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ORIGINAL ARTICLE

Cytogenetic and molecular investigation of autism cases seen at a University outpatient ward in South Brazil

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Abstract

Introduction: The term syndromic autism is used to refer to cases of autism spectrum disorder (ASD) that have a defined cause. A complementary investigation method for the etiological determination of ASD is neurogenetic investigation, and autism can have a hereditary behavior in up to 90% of cases. **Objective:** The purpose of this article was to evaluate the occurrence of syndromic autism and the genetic characteristics involved in patients with ASD. **Methods:** An observational, cross-sectional and retrospective study was carried out using data collected from the medical records of 135 children, aged between 2 and 15, who underwent genetic investigation and were diagnosed with ASD by the UNISUL Maternal and Child Ambulatory in Tubarão, Santa Catarina Brazil. **Results:** According to the results of the genetic tests, 20% of the patients who underwent some type of genetic investigation changed and were included in the group of atypical autists. Among the patients who underwent the CGH Array exam 70.97% had some genetic alteration found, among those who underwent the karyotype 5.26% had changes, followed by 2.31% of the changes in the patients who underwent Fragile X DNA test. It was found that the presence of dysmorphism and microcephaly had statistical significance in the probability of alteration in the investigation. **Conclusion:** In the etiological assessment of autism spectrum disorder is important to carry out the genetic test. The karyotype, Fragile X DNA test and CGH Array detected the etiology in 20% of the children studied. In patients with some dysmorphism, the tests appear to be more sensitive.

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INTRODUCTION

Autism Spectrum Disorder (ASD) is an evolutionary-behavioral disorder characterized by persistent impairment in social interaction and reciprocity, combined with the presence of a restricted, repetitive and stereotyped pattern of behaviors, interests or activities[1,2].

According to epidemiological data, there has been an increase in ASD prevalence rates over the years. If in the 1970s there was talk of one case for every 5,000 children; in 2004 the prevalence was one case in 125, increasing to one case in 110 in 2006, one case in 88 in 2008, one case in 68 in the year 2012 and one case in every 59 in 2014[3]. Finally, in 2020, in its last survey, with data referring to 2016, the Center for Disease Control (CDC) of the United States, found a prevalence of one case for every 54 children in the United States, with a ratio of 4 affected male individuals for every female case[4].

Among the etiological factors, genetic predisposition, prenatal clinical characteristics, obstetric factors as well as several environmental factors have already been implicated as possible causes of autism. Despite scientific advances, the precise pathophysiological mechanisms are still unknown, with no defined cause, no preventive means or curative treatment yet existing[3].

Due to this enormous clinical heterogeneity, the presence of multiple signs and symptoms and associated comorbidities, ASD can be considered a syndrome and not a disease in itself; and it may have multiple etiologies. The term syndromic autism or secondary autism is used, then, to refer to cases of ASD that have a definite cause, as opposed to idiopathic autism, which cause remains unknown[5].

In this regard, a complementary investigation method that has stood out for the etiological determination of ASD is the neurogenetic investigation.

From a neurogenetic point of view, it is estimated that ASD may have a hereditary influence in about 50 to 90% of cases, which demonstrates the importance of genetic factors in the pathogenesis of the disease[6]. Family and twin studies provide evidence for the genetic etiology of autism, showing an increased risk of recurrence of the condition of approximately 3 to 8% in families with an autistic child, and concordance for the diagnosis of autism in monozygotic twins of at least 60% if they use strict criteria for autism, 71% for the autistic spectrum and up to 92% when considering a broader picture of language/socialization disorders[7].

Thus, the clinical and genetic characterization of patients with ASD is of great importance to obtain a better treatment planning, to offer the possibility of genetic counseling to the patients' relatives and also for a better understanding of the pathophysiology and etiology of this disorder. Through this study, we sought to evaluate the occurrence of syndromic autism and its genetic alterations through the review of data from the medical records of patients with Autistic Spectrum Disorder - ASD treated at the Maternal-Child Neurogenetics Ward at the University of Sourth Santa Catarina.

METHODS

We ran an observational, cross-sectional and retrospective study through the analysis of medical records, with pediatric patients aged 2 to 15 years, treated at the Maternal-Child Neurogenetics Ward at the University of Sourth Santa Catarina.(UNISUL), located in the municipality of Tubarão.

Through a review of data from medical records, this study used information regarding the family, gestational and clinical history of each patient, in addition to data from genetic investigation tests, such as karyotype, search for the X-fragile gene and genomic hybridization comparison (GHC Array). The sample was of the census type with patients selected through the medical records of the neurogenetic research clinic of the MCN, which resulted in a total of 135 children who met the inclusion criteria.

We included the medical records of patients who met the diagnostic criteria for ASD, aged 2 to 15 years, of both genders, who underwent some complementary genetic investigation testing. The exclusion criteria would be medical records in which it was not possible to prove the reliability of the data or those that did not contain enough information to meet the objectives of the study. No medical records met these characteristics.

The ASD diagnosis of each patient included in the outpatient clinic was performed by pediatric neurologists and was based on the DSM-5 diagnostic criteria. As for the severity of autism, the classification was performed using the Childhood Autism Rating Scale (CARS) in its translated and validated version for Portuguese.

The variables in this study included age, sex, presence of microcephaly, macrocrania, dysmorphism, parents' age at conception, treatment for infertility or *in vitro* fertilization, family history of speech delay, intellectual disability, psychiatric disorder, autism spectrum disorder, bipolar mood disorder and schizophrenia, consumption of alcohol, tobacco or narcotics during pregnancy, use of medication in general, gestational antidepressants and anticonvulsants, occurrence of gestational infection. The results of complementary investigation tests were also collected, namely, magnetic resonance imaging of the skull, karyotype, comparative genomic hybridization by microarray (CGH Array) and polymerase chain reaction (PCR) for fragile X.

Genetic tests were evaluated in two ways. Initially regarding the presence or absence of alterations and later in a polytomous qualitative way in the description of its findings.

The Excel software was used to create the database and Stata 16.0 software for data analysis. For data description, we used measures of central tendency and dispersion for quantitative variables, and percentages for qualitative variables. The existence of an association between the presence of the outcome of interest according to the other exposure variables was assessed using the chi-square test. To compare the mean values associated with the primary outcome variable with the other exposure variables, we used the Student's t test. The significance level used in the study was 5% (p<0.05). Normality was identified by the Shapiro-Wilk test.

RESULTS

We collected information from 135 children diagnosed with MCN and who underwent a genetic test, seen at the Neurogenetic Research Outpatient Clinic belonging to the MCN of UNISUL, Tubarão campus. The results showed a predominance in males (91.85%) compared to females (8.15%), following the trend of male prevalence of the disease.

Regarding the age at the first consultation, the median found (p50%) was 6 years of age, ranging from 2 to 15 years old. The value of P25=3 years and P75=9 years.

Regarding the conception history of the patients, treatment for infertility was performed by 5.19% of the mothers, and of these, 2.96% underwent *in vitro* fertilization.

As for the family history of clinical past, the present study verified the percentage of occurrence of a past history of neuropsychiatric disorders among the relatives of the studied patients. There was a predominance of familial psychiatric disorder in 70 cases (51.85%). Family history of delayed speech development was also a significant finding among the reviewed data. Among the 135 children evaluated, 66 medical records reported a family history of speech delay, totaling a percentage of 48.89%. Intellectual disability was present in 64 cases (47.41%), and autism spectrum disorder in 42 cases (31.11%). The least representative family disorders were bipolar mood disorder in 28 cases (20.74%), and familial schizophrenia in 21 cases (15.56%).

One of the study criteria was the selection of medical records of patients who had undergone some genetic evaluation through complementary tests. The tests performed were: karyotype, PCR for fragile X and CGH Array. The genetic test that showed the most alterations was the CGH Array, with 70.97% (N=22) of the altered tests, followed by the karyotype with a much lower percentage, 5.26% (N=7) and the PCR for X frail: 2.31% (N=3).

Subsequently, all medical records of patients with ASD who had alterations in their genetic investigation tests were selected: karyotype, PCR for fragile X or in the CGH Array, patients who had genetic alterations considered normal in the general population were excluded from the group. Thus, it was possible to define a group of autistic people considered atypical, that is, those who can be considered with autism of genetically determined etiology. According to the results from the genetic tests, 20% of the patients who underwent some type of genetic investigation were included in the atypical autistic group, as shown in Table 1.

Table 1. Frequency of patients with some altered genetic exam. MCN/ University of South Santa Catarina - Tubarão, 2020.

Results from genetic tests	n (%)
Without changes	108 (80.00%)
With changes	27 (20.00%)
Total	**135 (100%)**

From the group formed by patients with genetically determined ASD, clinical data were crossed in order to check whether, by comparing the groups, with normal and abnormal genetic tests, it would be possible to find a clinical pattern.

Treatment data for infertility, IVF, intellectual disability in the family, familial psychiatric disorder, familial bipolar mood disorder, familial autism spectrum disorder, speech delay, alcohol use, smoking, medications, antidepressants, anticonvulsants, gestational infection showed no significant association when comparing groups of patients with genetically determined autism versus normal tests.

As for phenotypic changes, some children were classified in the neuropediatric chart as having some type of dysmorphism, macrocrania or microcephaly. The study found that 19.26% of patients (N=26) had some dysmorphism, followed by macrocrania with 23.70% (N=32) and microcephaly with 5.19% (N=7). These data were crossed comparing the groups with normal and abnormal genetic tests. Dysmorphism and microcephaly were statistically significant and are shown in Table 2.

Table 2 shows that 46.15% (N=12) of patients with dysmorphism had some alteration in genetic tests, the rate of patients without dysmorphism who had some alteration was 13.76% (N=15). Pearson's chi-square test was applied with a value of P=0.000, demonstrating a greater tendency to detect genetic autism in dysmorphic patients. Similarly, among patients with microcephaly, 57.14% (N=4) had some genetic investigation test altered, compared to 17.97% (N=23) of patients without microcephaly who had some alteration, the p value found was P=0.012.

After evaluating the presence or absence of alterations in the genetic tests, the description of their findings

Table 2. Association between an altered genetic test with dysmorphism and microcephaly. MCN/University of South Santa Catarina - Tubarão, 2020.

	Genetic test	
	Without changes	With changes
Dysmorphism		
Yes	14 (53.85%)	12 (46.15%)
No	94 (86.24%)	15 (13.76%)
Microcephaly		
Yes	3 (42.86%)	4 (57.14%)
No	105 (82.03%)	23 (17.97%)

Dysmorphism *p*=0.000
Microcephaly *p*=0.012

was checked. Table 3 shows the results found in the CGH Array exam.

DISCUSSION

In our study, there was a high male predominance in the sample, data that corroborate other published studies. Like other neurobehavioral disorders that have multifactorial causes, ASD has a typically male predominance, with a ratio that varies from 4 to 11 male cases for each female case[8].

This difference in prevalence with a male predominance for ASD may represent a difference in the clinical presentation of autism, leading to a referral bias. According to Mandy et al. male individuals with ASD generally have a higher frequency of externalizing behaviors than girls, such as hyperactivity and aggressive behavior, while female patients tend to present a higher frequency of internalizing symptoms such as depression and social isolation[9].

Except for the bias resulting from the referral process, genetic studies seem to demonstrate that female individuals have a higher genetic threshold, that is, exclusively female genes seem to offer a protective factor for autism[10].

In our study, we also found that the age at the first consultation ranged from two to 15 years, with a median of 6 years of age. One of the fundamental pillars in the clinical management of ASD is the early diagnosis, unfortunately, the data found seem to corroborate a typically Brazilian reality, where despite the symptoms appearing in general before the age of 3 years, the average age of diagnosis is between 6 to 8 years[11]. This delay in diagnosis leads to greater morbidity and prevents the institution of early intervention, a set of interdisciplinary therapeutic modalities that aim to increase the child's social and communication development potential, protect intellectual functioning by reducing damage, improve quality of life and direct competences for their future autonomy[12].

Regarding preconception risk factors, we found in our sample that around 5% of the cases were generated after

Table 3. Description of the genetic findings form patients with altered CGH Array. MCN/University of South Santa Catarina - Tubarão, 2020.

Genetic changes found in the CGH Array test
LOH region (heterozygous loss region) in chromosome 4 region q13.3q21.22
Microduplication of 10.949 or 10.9 Mb in chromosome 3 region p25.3-p26.3, linear position 61.891-11.011.240
Genetic material gain in chromosome 7,region p21.3-p22.3 (7p21.3-p22.3 chromosome syndrome)
Loss of genetic material with important microdeletion of chromosome 22 (q13.31-13.33) (Phelan-Mcdermid syndrome)
Gain of genetic material in chromosome 7, short arm of chromosome 7 in region 7p22.1
Microduplication of chromosome 16 region q24.1 with 237 Kpb
Microduplication of 433 kb in chromosome 15 region q13.3 involving the CHRNA7 gene.
Microduplication of 2.668 kbp in chromosome 15 region q24.1-q24.2
Pathogenic CNV found in region 5q14.3 (haploinsufficiency syndrome by MEF2C of dominant autosomal inheritance). Additionally, one CNV of uncertain significance found on region 4q28.3
Microdeletion of 573 kbp in chromosome 1 region q21.1; Identified in region LOH in chromosome 8 region q13.3-q22.1
Microdeletion of 2.882 kbp in chromosome 22 in region q11.21 characterizing the 22q11.2 deletion syndrome (DiGeorge syndrome)
Important microdeletion in chromosome 15 region q11.2-q13.1 (Prader-Willi or Angelmann syndrome)
Terminal deletion of the chromosome 12 long arm [arr 12q24.31--q24.33]
Microduplication of chromosome 17, region p13.3 [arr 17p13.3]
Terminal deletion of the chromosome 9 short arm , affecting band p24.3. Interstitial duplication of the chromosome 9 short arm affecting bands p24.3-p13.1
Loss of 244Kb from chromosome band 2p14.3 (VOUS – Variant of uncertain clinical significance)
Loss of 113kbp in chromosome 3 region q26.33 (microdeletion 3q26.33)
Identification of microdeletion of 79 kpb in chromosome 7 region q31.33 gene GRM8 which codes protein MGLUR8
Detected the loss of 1 312 kb copy in the long arm of chromosome X, including genes SPRY3 and VAMP7
156kbp gain in chromosome 1 - 662kbp gain in chromosome 7 and 302kbp gain in chromosome 16 region p11.2
312 kbp 11q23.3 microduplication
Microdeletion in gene AUTS2 in chromosome 7q11.2 and microdeletion in gene OTOGL in chromosome 12q21.31

infertility treatment and around 3% after *in vitro* fertilization. According to a meta-analysis performed by Liu et al. the use of assisted reproductive technology (defined as the use of any technology or medication with the aim of achieving pregnancy) is associated with an increased risk of developing autism in the offspring[13].

The study published by Sandin et al., after evaluating more than 6,500 children born in Sweden between 1982 and 2007 through *in vitro* fertilization, found that 103 (1.5%) had autism[14]. This high prevalence of autism, associated with assisted reproduction technology, seems to be related to epigenetic factors induced by exposure to high doses of hormones, preparation, manipulation and freezing of gametes and embryos[14].

In the evaluated medical records, there was also a relationship between ASD and family history of neuropsychiatric disorders such as general psychiatric conditions, intellectual disability, autism, bipolar mood disorder and schizophrenia. In a population cohort study carried out by Xie et al. evaluated more than 55,000 cases of ASD, it was found that the presence of a first-degree relative with intellectual disability, depression, anxiety, bipolar mood disorder or schizophrenia increased the risk of ASD by up to 9 times. And this influence could extend to fourth-degree relatives[15].

Finally, considering the other gestational clinical aspects, we noticed in the evaluated medical records that gestational infection was present in almost 26% of the cases, use of antidepressants in 7%, tobacco in 6%, alcohol in 4% and anticonvulsants in 2%. At some point, all these factors such as gestational infection, use of antidepressants during pregnancy [16], cigarettes, alcohol and anticonvulsants[17] have already been implicated as risk factors for ASD.

According to the results from the genetic tests found in the present study, 20% of the patients who underwent some type of genetic investigation had some alteration, being included in the group of atypical autistics. These data are similar to those from other studies that estimate that autism associated with genetic alterations such as chromosomal variations, specific copy number variants and monogenic conditions is between 15 and 25% of cases[18].

The genetic test that demonstrated the highest detection of any genetic alteration was the CGH Array. This test offers significant sensitivity for the identification of submicroscopic chromosomal abnormalities and is one of the most used techniques in daily practice[19]. Of the 31 patients who underwent the test in this study, 22 had some type of alteration, whether it was already described in the literature as related to ASD or not. Comparatively, most of the studies evaluating the use of microarray techniques in the etiological investigation of patients with ASD, found a lower percentage of alterations, around 10%[20]. It is possible that this difference reflects the profile of the university clinic studied. As it is a public clinic, linked to the unified healthcare system (SUS), the financial difficulties of patients constitute a barrier to carrying out complementary exams. Another factor seems to be the availability of a medical geneticist to guide the tests. Thus, both facts corroborate for a much more rational indication of the exams.

Regarding the other genetic studies, Çop et al, investigating a population of 96 children with ASD, found alterations in 9.7% of the karyotype exams and 1.5% of the PCR exams for fragile X syndrome[21]. Our data showed approximate results, with 5.3% of alterations in karyotype exams and 2.3% in searches for the detection of fragile X syndrome.

The current study demonstrated a greater tendency to detect genetic autism in dysmorphic patients, with a value of *P*=0.000. Altogether 46.15% of patients with dysmorphism showed some alteration in genetic tests compared to 13.76% of patients without dysmorphism. A 2011 study showed concordant results, in which dysmorphic appearance and lower IQ were considered factors directly related to changes in genetic tests[22].

Similarly, patients with microcephaly also showed a greater tendency to detect alterations in genetic tests. A total of 57.14% of the microcephalic patients had some altered genetic investigation test, compared to 17.97% of the patients without microcephaly, the p value found was *P*=0.012. Genetic alterations that predispose to autism and alter the phenotype causing microcephaly were found in the literature. Copy number variation in Del1q21.1, Del2q23.1, Dup16p11.2 and chromosomal alterations in SYNGAP1(6p21.3), SCN2A(2q24.3), DYRK1A (21q22.13), are examples of genetic alterations that lead to concomitantly with autism and microcephaly[23].

Finally, through genetic investigation, in several evaluated patients, it was possible to diagnose specific genetic syndromes that may contribute to the pathophysiological understanding of autism. (Table 4)

Although individually rare, each of these described conditions may provide clues about regions or candidate genes related to the pathophysiology of autism. Future studies may enable a better understanding of autism and the possibility of more promising treatments.

As limitations to the study, only a small proportion of families with autistic children seek or have access to genetic counseling, generating a low number of patients studied. In addition, low income, which is a predominant characteristic of the patients evaluated at the public clinic in question, makes it difficult to carry out additional tests. The test that most detected genetic alterations, the CGH Array, is also the most expensive and was performed by only 31 of the 135 patients.

CONCLUSION

The recent increase in autism spectrum disorder diagnoses justifies the importance of searching for the etiology of the syndrome. In this context, we found that genetic investigation can guide families of children with ASD in about 20% of cases. This percentage seemed to be more relevant in children who have some dysmorphism, especially microcephaly.

Table 4. Regions or genes altered in the study patients, associated with the physiology of autism.

Phelan-Mcdermid syndrome (1 case)	50% of patients with the syndrome have ASD and other neuropsychiatric phenotypes such as intellectual disability, severely delayed or absent language[24].
DiGeorge syndrome (1 case)	Between 20% and 50% of those diagnosed have autism. Additionally, 60% of adults will have a psychiatric diagnosis such as schizophrenia, attention deficit hyperactivity disorder, and anxiety disorders[25].
Prader-Willi ou Angelmann syndrome (1 case)	This disorder is related to autism in about 30% of cases, in addition there are frequent reports of speech delay (67%), motor delay (42%), dysmorphism (39%) and changes in the magnetic resonance of the skull (43%) related to it[20].
Haploinsufficiency by dominant autosomal inheritance MEF2C syndrome (1 case)	is related to ASD in about 40% of the cases, in addition to severe intellectual disability with absence of speech, in up to 90% of the cases alterations are observed in the magnetic resonance of the skull[21].
Microduplication in region q13.3 of chromosome 15 (1 case)	Autistic patients between 40 and 85% of the cases[26].
Microduplication inregion p25.3 of chromosome 3 (1 case)	23.8% of patients had autistic features, in addition to intellectual disability and facial dysmorphism[27].
Microduplication in region q24.1-q24.2 of chromosome 15 (1 case)	studies demonstrate that changes in copy number in the region have been implicated in autism and intellectual disability[28].
Microdeletion in region q21.1 of chromosome 1 (1 case)	A relationship with autism may be related to microcephaly in 50% of cases and mild intellectual disability in 30% of cases[29].
Microdeletion of gene AUTS2 (1 case)	mutations in this gene have already been described in association with autism, intellectual disability and microcephaly[30].
Gain in chromosome 16 region p11.2 (1 case)	This gene has been reported in ASD cohorts with a frequency of 1% and its expressivity is related to neurobehavioral abnormalities that include intellectual disability and somatic abnormalities[31].

Several types of genetic alterations were found in the studied patients. Indeed, other genetic studies have revealed the involvement of hundreds of gene variants in autism, indicating that genetic heterogeneity is a hallmark of autism.

We emphasize here, despite the technical and economic difficulties, the importance of an adequate investigation of each patient within the autism spectrum. Finding a genetic reason for autism seems to have several beneficial effects: it allows family members to have access to adequate genetic counseling enabling better family planning, it brings a better understanding of the natural history of the specific condition, it enables the provision of more personalized healthcare services, identifies underlying medical risks associated with the specific diagnosis and allows family members to access support associations and help improve their quality of life.

Autism neurogenic investigation exams should be offered to all families but consider a more rational use due to difficult access, genetic investigation should prioritize those patients who have some type of dysmorphism and be started with simpler exams such as the karyotype and further supplemented as needed.

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