Array-CGH: importance in the study of developmental delays in pediatrics

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Introduction. Global developmental delay (GDD) is an intellectual and adaptive impairment in infants under 5 years of age who fail to meet expected developmental milestones. Intellectual disability is characterized by limitation in intellectual function and adaptive behavior, with onset in childhood. Frequent identifiable causes of GDD and intellectual disability are chromosomal imbalances. The array comparative genomic hybridization (aCGH) has contributed to improve the detection rate of genetic abnormalities and is considered the first-tier genetic test for unexplained intellectual disability.

Aim. To analyze the results of a genetic study by aCGH due to GDD or intellectual disability in pediatric patients.

Patients and methods. Retrospective analysis of pediatric patients followed in outpatient, which underwent a genetic study by aCGH, from 2012 to 2017.

Results. 215 patients were studied by aCGH. Of the total, 64.2% were investigated for intellectual disability and 35.8% for GDD. A 23.3% presented aCGH deletions or duplications, 56% for intellectual disability and 44% for GDD, with chromosomes 16, 22, 2 and 1 being the most implicated.

Conclusion. Our study demonstrated a higher prevalence in males, according to previously published reports. The rate of detection abnormalities classified as pathogenic was higher than in other studies.

Key words. Array comparative genomic hybridization. Genetic diagnosis. Global development delay. Intellectual disability. Neurodevelopment. Pediatrics.

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Introduction

Global developmental delay (GDD) is an intellectual and adaptive impairment in infants and young children under 5 years of age who fail to meet expected developmental milestones in multiple areas of functioning. Not all children with GDD will have criteria for ID in future [1].

Intellectual disability (ID), classified by *Diagnostic and statistical manual of mental disorders, fifth edition* (DSM-5), is a neurodevelopment disorder more prevalent in male (1.2-1.6:1), characterized by limitation in intellectual function, confirmed by standardized psychometric test, and at least one area of adaptive behavior: conceptual, social and practical, with onset in childhood and presenting before 18 years of age [1,2]. It is a major public health problem because affects 1-3% of the population [1,3]. The term ID replaced the designation of 'mental retardation' [4].

Important risk factors for ID include low level of maternal education, advanced maternal age, and poverty [1].

The etiology of GDD and ID includes prenatal causes: genetic disorders (> 50%; 15% chromosomal abnormalities as trisomy 21), inborn errors of metabolism (3%), maternal diseases, congenital infections, brain disorders and intrauterine exposure to alcohol, toxins or teratogens (phenytoin, valproate); perinatal: encephalopathy due to intrapartum asphyxia, intracranial hemorrhage; postnatal: traumatic and hypoxic brain injuries, infections, demyelinating diseases, epilepsy, metabolic diseases, intoxications by lead or mercury and radiation. A minority is due to environmental factors (malnutrition) [1,2].

The etiology remains unknown in most of the cases [5,6]. With the advent of next-generation sequencing techniques, it is possible that this percentage will decrease.

There are medical and physical conditions commonly associated with ID as cerebral palsy, congenital heart disease, endocrine abnormalities, obesity, eating disorders, anxiety, seizures, sleep disorders, attention deficit hyperactivity disorder or autism [1].

The approach includes a detailed history (prenatal and perinatal problems, developmental progress, behavioral, social and educational history; three-generation family history; consanguinity; medical problems; medication), physical examination (somatometry, neurologic examination, dysmorphic features, cutaneous findings, skeletal changes) and the availability of standardized intelligence tests and other specific tests [3],

The array comparative genomic hybridization (aCGH), which detects submicroscopic cytogenetic abnormalities, mostly not identified by high resolution karyotype, has successfully contributed to improve the detection rate of genetic abnormalities and is considered the first-line genetic test for unexplained ID. Besides that, the use of aCGH has led to the identification of approximately 50 recurrent copy number variations (CNVs) that are found in the general population but detected with increased frequency in individuals with ID, autism, epilepsy or schizophrenia [1,3].

Other tests that should be considered if there are specific features in the history or abnormal findings on physical examination, includes electroencephalography, magnetic resonance imaging, fragile X syndrome test (the most prevalent form of inherited ID in males), metabolic screening, karyotype analysis (used in cases of suspicion of mosaicism or to clarify rearrangements identified in the aCGH, for example marker chromosomes or derivative chromosomes resulting from a translocation or inversion present in one of the parents), fluorescence in situ hybridization (FISH), and more recently the next-generation sequencing (NGS) including whole exome sequencing, whole genome sequencing and NGS specific gene panels [2,3].

The aim of this study was to evaluate the results of a genetic study using aCGH in GDD or ID pediatric patients.

Patients and methods

We performed a retrospective and descriptive analysis, based on review of digital clinical records of pediatric patients followed in outpatient at a Portuguese 3 level hospital, which underwent a genetic study by aCGH, from the years 2012 to 2017. Analyzed demographic variables included age and gender, past history, the reason for referral and the result of the genetic study. aCGH was performed using the Agilent 4x180K platform and cytogenomics 4.0.2.21. CNVs were classified as pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign and benign, in accordance with the American College of Medical Genetics Standards (ACMGS) and *Guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013* [7].

This study was approved by the Ethics Committee of Centro Hospitalar e Universitário de São João.

The statistical analysis of the collected data was performed with recourse to Microsoft Office Excel.

Results

In this study 215 patients were studied by aCGH, 120 (55.8%) male and 95 female.

Of the total, 35.8% were investigated for GDD and 64.2% for ID, with 3.8 and 7.4 years old of median age at the moment of the hospital referral, respectively.

This complementary exam was requested by clinical geneticist in 92 (42.8%) cases, developmental behavioral pediatricians in 71, neuropediatrics in 46, metabolic diseases clinicians in three cases, child and adolescent psychiatrists in two cases and one case by pediatrics, besides that 73.5% were observed in an outpatient genetic clinics.

There were 39 (18.1%) of patients with congenital malformations as ventricular septal defect, cataract or cleft lip and palate, 36 with dysmorphic signs, mainly facial, 36 with epilepsy and 18 with autism spectrum disorder.

Of these patients, 50 (23.3%) presented deletions or duplications CNVs, 56% for ID and 44% for GDD, classified as pathogenic or likely pathogenic, with chromosomes 16 (n = 8), 22 (n = 6), 2 (n = 5) and 1 (n = 5) being the most implicated. Table I and II shows patients' data and aCGH results, table II shows the CNVs found and the parent's study, if available. In these cases, 48% of the parents were investigated in a genetics outpatient, and 24% (n = 12) had the same change reported in their children's aCGH.

When aCGH was normal, subsequent or complementary investigation revealed etiology in 31 cases (14.4%).

Discussion

The history and physical examination identify the etiology of ID in 17 to 34 percent of cases [1,8]. The exhaustive etiological investigation of individuals with ID entails large economic, family or individual

Table II. Patient's data (*n* = 215).

		n	%
Sex	Male	120	55.8
	Female	95	44.2
Cause of investigation	Global developmental delay	77	35.8
	Intellectual disability	138	64.2
aCGH requested by	Clinical geneticist	92	42.8
	Development behavioral pediatricians	71	33.0
	Neuropediatrics	46	21.4
	Metabolic diseases clinicians	3	1.4
	Adolescent psychiatrists	2	0.9
	Pediatrics	1	0.5
Genetic counseling		158	73.5
Past medical history	Congenital malformations	39	18.1
	Dysmorphic signs	36	16.7
	Epilepsy	36	16.7
	Autism spectrum disorder	18	8.4
aCGH with deletions or duplications CNVs		50	23.3

aCGH: array comparative genomic hybridization; CNVs: copy number variations.

Table II. aCGH with deletions or duplications CNVs (*n* = 50).

		n	%
aCGH association	Global developmental delay	28	56
	Intellectual disability	22	44
CNVs classification	Pathogenic	20	40
	Likely pathogenic	29	58
	Variant of uncertain significance	1	2

aCGH: array comparative genomic hybridization; CNVs: copy number variations.

costs [9]. However, after the clinical investigation, aCGH study is most helpful for the etiological diagnosis of GDD or ID and consequently, for better management, prognostic definition and genetic counseling, including the planning of reproductive choices and the use of prenatal diagnosis and preimplantation genetic testing. A specific diagnosis provides patients and pediatricians with information about expected natural history and avoids the need for other expensive and invasive tests. Furthermore, it is expected that the new knowledge generated by identifying specific diagnoses bring on new specific treatments [3,6].

aCGH study provides a genome-wide scan of CNVs (microdeletions and microduplications), involving hybridization of a patient's DNA onto predetermined targets representative of the whole genome (in this case synthetic oligonucleotide probes) spotted onto glass slides and subsequentially scanning and analysis of the fluorescence ratio profiles with a specific software.

To determine the clinical significance CNVs findings were compared with Database of Genomic Variants (DGV) and Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources (DECIPHER) data previously reported. Besides that, clinical data were compared with the literature (OMIM, ECARUCA, Orphanet) trying to establish an association between the data [10].

Our study demonstrated a higher prevalence in males, according to previously published reports [1]. The presence of epilepsy (16.7%) and autism spectrum disorder (8.4%) was lower than in other published studies (22.2% and 10.1%, respectively) [11].

The rate of detection abnormalities classified as pathogenic was higher (23%) than in other studies (15-20%) [1,12].

The onset (3 years-old) of etiological investigation in the pre-school was similar to other centers [2].

In patients with moderate to severe ID in whom other standard tests (including aCGH, fragile-X in male patients, responsible for less than 1% of ID [13], and *MECP2* gene in female patients) have failed to identify the cause, NGS should be considered using trio-based whole exome sequencing [3].

However, there are causative genetic variants that are not detected by NGS such as large-scale genomic rearrangements or trinucleotide repeat expansions or because the related pathogenic variants are located in genes that are yet to be associated with ID or in regulatory regions whose role has not yet been recognized or due to epigenetic processes not detected by NGS [3].

Table III. CNVs found and the parent's study (*n* = 50).

Case	Sex	Gene	Phenotype/other clinical	Cause	Additional findings
1	F	*arr2q24.2(161,967,633-163,483,133)x1*	Hypotonia	GDD	*De novo* alteration
2	F	*arr 6p25.3 (266,079-378,956)x1*	Cranial asymmetry, maternal ID	GDD	Ø
3	F	*arr 3q29(192,759,379-197,845,254)x3*	Facial dysmorphia	GDD	Ø
4	F	*arr 16p13.11(15,048,751-16,292,235)x1*	Facial dysmorphia	GDD	*De novo* alteration
5	F	*arr 20q13.33(60,929,614-62,087,852)x3*	Epilepsy	GDD	Inherited from male progenitor
6	F	*arr 3p25.3(9,340,049-10,344,052)x3*	Finger pads, epicantus	GDD	*De novo* alteration
7	M	*arr 1q22-1q23.1(156,132,786-157,120,342)x3*	Macrocephaly	GDD	Ø
8	F	*arr21q22.12(37,484,659-37,612,992)x3*	Facial dysmorphia	GDD	Ø
9	M	*arr 22q11.21(18,651,614-21,464,119)x1*	Facial dysmorphia	GDD	*De novo* alteration
10	M	*arr 5p15.2 (11,472,074-11,679,358)x1*	–	GDD	Ø
11	F	*arr 18q21.2(52,942,337-53,141,098)x1*	Hypotonia, facial dysmorphia	GDD	*De novo* alteration
12	M	*arr 1q23.1 (161,967,426-162,280,549)x3*	–	GDD	Inherited from female progenitor
13	M	*arr 15q23-q24.1(72,429,509-74,343,898)x1*	Macrocephaly	GDD	*De novo* alteration
14	F	*arr 2p12-p11.2(77,919,423-87,060,262)x1*	Facial dysmorphia, epilepsy	GDD	Ø
15	M	*arr15q11.2(22,765,628-23,208,901)x1*	Autism spectrum disorder	GDD	Ø
16	F	*arr 16q24.3(89,325,387-89,559,189)x1*	Facial dysmorphia, deafness	GDD	Ø
17	M	*arr16p11.2(29,652,999-30,198,600)x1*	–	GDD	Ø
18	F	*arr 6p22.3(15,361,204-15,397,836)x1*	Strabismus, hyperthyroidism	GDD	*De novo* alteration
19	M	*arr 22q11.21(18,909,044-19,147,457)x3*	Macrocephaly, autism spectrum disorder, inverted nipples	ID	*De novo* alteration
20	M	*arr 2q33.1(200,119,529-200,556,471)x3*	–	GDD	*De novo* alteration
21	M	*arr16p13.3 (6,889,408-6,964,191)x1*	–	ID	Inherited from female progenitor
22	M	*arr 17q12(34,450,405-36,243,028)x1*	Renal pathology	ID	Ø
23	M	*arr 2p16.3(51,193,626-51,476,523)x1*	Attention-deficit/hyperactivity disorder	ID	Inherited from female progenitor
24	M	*arr 8p23.1(8,100,384-11,860,569)x3*	–	ID	Ø
25	F	*arr16p11.2(29,652,999-30,198,600)x1*	Syndactyly	ID	Ø
26	M	*arr 19q13.32-q13.33(47,773,137-48,254,624)x3*	Family history of ID	ID	Ø
27	F	*arr 9q33.1(119,501,358-119,548,870)x1*	Father history of ID	ID	Inherited from female progenitor

Table III. CNVs found and the parent's study (n = 50). *(cont.)*.

Case	Sex	Gen	Phenotype/other clinical	Cause	Additional findings
28	M	*arr 1q43(239,855,264-239,912,160)x1*	Hypotonia	ID	Ø
29	F	*arr 17p11.2(16,757,564-20,463,361)x3*	Heart disease	ID	*De novo* alteration
30	M	*arr 2q33.3(207,639,004-207,657,132)x1*	Epilepsy, strabismus	ID	Ø
31	M	*arr 1q43(237,381,873-237,497,031)x1*	Hypotonia, microcephaly, epilepsy	ID	Ø
32	M	*arr15q11.2(22,815,306-23,059,073)x1*	Epilepsy, clubfoot	ID	Ø
33	F	*arr 22q11.21(18,894,835-21,464,119)x1*	Cleft lip and palate	ID	Ø
34	F	*arr 7q11.23(74,090,390-76,214,077)x3*	Strabismus, cataract	ID	*De novo* alteration
35	M	*arr 8p21.3(22,222,050-22,370,282)x3*	Hemiparesis	ID	*De novo* alteration
36	F	*arr 22q13.33 (50,425,989-50,579,476)x1*	Sister history of ID	ID	Ø
37	F	*arr 22q13.33 (50,425,989-50,579,476)x1*	Sister history of ID	ID	Ø
38	M	*arr16p11.2(29,133,676--30,198,600)x1*	–	ID	Inherited from female progenitor; also present in the brother
39	F	*arr 7q11.23(75,160,961-76,214,077)x1*	Facial dysmorphia, epilepsy, macrocephaly	ID	Ø
40	F	*arr 20q13.33(61,645,627-62,147,345)x3*	Attention-deficit/hyperactivity disorder	ID	Ø
41	F	*arr 16p13.11 (14,968,855-16,292,235)x3*	Facial dysmorphia, pectus excavatum	ID	Inherited from male progenitor
42	F	*arr 7q11.21(62,460,665-63,412,662)x3, 8p11.23p11.21(37,228,320-43,396,776)x3, 10p11.21p11.1(35,841,635-39,076,591)x3*	Mosaic variegated aneuploidy syndrome	ID	Ø
43	F	*arr15q11.2(22,765,628-23,208,901)x1*	–	ID	Inherited from female progenitor; also present in the brother
44	M	*arr 17q12(34,817,422-36,209,228)x3*	–	ID	Inherited from female progenitor
45	F	*arr 1q21.1(145,632,334-145,833,054)x1, 8p21.3(22,222,050-22,370,282)x3*	–	ID	Ø
46	M	*arr 5p13.2(37,351,249-37,439,604)x3, 22q11.21(18,894,835-19,010,508)x1*	Facial dysmorphia, autism spectrum disorder	ID	dup 5p13.2 (inherited from female progenitor); del 22q11.21 (inherited from male progenitor)
47	F	*arr Xp22.31-q11.2(7,867,300-61,931,689)x3*	Facial dysmorphia, epilepsy	GDD	Inherited from female progenitor
48	M	*arr 16q24.1-24.2(86,725,387-87,845,741)x1*	Epilepsy	GDD	Ø
49	M	*arr 19p13.2(12,615,605-12,814,116)x3*	Autism spectrum disorder	GDD	Inherited from male progenitor
50	F	*arr 8p23.1-pter(176,814-6,939,296)x3, 11q24.2-qter(124,518,113-134,927,114)x1*	Facial dysmorphia, heart disease	ID	Ø

F: female; GDD: global developmental delay; ID: intellectual disability; M: male.

References

1. Pivalizza P, Lalani S, Firth HV, Bridgemohan DC. Intellectual disability in children: definition, diagnosis, and assessment of needs. UpToDate 2018. URL: http://www uptodate com/contents/intellectual-disability-in-children-definition-diagnosis-and-assessment-of-needs. [21.02.2018].
2. Pereira C, Martins R, Bandeira de Lima C, Baptista M, Sousa A. Perturbação do desenvolvimento intelectual/incapacidade intelectual: experiência de um centro de neurodesenvolvimento de um hospital de nível III. Acta Pediatr Port 2017; 48: 304-11.
3. Amor DJ. Investigating the child with intellectual disability. J Paediatr Child Health 2018; 54: 1154-8.
4. American Psychiatric Association. Diagnostic and statistical manual of mental disorders, fifth edition (DSM-5). Washington DC: American Psychiatric Publishing; 2013.
5. Oliveira R, Rodrigues F, Venâncio M, Saraiva JM, Fernandes B. Avaliação e investigação etiológica do atraso do desenvolvimento psicomotor/défice intelectual. Saúde Infantil 2012; 34: 5-10.
6. Vissers LE, Gilissen C, Veltman JA. Genetic studies in intellectual disability and related disorders. Nat Rev Genet 2016; 17: 9-18.
7. South ST, Lee C, Lamb AN, Higgins AW, Kearney HM. ACMG standards and guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013. Genet Med 2013; 15: 901-9.
8. Moeschler JB, Shevell M. Clinical genetic evaluation of the child with mental retardation or developmental delays. Pediatrics 2006; 117: 2304-16.
9. Lopes A, Correia C, Guardiano M, Dória S, Guimarães M. Défice cognitivo: até onde investigar. Acta Pediatr Port 2011; 42: 225-7.
10. Arican P, Dundar NO, Ozyilmaz B, Cavusoglu D, Gencpinar P, Erdogan KM, et al. Chromosomal microarray analysis in children with unexplained developmental delay/intellectual disability. J Pediatr Genet 2019; 8: 1-9.
11. Oeseburg B, Dijkstra GJ, Groothoff JW, Reijneveld SA, Jansen DEC. Prevalence of chronic health conditions in children with intellectual disability: a systematic literature review. Intellect Dev Disabil 2011; 49: 59-85.
12. Beaudet AL. The utility of chromosomal microarray analysis in developmental and behavioral pediatrics. Child Dev 2013; 84: 121-32.
13. Coffee B, Keith K, Albizua I, Malone T, Mowrey J, Sherman SL, et al. Incidence of fragile X syndrome by newborn screening for methylated FMR1 DNA. Am J Hum Genet 2009; 85: 503-14.

Relevancia de los *arrays* de hibridación genómica comparada en el estudio de los retrasos del desarrollo en pediatría

Introducción. El retraso general del desarrollo (RGD) constituye un trastorno intelectual y del comportamiento adaptativo que aparece en los niños menores de 5 años que no consiguen alcanzar los hitos del desarrollo normal. La discapacidad intelectual se caracteriza por la limitación en el funcionamiento intelectual y en el comportamiento adaptativo, surgida en la infancia. Entre las causas frecuentes y reconocibles del RGD y de la discapacidad intelectual se encuentran los desequilibrios cromosómicos. Los *arrays* de hibridación genómica comparada (aCGH) han contribuido a mejorar la tasa de detección de las anomalías genéticas y ya se consideran la prueba genética de elección para la discapacidad intelectual de origen desconocido.

Objetivo. Analizar los resultados del estudio genético con aCGH motivado por un RGD o una discapacidad intelectual en pacientes pediátricos.

Pacientes y métodos. Análisis retrospectivo de pacientes pediátricos sometidos a seguimiento ambulatorio que fueron objeto de un estudio genético con aCGH entre 2012 y 2017.

Resultados. El número de pacientes sometidos al estudio con aCGH ascendió a 215. Del total, el 64,2% fueron investigados por discapacidad intelectual, y el 35,8%, por RGD. El 23,3% presentó deleciones o duplicaciones en la aCGH; el 56%, por la discapacidad intelectual; y el 44%, por el RGD, y los cromosomas 16, 22, 2 y 1 fueron los implicados con más frecuencia.

Conclusión. El presente estudio demuestra la mayor prevalencia de ambos en el sexo masculino, en consonancia con otras publicaciones precedentes. La tasa de detección de las anomalías clasificadas como patógenas resultó superior a la notificada en otros estudios.

Palabras clave. *Array* de hibridación genómica comparada. Diagnóstico genético. Discapacidad intelectual. Neurodesarrollo. Pediatría. Retraso general del desarrollo.