WHEN SHOULD SINGLE GENE, GENE PANEL AND EXOME SEQUENCING BE APPLIED IN STRUCTURALLY ABNORMAL FETUSES?

Montse Pauta[1], Berta Campos[2], Maria Segura-Puimedon[2], Gemma Arca[3], Alfons Nadal[4], Albert Tubau[5], Silvia Pina[6], Edda Marimon[7], Lourdes Martín[8], Eva López-Quesada[9], Joan Sabrià[7], Begoña Muñoz[10], Esperanza Garcia[11], Fernanda Paz Y Miño[12], Virginia Borobio[12], Elisenda Eixarch[12,13], Antoni Borrell[1,12]

[1]BCNatal, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Catalonia, Spain. [2]qGenomics. Esplugues de Llobregat, Catalonia, Spain. [3]DepartmentofNeonatology, BCNatal, Hospital Clinic Barcelona, Barcelona, Catalonia, Spain [4]Department of Pathology, Hospital Clínic Barcelona, Barcelona, Catalonia, Spain.[5]Department of Obstetrics and Gynecology, Son Llàtzer Hospital, Palma de Mallorca, Spain.[6]Department of Obstetrics and Gynecology, Parc Taulí Hospital. Sabadell, Catalonia, Spain. [7]BCNatal, Department of Obstetrics and Gynecology. Hospital Sant Joan de Déu, Esplugues de Llobregat, Catalonia, Spain. [8]Department of Obstetrics and Gynecology, Hospital Joan XXIII de Tarragona, Catalonia, Spain. [9] Department of Obstetrics and Gynecology, Hospital Mútua de Terrassa, Terrassa, Catalonia, Spain. [10] Department of Obstetrics and Gynecology,Hospital Sant Joan de Reus, Reus, Catalonia, Spain. [11]Department of Obstetrics and Gynecology, Hospital Consorci Sanitari de Terrassa, Terrassa, Catalonia, Spain. [12]BCNatal, Department of Obstetrics and Gynecology. Hospital Clínic de Barcelona, Barcelona, Catalonia, Spain. [13]Centre for Biomedical Research on Rare Diseases (CIBER-ER), Madrid, Spain.**

INTRODUCTION

The advent of high-throughput **Next-Generation Sequencing (NGS)** has allowed to sequence the whole human genome in a single reaction. Our group initially applied the NGS technology to diagnose monogenic disorders, using **single gene studies** when a specific fetal anomaly pattern was attributed to a particular gene, or a **gene panel** if the fetal anomaly pattern was probably caused by several genes. Since 2016, when the observed fetal phenotype did not overlap with a previously described phenotype, we applied the "**solo-clinical exome sequencing (CES)",** which entails sequencing the fetus alone, and interpreting the OMIM genes.

OBJECTIVE

To assess the diagnostic yield of different Next-Generation Sequencing (NGS) studies: single gene, gene panel or "solo" clinical exome sequencing (solo-CES), in fetuses with structural anomalies, normal chromosomal microarray analysis (CMA) in the absence of a known familial mutation

RESULTS

During the study period (2015-2019), 118 NGS studies were performed in 108 structurally abnormal fetuses with a normal CMA. Overall diagnostic yield accounted for 35% (41/118) of samples and 38% (41/108) of the fetuses. Diagnostic yield in gene panels was 32% (12/37), similar to 39% (28/71) in solo-CES. Single gene NGS studies were abandoned in 2018 because of the observed low yield (10%: 1/10).

1. Gene Panel (32%: 12/37)			
Gene Panel type	Number of Included Genes	Definitive Diagnoses (N/N)	Diagnostic Yield (%)
1.1. RASopathies-Hydrops Panel	130	5/15	33%
1.2.C.A.K.U.T Panel	140	4/5	80%
1.3. Osteogenesis Imperfecta Panel	22	1/4	25%
1.4. Tuberous Sclerosis Panel	2	1/3	33%
1.5. Craniosynostosis Panel*	11	1/3	33%
1.6. Lissencephaly Panel**	24	0/2	0%
1.7. Cornelia de Lange Panel	5	0/1	0%
1.8. Visceral myopathy Panel	12	0/1	0%
1.9. Cardiomyopathy Panel	119	0/1	0%
1.10. Fanconi Panel§	22	0/1	0%
1.11. CHARGE Panel	2	0/1	0%
2. Solo Clinical Exome Sequencing (39%: 28/71)			
Indication		Definitive Diagnoses (N/N)	Diagnostic Yield (%)
2.1. Multisystem Anomalies		19/43	44%
2.2. Recurrent Anomalies		3/11	27%
2.3. Skeletal Dysplasia		5/6	83%
2.4. Increased Nuchal Translucency		0/4	0%
2.5. Complex CNS Anomalies		1/4	25%
2.6. Complex Cardiac Defects		0/3	0%

MATERIAL AND METHODS

In specific fetal anomalies, **a gene panel** was preferred. "Gene panel" was defined as the test that analyzes by NGS multiple genes at once, ranging from 2 to 200 in our study, associated to a specific fetal phenotype. **Solo-CES** was consistently offered in: a) Multisystem anomalies (at least 2 major anomalies from different anatomical systems); b) Recurrent anomalies (the same major anomaly in consecutive pregnancies); and c) Severe skeletal dysplasias with at least bilateral upper and/or lower limbs micromelia below-3 SD, not resembling osteogenesis imperfect.

During the last 17 months (July 2018- November 2019) of the study, pregnancies 115 affected by multisystem or recurrent structural fetal anomalies from 7 further 116 participating centers were also recruited and studied by means of CES.

Pathogenicity of variants identified in genes that were related to the index case phenotype or overlapping phenotypes was assessed following the American College of Medical Genetics and Genomics (ACMG) guidelines

GENE PANEL.

Gene	Genomic Variants (nucleotide)	ACMG Variant Type	Zygosity	Associated Conditions (Inheritance Pattern)
1.1.RASOPATHIES-HYDROPS PANEL				
FOXS2	NM_005251: c.461_463delACA*	L.Pat.	Het.	Lymphedema-distichiasis syndrome (AD, *de novo*)
KRAS	NM_033360: c.65A>G	Pat.	Het.	Noonan syndrome (AD, *de novo*)
RIT1	NM_006912.5: c.245T>G	Pat.	Het.	Noonan syndrome (AD, *de novo*)
RIT1	NM_006912.6: c.245T>G	Pat.	Het.	Noonan syndrome (AD, *de novo*)
PIEZO1	NM_001142864: c.1490delG* ; c.5289C>G*	Pat. /Pat.	Comp. Het.	Hereditary Lymphedema III (AR)
1.2. C.A.K.U.T. PANEL				
NEK8	NM_178170.3: c.325_327delTTC	L.Pat.	Hom.	Renal-hepatic-pancreatic dysplasia 2 (AR)
NPHP3	NM_153240.5: c.434_437delAAAG	Pat.	Het. UPD	Renal-hepatic-pancreatic dysplasia 2 (AR)
TTC8	NM_144596.3: c.69delC ; c.799-?_909+*	Pat./Pat.	Comp. Het.	Bardet-Biedl syndrome (AR)
PKHD1	NM_138694.3: c.842G>A* ; c.1529delG	L. Pat/ Pat.	Comp. Het.	Polycystic Kidney Disease (AR)
1.3. OSTEOGENESIS IMPERFECTA PANEL				
COL1A2	NM_000089: c.2350-2A>G*	L.Pat.	Het.	Osteogenesis imperfecta (AD, *de novo*)
1.4. TUBEROUS SCLEROSIS PANEL				
TSC2	NM_000548.3: c.2251C>T	Pat.	Het.	Tuberous Sclerosis (AD, *de novo*)
1.5. CRANIOSYNOSTOSIS PANEL				
FGFR2	NM_000141.4: c.1024T>G	L.Pat.	Het.	Pfeiffer syndrome (AD, *de novo*)

SOLO CLIINCAL EXOME SEQUENCING

Gene	Genomic Variants (nucleotide)	ACMG Variant Type	Zygosity	Associated Conditions (Inheritance Pattern)
2.1. MULTISYSTEM ANOMALIES				
ASCC1	NM_001198798: c.157dupG	L.Pat.	Hom.	Spinal muscular atrophy with congenital bone fractures 2 (AR)
PKD1L1	NM_138295: c.2276A>T	VUS	Hom.	Visceral Heterotaxy 8 (AR)
RSPO2	NM_178565: c.277T>A*	VUS	Hom.	Tetraamelia syndrome (AR)
SUCLG1	NM_003849: c.626C>A	L.Pat.	Hom.	Mitochondrial depletion syndrome 9 (AR)
EVC	NM_153717: c.721A>T ; c.2041G>T*	Pat./Pat.	Comp. Het.	Ellis-Van Creveld syndrome (AR)
PCNT	NM_006031: c.2282G>A ;c.4675A>T*	VUS/VUS	Comp. Het.	Microcephalic osteodysplastic primordial dwarfism type II (AR)
COL27A1	NM_032888: c.2548G>A ; c.3249+1G>T*	L.Pat./L.Pat.	Comp. Het.	Steelsyndrome (AR)
DHCR7	NM_001360: c.1228G>A ;c.452G>A	Pat./Pat.	Comp. Het.	Smith-Lemli-Opitz syndrome (AR)
EFTUD2	NM_001142605: c.2287_2314del*	L.Pat.	Het.	Mandibulofacial dysostosis with microcephaly (AD, *de novo*)
EFTUD2	NM_001142605: c.146_168del	Pat.	Het.	Mandibulofacial dysostosis, Guion-Almeida type (AD, *de novo*)
ASXL1	NM_015338: c.1582C>T*	L.Pat.	Het.	Bohring-Opitz syndrome (AD, *de novo*)
KMT2D	NM_003482: c.13450C>T	Pat.	Het.	Nabuki syndrome (AD, *de novo*)
KMT2D	NM_003482.3: c.13450C>T	Pat.	Het.	Nabuki syndrome (AD, *de novo*)
TCF4	NM_001083962: c.1733G>T	L.Pat.	Het.	Pitt-Hopkins syndrome (AD, *de novo*)
BRAF	NM_004333: c.1785T>G	Pat.	Het.	Cardiofaciocutaneous syndrome (AD *de novo*)
SLC25A24	NM_013386: c.992C>G*	VUS	Het.	Fontaine syndrome (AD, *de novo*)
GLI2	NM_005270: c.3323G>T	VUS	Het.	Holoprosencephaly 9 (AD) (inherited from a parent affected with a microform)
SIX3	NM_005413: c.110G>A*	VUS	Het.	Holoprosencephaly 2 (AD) (inherited from a parent affected with a microform)
KDM5C	NM_001146702: c.3923C>T	VUS	Het.	Intellectual disability syndromic, Claes-Jensen type (X-linked)
2.2. RECURRENT ANOMALIES				
SEC23B	NM_001172745: c.716A>G	L.Pat.	Hom.	Congenital dyserythropoietic anemia type II (AR)
ASPM	NM_018136.4: c.7551T>G	L.Pat.	Het.	Microcephaly with simplified gyral pattern (AR)
DOK7	NM_001164673: c.230C>T ; NM_173660: c.532+4A>G*	L.Pat./L.Pat.	Comp. Het.	Fetalakinesia deformation sequence (AR)
2.3. SKELETAL DISPLASIAS				
COL2A1	NM_001844.4: c.3819G>A*	L.Pat.	Het.	Spondyloepiphyseal dysplasia congenita (AD, de novo)
CANT1	NM_138793: c.1037C>T	L.Pat.	Hom.	Desbuquois dysplasia 1 (AR)
HSPG2	NM_001291860: c.6823G>A ; c.3581G>T*	VUS/VUS	Comp. Het.	Schwartz-Jampel syndrome (AR)
DYNC2H1	NM_001080463: c.6128G>A;c.8343A>G* ; NM_001377: c.12439C>T*	L. Pat/ L.Pat /VUS	Comp. Het.	Short-rib thoracic dysplasia 3 with or without polydactyly syndrome (AR)
RMRP	NR_003051.3: n28G>A (;) NR_0030513: n.71A>G	L.Pat./L.Pat.	Comp. Het.	Anauxetic dysplasia (AR)
2.5. CENTRAL NERVOUS SYSTEM COMPLEX ANOMALIES				
CHD7	NM_017780: c.5839C>T	Pat.	Het.	CHARGE syndrome (AD, *de novo*)

CONCLUSION

- NGS provided a definitive diagnosis in 38% of fetuses with selected structural anomalies and normal CMA result, which is crucial to establish the prognostic profile and to estimate the recurrence risk for future pregnancies.

- The "solo-CES" approach, which entails sequencing the fetus alone, and interpreting only the OMIM genes, gives similar diagnostic yields to that obtained by "trio-WES" testing