Genetic analysis algorithm for the study of patients with Multiple Congenital Anomalies and isolated Congenital Heart Disease

Marisol Delea[1], Lucia. S. Massara[2], Lucia D. Espeche[1], M. Paz Bidondo[1], Jaen Oliveri[2], Paloma Brun[2], Mónica Fabro[3], Micaela Galain[3], Cecilia S. Fernández[3], Melisa Taboas[1], Carlos D. Bruque[1], Emilio Kolomenski[7], Agustín Izquierdo[8], Ariel Berenstein[9], Pablo Barbero[1], Viviana Cosentino[4], Celeste Martinoli[5], Mariana Vilas[6], Mónica Rittler[6], Rodrigo Mendez[1], Lilian Furforo[6], Rosa Liascovich[1], Boris Groisman[1], Sandra Rozental [1], Liliana Dain [1,7]* and the PID ACM-CC taskforce.

[1]Centro Nacional de Genética Médica- ANLIS, C.A.B.A. Argentina; [2]Hospital de Alta Complejidad en Red El Cruce – SAMIC, Pcia. de Buenos Aires, Argentina; [3]Novagen, C.A.B.A. Argentina; [4]Hospital Interzonal General de Agudos Luisa Cravenna de Gandulfo, Provincia de Buenos Aires, Argentina; [5]Hospital Sor Maria Ludovica, La Plata, Provincia. de Buenos Aires Argentina; [6]Hospital Materno Infantil Ramón Sardá, C.A.B.A, Argentina; [7]Departamento de Fisiología, Biología Molecular y Celular, Instituto de Biociencias, Biotecnología y Biología Traslacional (iB[3]), Facultad de Ciencias Exactas y Naturales- UBA, C.A.B.A, Argentina; [8] Centro de Investigaciones Endocrinológicas¨Dr. Cesar Bregada, C.A.B.A, Argentina; [9] Instituto Multidisciplinario de Investigaciones en Patologias Pediatricas, C.A.B.A, Argentina. ***marisoldelea@gmail.com,** ldain@fbmc.fcen.uba.ar

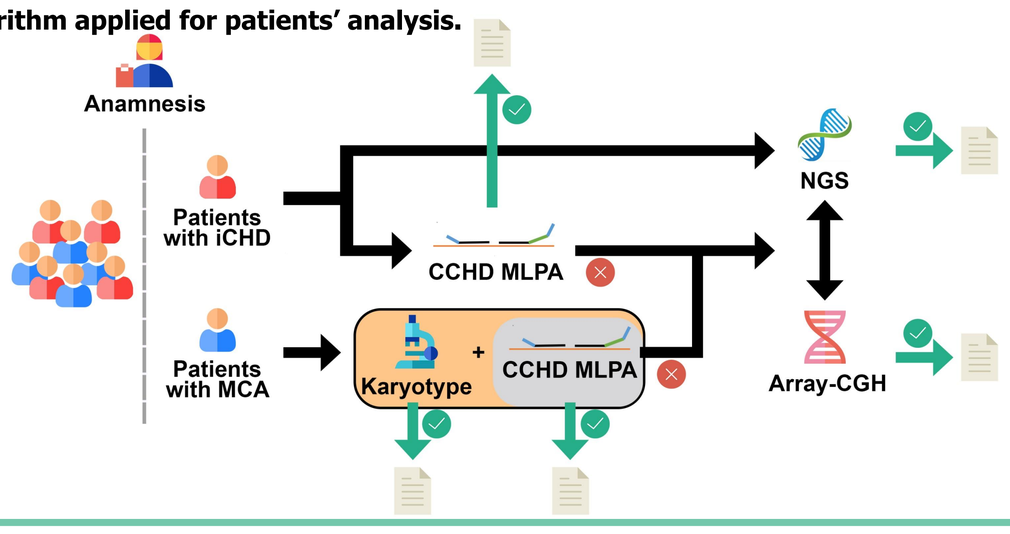
Introduction

Congenital anomalies (CA) are morphological and/or functional disorders of prenatal origin resulting from morphological disturbances in the process of human development. CA affect 3% to 5% of newborns and represent the second leading cause of infant mortality in Argentina, after perinatal conditions. Newborns presenting multiple congenital anomalies (MCA) have a prevalence of 2.26/1,000 births whereas congenital heart defects (CHD) are the most frequent CA, with a prevalence at birth of 4.06/1,000 births[1]. The etiology of these defects is widely recognized as heterogeneous, with contribution of genetic (~40%) and environmental/maternal factors (~5-10%). Nevertheless, in 50% of the cases, the etiology remains unknown. Although largely studied in several populations, there are few studies on the genetic contribution of CA in Latin America.

Objective

The aim of this study was to identify the genetic causes in Argentinean patients with MCA and isolated CHD (iCHD).

Algorithm applied for patients' analysis.

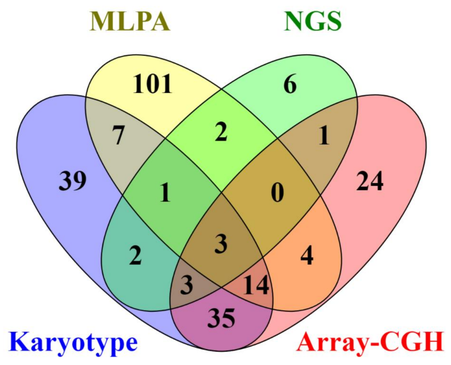


Methods

- **Patients:** 174 MCA and 194 iCHD born between June 2015 and August 2019 in 13 public hospitals participating in the National Network of Congenital Anomalies of Argentina (RENAC) and patients up to 16 years attending at the Genetic Services of Hospital Sor María Ludovica and Hospital El Cruce, Buenos Aires Province.
- **DNA** was extracted from peripheral blood by standard procedures.
- **Cytogenetic Analysis** was performed on GTW-banded metaphases in MCA patients
- **Multiple-dependent Ligation Probe Amplification (MLPA,** SALSA P250 kit, MRC-Holland) was performed in 137 patients with conotruncal CHD (CCHD) or 22q11 deletion syndrome (22q11DS) phenotypes.
- **Array-CGH** (Agilent 8X60K) was performed in 89 MCA selected patients.
- **Next generation sequencing** was performed in 18 patients with suspected syndromes and/or family history by TruSight® Cardio Sequencing kit (Illumina, n=6) or Whole exome sequencing (WES) (Agilent SureSelect Human All Exon V6 and V7 kit ,n=12) followed by an *in-silico* selection of candidate genes for variant analysis.

Results

A total of 276 patients were studied by at least one technique. The Venn diagram shows the 252 successfully analyzed samples (145 MCA and 107 iCHD).



Cytogenetic Analyses

A total of 104/174 patients with MCA were successfully karyotyped.

Karyotype analysis

Karyotype	N	N (%)
46,XX	51	90 (86.5)
46, XY	39	
47,XY,+13	1	
47,XY,+18 or 47,XX,+18	6	
47,XXX/47,XX+14[a]	1	
46,XY,t(1;2)(q25;q21) [a]	1	
46,XX,t(11;17)(p10;p10)	1	14 (13.4)
46,XX,del(15)(q11.2q13)	1	
46,XX,?del(21)	1	
47,XX,+mar	1	
46,XY,?trp(8)(p21.1p21.2)	1	
Total	104	104 (100)

a:These patients were also analyzed by array-CGH, see below

MLPA

A total of 132/137 were successfully analyzed: 27 patients (20.5%) presented at least one chromosome imbalance.

MLPA analysis

	CCHD MCA	iCCHD	Suspected 22q11DS	Total
Normal	18	60	27	105
Del 22q11 (3Mb)	5	13	3[a]	21
Del 22q11 (1.5Mb)	1	2	-	3
Dup 22q11 (1.5Mb)	1	1	-	2
Del 22q11.2 (*TBX1*)	-	1	-	1
Total	25	77	30	132

Del: Deletion Dup: duplication. CCHD: Conotruncal CHD, MCA: Multiple Congenital anomalies; iCCHD. isolated CCHD: 22q11DS: 22q11 deletion syndrome. (without CCHD); a: These 3 patients had an iCHD. these results are partialy reported else where [2].

Next Generation Sequencing (NGS)

Of the 18 selected samples analyzed, 12 presented clinically relevant nucleotide variants (67%).

Clinically relevant genetic variants detected by NGS

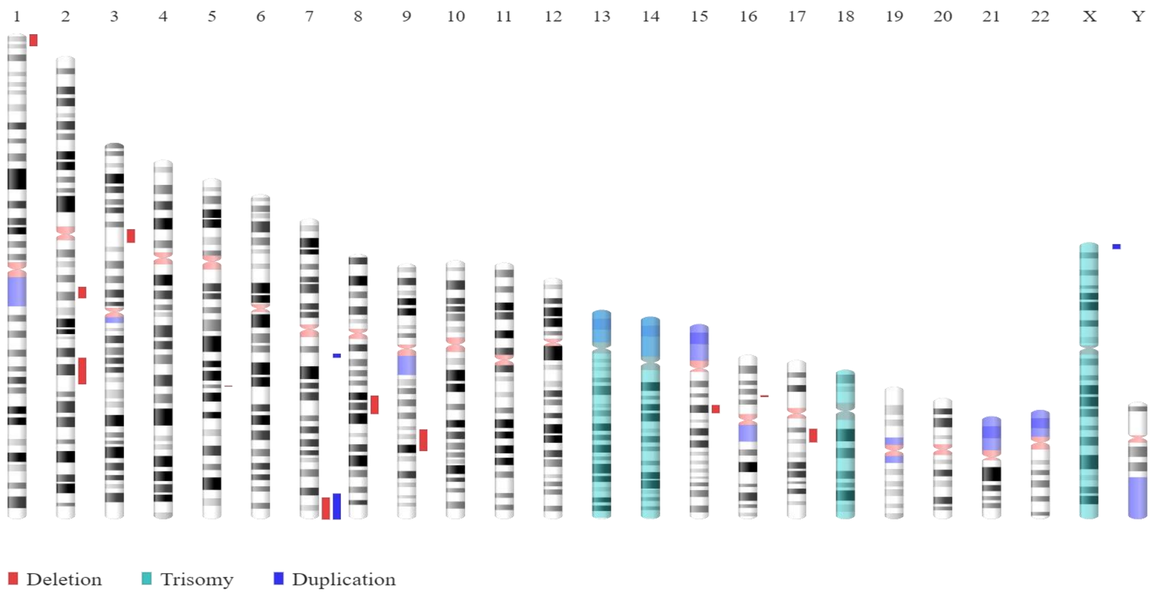
Gene	ACMG classification	Protein change	Phenotype
SHH	Likely Pathogenic	p.His270Tyr[a]	MCA
MYH11	Pathogenic	p.?[a,b]	MCA
PTPN11	Pathogenic	p.(Ala461Thr)	MCA[c]
FOXL2	Likely Pathogenic	p.(Tyr215Cys)	MCA
PTPN11	Pathogenic	p.Asn308Asp	MCA
EP300	Pathogenic	p.(Gln2361Ter)[a]	MCA[d]
PTPN11	Pathogenic	p.(Asp61Asn)	MCA[c]
KAT6B	Pathogenic	p.(Thr1525IlefsTer25)	MCA[e]
MYBPC3	Likely Pathogenic	p.(Arg726Cys)	MCA/iCHD[c]
RAF1	Pathogenic	p.(Ser257Leu)	iCHD[c]
MYH7	Likely Pathogenic	p.(Asn224Ile)[a]	iCHD[c]

iCHD: isolated Congenital Heart Disease MCA: Multiple Congenital Anomalies ACMG: American College Medical Genetics and genomics; a: Novel; b: This variant is a deletion of a splice acceptor site. c: Analyzed by TruSight® Cardio Sequencing kit. d: This patient also presented a 0.02 Mb pathogenic deletion at 5q22.2. e:Already described [3].

Array-CGH :

A total of 84/89 selected samples were successfully analyzed by a-CGH. We found 17 clinically relevant Copy Number Variations (CNVs) in 16 patients (19%).

Ideogram of clinically relevant CNVs found by a-CGH.



Clinically relevant CNVs found by array-CGH

ACMG classification	Patients	Unbalances	Size (Mb)	MIM #
Pathogenic	14	Del 1p36.33p36.23, Dup 7q35q36.3[a,b]	7.10,12.2	607872
		Del 2q24.2q31.1	13.73	-
		Del 2q14.2q14.3	7	612345
		Del 5q22.2[c]	0.02	-
		Del 7q36.1q36.3[a]	10.06	-
		Dup 7q11.23	1.27	609757
		Del 8q21.11q21.3[b,d]	11.19	614230
		Del 9q22.2q31.1	12	-
		T13[a]	-	-
		Del 15q14	6.22	616898
		Del 16p12.2	0.57	136570
		T18[a]	-	-
		Dup Xp22.33	1.7	-
		TX,T14[e]	-	-
Likely Pathogenic	2	Del 3p21.31	4.1	-
		Del 17q25.3	0.50	-

ACMG: American College of Medical Genetics and Genomics; Dup: Duplication, Del: Deletion, T: Trisomy. a:Cytogenetic study failed. b: Parents presented a normal karyotype. c: This patient was studied by NGS see below. d:This patient presented a 46,XY,t(1;2)(q25;q21) karyotype. e: This patient presented a mosaic doble trisomy when validated by a cytogenetic study already described [4].

Conclusions

- Using an algorithm that combines molecular techniques with clinical and genetic evaluation, we determined a genetic cause in 66 patients with an overall diagnostic yield of 26.2%, similar to other studies in different populations.
- 40% of cases did not have a karyotype due to culture failure or difficulties in samples 'referral. In these cases it is important to apply array-CGH analysis to overcome technical difficulties in cytogenetic studies and for the detection of clinically relevant CNVs.
- 22% of cases with iCCHD presented imbalances in the 22q11 region. In these cases early diagnosis and interventions are key to prevent clinical complications.
- The diagnostic yield of CMA, as a second or third-tier test for MCA patients from the Argentinian public health system was 19%.
- Details on patient's phenotypes and family history were key to achieve genetic diagnosis in 67% of cases using NGS approaches.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

****PID Task Force: CNGM:** Buzzalino N, Castro T. **Htal. El Cruce**. Antonietti L,. Arrospide N, Scandizzo E, Qualina V, Romero E. Anoni P , Ortega D. **Htal. Evita Pueblo:** Tomasoni T, Luna L, Zalazar ML, Stremiz DE. **Htal. Iriarte**: Monti F, Flores Y, Carballido GL. **Htal. Melendez:** Heevel V, Gomez V, Molina N. **Htal. Alende:** Cuesta C. **Htal. A. Goitía:** Vera V, Vilardo MA **Htal. Eurnekián:** Garello J, Marques V, Marquez MA. **Htal. Evita:** Raggio M. **Htal. L. Gandulfo:** Mangiante O, Amor D. **Htal. N. López**: Jewtuszyk M, Val S. **Htal Materno Infantil "Dr. Garlos Gianantonio":** Senra BC. **Htal. Presidente Perón**: Izzo N, Brautigam MP. **Maternidad R. Sarda:** Fernández G, Arbones MC. **Htal. Sor Maria Ludovica:** Cecotti N.

Fundings: National Agency for the Promotion of Science and Technology (Grant ID: PID 2012-0060)

Refererences

1. Groisman, B (2013). RENAC: Registro Nacional de Anomalías Congénitas de Argentina. Archivos Argentinos de Pediatría, 111(6), 0–0.
2. Delea, M., (2018). Genetic Imbalances in Argentinean Patients with Congenital Conotruncal Heart Defects. Genes, 9(9). https://doi.org/10.3390/genes9090454
3. Mendez, R (2020). A novel pathogenic frameshift variant of KAT6B identified by clinical exome sequencing in a newborn with the Say–Barber–Biesecker–Young–Simpson syndrome. Clinical Dysmorphology, 29(1), 42.
4. Massara, L. S. (2019). Double Autosomal/Gonosomal Mosaic Trisomy 47,XXX/47,XX,+14 in a Newborn with Multiple Congenital Anomalies. Cytogenetic and Genome Research, 159(3), 137–142.