



Research Note

Array-CGH analysis in patients with intellectual disability and/or congenital malformations in Brazil

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ABSTRACT. In several patients, intellectual disability and/or congenital malformation may be attributed to chromosomal changes. In this study, we conducted an array-CGH test of 200 patients from the Northeast of Brazil with intellectual disability and/or congenital malformation. Blood samples were collected from the proband and from their parents when possible. DNA was extracted and investigated using the array-CGH test. Findings were evaluated for the pathogenicity in databases of benign and pathogenic changes (ISCA, UCSC, DGV, and DECIPHER). Forty-seven copy number variations (CNVs) were identified in 43/200 (21.5%) patients, including 25/98 (25.5%) in males and 22/102 (21.57%) in females. We considered 33 of these to be clinically significant, reaching a diagnosis rate of 16.5%. The sizes of the CNVs varied from 102 kb to 24 Mb in deletions and from 115 kb to 140 Mb in duplications. In 10/47 (21.3%) patients, the rearrangement involved a sex chromosome. Thirty-nine patients had one chromosomal aberration, while 2 concomitant abnormalities were detected in 4 patients.

Ten of 47 CNVs (21.3%) were > 5Mb in size. Fifteen patients had CNVs related to known syndromes. This research highlights the contribution of submicroscopic chromosomal changes to the etiology of intellectual disability and/or congenital malformation, particularly the implication of chromosomal abnormalities detected using an array-CGH test, with a high rate of 16.5%. Thus, our results support the use of array-CGH replacing standard karyotype as the first-tier cytogenetic diagnostic test for patients with multiple congenital anomalies and/or intellectual disability.

Key words: Array-CGH; Congenital malformation; Intellectual disability; Northeastern Brazilian patients

INTRODUCTION

Epidemiological studies suggest that intellectual disability (ID) affects 2-3% of the general population worldwide and may occur with or without multiple congenital anomalies (MCA) (Rauch et al., 2006; Galasso et al., 2010; Pani et al., 2010; Ropers, 2010).

Over the past 50 years, conventional karyotyping has been successfully used to identify chromosomal abnormalities. Karyotype has the advantage of surveying the entire genome at once, but the resolution of cytogenetic analysis is limited to approximately 5-10 Mb. In the past 20 years, new molecular techniques have been introduced and smaller structural variations among submicroscopic changes so-called copy number variations (CNVs) can only be identified through molecular approaches, such as fluorescent *in situ* hybridization and multiplex ligation-dependent probe amplification and microarray techniques.

The microarray technique, introduced in 2003 to investigate genome-wide chromosome imbalances in patients with ID and/or MCA, can detect up to 99% of all chromosomal changes (Vissers et al., 2003; Hochstenbach et al., 2009).

Because of its high diagnostic yield, effectiveness and health-care possibilities, array-based screen for CNVs was recommended in 2010 by the American College of Medical Genetics and Genomics as the preferred first clinical genetic diagnostic test for patients with developmental delay, ID or MCA (Newman et al., 2007; Wordsworth et al., 2007; Gijsbers et al., 2009; Manning et al., 2010; Miller et al., 2010; Regier et al., 2010; South and Brothman, 2011; Bartnik et al., 2014). In addition, microarray analyses provide accurate diagnosis in approximately twice as many cases as classical karyotyping and fluorescent *in situ* hybridization (Gijsbers et al., 2009; Hochstenbach et al., 2009; Ahn et al., 2010; Miller et al., 2010). In recent years, approximately 15-20% of ID cases are caused by submicroscopic CNVs (Zahir and Friedman, 2007; Gijsbers et al., 2009).

In Brazilian studies, genome changes investigated by array techniques have been detected in up to 25% of patients with idiopathic syndromic ID (Krepischi-Santos et al., 2006; Hochstenbach et al., 2011).

Here, we present a pioneering study of array-CGH in a group of Northeastern Brazilian patients with ID and/or MCA with no etiological diagnosis.

MATERIAL AND METHODS

From April 2010 to July 2014, a total of 200 patients were referred for genetic diagnosis by medical genetic centers from the Northeastern states of Brazil. Our total group consisted of

98 (49%) males and 102 (51%) females. Inclusion criterion was ID without a defined etiology, associated or not with MCAs.

Sample collection and DNA extraction

Blood samples were collected from the patients and their parents, when available. DNA was extracted from peripheral blood leukocytes using a salting-out method according to standard protocols.

Array-CGH

Investigation was performed using array-CGH with 44 K (N = 1), 60 K (N = 180), and 180 K (N = 19) oligo-chips (Agilent Technologies, Santa Clara, CA, USA). DNA digestion, labeling, and hybridization were performed following the manufacturer instructions.

Analysis and CNV classification

All chromosomal abnormalities identified, including deletions and duplications of at least 3 adjacent probes or of a minimum region of 300 kb, were compared with genomic variants described in databases of benign and pathogenic changes to define their possible pathogenicity (DGV <http://projects.tcag.ca/variation/>, UCSC Genome Browser <http://www.genome.ucsc.edu/>, ISCA <https://www.iscaconsortium.org/>, and DECIPHER <http://decipher.sanger.ac.uk/>). Furthermore, gene content was also evaluated using databases such as the NCBI Gene Database, GeneCards, and OMIM.

CNVs were classified as described by Miller et al. (2010) and were classified into 4 groups: I) causatives, when associated with known microdeletion/microduplication syndromes; II) probably causative, when the CNV was not previously associated with known microdeletion/microduplication syndromes, but contained genes known to cause a syndrome of a clear phenotype (Ahn et al., 2013) or because similar CNVs were already described in patients from other studies; III) non-causative (benign); IV) VOUS, variants of unknown clinical significance.

RESULTS

Samples from 200 children with ID and/or MCA were analyzed. CNVs were identified in 43/200 (21.5%) patients (Figure 1); 25/98 (25.5%) males and 22/102 (21.57%) females. The CNVs included a total of 27 deletions and 20 duplications. In 10/47 (21.3%) patients, the rearrangement involved a sex chromosome. Thirty-nine patients (19.5%) had a chromosomal aberration, whereas 2 concomitant abnormalities (i.e., 1 duplication and 1 deletion) were detected in 4 patients (2%). Ten of 47 CNVs (21.3%) were >5 Mb in size.

A total of 33 causative or probably causative CNVs were detected (16.5%). Among these, microdeletion/microduplications related to known syndromes were identified in 15 patients (7.5%). These syndromes included: chromosome 16p11.2 deletion syndrome; Miller-Dieker lissencephaly syndrome; chromosome 14q11-q22 deletion syndrome; CHARGE syndrome; chromosome 1q41-q42 deletion syndrome; Williams-Beuren syndrome (two 2 cases); Ritscher-Schinzel syndrome; chromosome 7q11.23 duplication syndrome; chromosome 15q13.3 deletion syndrome; Wolf-Hirschhorn syndrome; neurofibromatosis; ichthyosis; and Klinefelter syndrome (Table 1).

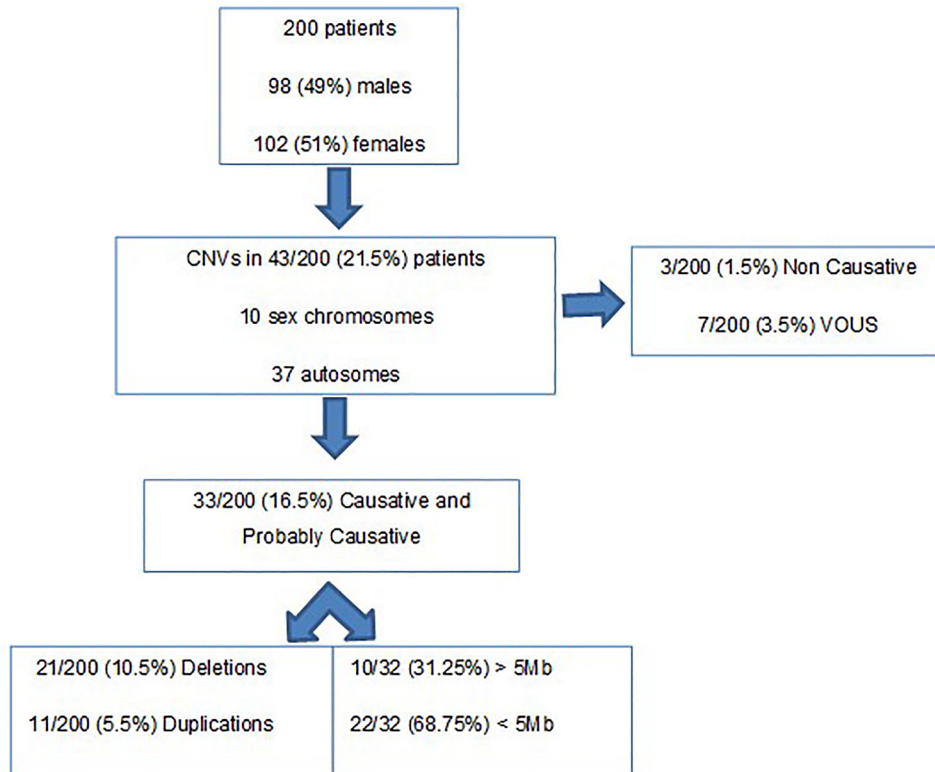


Figure 1. Flow diagram showing the number of patients studied and their respective CNVs.

Table 1. Array-CGH results of 15 Brazilian patients with causative CNVs associated with known microdeletion/microduplication syndromes (group I).

Patient	Gender	Resolution	Locus (Hg 18)	Size	Associated syndrome/OMIM
1	M	60 k	array 1q41q42 (221.105.086-222.351.150)x1	1.3 Mb	1q41q42 Deletion syndrome (MIM ID #612530)
2	F	60 k	array 4p16.3-p16.1 (62.320-6.698.980)x1/array 12p13.33-p13.32 (100.698-10.177.433)x3	6.6 Mb/10 Mb	Wolf-Hirschhorn syndrome (MIM ID #194190)
3	F	60 k	array 7q11.23 (72.652.411-74.136.224)x1	1.5 Mb	Williams-Beuren syndrome (MIM ID #194050)
4	F	60 k	array 7q11.23 (72.404.248-73.831.333)x1	1.4 Mb	Williams-Beuren syndrome (MIM ID #194050)
5	M	60 k	array 7q11.23 (72.382.783-73.777.467)x3	1.5 Mb	7q11.23 Duplication syndrome (MIM ID #609757)
6	F	60 k	array 8q12.1-q12.2 (61.282.418-61.831.475)x1	549 kb	CHARGE syndrome (MIM ID #214800)
7	F	180 k	array 8q24.13 (126.051.391-126.389.649)x3	338 kb	Ritscher-Schinzel syndrome (MIM ID #220210)
8	M	60 k	array 14q12-q13.3 (31.097.626-36.478.503)x1	5.4 Mb	14q11-q22 Deletion syndrome (MIM ID #613457)
9	M	60 k	array 15q13.2-q13.3 (28.742.018-30.231.385)x1	1.48 Mb	15q13.3 Microdeletion syndrome (MIM ID #612001)
10	F	60 k	array 16p11.2 (29.581.468-30.098.094)x1	500 kb	16p11.2 Deletion syndrome (MIM ID #611913)
11	M	60 k	array 2q13 (110.219.763-110.322.064)x1/ array 17p13.3 (867.382-1.592.841)x1	725 kb/ 102 kb	Miller-Dieker syndrome (MIM ID #247200)
12	M	60 k	array 16p12.1 (21.907.260-22.315.593)x1	~400 kb	Recurrent 16p12.1 microdeletion (MIM ID #136570)
13	M	60 k	array 17q11.2 (26.057.789-27.351.206)x1	~1.3 Mb	Neurofibromatosis (MIM ID #162200)
14	M	60 k	array Xp22.31 (110.777.959-111.374.876)x1/ array 7q31.1 (110777959-111374876)x1	~1.5 Mb/ 700 kb	Ichthyosis (MIM ID #308100)
15	M	60 k	array Xp22.33-q28 (701-154.832.671)x2	XXY	Klinefelter syndrome (MIM ID #312300)

In 18 patients (9%), we detected probably causative CNVs that had not previously been associated with any known syndrome (Table 2).

Table 2. Array-CGH results of 18 Brazilian patients with probably-causative CNVs, not previously associated with known microdeletion/microduplication syndromes (group II).

Patient	Gender	Resolution	Locus (Hg 18)	Size
16	F	44 k	array 2p25.3 (20.141-2.071.858)x1	~2 Mb
17	M	60 k	array 7q21.11 (79.320.563-84.313.060)x1	4.9 Mb
18	M	60 k	array 9q34.11 (130.097.894-132.378.474)x1	2.2 Mb
19	M	60 k	array 11q13.1 (63.365.945-64.625.636)x1	~1.27 Mb
20	F	60 k	array 13q21.33-q22.3 (67.907.119-77.776.997)x1	9.8 Mb
21	F	180 k	array 14q32.31-q32.33 (102.717.528-107.287.649)x3	4.5 Mb
22	F	60 k	array 15q11.2-q13.1 (18.946.017-26.830.949)x3	8 Mb
23	F	60 k	array 15q26.3 (97.606.456-100.201.002)x1	2.6 Mb
24	M	60 k	array 16q11.2-q22.3 (45.058.116-70.424.463)x3	25 Mb
25	F	60 k	array 17p13.3 (2.340.484-2.516.432)x1	175 kb
26	M	60 k	array 18p11.32-p11.21 (145.520-14.071.934)x1	13.9 Mb
27	M	60 k	array 18q21.2 - q23 (51.915.019-76.083.258)x1	~24 Mb
28	F	60 k	array 18q12.3-q22 (38.288.660-67.206.375)x3	~30 Mb
29	F	60 k	array 22q11.22-11.23 (21.386.362-23.318.602)x3	~1.9 Mb
30	M	60 k	array Xq13.2-q13.3 (73.422.371-74.266.467)x2	~800 kb
31	M	60 k	array Xq24 (118.593.371-118.725.211)x1	130 kb
32	M	60 k	array Xp22.31 (8.393.893-8.649.904)x2	~250 kb
33	F	60 k	array Xq27.2-q28 (140.565.997-154.886.088)x1/ array Xp22.33-q27. (701-140.385.067)x~3	14 Mb /140 Mb

DISCUSSION

Several studies have shown that it is essential for patients with ID and/or MCA to obtain a definitive diagnosis to receive early medical interventions, better clinical management (Coulter et al., 2011; Riggs et al., 2014), and proper genetic counseling (Pina-Neto, 2008).

In this study, we identified CNVs in 43 of 200 patients (21.5%), 33 of which were considered to be clinically significant, bringing the causative diagnosis rate to 16.5%.

This is the first report of a group of patients from Northeastern Brazil with ID and/or MCA using array-CGH to detect CNVs. Although the selection criteria for array-CGH investigations are highly variable, similar reports in the Brazilian population showed approximately the same detection rate of pathological CNVs. Among 95 Brazilian syndromic patients, Krepischi-Santos et al. (2006) accounted for 17% of CNVs to be causally related to abnormal phenotypes. Pereira et al. (2014) detected 22% of pathogenic CNVs in 15 patients from Central Brazil who had IDs.

In our study, among the 33 causative and probably causative CNVs (Tables 1 and 2), only 8 patients (4%), who had CNVs larger than 5 Mb that could have been detected by karyotype, had this test been offered previously. Thus, the remaining 25 patients would be undiagnosed after conventional cytogenetics, indicating the major contribution of array-CGH for diagnosing these patients.

Interestingly, Klinefelter syndrome was not clinically suspected. This patient was referred at 10 years old, in the prepubertal stage, when hypogonadism had not been characterized. Although his height was in 90th percentile, the parents complained of poor school performance, attention deficit hyperactivity disorder, and bipolar disorder. In this scenario, Klinefelter syndrome was not clinically suspected. Because of the patient's age, it was not possible to diagnose hypogonadism. The unique features related to Klinefelter observed in this patient were height in the 90th percentile and poor school performance. The main feature of this patient was ID and psychiatric disorder.

Patient 33 had a karyotype revealing an extra rearranged X chromosome, suggesting a 47,XXX. However, the clinical phenotype was not completely compatible with the described features of triple X females (Butnariu et al., 2013). The array-CGH test was essential for defining the break points and showed that although she indeed had 3 copies of *SHOX* (Xp22.33), which may explain her high stature (180 cm), she had only 1 copy of *FOP1* (Xq28), a gene related to premature ovarian failure, which may have caused her hypogonadism hypogonadotropic (Rosa et al., 2008).

Eighteen CNVs were classified as probably causative (Table 2). Although they are not related to any previously described syndrome, typically a gene within the CNV is related to a similar phenotype. For example, patient 25 showed a 175-kb interstitial deletion in 17p13.3, which only covers the *LIS1* gene (MIM ID #601545). This likely explains the patient's clinical phenotype (Dobyns et al., 1993; Neer et al., 1993). Patient 31 showed a 130-kb interstitial deletion in Xq24, to which *UBE2A* is mapped (MIM ID #312180). Genomic deletions or point mutations in *UBE2A* have been described in individuals with ID and dysmorphic facial features (Budny et al., 2010). Patient 32 showed a 250-kb interstitial duplication in Xq22.31 involving *KAL1* (MIM ID #300836), which causes ID and MCA in patients when duplicated (Sowińska-Seidler et al., 2015).

Some CNVs were also classified as probably causative because they have been described in other patients with similar clinical characteristics. For example, patients 16, 21, and 24 had a terminal deletion at 2p25.3 (Stevens et al., 2011; Bonaglia et al., 2014; Doco-Fenzy et al., 2014), a terminal duplication at 14q32.31-q32.33, and an interstitial deletion of 500 kb at 16p11.2, respectively.

Among the CNVs classified as causative (N = 33), a greater number of deletions than duplications (22 vs 11) was observed. As previously described by Menten et al. (2006), this observation can be explained in a few ways: there is a greater chance that some duplications are missed technically, duplications generally cause a milder phenotype leading to a selection bias, and the biological frequency of a deletion event is higher than that of a duplication event. Similarly, at the chromosomal level, chromosomal trisomies or supernumerary marker chromosomes are better tolerated than autosomal monosomies (Liehr et al., 2011).

CNVs detected in patients 34, 35, and 36 were classified as non-causative (Table 3) because there have been several descriptions of other patients with variations in the same chromosomal segment in databases, and in some cases, the variations were inherited from a normal parent.

Table 3. Array-CGH results of the 3 Brazilian patients with non-causative CNVs - benign (group III).

Patient	Gender	Resolution	Locus (Hg 18)/ inheritance	Size
34	F	60 k	array 6q11.1 (62.354.703-62.935.334)x3/mat	~1 Mb
35	F	60 k	array 9p24.3 (194.090-371.572)x3/mat	177 kb
36	M	60 k	array 15q26.3 (99.753.088-100.080.964)x3/mat	~480 kb

For instance, *DOCK8* (MIM ID #611432) maps within the 177-kb interstitial duplication in 9p24.3 in patient 35. While deletions of the same region have been described in patients with ID (Griggs et al., 2008; Di Gregorio et al., 2014), the effect of duplication of *DOCK8* has not been elucidated. Mental disabilities have been described in patients with duplications in the same genomic segment, but in all cases the duplication was inherited from an unaffected parent. Furthermore, the DGV database reports the occurrence of duplication in this segment in individuals in the general population. Therefore, this variant may contribute to the patient's clinical status; however, the presence of the variant in an unaffected individual clearly indicates that this finding is not sufficient to explain the clinical manifestations, and thus this CNV was classified as not-causative.

Seven CNVs (3.5%) were classified as VOUS (Table 4) because literature data or inheritance patterns did not enable clear determination of its pathogenicity. Patient 37 showed a terminal deletion in the short arm of chromosome 4, affecting band 4p16. Although this CNV overlaps with the region associated with Wolf-Hirschhorn syndrome, it is smaller than that reported in patients with the syndrome and does not appear to involve the critical region. There have been no

descriptions of patients with deletions involving only *PDE6B*, as was observed in this patient. This girl has IDs but she did not have the typical facial characteristics associated with Wolf-Hirschhorn syndrome.

Table 4. Array-CGH results of the six Brazilian patients with variants of unknown clinical significance (VOUS; group IV).

Patient	Gender	Resolution	Locus (Hg 18)/inheritance	Size
37	F	60 k	array 4p16 (61.352-799.952)x1/indeterminate	~800 kb
38	F	60 k	array 11q24.2 (126.467.319-126.699.302)x3/indeterminate	231 kb
39	F	60 k	array 16p13.11 (15.399.618-15.514.494)x3/indeterminate	115 kb
40	F	60 k	array 19p13.3 - p13.12 (13.471.747-14.684.081)x1/indeterminate	1.2 Mb
41	F	180 k	array Xq21.1 (80.364.877-80.653.581)x3/indeterminate	289 kb
42	M	60 k	array Xp22.31 (7.594.433-8.057.652)x2/indeterminate	~500 kb
43	M	60 k	array Xp22.31 (6.462.695-8.057.511)x2/indeterminate	~1.6 Mb

Patient 43 contained a duplication of ~1.6 Mb in Xp22.31, as reported in another patient by Faletra et al. (2012). Similarly, variants were observed both in normal individuals and in individuals with clinical conditions associated with ID. Several of the affected individuals inherited the variant from normal parents, showing that this duplication alone is not sufficient to cause the clinical condition, but may determine a predisposition to it.

Although modern molecular cytogenetic methods, including array-CGH, show great potential for identifying novel chromosomal syndromes, there are still several diagnostic challenges and unexplored questions, particularly regarding the role of CNVs in the pathogenesis of complex human genetic diseases. Variants of uncertain clinical significance should be reviewed periodically. Thus, with the description of new cases with the same CNV, it will be possible to elucidate the relationships between the variant and the patient's condition.

A high percentage of ID and MCA are caused by submicroscopic chromosomal imbalances.

Microarray molecular screening of the whole genome is increasingly thought to have clinical impact, replacing standard karyotyping as the first-tier cytogenetic diagnostic test. This may help families and their assistant physicians in reaching an accurate diagnosis, bringing closure to their postnatal search for an explanation regarding the ID and/or MCA.

Conflicts of interest

The authors declare no conflict of interest.

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