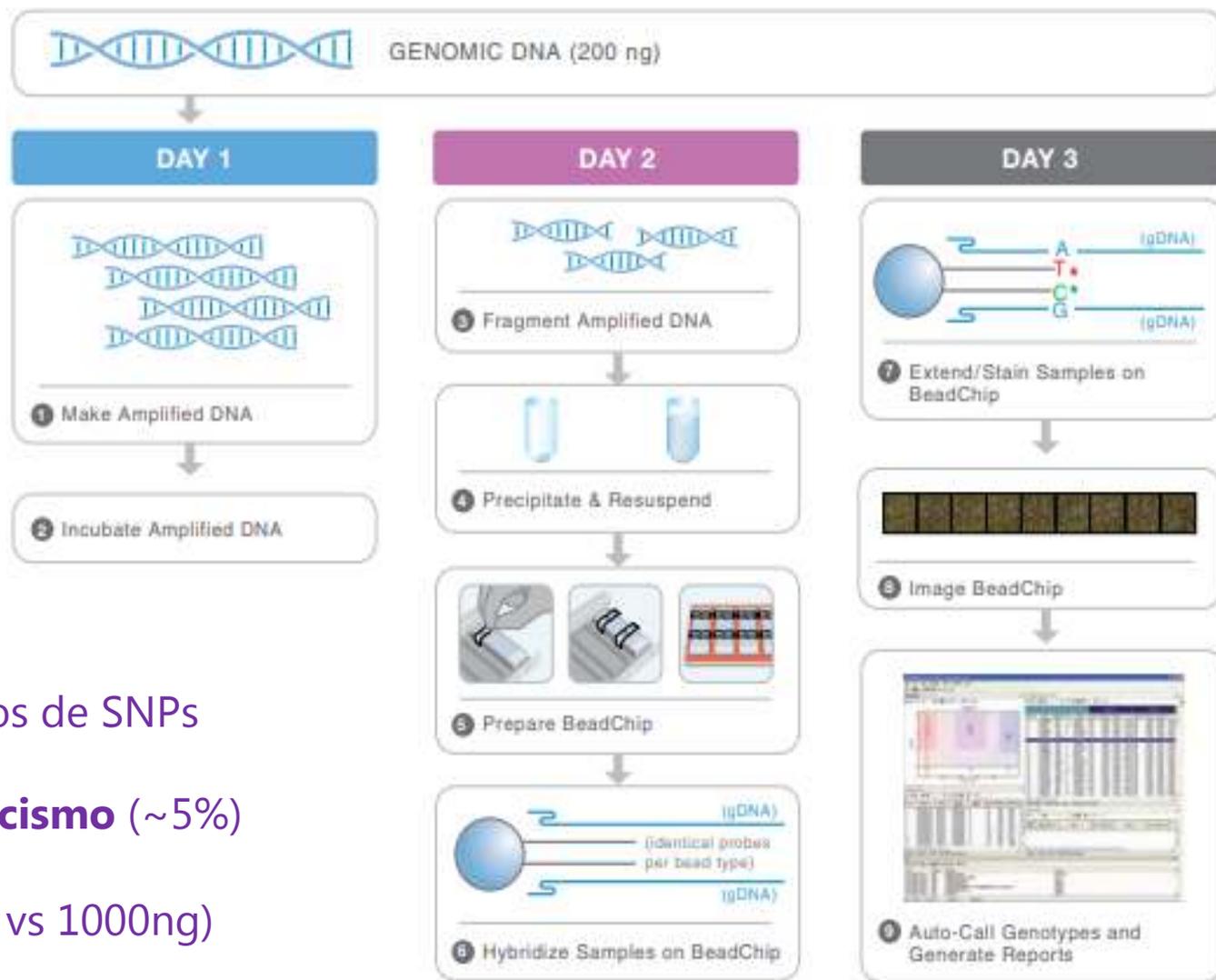
Síndrome de Williams

- ✓ Hibridación comparativa: Muestra vs referencia
- ✓ Se analiza **ratio** entre muestra y referencia → **n° de copias**
- ✓ **Regiones ISCA** con resolución de entre 20-50Kb
- ✓ Sondas de oligos y SNPs (180K), sondas de oligos (60K)
- ✓ Mosaicos > **20%**
- ✓ UPD mayores a **10Mb**



Nextseq 550

CytoScan: Array de SNPs



- ✓ **Muestra vs matriz** de polimorfismos de SNPs
- ✓ Paso de amplificación
- ✓ Detección de **bajo grado de mosaicismo** (~5%)
- ✓ DUP mayores a ~**3Mb**
- ✓ **Input de ADN es más bajo** (200ng vs 1000ng)
- ✓ Análisis con BlueFuse

* Indicates Stain in Red Channel
* Indicates Stain in Green Channel

Clasificación & Interpretación

ACMG PRACTICE GUIDELINES

American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants

Hutton M. Kearney, PhD¹, Erik C. Thorland, PhD², Kerry K. Brown, PhD³,
Fabiola Quintero-Rivera, MD⁴, and Sarah T. South, PhD⁵, A Working Group of the American College of
Medical Genetics (ACMG) Laboratory Quality Assurance Committee

Disclaimer: These ACMG Standards and Guidelines are developed primarily as an educational resource for clinical

RECOMMENDATIONS FOR SYSTEMATIC EVALUATION AND CLINICAL INTERPRETATION OF CNVs

1. **Familiarization with well-established contiguous gene syndromes**
2. **Consideration of CNV size**
3. **Consideration of genomic content in CNV interval**
 - Genes with reported pathogenic mutations in the medical literature*
 - Genes with no reported mutations in the medical*
 - No genes in interval*
4. **Comparison of CNV with internal and external Databases:**
 - Dosage of copy number imbalance reported in the general population studies.*
 - Size of the reported benign CNV relative to the CNV in question.*
 - Sex of individual in database relative to patient sex.*
 - Validity of the CNV reported in general population databases.*
 - Clinical characterization of "normal" individuals*

BENIGNA

PROBABLEMENTE
BENIGNA

VOUS

PROBABLEMENTE
PATOGENICA

PATOGENICA

Database of Genomic Variants

A curated catalogue of human genomic structural variation

About the Project Downloads Links Statistics FAQ
Genome Browser Query Tool Submissions Contact Us Training Resources

**DB resultados de estudios de individuos
controles en un formato normalizado**

OMIM®

Online Mendelian Inheritance in Man®

An Online Catalog of Human Genes and Genetic Disorders

Updated October 17, 2018

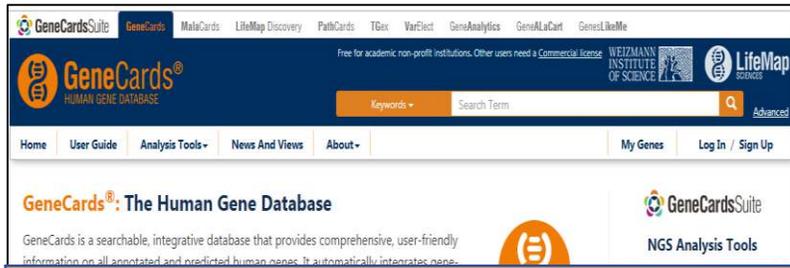
Catálogo de genes y enfermedades



Genome Browser

Jump to position, gene or band

DB de CNVs con fenotipo asociado



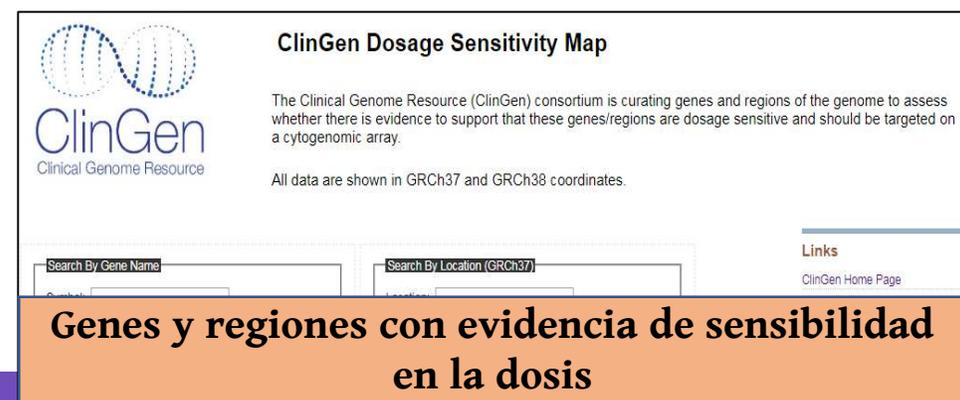
Info de cada gen de + de 100 WEBS



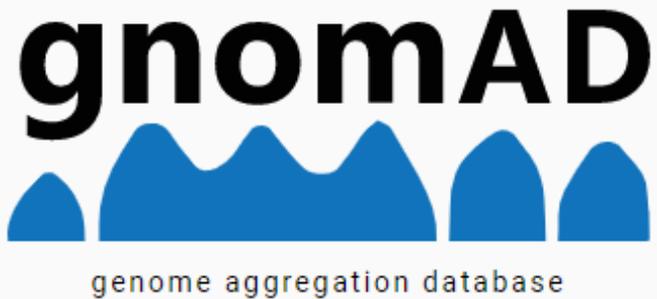
**Revisiones expertas de enfermedades en un formato
estandarizado y con un enfoque muy clínico y aplicado**



Smes conocidos/ familiares y profesionales



**Genes y regiones con evidencia de sensibilidad
en la dosis**



gnomAD v2.1.1 ▾ Search by gene, region, or variant

Please note that gnomAD v2.1.1 and v3 contain largely non-overlapping samples and both datasets must be used to capture the full set of variation across gnomAD. For more information, see the FAQ ["Should I switch to the latest version of gnomAD?"](#)

Examples - Gene: [PCSK9](#), Variant: [1-55516888-G-GA](#)

Structural variants

- gnomAD SVs v2.1: Structural variant (SV) calls generated from a set of WGS samples that largely overlaps those in gnomAD v2.1. This current SV release includes **10,847 unrelated genomes**.
- gnomAD SVs v2.1 (controls): Only samples from individuals who were not selected as a case in a case/control study of common disease. **5,192 genomes**.
- gnomAD SVs v2.1 (non-neuro): Only samples from individuals who were not ascertained for having a neurological condition in a neurological case/control study. **8,342 genomes**.

Update 2019: ACMG standards for CNVs!!



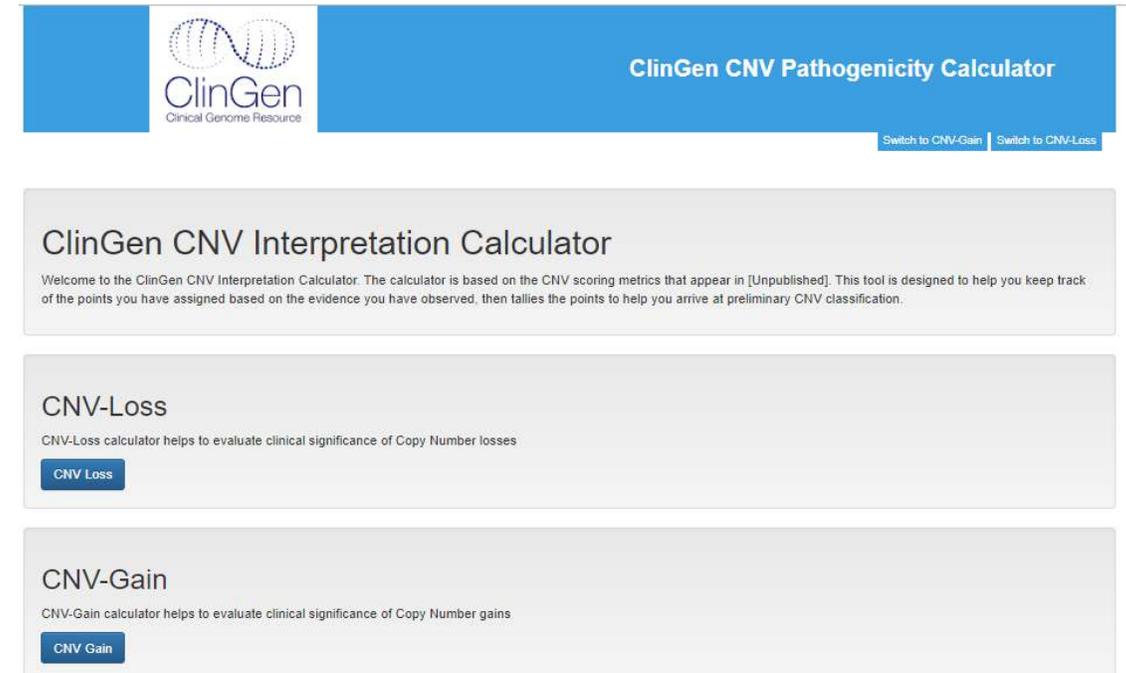
Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen)

Erin Rooney Riggs, MS, CGC¹, Erica F. Andersen, PhD^{2,3}, Athena M. Cherry, PhD⁴, Sibel Kantarci, PhD⁵, Hutton Kearney, PhD⁶, Ankita Patel, PhD⁷, Gordana Raca, MD, PhD⁸, Deborah I. Ritter, PhD⁹, Sarah T. South, PhD¹⁰, Erik C. Thorland, PhD⁵, Daniel Pineda-Alvarez, MD¹¹, Swaroop Aradhya, PhD^{4,11} and Christa Lese Martin, PhD¹

Disclaimer: This technical standard is designed primarily as an educational resource for clinical laboratory geneticists to help them provide quality clinical laboratory genetic services. Adherence to this standard is voluntary and does not necessarily assure a successful medical outcome. This standard should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the clinical laboratory geneticist should apply his or her own professional judgment to the specific

Riggs, E.R., Andersen, E.F., Cherry, A.M. *et al.* *Genet Med* (2019)

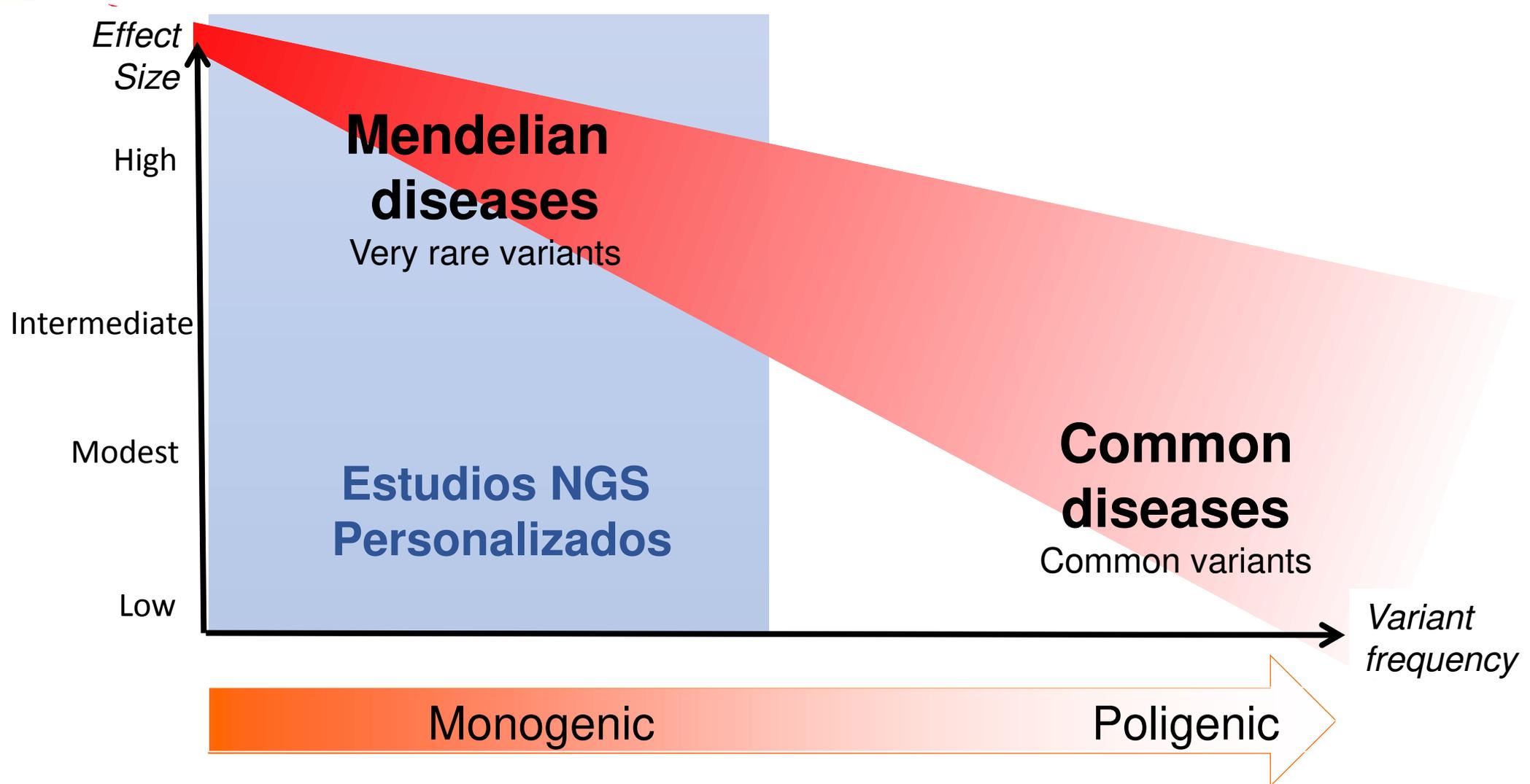
- ❖ Quantitative, evidence-based scoring framework
- ❖ Five-tier classification system
- ❖ “Uncoupling” the evidence-based classification its potential implications for a particular individual.



The screenshot shows the ClinGen CNV Pathogenicity Calculator interface. At the top, there is a blue header with the ClinGen logo and the text 'ClinGen CNV Pathogenicity Calculator'. Below the header, there are two buttons: 'Switch to CNV-Gain' and 'Switch to CNV-Loss'. The main content area is titled 'ClinGen CNV Interpretation Calculator' and includes a welcome message. Below this, there are two sections: 'CNV-Loss' and 'CNV-Gain'. Each section has a sub-header and a brief description of the calculator's purpose, followed by a button labeled 'CNV Loss' or 'CNV Gain'.

<http://cnvcalc.clinicalgenome.org/cnvcalc/>

Anomalías Monogénicas



OPEN ACCESS

Edited by:
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INSERM U1127 Institut du Cerveau et
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Alvina Jordanova,
University of Antwerp, Belgium
Caroline News,
Institut National de la Santé et de la

Diagnostic Yield and Treatment Impact of Targeted Exome Sequencing in Early-Onset Epilepsy

Michelle Damos^{1*}, Ilaria Guella^{2*}, Conrado DeGuzman¹, Marna B. McKenzie², Sarah E. Buerki^{1,3}, Daniel M. Evans², Eric B. Toyota¹, Cyrus Bootman¹, Linda L. Huh¹, Anita Datta¹, Aspasia Michoulas¹, Kathryn Selby¹, Bruce H. Bjornson¹, Gabriella Horvath⁴, Elena Lopez-Rangel², Clara D. M. van Kameboek^{4,5}, Ramona Salvarinova⁶, Erin Slado¹, Patrice Eydoux^{6,7}, Shaïm Adam⁸, Margot I. Van Allen⁹, Tanya N. Nelson^{6,8}, Cornelia Bolbocean^{11,12}, Mary B. Connolly¹ and Matthew J. Farrer²

¹Division of Neurology, Department of Pediatrics, University of British Columbia and BC Children's Hospital, Vancouver, BC, Canada; ²Department of Medical Genetics, Centre for Applied Neurogenetics (CAN), University of British Columbia, Vancouver, BC, Canada; ³Division of Neuropediatrics, University Children's Hospital Zurich, Zurich, Switzerland; ⁴Division of Neurology, Department of Pediatrics, BC Children's Hospital, Vancouver, BC, Canada; ⁵Department of Pediatrics, BC Children's Hospital, Vancouver, BC, Canada; ⁶INSERM U1127 Institut du Cerveau et de la Moelle épinière, France; ⁷Université Claude Bernard Lyon 1, France; ⁸University of Antwerp, Belgium; ⁹Caroline News, Institut National de la Santé et de la

Clinical whole-exome sequencing for the diagnosis of rare disorders with congenital anomalies and/or intellectual disability: substantial interest of prospective annual reanalysis

Sophie Nambot, MD^{1,2,3,4}, Julien Thevenon, MD, PhD^{1,3,4}, Paul Kuentz, MD, PhD^{2,3,4}, Yannis Duffourd, MSc^{3,4}, Emilie Tisserant, PhD^{3,4}, Ange-Line Bruel, PhD^{3,4}, Anne-Laure Mosca-Boidron, MD^{2,3,4}, Alice Masurel-Paulet, MD^{1,3}, Daphné Lehalle, MD¹, Nolwenn Jean-Marçais, MD^{1,3}, Mathilde Lefebvre, MD^{1,2}, Pierre Vabres, MD, PhD^{3,4}, Salima El Chehadeh-Djebbar, MD¹, Christophe Philippe, MD, PhD^{2,4}, Frederic Tran Mau-Them, MD^{2,4}, Judith St-Onge, DEC^{2,4}, Thibaud Jouan, BSc^{2,3,4}, Martin Chevarin, HNC^{2,3,4}, Charlotte Poé, BSc^{2,3,4}, Virginie Carmignac, PhD⁴, Antonio Vitobello, PhD^{2,3,4}, Patrick Callier, MD, PhD^{2,3,4}, Jean-Baptiste Rivière, PhD^{2,3,4}, Laurence Faivre, MD, PhD^{1,3,4}, Christel Thauvin-Robinet, MD, PhD^{1,2,3,4} and Orphanomix Physicians' Group⁸

European Journal of Human Genetics (2018) 26:644–651
<https://doi.org/10.1038/s41431-018-0099-1>



ARTICLE



Exome sequencing has higher diagnostic yield compared to simulated disease-specific panels in children with suspected monogenic disorders

Oliver James Dillon^{1,2} · Sebastian Lunke¹ · Zornitza Stark^{1,2} · Allison Young¹ · Natalie Thorne³ · Melbourne Genomics Health Alliance · Clara Gall^{4,5} · Susan M. White^{1,2} · Tiong Yang Tan^{1,2}

Received: 1 September 2017 / Revised: 11 December 2017 / Accepted: 11 January 2018 / Published online: 16 February 2018
© European Society of Human Genetics 2018

Abstract

As test costs decline, whole-exome sequencing (WES) has become increasingly used for clinical diagnosis, and now represents the primary alternative to gene-panel testing for patients with a suspected genetic disorder. We sought to compare the diagnostic yield of singleton-WES with simulated application of commercial gene panels in children suspected of having



Caso Índice



Estudios en trío



Frequency of disorder

Rare
($<1/10.000$)
Charge Syndrome



1 gene

Low frequency
($1/10.000-1/100$)
Noonan Syndrome



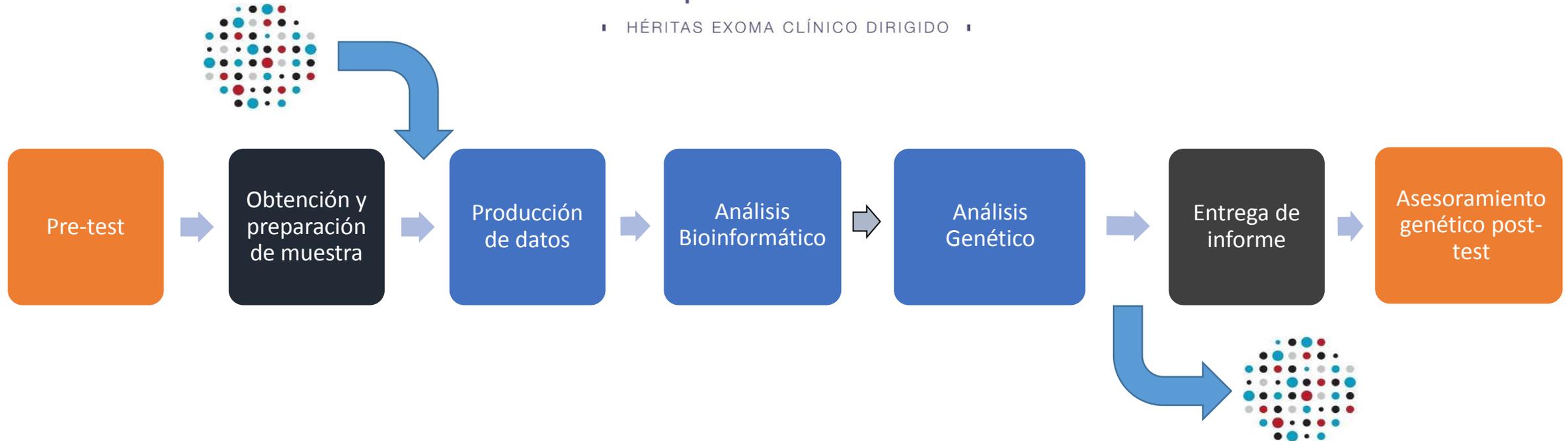
2-100 genes

Common
($>1/100$)
Intellectual disability

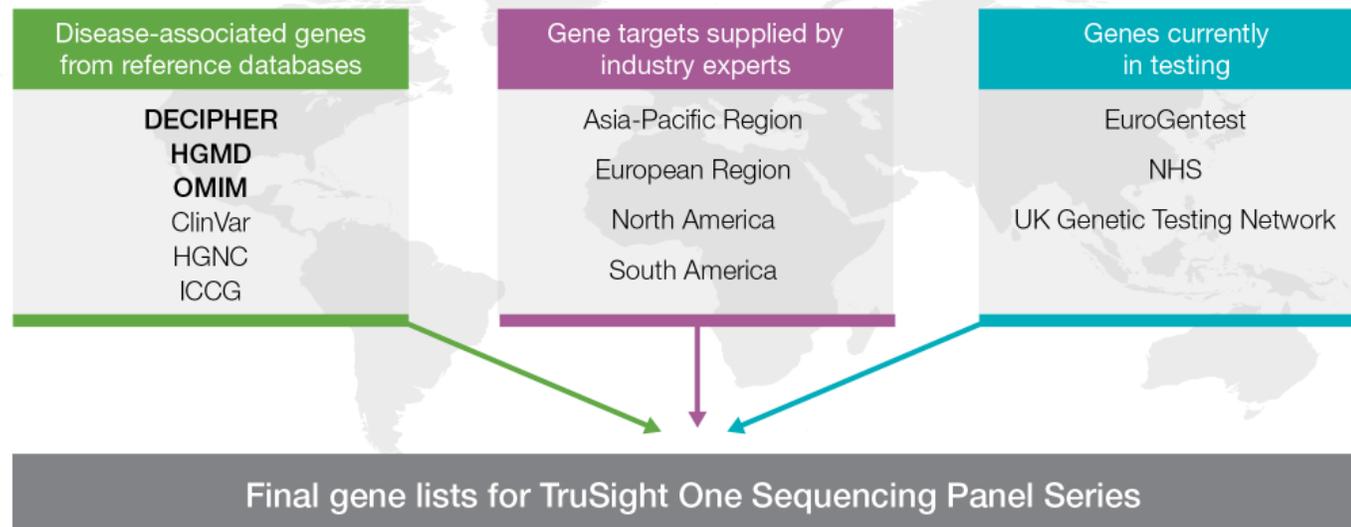


>100 genes


fOCUS
HÉRITAS EXOMA CLÍNICO DIRIGIDO



The TruSight One Expanded Sequencing Panel



Exoma clínico

Solo aquellos genes relevantes (o potencialmente) clínicamente

**6704 genes
(86.000 exones)**

Table 1: TruSight One Sequencing Panel Specifications

Parameter	TruSight One Expanded
Cumulative Target Region Size	16.5 Mb
No. of Target Genes	6704
No. of Target Exons	~86,000
Probe Size	80-mer
No. of Probes	183,809
Minimum Coverage ^a	> 20
Average Coverage	> 100

a. 95% of target regions typically covered at > 20 (higher percent coverage possible with fewer samples per run)

ANÁLISIS BIOINFORMÁTICO



- ✓ Bioinformáticos / Científico de Datos
- ✓ Pipeline bioinformático propietario Héritas basado en GATK (*Broad Institute*)

Interpretación de variantes



✓ **OMIM** ($\cong 4000$ genes, > 6200 fenotipos genes)

✓ **Databases ExAc / GnomAD**

Estandarización de diversos datasets de proyectos de secuenciación NGS existentes

✓ **Clasificación de variantes (ACMG)**



- ✓ **Bening**
- ✓ **Likely-bening**
- ✓ **Variant of unknown significance**
- ✓ **Likely-patogenic**
- ✓ **Pathogenic**

	← Strong	Benign	Supporting	Supporting	Moderate	Pathogenic	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 In-frame indels in repeat w/out known function BP3	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 effect BP6		Missense in gene with low rate of benign variants PP2	Mutational hot spot or well-studied functional domain without benign variation PM1		Well-established functional studies show a deleterious effect PS3	
Segregation data		Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data			
De novo data					De novo (without paternity & maternity confirmed) PM6		De novo (paternity and maternity confirmed) PS2	
Allelic data			Observed in <i>trans</i> with a dominant variant BP2 Observed in <i>cis</i> with a pathogenic variant BP2		For recessive disorders, detected in <i>trans</i> with a pathogenic variant PM3			
Case data			Reputable source w/out shared data = benign BP6					
Other data			Found in case with an alternate cause BP5					Patient's phenotype or FH highly specific for gene PP4

Programas de Controles externos para validación de estudios NGS





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