Conjecturas

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Long contiguous stretches of homozygosity identified by chromosomal microarray analysis in a population with intellectual disability and autism spectrum disorder from Central Brazil

Longos trechos contíguos em homozigose identificados por análise cromossômica por microarranjos em uma população com deficiência intelectual e transtorno do espectro autista do Brasil Central

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ABSTRACT

Long continuous stretches of homozygosity (LCSH) are uninterrupted regions of homozygous alleles originated by consanguinity, uniparental disomy (UPD), DNA repair mechanisms, and ancestral haplotypes. We identified genomic LCSHs regions by CMA, evaluating their frequencies and origin's mechanism in patients with intellectual disability and/or autism spectrum disorder from the public health service in Central Brazil. In 90% of patients, at least one LCSH was identified with a total of 265 LCSHs observed. Of 265 LCSHs, 59% were recurrent, observed in chromosomes 11, 16, and X, and 41% were non-recurrent and observed in the X and autosome chromosomes except chromosome 19. Non-recurrent LCSHs were originated by consanguinity in 9.7% of patients, by possible DNA repair or ancestral haplotype mechanisms in 85.4% of patients, and by a possible segmental UPD in 4.9% of patients. Our findings showed the utility of high SNP density CMA in identifying LCSHs, highlighting the importance of characterizing the profile of LCSHs in the Brazilian population, especially from Central Brazil population.

Keywords: homozygous alleles, microarray, neurodevelopmental disorders

RESUMO

Longos trechos contínuos em homozigose (LCSH) são regiões ininterruptas de alelos homozigotos originados por consanguinidade, dissomia uniparental (UPD), mecanismos de reparo de DNA e haplótipos ancestrais. Identificamos regiões genômicas de LCSHs através do CMA, avaliando suas frequências e mecanismo de origem em pacientes com deficiência intelectual e/ou transtorno do espectro autista do serviço público de saúde do Brasil Central. Em 90% dos pacientes, pelo menos um LCSH foi identificado com um total de 265 LCSHs observados. De 265 LCSHs, 59% eram recorrentes, observados nos cromossomos 11, 16 e X, e 41% eram não recorrentes e observados nos cromossomos X e autossomos, exceto no cromossomo 19. LCSHs não recorrentes foram originados por consanguinidade em 9,7% dos pacientes, por possível reparo de DNA ou mecanismos de haplótipos ancestrais em 85,4% dos pacientes e por possível UPD segmentar em 4,9% dos pacientes. Nossos achados mostraram a utilidade do CMA de alta densidade de SNPs na identificação de LCSHs, destacando a importância de caracterizar o perfil de LCSHs na população brasileira, especialmente na população do Brasil Central.

Palavras-chave: alelos homozigotos, microarranjo, transtornos do neurodesenvolvimento

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INTRODUCTION

Long contiguous stretches of homozygosity (LCSH) are defined as a neutral chromosomal region of homozygous alleles and are characterized by regions of low recombination in the genome. The mechanism of origin and the consequences of LCSHs are poorly understood, hindering the interpretation of such genomic alterations. However, the presence of LCSH may indicate parental consanguinity, uniparental disomy (UPD), replicative DNA repair mechanism, and ancestral haplotypes (IOUROV et al., 2015; CHAVES et al., 2019).

The LCSHs ≥ 5Mb distributed throughout several chromosomes suggests consanguinity between biological parents of an individual (KEARNEY; KEARNEY; CONLIN, 2011). The consanguinity, defined as the kinship of two individuals having a common ancestor, could be influenced by religion, culture, and ethnicity. Also, inbreeding could increase the incidence of recessive disorders (IQBAL; VAN BOKHOVEN, 2014; CEBALLOS et al., 2018; OMAR; KOKAB, 2019). Although LCSHs are common for inbred populations, higher frequency of homozygous regions could also be found in the genome of outbred populations, characterizing regions of reduced recombination rates (CHAVES et al., 2019).

Uniparental disomy is defined by the presence of both homologs of a whole chromosome or chromosome segment inherited from only one parent. The formation of UPD could be the result of chromosomal rescue, a cellular mechanism to normalize the number of chromosomes in gametic cells. UPD is an important mechanism that can lead to human diseases and even human zygote unviability, particularly, when it involves chromosome regions with imprinted genes. However, it is currently difficult to estimate the frequency of UPD responsible for patients' phenotypes (HOPPMAN et al., 2018; WAGGONER et al., 2018; CHAVES et al., 2019).

The exposure to toxic agents, errors in chromosome segregation, and failure in DNA replication are events that can result in DNA double-strand breaks (DSBs). The repair of DSBs is fundamental for cell survival and prevents genome instability, therefore, the importance of cellular repair mechanisms to restore cell viability (SAKOFSKY; MALKOVA, 2017). In vitro, studies using human cell lines have demonstrated the DNA repair mechanisms potentially contribute to the LCSHs formation in neurodevelopmental disorders (SAKOFSKY; MALKOVA, 2017; CHAVES et al., 2019).

Neurodevelopmental disorders (NDD) consist of a vast and heterogeneous range of conditions with symptom onset during childhood and affect brain development (THAPAR; COOPER; RUTTER, 2017; ALABAF et al., 2019;). This group of disorders is characterized by impairment in cognition and motors skills, communication, and/or behavior, which included developmental delay (DD), intellectual disability (ID), autism spectrum disorder (ASD), schizophrenia, attention-deficit/hyperactivity disorder (ADHD), and epilepsy (APA, 2013; ISMAIL; SHAPIRO, 2019).

Considering the contribution of genetic etiology for these disorders, the American College of Medical Genetics and Genomics (ACMG) and the European Guidelines recommended the Chromosomal Microarray Analysis (CMA) as the first-tier test for the diagnostic of individuals with ID, GDD, ASD, and congenital anomalies (WAGGONER et al., 2018; SILVA et al., 2019). The CMA has been widely applied as a sophisticated approach for cytogenomic diagnose with high resolution, based on the distribution of polymorphic and non-polymorphic markers covering the whole human genome of medical interest, enabling the identification of LCSHs regions. Nevertheless, the clinical significance and interpretation of homozygosity regions are still challenging. Our knowledge about the frequency of LCSH in the Brazilian population is reduced, especially in Central Brazil, since the CNVs (Copy Number Variations) and regions with LCSH have ethnically specific occurrences and frequency distributions (ALI et al., 2020).

Herein, we report out the CMA analysis in a cohort of patients with ID and/or ASD referred by doctors from the public health system in Central Brazil to identify and classify the LCSHs according to the origin's mechanism and estimate their population frequency.

MATERIALS AND METHODS

Cohort of patients

The cohort was comprised of 100 patients with intellectual disability and/or autism spectrum disorder. Assistant physicians from Goiás state public health system referred each patient to the genetic services at the Laboratory of Human Cytogenetics and Molecular Genetics of the State Health Secretary and the Replicon Research Group of the School of Life and Medical Sciences of the Pontifical Catholic University of Goiás in Central Brazil.

The study was approved by the Ethics Committee on Human Research from the Pontifical Catholic University of Goiás under the number CAAE: 0051.0.168.000-11 and it followed the ethical principles of the Declaration of Helsinki. Parents of all patients signed written informed consent.

Chromosomal Microarray Analysis

Genomic DNA was isolated from whole blood using Illustra Blood Genomic Prep Mini Spin Kit (GE Healthcare Life Sciences, Piscataway, New Jersey, USA), following the manufacturer's instructions.

The CMA was carried out in the patients and their biological parents using the GeneChip® CytoScanHDTM array (Thermo Fisher Scientific, EUA), a genotyping matrix composed of polymorphic and non-polymorphic markers, following the manufacturer's recommendations. The array contains 750,000 SNP markers and about 2 million non-polymorphic markers allowing the identification of Copy Number Variants (CNV) and loss of heterozygosity (LOH). The array provided a robust coverage to identify genomic variations with ≥ 99% sensitivity. CMA analyzes were done using the Chromosome Analysis Suite 3.0 (ChAS®) software (Affymetrix, Santa Clara, USA).

LCSHs analysis

For LCSH analyses, we removed all CNVs of loss because it could be considered as homozygosity. The analyses of LCSHs were done in both autosomal and X chromosomes. Although X chromosome LCSHs are relatively common and indicate the low rate of recombination, we decided to include this chromosome in our analysis because X chromosome's variations are frequently reported as the cause of ID and ASD.

The LCSH threshold was set at a minimum 3Mb and ≥ 2% of the size of LCSHs about to the total chromosome size. The LCSHs were classified according to Chaves et al. (2019) and, thus, it would be considered recurrent LCSHs if observed in ≥ 5% of the study group. On the other hand, LCSHs detected with frequency < 5% were considered as non-recurrent.

To determine the formation mechanism of LCSHs, we considered:

(1) LCSH ≥ 5Mb, distributed throughout several chromosomes, their origin was considered by consanguinity. To estimate the degree of the relationship between the

patient's biological parents, we applied the Inbreeding Coefficient (F) proposed by Kearney et al. (2011).

(2) LCSHs ≥ 10Mb, restricted to one single or few chromosomes, the origin was regarded as a possible UPD;

(3) LCSHs < 10Mb, distributed throughout one or few chromosomes, the origin was considered a possible DNA repair or ancestral haplotypes.

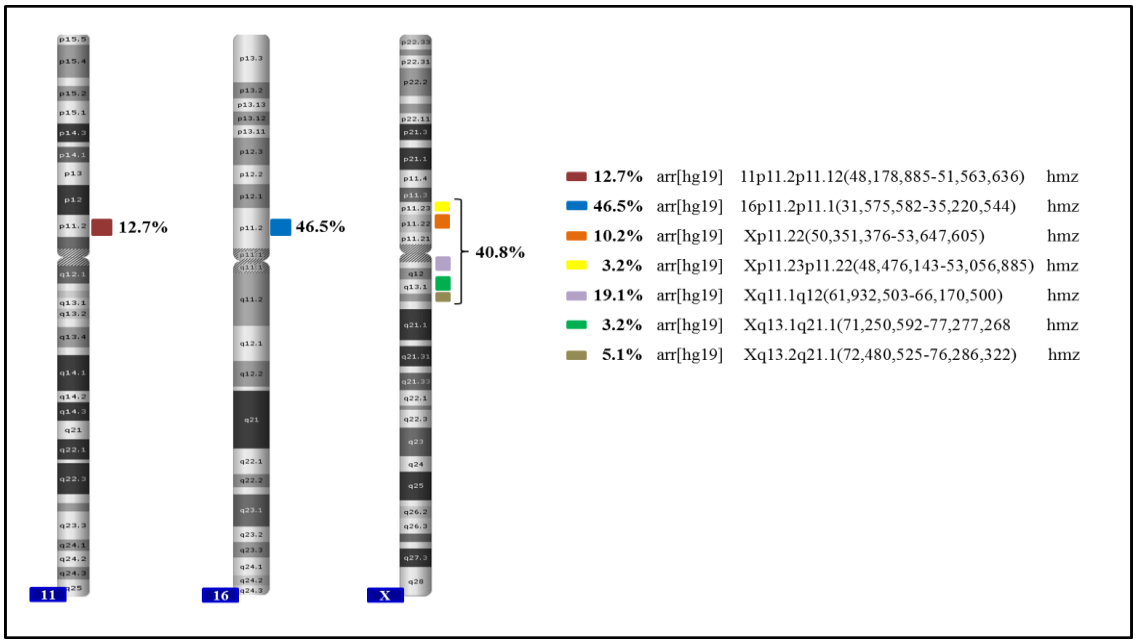
RESULTS

In total, 100 patients participated in the current study, with an average age of nine years old. About 80 (80%) patients had clinical diagnosis of intellectual disability while 20 (20%) patients were diagnosed within the autism spectrum disorder. The cohort was comprised of 54 (54%) males and 46 (46%) females. Consanguinity was reported for four (4%) patients.

From 100 patients, 10 (10%) showed no evidence of LCSHs, and 90 (90%) exhibited one or more LCSHs ≥ 3Mb. A total of 265 LCSHs were observed in 90 patients, of which 59% (157/265) were recurrent, and 41% (108/265) were considered non-recurrent.

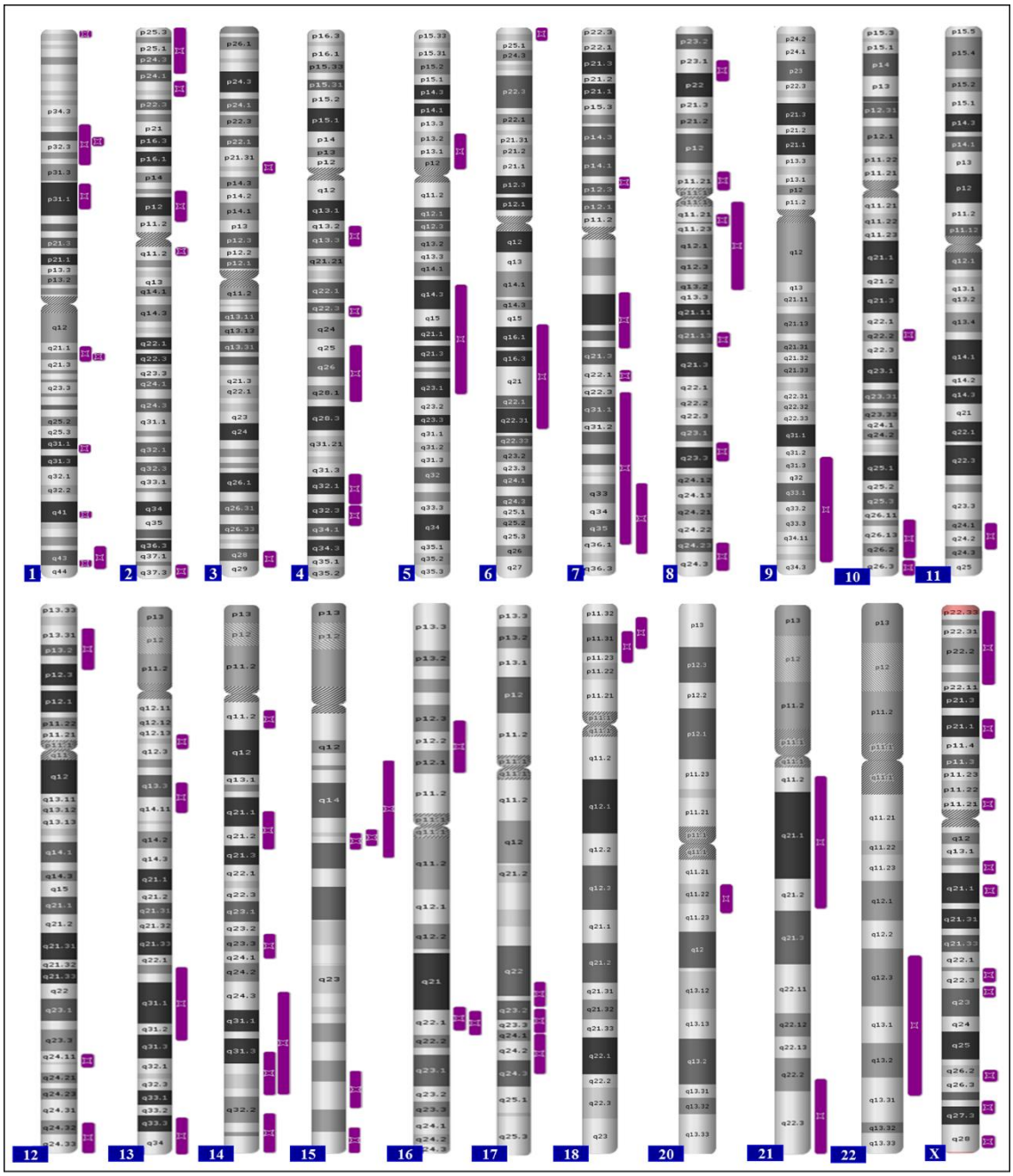
The distribution of 157 recurrent LCSHs was observed in 54% (49/90) patients, and the LCSHs were detected in seven different regions from chromosomes 11, 16, and X (Figure 1). On the other hand, 108 non-recurrent LCSHs were observed in 46% (41/90) patients and distributed in 84 different regions from the X chromosome and autosome chromosomes except chromosome 19 that was not detected any LCSH (Figure 2).

Figure 1 – Ideogram is showing the distribution of recurrent LCSHs on chromosomes 11, 16, and X from a cohort of patients with ID and/or ASD.



Source: collection of the authors.

Figure 2 – Ideogram is showing the distribution of 108 non-recurrent LCSHs from a cohort of patients with ID and/or ASD.



Source: collection of the authors.

From 108 non-recurrent LCSHs, 53.7% (58/108) were originated by consanguinity and were distributed across the genome of 9.7% (4/41) of patients with intellectual disability. After calculating the Inbreeding Coefficient (F), we estimated that 4.85% of the biological parents have third degree of inbreeding (first cousins or half-uncle with niece) and 4.85% of the biological parents have fourth degree of inbreeding (first cousins once removed).

We observed 1.85% (2/108) non-recurrent LCSHs originated by a possible segmental UPD and were distributed in the genome of 4.9% (2/41) male patients. A patient with ID showed one LCSH of 10.19Mb at 12p13 region, and another patient with ASD showed one LCSH of 11.83Mb at 5p13.2 region.

We detected 43.5% (47/108) non-recurrent LCSHs suggestive of DNA repair mechanism or ancestral haplotypes and were distributed in the genome of 85.4% (35/41) of patients with ID/ASD. Around 57.5% (27/47) non-recurrent LCSHs were identified in autosome chromosomes and 42.5% (20/47) were observed in the X chromosome, comprising mainly the regions 3p21.31, 10q22.1, 15q15.2, Xp11.21, Xq21.1, Xq22.2, Xq22.3, and Xq26.2.

DISCUSSION

In the present study, we observed a total of 265 LCSHs in 90 patients with ID/ASD, in which 108 LCSHs were considered as non-recurrent, and 157 LCHSs were considered as recurrent. For LCSHs at 11p11.2 and 16p11.2 regions classified as recurrent in our dataset, both have been found common LCSHs in a South Brazil study (CHAVES et al., 2019). These two regions were considered frequent in affected and non-affected patients reported by Wang et al. (2015). Both regions could represent a possible ancestral haplotype unrelated to the development of ID/ASD.

Recurrent LCSHs observed in the X chromosome involved five different regions. Xp11.23p11.22, Xp11.23p11.22, Xq11.1q12, Xp11.22, and Xq13.2q21.1. The Xp11.23p11.22 recurrent LCSH was also reported by Wang et al. (2015) as a common region and with no clinical relevance. Pajusalu et al. (2015) also reported the Xq13.1q21.1 recurrent LCSH and considered this region as a benign polymorphism. On the other hand, Xq11.1q12, Xp11.22, and Xq13.2q21.1 regions had never been reported before associated with LCSHs presented in patients with ID/ASD, being the current study a pioneer report of LCSHs in these regions in patients with these neurodevelopmental disorders. Several studies do not recommend X chromosome LCSHs analysis because of male hemizygosity and a high percentage of homozygosity in female X chromosomes due to the low recombination rate (SUND et al., 2013). However, we decided to analyze LCSHs in the X chromosome from females because rearrangements involving this chromosome have already been strongly associated with one of the causes of ID/ASD development.

In our cohort from Goiás, Central Brazil, the 10q22.1 region was classified as non-recurrent LCSH. Differently, a recent Brazilian study considered this same region prevalent in the population of Santa Catarina (CHAVES et al., 2019). The Brazilian population is heterogeneous and highly mixed within the boundaries of a country with continental dimensions. Thus, the divergence in the frequency of this LCSH could be attributed to regional differences between studied populations, emphasizing the need to investigate, catalog, and generate population specific LCSH databases for each region from the Brazilian population (KU et al., 2011; PAJUSALU et al., 2015).

LCSHs are more common than previously thought (LI et al., 2006) and has been observed in genomic regions with low recombination rates. Thus, it is believed that these regions' location is similar across different populations (GIBSON; MORTON; COLLINS, 2006). Additionally, it is essential to highlight a cautionary note before stating that recurrent LCSHs have no clinical significance in an affected population, one should know its frequency in similar unaffected population (CHAVES et al., 2019). However, considering the complexity and multifactorial etiology of ID/ASD, we could not completely disregard the role of recurrent LCSHs in patients with these disorders.

The 108 non-recurrent LCSHs observed in our study were identified in the X chromosome and all autosome chromosomes except chromosome 19. The absence of LCSH in chromosome 19 has also been described by Li et al. (2006). The chromosomes 1, 8, and 14 showed the largest number of non-recurrent LCSHs, respectively, in contrast to Pajusalu et al. (2015). They reported that the chromosomes with the largest number of non-recurrent LCSHs were 2, 3, and 1, respectively. The regions on chromosome X, which presented the largest number of non-recurrent LCSHs were: Xp11.21, Xp21.1, Xq26.2, Xq28, Xq21.1, Xq22.2, and Xq22.3. Our study is the first to report LCSHs the above-mentioned X chromosome regions in association with ID/ASD, except the Xp11.21, which was previously described in a recent study considered this region with no clinical relevance (BARRIE et al., 2018).

The non-recurrent LCSHs originated by parental consanguinity were found in 9.7% (4/41) of the patients' genomes, and the inbreeding coefficient suggested kinship of third to fourth degree between the progenitors. According to a review study of Ceballos et al. (2018), our results demonstrated that at least 10% of the global population are the offspring of consanguineous marriages. Nevertheless, a Brazilian study observed some degree of inbreeding in 26.5% of the Santa Catarina population from South Brazil

(CHAVES, 2018). These differences regarding consanguineous marriages world-wide may reflect specific cultural features of each regional population. In Central Brazil, marriages among first cousins are relatively common and socially accepted. Clinically, homozygosity mapping could help diagnose autosomal recessive disorders, especially in inbred families, where the risk of developing these disorders is modulated by the degree of kinship of the parents (SUND et al., 2013).

Around 85.4% of our patients showed that non-recurrent LCSHs originated by possible DNA repair or ancestral haplotypes. Several LCSHs were detected in the autosomes. However, the X chromosome had a more significant number of different regions with non-recurrent LCSHs. This result was already expected mainly because of the low rate of recombination observed in X chromosomes.

Establishing the DNA repair or ancestral haplotype as mechanisms of formation of LCSHs proved challenging mainly due to the absence of the populational frequency and spectrum of LCSHs from healthy Brazilians and because the pathogenic implication of LCSHs associated with specific phenotypes and ancestral haplotypes have never yet been described (CHAVES, 2018). An important repair pathway as the break-induced replication (BIR) could potentially contribute to loss of heterozygosity and the formation of complex chromosomal rearrangements in neurodevelopmental disorders (SAKOFSKY; MALKOVA, 2017). Finally, it is outstanding how the DNA repair mechanism could lead to the formation of complex genomic rearrangements in patients with ID/ASD (CARVALHO; LUPSKI, 2016; SAKOFSKY; MALKOVA, 2017) and this area requires a lot of studies to make sense of the actual contribution of DNA repair to the overall outcome of ID/ASD phenotypes.

Non-recurrent LCSHs observed on chromosomes 5 and 12 originated by a possible segmental UPD were identified in 4.9% of the patients, and both chromosomes have not yet been associated with any imprinting syndromes. However, methylation tests and/or microsatellites analysis to confirm whether the origin of these non-recurrent LCSHs is by UPD have not been performed, which makes our results speculative and hypothetical, and will require further molecular investigation.

A previous study suggested that the UPD prevalence is variable in the general population or affected cohorts and cannot be comparable due to differences in methodological approaches and limitations from the samples. Additionally, they also highlighted that UPD is a rare cause of ID and developmental delay (SCHROEDER et

al., 2014). These results are corroborating with ours, that showed the frequency of LCSH originating by possible UPD is lower than that of LCSHs originating by other mechanisms in patients with ID/ASD.

Homozygosity mapping in individuals from different affected or healthy populations can provide information about demographic and evolutionary history differences between healthy populations. Also, it can provide evidence of the impact of these regions on the health of the affected population.

CONCLUSION

To our knowledge, there is a scarcity of LCSHs data in healthy or NDD in the Brazilian population. In this sense, our results are relevant, showing the importance of the profile of LCSHs in the Brazilian population, especially from the State of Goiás, and provide information about LCSHs influence in neurodevelopmental disorders. Here we propose a national collaborative study among all users of genomic arrays should be undertaken to elaborate a populational catalog of LCSH variation in humans, which could prove itself useful to future studies investigating the variation associated with human diseases, such as ID/ASD.

The LCSHs investigation in our study allowed us to characterize recurrent and non-recurrent LCSHs regions and infer the potential mechanisms of LCHSs formation. Additionally, our findings highlighted the utility of high SNP density CMA in the identification of LCSHs. Finally, it enabled the understanding of the influence of LCSHs in the susceptibility regions that harbor genes involved in the central nervous system development as well as within regions related to recessive monogenic disorders. Nevertheless, it is essential to note that the presence of LCSH alone does not establish a definitive diagnostic result and for the diagnoses strategies using next-generation sequencing (NGS) approaches to increase the clinical impact on the diagnostic of regions of homozygosity are still required.

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