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Centro de Referencia Perinatal Oriente

Facultad de Medicina, Universidad de Chile

Uso y rendimiento de NGS en diagnóstico prenatal

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Introducción

Anomalías congénitas: 3% de ecografías

Cariotipo, QF-PCR y CMA identifican 40% de anomalías con origen genético

Sin embargo, 70 - 80% de fetos con cariotipo normal tienen CMA normal (Hilman, 2013) -> ¿enfermedades monogénicas?

Shaffer LG et al. Detection rates of clinically significant genomic alterations by microarray analysis for specific anomalies detected by ultrasound. Prenat Diagn. 2012;32:986–95.

Hillman SC et al. Use of prenatal chromosomal microarray: prospective cohort study and systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2013;41:610–20.

Método de elección para enfermedades monogénicas es secuenciación masiva en paralelo:

- Paneles (dirigidos a motivo de indicación)
- Secuenciación de exoma (clínico o completo)
- Secuenciación de genoma completo

Indicaciones

- Anormalidad(es) ecográficas sugerentes de origen monogénico (al menos 20% de rendimiento esperado*)
- Anomalías múltiples con cariograma/CMA negativos
- Agregación familiar de fenotipo (feto previo con anomalías o hijo previo afectado)

* Se pueden considerar con rendimientos entre 10 y 20%

Abulí, A. et al. (2024). Guidelines for NGS procedures applied to prenatal diagnosis by the Spanish Society of Gynecology and Obstetrics and the Spanish Association of Prenatal Diagnosis. *Journal of medical genetics*, *61*(8), 727–733.

Table 1 Clinical indications and estimated diagnostic yield for prenatal NGS (ordered from higher to lower diagnostic yield)

Clinical indication	Diagnostic yield of NGS (%)	Reference
Bilateral hyperechogenic, dysplastic or polycystic kidneys	64	22
Skeletal dysplasia	53	23
Recurrent anomaly	40	24
Fetal akinesia deformation sequence	37	23
Craniosynostosis	38	22
Multiple anomalies involving various systems	33	25
Central nervous system anomalies (except single anomalies)	32	26
Non-isolated nuchal translucency	26	23
Non-immune hydrops fetalis	22	23

NGS, next-generation sequencing.

Table 2 Anomalies with a diagnostic yield of >10% may also be considered

Clinical indication	Diagnostic yield of NGS (%)	Reference
Single CNS anomaly	16	26
Severe early onset fetal growth restriction	12	27
Isolated heart defect	11	23

CNS, central nervous system; NGS, next-generation sequencing.

Abulí, A. et al. (2024). Guidelines for NGS procedures applied to prenatal diagnosis by the Spanish Society of Gynecology and Obstetrics and the Spanish Association of Prenatal Diagnosis. *Journal of medical genetics*, *61*(8), 727–733.

Clinical guidelines
Position statement

Guidelines for NGS procedures applied to prenatal diagnosis by the Spanish Society of Gynecology and Obstetrics and the Spanish Association of Prenatal Diagnosis

Anna Abulí [1, 2], Eugenia Antolín [3], Antoni Borrell [4], Maria Garcia-Hoyos [5], Fe García Santiago [6], Irene Gómez Manjón [7], Nerea Maíz [8, 9], Cristina González González [10], Laia Rodríguez-Revenga [11, 12], Irene Valenzuena Palafoll [1], Javier Suela [13]

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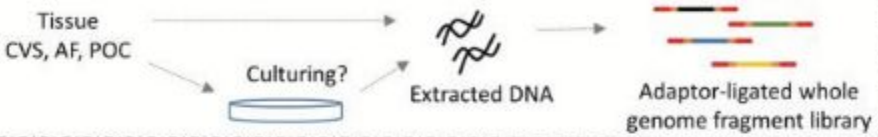
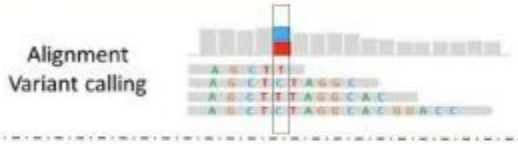
Although we have provided explanations for different NGS diagnostic approaches (panels, ES, WGS), we strongly recommend the use of ES in prenatal diagnosis for the reasons previously detailed. Therefore, all considerations regarding clinical considerations, reported findings and genetic counselling will be based on the use of ES.

Trio ES (parents and fetus) is recommended in prenatal diagnosis because it allows for filtering out many non-informative variants and it speeds up response time.[18 19]

Recomendaciones actuales: preferir exoma completo por sobre otras alternativas (idealmente exoma trío)

Consideraciones técnicas

Workflow Step	Consideration
1. Sample processing and library preparation 	**DNA quality and Quantity** **Turnaround Time** **Maternal Cell Contamination**
2. Sequencing and primary bioinformatics analysis 	**Variant type: SNVs and indels** **Allele fractions: CPM, MCC, twins effects**
3. Variant filtration  General population Disease databases Mode of inheritance Phenotype?	**Lack of prenatal phenotyping** **Absence of genotype-phenotype information** **"*De novo*" filters and MCC**
4. Interpretation and reporting  Allele frequency Gene-disease association Functional and segregation data Clinical information	**Lack of prenatal phenotyping** **Absence of genotype-phenotype information** **Variants of Uncertain Clinical Significance** **Primary versus secondary findings** **Implications to other family members**
5. Counseling  Pregnancy outcomes Intervention options Uncertainties	**Penetrance** **Age of onset** **Ethical issues**

Método en general es similar a postnatal, pero con algunas consideraciones

Abou Tayoun, A. N., Spinner, N. B., Rehm, H. L., Green, R. C., & Bianchi, D. W. (2018). Prenatal DNA Sequencing: Clinical, Counseling, and Diagnostic Laboratory Considerations. *Prenatal diagnosis*, *38*(1), 26–32. https://doi.org/10.1002/pd.5038

Preparación de librería

Similar a cualquier muestra postnatal si la calidad es adecuada

- Fragmentación de ADN genómico (ultrasonicación o procesos enzimáticos), luego ligación de adaptadores

Kits hasta 50 ng de ADN input

NGS en muestra de líquido amniótico

Líquido amniótico contiene poblaciones heterogéneas de células derivadas del feto

A diferencia de BVC, tiene menor tasa de contaminación materna y no tiene mosaicismo placentario confinado

Abou Tayoun, A. et al. (2018). Prenatal DNA Sequencing: Clinical, Counseling, and Diagnostic Laboratory Considerations. *Prenatal diagnosis*, *38*(1), 26–32.

NGS en muestra de líquido amniótico

Contaminación por ADN materno estimada en 0.3 - 0.7% del total de muestras

Se cree que provendría de células de piel materna

- Se sugiere descartar primeros mL de muestra

Cultivo podría probabilidad de contaminación (sin embargo, no se hace de rutina)

Nagan N, Faulkner NE, Curtis C, et al. Laboratory guidelines for detection, interpretation, and reporting of maternal cell contamination in prenatal analyses a report of the Association for molecular pathology. J Mol Diagn 2011;13:7–11.

Mosaicismo en BVC

- Mosaicismo en 1 - 2.5% de BVC por muestra materna
- Cultivar BVC no mejora outcomes y aumenta tiempo de proceso
- Disección cuidadosa de BVC mejora resultados
  - De todas maneras, debiera evaluarse con SNPs o STRs entre sangre materna y muestra
- En caso de detectarse, se recomienda estudio en líquido amniótico

Abou Tayoun, A. et al. (2018). Prenatal DNA Sequencing: Clinical, Counseling, and Diagnostic Laboratory Considerations. *Prenatal diagnosis*, *38*(1), 26–32.

Otras limitaciones

NGS detecta indels hasta 25 - 50 bp de manera confiable

- No confiable clínicamente para CNVs a nivel de exón, expansión de repeticiones o rearreglos estructurales
- WGS es más sensible que WES para detección de CNVs y rearreglos

Interpretación limitada a regiones codificantes +/- 2-20 bp de límites codificantes

Detección de variantes missense, indels y CNVs generalmente > 30 kb es diagnóstica

Abou Tayoun, A. et al. (2018). Prenatal DNA Sequencing: Clinical, Counseling, and Diagnostic Laboratory Considerations. *Prenatal diagnosis*, *38*(1), 26–32.
Gordeeva, V. (2021). Benchmarking germline CNV calling tools from exome sequencing data. *Scientific reports*, *11*(1), 14416.

Otras limitaciones

Asociaciones fenotipo-genotipo prenatal están mucho menos descritas y son variables en el tiempo

- Por ende, es más difícil determinar patogenicidad de variantes

Abou Tayoun, A. et al. (2018). Prenatal DNA Sequencing: Clinical, Counseling, and Diagnostic Laboratory Considerations. *Prenatal diagnosis*, *38*(1), 26–32.

› Ultrasound Obstet Gynecol. 2022 Jun;59(6):723-730. doi: 10.1002/uog.24842.

Evolving fetal phenotypes and clinical impact of progressive prenatal exome sequencing pathways: cohort study

F Mone [1], H Abu Subieh [2], S Doyle [3], S Hamilton [3], D J Mcmullan [3], S Allen [3], T Marton [4], D Williams [3], M D Kilby [5 6]

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En fetos con variante patogénica causal de fenotipo y que el embarazo alcanzó el tercer trimestre, se encontró anomalías adicionales en 73.3% de casos

- Hidrops (27%), artrogriposis (27%) y anomalías cerebrales (18.2%)

Mone, F. et al. (2022). Evolving fetal phenotypes and clinical impact of progressive prenatal exome sequencing pathways: cohort study. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *59*(6), 723–730. https://doi.org/10.1002/uog.24842

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Considerations for using prenatal NGS diagnostic tests

- Prenatal phenotypic relation to most genetic conditions have not been extensively reported. The application of prenatal NGS diagnostic tests will improve our knowledge in prenatal genotype-phenotype conditions (phenotype expansion).[16] It is crucial to take this into account when analysing the variants identified as potentially causatives.
- The gestational age at which NGS diagnostic tests are performed should be considered, since fetal phenotype might evolve over time. If new clinical information becomes available, either during pregnancy, postnatally or through necropsy findings after termination of pregnancy or fetal demise, exome reanalysis should be considered.

Abulí, A. et al. (2024). Guidelines for NGS procedures applied to prenatal diagnosis by the Spanish Society of Gynecology and Obstetrics and the Spanish Association of Prenatal Diagnosis. *Journal of medical genetics*, *61*(8), 727–733.

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Table 2. Revised Karyotypes from Whole-Genome Sequencing

Subject ID	Clinical Interpretation	Revised Karyotype from Sequencing	Diagnosis
1	46,XY,t(9;16)(q22;p11)	46,XY,t(9;16)(q22.**33**;p1**2.1**)	Autism spectrum disorder
2	46,XY,t(3;6)(q26.2;q16.2)	46,XY,t(3;6)(q26.**32**;q16.**3**)	Autism spectrum disorder
3[a]	46,XX,inv(5)(p12q13.1)	46,XX,inv(5)(p1**4.2**q1**4.3**)	Global developmental delay, hypotonia, seizures
4	46,XY,t(6;9)(q16.2;q13)	46,XY,**inv(6)(q16.1q16.1)**t(6;9)(q16.**1**;q**21.3**)	Autism spectrum disorder
5a, b	46,XY,t(3;18)(q13.3;q21.3)	46,XY,t(3;18)(q13.3**2**;q21.**2**)	Global developmental delay, multiple congenital anomalies

Karyotype analyses for each subject included in whole-genome sequencing. Karyotyping was performed at various sites, including referring clinics. In all subjects, a revision to the clinical interpretation was required after sequencing. Subjects included in the CapBP experiment were required to have cytogenetic analyses that previously localized the breakpoint to less than one megabase, so further revision was not necessary.

[a] Subject is DGAP218, see Developmental Genome Anatomy Project (Web Resources).

Rearreglos son detectables por WGS. Sin embargo, sin validación clínica entre laboratorios

Talkowski, M. E. (2011). Next-generation sequencing strategies enable routine detection of balanced chromosome rearrangements for clinical diagnostics and genetic research. *American journal of human genetics*, *88*(4), 469–481.

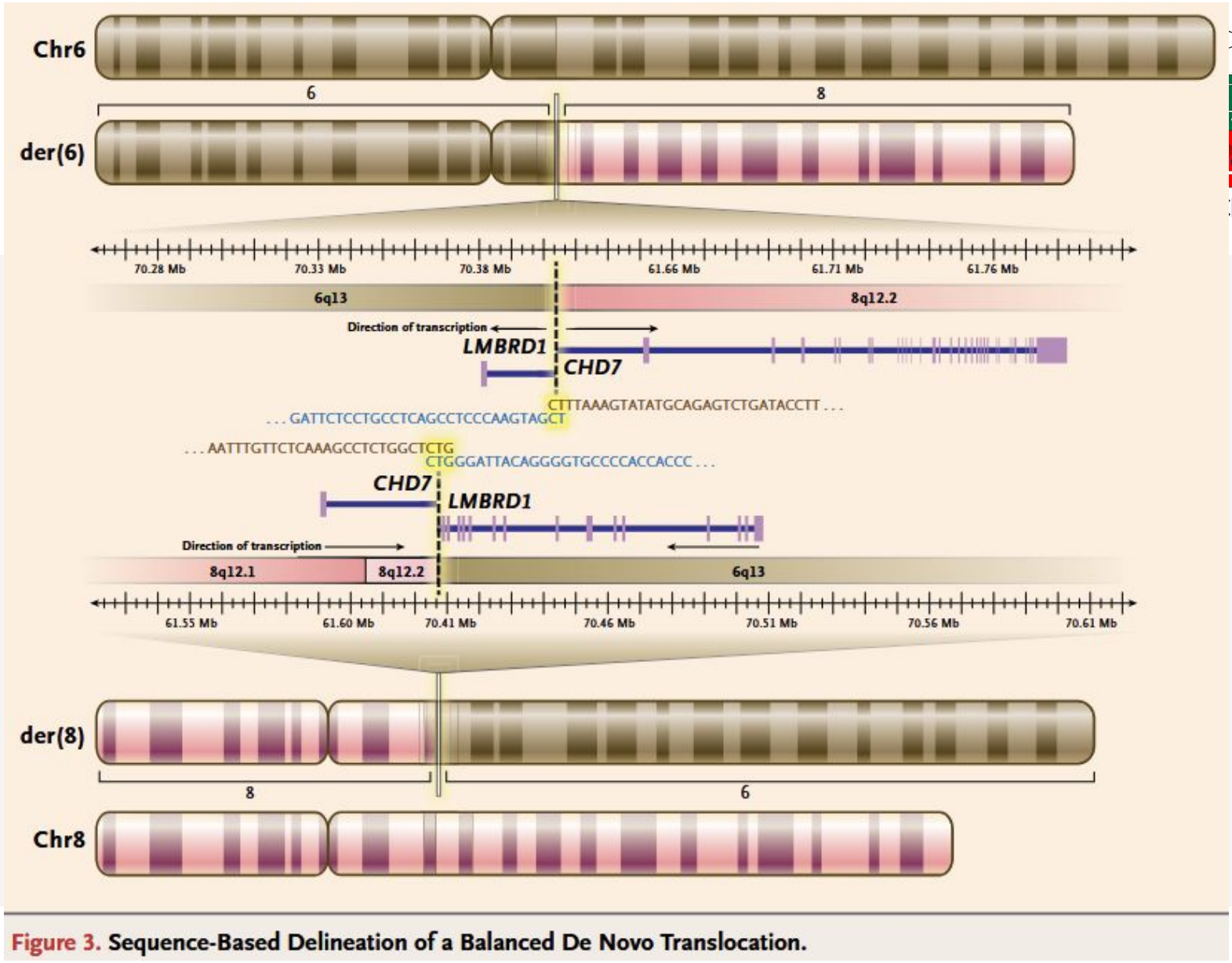


Figure 3. Sequence-Based Delineation of a Balanced De Novo Translocation.

Talkowski, M. E. et al. (2012). Clinical diagnosis by whole-genome sequencing of a prenatal sample. *The New England journal of medicine*, *367*(23), 2226–2232.

Hallazgos incidentales

ACMG y CCMG: recomiendan reportar variantes LP o P en genes con alta penetrancia que se asocian a enfermedades moderadas o severas de inicio en la niñez

Se recomienda no reportar estado de portador

Si se realiza estudio trio, recomiendan cuidado en consejería pre test respecto a informe de hallazgos en progenitores

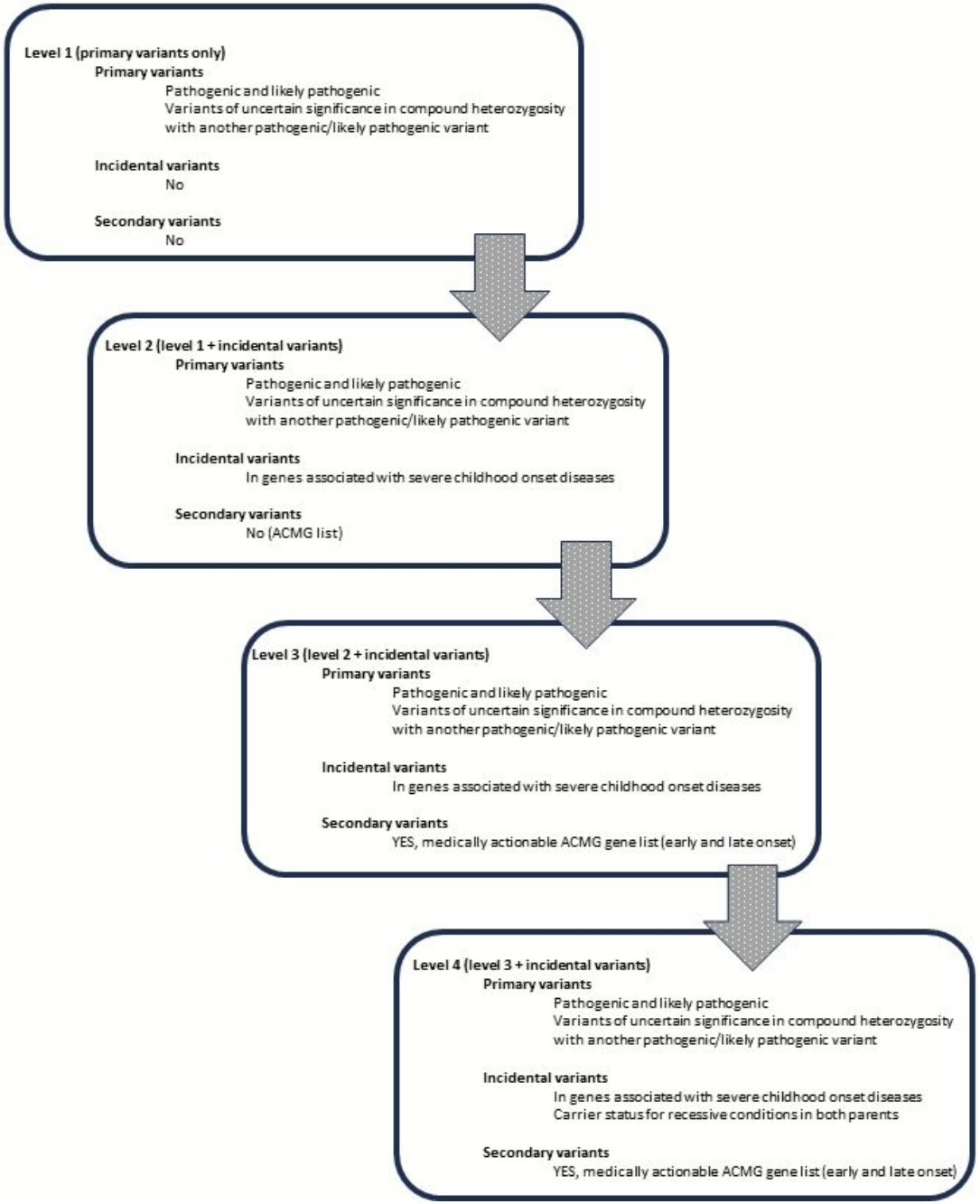
Abulí, A. et al. (2024). Guidelines for NGS procedures applied to prenatal diagnosis by the Spanish Society of Gynecology and Obstetrics and the Spanish Association of Prenatal Diagnosis. *Journal of medical genetics*, *61*(8), 727–733.

Hallazgos secundarios

ACMG sugiere reporte de hallazgos secundarios en escenario prenatal

Otros no recomiendan hacerlo (NHS, 2020; European Society of Human Genetics, 2021; Canadian College of Medical Geneticists, 2022), pero dejan a criterio final del clínico

Abulí, A. et al. (2024). Guidelines for NGS procedures applied to prenatal diagnosis by the Spanish Society of Gynecology and Obstetrics and the Spanish Association of Prenatal Diagnosis. *Journal of medical genetics*, *61*(8), 727–733.



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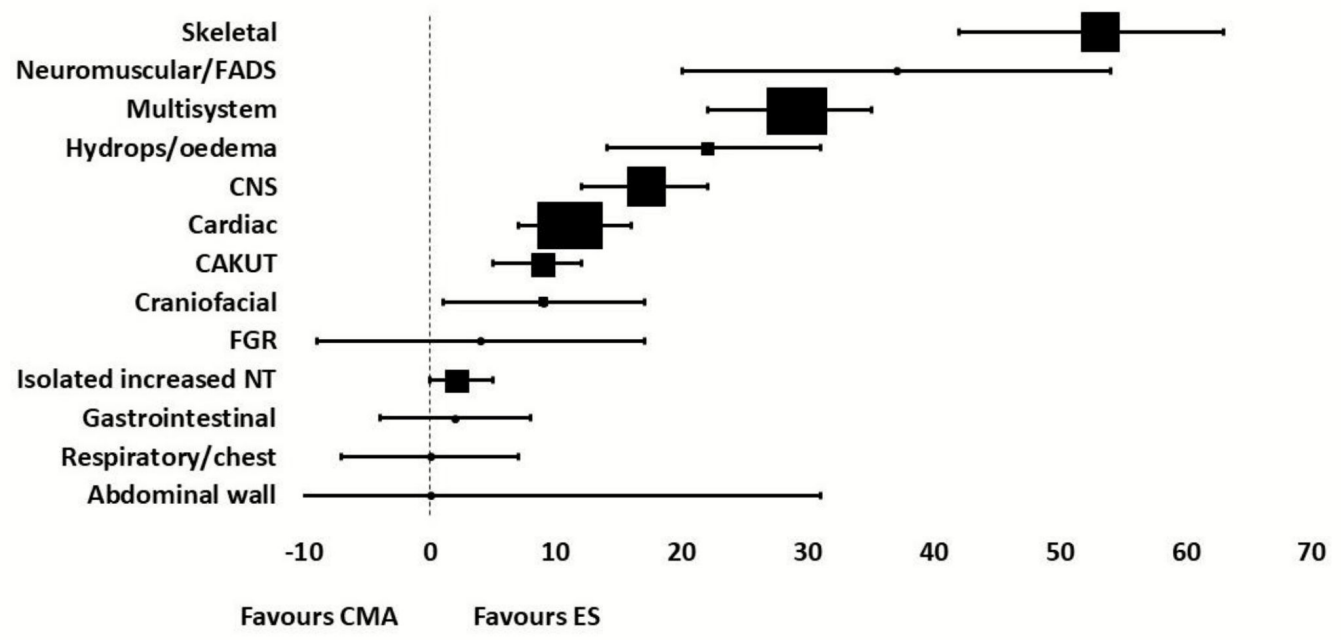
Propuesta de modelo escalonado para reporte de resultados prenatales

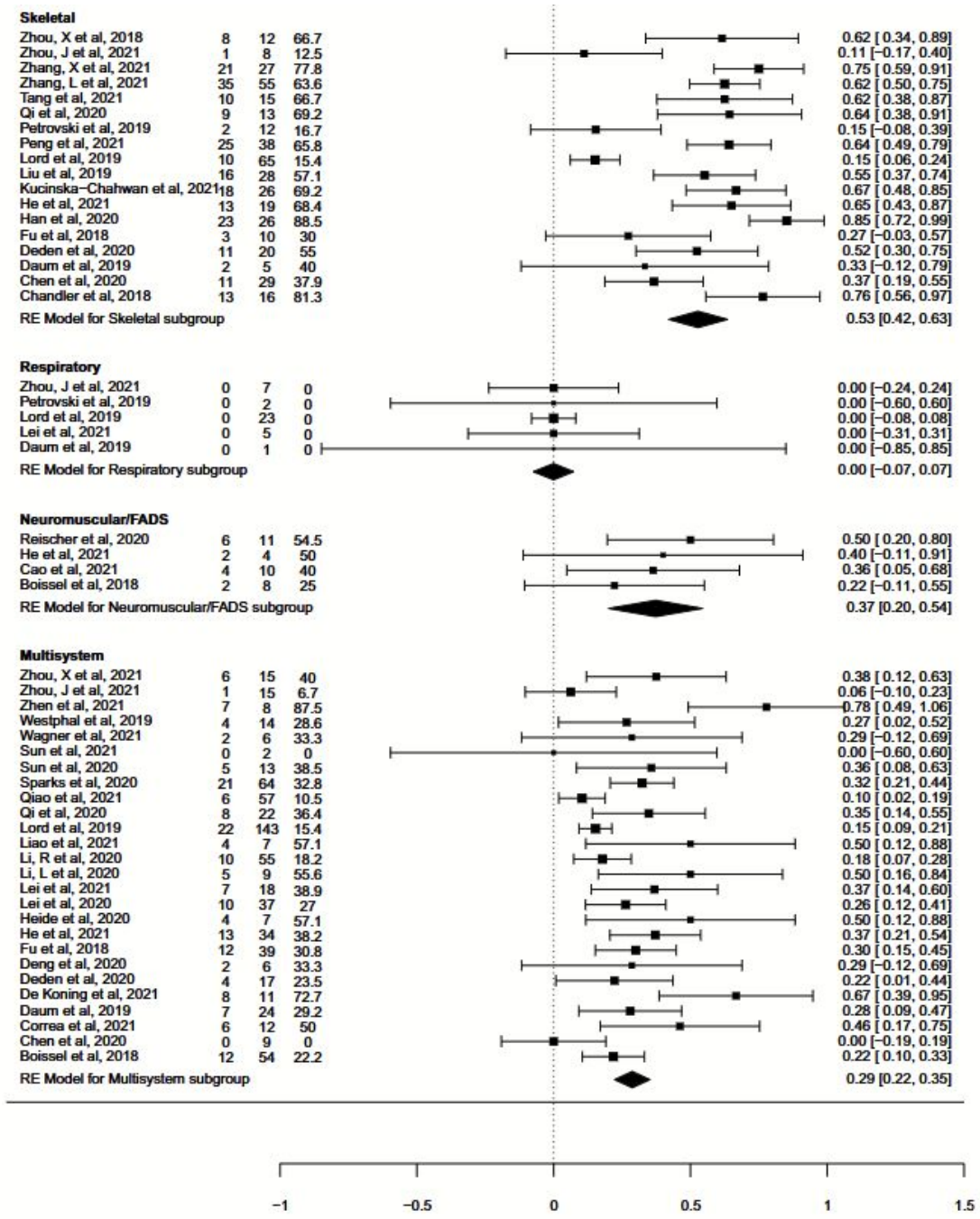
Abulí, A. et al. (2024). Guidelines for NGS procedures applied to prenatal diagnosis by the Spanish Society of Gynecology and Obstetrics and the Spanish Association of Prenatal Diagnosis. *Journal of medical genetics*, *61*(8), 727–733.

Rendimientos

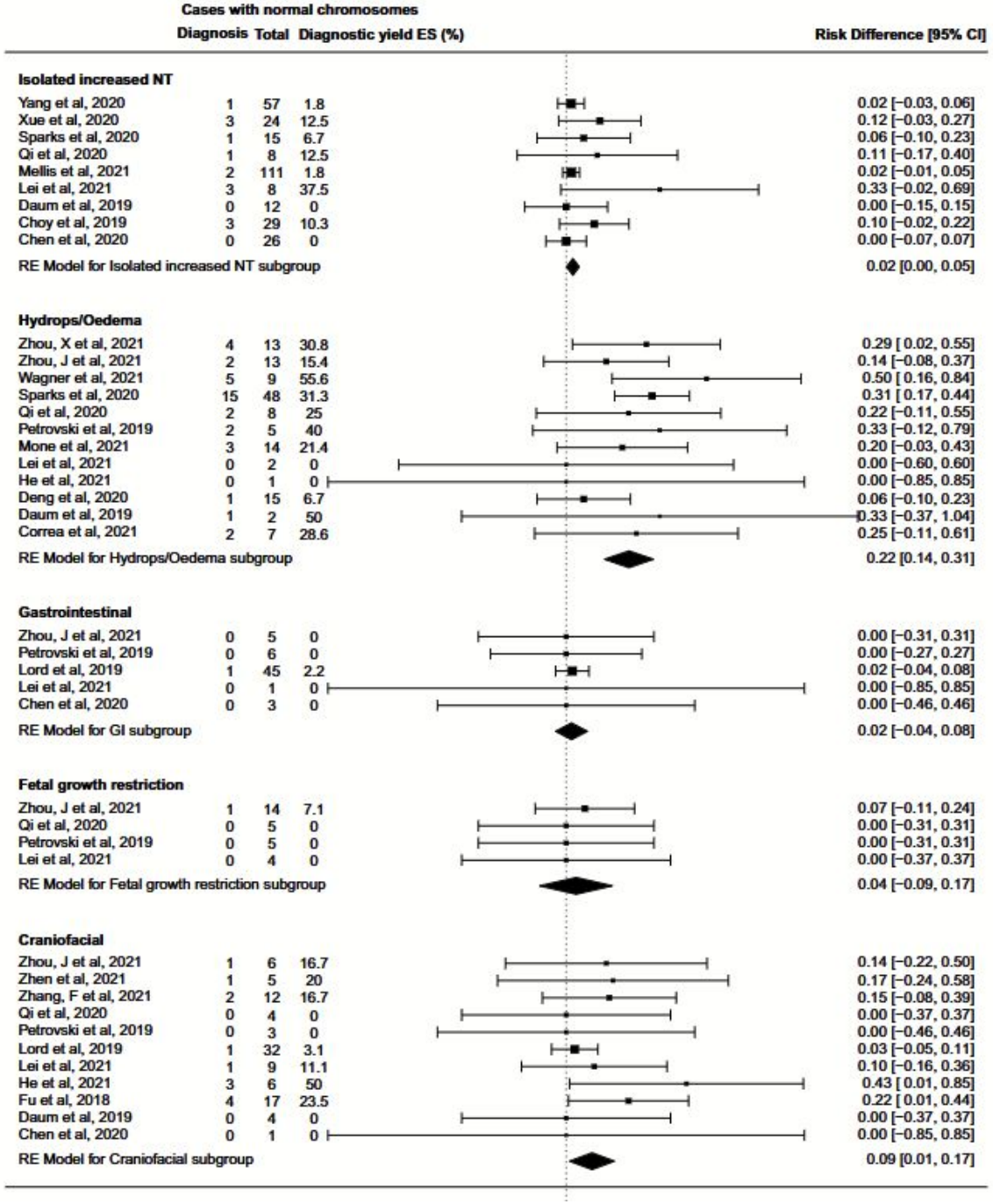
WES: rendimiento adicional a cariograma + CMA es variable según motivo de indicación:



Mellis, R. et al. (2022). Diagnostic yield of exome sequencing for prenatal diagnosis of fetal structural anomalies: A systematic review and meta-analysis. *Prenatal diagnosis*, *42*(6), 662–685.



Mellis, R. et al. (2022). Diagnostic yield of exome sequencing for prenatal diagnosis of fetal structural anomalies: A systematic review and meta-analysis. *Prenatal diagnosis*, *42*(6), 662–685.



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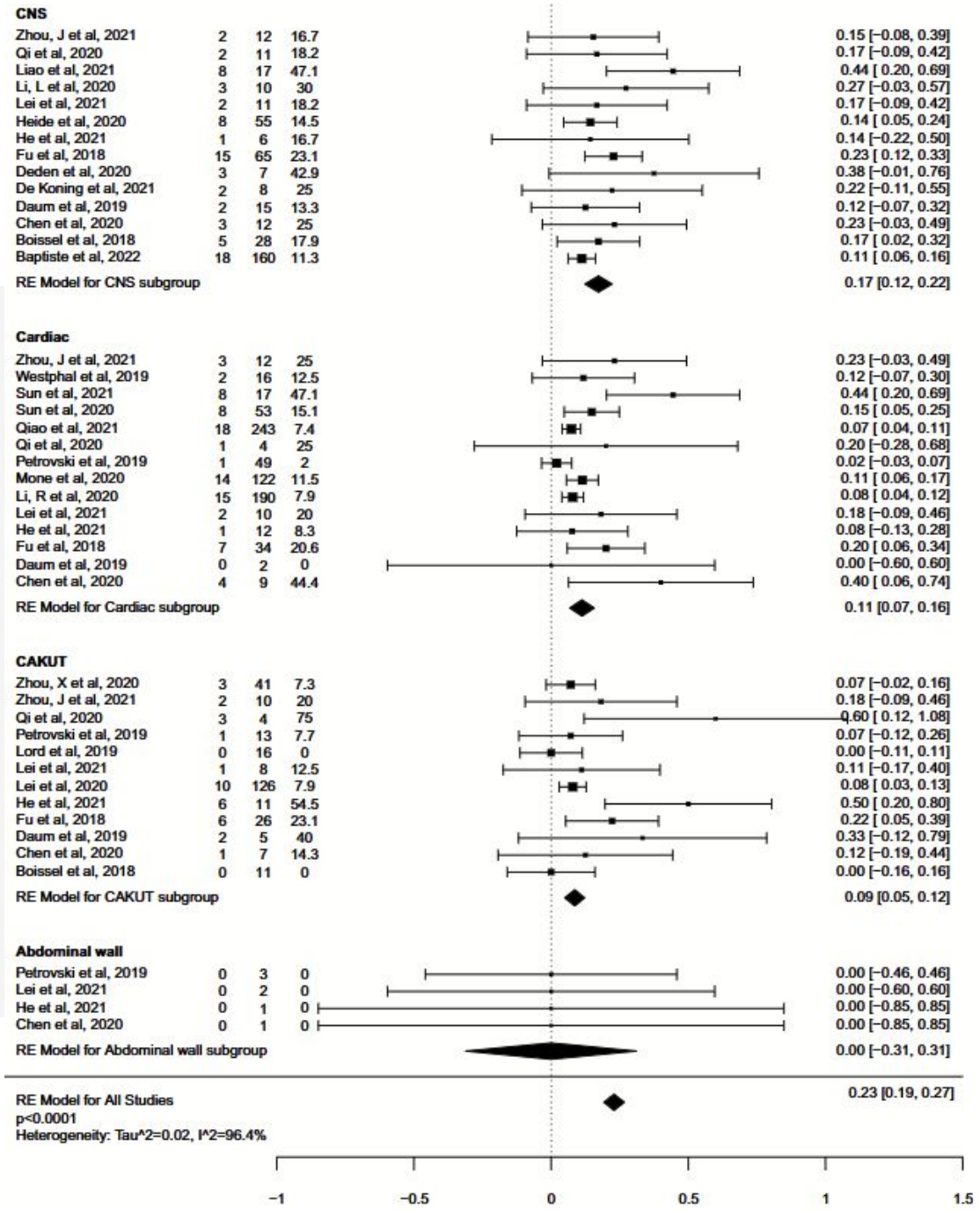
Mellis, R. et al. (2022). Diagnostic yield of exome sequencing for prenatal diagnosis of fetal structural anomalies: A systematic review and meta-analysis. *Prenatal diagnosis*, *42*(6), 662–685.



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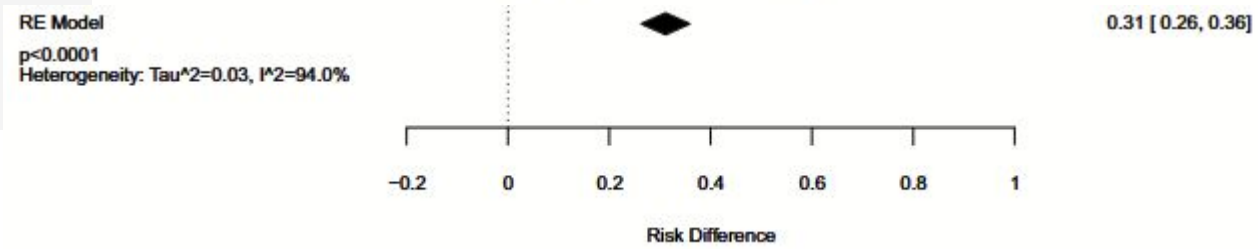
Mellis, R. et al. (2022). Diagnostic yield of exome sequencing for prenatal diagnosis of fetal structural anomalies: A systematic review and meta-analysis. *Prenatal diagnosis*, *42*(6), 662–685.

TABLE 3 Pooled effect size for incremental diagnostic yield of ES over CMA in different phenotypic groups

Phenotypic group	Cases analysed	Pooled estimated diagnostic yield [95% CI], *p*-value
Skeletal	424	53% [42%–63%], $p < 0.0001$
Neuromuscular/Fetal akinesia deformation sequence (FADS)	33	37% [20%–54%], $p < 0.0001$
Multisystem	698	29% [22%–35%], $p < 0.0001$
Hydrops/Oedema	137	22% [14%–31%], $p < 0.0001$
Central nervous system (CNS)	417	17% [12%–22%], $p < 0.0001$
Cardiac	773	11% [7%–16%], $p < 0.0001$
Craniofacial	99	9% [1%–17%], $p = 0.02$
Congenital anomalies of kidneys and urinary tract (CAKUT)	278	9% [5%–12%], $p < 0.0001$
Fetal growth restriction	28	4% [–9 to 17%], $p = 0.59$
Isolated increased nuchal translucency (NT)	290	2% [0%–5%], $p = 0.04$
Gastrointestinal	60	2% [–4 to 8%], $p = 0.5$
Respiratory/Chest	38	0 [–7 to 7%], $p = 1$
Abdominal wall	7	0 [–31% to 31%], $p = 1$

Note: Phenotypic groups refer to fetuses with one or more anomalies in a single body system. Fetuses with anomalies in more than one system are classified as 'Multisystem'. The 'isolated increased NT' group contains a mixture of (i) cases with isolated increased NT at presentation where it was unspecified whether additional anomalies developed later in pregnancy, and (ii) cases where isolated increased NT remained isolated throughout pregnancy. The bold and underlined formatting was just for emphasis in defining the two sub-groups.

Abbreviations: CMA, chromosomal microarray; ES, exome sequencing.



Riesgo agrupado: 0.31

Mellis, R. et al. (2022). Diagnostic yield of exome sequencing for prenatal diagnosis of fetal structural anomalies: A systematic review and meta-analysis. *Prenatal diagnosis*, *42*(6), 662–685.

WGS: rendimiento

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Summary of fetal exome sequencing publications with >5 fetuses included

First author	Number of cases	Cohort description	Proband vs. trio	Pathogenic variant	Likely pathogenic variant
Yang et al., 2014	11	Terminated anomalous fetus	Trio	6 of 11 (54%)	—
Carss et al., 2014	30	Prenatal sonographic anomalies	Trio	3 of 30 (10%)	5 of 30 (16.7%)
Drury et al., 2015	24	Prenatal sonographic anomalies including NT ≥ 3.5mm	14 Proband10 Trio	5 of 24 (20.8%)	1 of 24 (4.2%)
Alamillo et al., 2015	7	Multiples sonographic anomalies termination or demise	Trio	3 of 7 (42.9%)	1 of 7 (14.3%)
Pangalos et al., 2017	14	Prenatal sonographic anomalies	Proband only	6 of 14 (42.9%)	—
Yates et al., 2017	84	Demise or termination	33 Proband/duo51 Trio/quad	17 of 84 (20%)	38 of 84 (45%)
Vora et al., 2017	15	Multiple sonographic anomalies	Trio	7 of 15 (46.7%)	1 of 15 (6.7%)
Fu et al., 2018	196	Prenatal sonographic anomalies	34 Proband13 Trio	47 of 196 (24%)	25 of 196 (12.8%)

WGS: rendimiento

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Table 2 Incremental yield of whole-genome sequencing compared with quantitative fluorescence polymerase chain reaction/ chromosomal microarray analysis with resolution of 50 kb

Anomaly group	*Incremental yield (%)*	*Heterogeneity (%)*
All		
All cases	26 (18–36)	86
Prenatal	16 (9–24)	85
Postnatal	39 (27–51)	53
Multisystem		
All cases	30 (19–43)	65
Prenatal	23 (12–36)	70
Postnatal	43 (27–59)	21
R21 selected		
All cases	32 (22–42)	70
Prenatal	22 (14–31)	65
Postnatal	48 (37–60)	0

Shreeve, N. et al. (2024). Incremental yield of whole-genome sequencing over chromosomal microarray analysis and exome sequencing for congenital anomalies in prenatal period and infancy: systematic review and meta-analysis. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *63*(1), 15–23.

Overview

Rapid prenatal exome sequencing (**R21** in the National Genomic Test Directory) is undertaken for at-risk pregnancies in which a genomic diagnosis would guide management of the fetus. At present, **R21** testing may only be requested by a clinical geneticist, usually following multidisciplinary team discussion with the fetal medicine team. Testing requires invasive prenatal sampling and involves exome sequencing of a nationally approved panel of genes known to cause prenatal genetic disorders.

What are the R21 testing eligibility criteria?

R21 testing can be requested when:

- a fetus presents with multiple anomalies affecting multiple systems; **and/or**
- the presentation is suggestive of an underlying monogenic disorder; **and**
- a molecular diagnosis may influence the management of the pregnancy or the baby in the immediate neonatal period.

National genomic test directory - NHS. Disponible en https://www.genomicseducation.hee.nhs.uk/genotes/knowledge-hub/r21-rapid-prenatal-exome-sequencing/

Some examples of presentations for which **R21** testing may be indicated include a fetus with:

- multiple anomalies in a number of different systems;
- a suspected skeletal dysplasia where intrauterine growth restriction has been excluded;
- large echogenic kidneys and a normal bladder;
- major central nervous system anomalies in which a neural tube defect has been excluded;
- multiple contractures (excluding bilateral isolated talipes);
- a nuchal translucency greater than 6.5 millimetres **and** at least one additional anomaly and in which the array comparative genomic hybridisation (CGH) is normal; and
- non-immune fetal hydrops (fluid/oedema in at least two compartments detected at or after routine second trimester scan) and a normal array CGH.

Please also refer to the test directory for the complete lists of eligibility and exclusion criteria.

National genomic test directory - NHS. Disponible en https://www.genomicseducation.hee.nhs.uk/genotes/knowledge-hub/r21-rapid-prenatal-exome-sequencing/

ORIGINAL ARTICLE

PRENATAL DIAGNOSIS WILEY

Exome sequencing in every pregnancy? Results of trio exome sequencing in structurally normal fetuses

Michal Levy[1,2,3] | Shira Lifshitz[1] | Mirela Goldenberg-Fumanov[1] | Lily Bazak[1] | Rayna Joy Goldstein[1] | Uri Hamiel[1,2] | Rachel Berger[1] | Shlomo Lipitz[2] | Idit Maya[1,2,3] | Mordechai Shohat[1,2,4]

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- Análisis retrospectivo, centro único. Fetos estructuralmente normales
- Exoma clínico
- 1825 exomas, 1020 casos de bajo riesgo con 28 fetos (2.7%) con variantes potencialmente patogénicas, con 64% de novo dominantes
- Terminación voluntaria del embarazo en 13 casos (1.2%), incluyendo 4/7 VUS

Levy, M. et al. (2025). Exome sequencing in every pregnancy? Results of trio exome sequencing in structurally normal fetuses. *Prenatal diagnosis*, *45*(3), 276–286.

Rendimientos - Obito

Quinlan-Jones (2019): 27 fetos abortados estructuralmente anormales con CMA y QF-PCR normal

- Rendimiento de exoma trío 37%
- 4 variantes de novo, 6 restantes heredadas AR o ligadas a X

Yates (2017): 84 fetos, exoma trío

- 20% positivos, 45% posibles, 9% gen candidato
- 24% positivos en WES trio, 14% en WES solo

Quinlan-Jones, E. et al. (2019). Molecular autopsy by trio exome sequencing (ES) and postmortem examination in fetuses and neonates with prenatally identified structural anomalies. *Genetics in medicine : official journal of the American College of Medical Genetics*, *21*(5), 1065–1073.

Otros

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J Perinat Med. 2022 Dec 13;51(6):769-774. doi: 10.1515/jpm-2022-0504. Print 2023 Jul 26.

Rapid diagnosis of intra-amniotic infection using nanopore-based sequencing

Piya Chaemsaithong [1 2 3 4], Roberto Romero [3 4 5 6 7 8], Pisut Pongchaikul [9 10 11],

Conclusiones

- NGS presenta rendimiento variable según la indicación clínica, y requiere criterio según recursos disponibles para indicar
- WGS promete gran rendimiento. Sin embargo, se necesita evidencia comparativa adicional para poder sugerir por sobre exoma