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Detection of small copy number variations (CNVs) in autism spectrum disorder (ASD) by custom array comparative genomic hybridization (aCGH)

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ABSTRACT

Autism spectrum disorder (ASD) has a strong genetic basis and advances in genomic scanning methods have resulted in the identification of the underlying alterations in about 30% of the cases. The overwhelming majority of these alterations are either sequencing variants or large copy number variations (CNVs). In this pilot study, we tested whether the use of a customized array comparative genomic hybridization (aCGH), targeting exons of 269 ASD candidate genes, would allow the identification of small potentially pathogenic CNVs (<100 Kb). We detected 10 rare, potentially pathogenic CNVs in nine out of 98 patients with idiopathic ASD, and none of 200 Brazilian controls. Two out of five CNVs identified among the non-syndromic cases, involving the genes *MBD2* and *SLC17A6*, were smaller than 100 Kb. In a subsequent screening of other 407 patients and 350 non-affected controls for CNVs involving *SLC17A6*, a gene without previous documentation in the literature of involvement with neurodevelopmental disorders, we found intragenic duplications in another proband but also in five controls. Of note, a commercial 500 K SNP-array did not detect the smallest gains in *SLC17A6*. Our results suggest that small CNVs contribute to the etiology of ASD and that customized CGH array has significant potential to improve the sensitivity for detecting this class of alterations.

Abbreviations: aCGH, array comparative genomic hybridization; CNV, copy number variation; MLPA, multiplex ligation probe amplification; NGS, next generation sequencing.

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1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by significant and persistent deficits in communication and social interaction, and by restricted and repetitive patterns of behavior, interests or activities (American Psychiatric Association, 2013). ASD is considered a common disorder with a strong genetic component, affecting one in 68 individuals and presenting a gender ratio of 4.2 boys–1 girl (Centers for Disease Control and Prevention, 2014; Devlin & Scherer, 2012; Fombonne, 2009; Gillberg, Cederlund, Lamberg, & Zeijlon, 2006).

High-throughput genomic approaches, as array comparative genomic hybridization (aCGH) and next generation sequencing (NGS), have revolutionized the knowledge on ASD genetics, unraveling a number of rare copy number variations (CNVs) and sequencing variants for which there is strong evidence for a causal role in the phenotype (Betancur, 2011; Hoischen, Krumm, & Eichler, 2014). Nevertheless, in more than 70% of the ASD patients, the underlying mutations remain to be identified (Betancur, 2011; Buxbaum et al., 2014). We have therefore raised the hypothesis that small CNVs (between 1 and 100 Kb), below the resolution of most of the commercial microarrays and still poorly detected by NGS, could contribute to the phenotype in a significant proportion of cases.

As a first step to address this question, we have performed a pilot study using a customized aCGH with high density of probes targeting exons of 269 ASD candidate genes, in order to screen for small potentially pathogenic CNVs among Brazilian patients with ASD.

2. Materials and methods

2.1. Patients

A total of 505 Brazilian patients with idiopathic ASD were included in the study. While 434 patients were referred to the Human Genome and Stem-Cell Research Center (HUG-CELL), Sao Paulo (most ascertained at the Institute of Psychiatry, University of Sao Paulo), 71 were ascertained at the *Hospital de Base*, Faculty of Medicine of Sao Jose do Rio Preto. ASD was diagnosed based on the fourth or fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM) by psychiatrists at the above-mentioned institutions, and evaluated following previously standardized criteria (Griesi-Oliveira et al., 2012; Moreira et al., 2014; Orabona et al., 2009). In most cases children's behavior was characterized by score questions of Childhood Autism Rating Scale (CARS) and/or by an interview adapted from the Autism Diagnostic Interview-Revised (ADI-R) (Griesi-Oliveira et al., 2012). At the HUG-Cell, the intelligence quotient (IQ) of ASD patients has been systematically assessed in the last two years by means of the Wechsler Intelligence Scale for Children-Third Edition (WISC-III) and of the Wechsler Adult Intelligence Scale-Third Edition (WAIS-III) (Nascimento & Figueiredo, 2002). For some patients, particularly for those who present severe verbal and interaction difficulties, non-verbal IQ calculations were based on the Raven's matrices standard (Campos, 2004).

Patients presenting dysmorphic features and/or congenital malformations were classified as syndromic. Patients with clinically recognizable, autism-related syndromes and metabolic disorders, such as Rett, Prader-Willi, Angelman, Williams, DiGeorge, Beckwith-Wiedmann, and Smith-Lemli-Opit syndromes, were not included in the present study. Exposition to teratogenic drugs or infections during pregnancy was also an exclusion criterion. Finally, all patients were negative for CNVs at 15q11-13, 16p11 and 22q13 through multiplex ligation probe amplification (MLPA) analysis with the SALSA MLPA KIT P343-B1 AUTISM-1 (MRC-Holland, Amsterdam, The Netherlands; Moreira et al., 2014), and all boys were negative for *FMR1* expansion (Haddad, Mingroni-Netto, Vianna-Morgante, & Pena, 1996).

Patients at HUG-CELL were 10.9 ± 7.8 years old at ascertainment, about 23% of them were syndromic, and 56 (13%) were probands of familial cases. The male–female ratio was 3.2–1, which may be accounted for by a higher proportion of patients with intellectual disability (ID) as compared to other studies (roughly 77% versus 55%) with reported male-female ratios closer to 4 to 1 (Centers for Disease Control and Prevention, 2014; Miles, 2015; Werlin & Geschwind, 2013).

Patients ascertained at the *Hospital de Base* were 12.1 ± 7.8 years old at ascertainment, and only 3% of them were syndromic. All 71 patients were sporadic cases, with a male–female ratio of 4.5–1 and an ID rate of 55%.

The Ethics Committee of Bioscience Institute, University of São Paulo approved this research. Individuals were included in this study only after written informed consent signature (by themselves or legal caregivers).

2.2. Array CGH design

A custom oligonucleotide array with approximately 60,000 features (Custom 8×60 K Microarray, Agilent Technologies, Santa Clara, CA, USA) was designed with the eArray web software using the filters: similarity score, probe score above 0.8 and probe $\geq$59 bp, in order to select the best-performing oligonucleotides from Agilent's library. The array, which was designed before 2010, comprises probes targeted to all exons, including those at 5′ and 3′ UTR regions, of 269 selected genes based on the following criteria: (i) genes previously reported as candidates for ASD and/or other neuropsychiatric disorders in at least two different studies; (ii) genes involved in the ASD-associated pathways and with similar functions to known candidate ASD genes; (iii) genes mapped in ASD known/candidate regions and encoding proteins with neurological functions. The array included over 33,000 tiling probes targeted to 4229 exons of these genes, with an average of eight probes per exon and at least three probes covering the overwhelming majority of exons. This design should provide increased sensitivity for