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AN INTEGRATIVE REVIEW OF GENETIC TESTING FOR THE DIAGNOSIS OF AUTISM SPECTRUM DISORDER

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ABSTRACT

The purpose of the study is to verify in the literature in a systematized way studies that prove the efficacy of genetic tests in the diagnosis of Autism Spectrum Disorder. Data were collected from the Research Databases of the Virtual Health Library and PubMed, using the combination of the following keywords: Genetic tests AND autistic spectrum disorder diagnosis. Articles from 1980 to 2020 were researched in the Portuguese, English and Spanish languages of national and international journals and included 17 full-text articles, in English, from PubMed to make up this study. The selection process of the studies was carried out by adapting the protocol to systematic PRISMA Flow Diagram reviews and characterized in a table. By the study selection method, the 17 articles were categorized into a table that was initially divided into five groups according to the genetic tests addressed. They were systematically subdivided into two definitive classes. The study gave relevance and visibility to the diagnostic yield of each test, in order to scientifically stimulate constant research for results that can positively influence the genetic counseling of each affected family. Thus, this Integrative Literature Review demonstrated the efficacy of genetic tests in search of the light of accuracy.

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INTRODUCTION

Autism Spectrum Disorder is defined as a neurogenetic disorder of polygenic heredity that has heterogeneity of symptoms corroborating the involvement of socialization and communication of patients. Previously, the disorder was described only as "autism", which was eventually modified to the spectrum by adding a group of diseases that have similar characteristics, such as (a) Autistic Disorder, also called classical autism; (b) Asperger's Syndrome; (c) Invasive Developmental Disorder; (d) Disintegrative Childhood Disorder; (e) Rett Syndrome, according to DSM-5 (2013). In this bias, affected individuals tend to present a stereotyped pattern of repetitive and restrictive behavior, such as limited obsessive interests, repetitive movements, repetition of meaningless words, difficulties with social interaction and sensory abnormalities due to speech delay or speech disorder (DSM-5, 2013). Autism Spectrum Disorder (ASD) has been described for approximately seventy years, with exponential growth in the number of cases reported since the 1990s affecting an average of 1% of the world population (UN - United Nations) (Lai *et al*., 2013). According to the Pan American Health Organization (PAHO), one in 160 children has ASD as this condition begins in childhood and tends to extend into adolescence and adulthood (PAHO, 2017). Nationally, there are still no official data on the occurrence of autism, since the law to include autism in the census of the Brazilian Institute of Geography and Statistics (IBGE) was recently sanctioned (2020). With this, Brazil is based on the UN estimate, being estimated, approximately 2 million individuals within the spectrum (Mello *et al*., 2013). In this sense, it is worth mentioning that according to the 2010 IBGE census, 454,706 children had ASD in Brazil (IBGE, 2010). Additionally, it is important to highlight that, according to the Center for Disease Control and Prevention, ASD affected, in 2015, 1 in 42 boys and, to a lesser extent, 1 in 198 girls indicating that there is a trend of higher involvement pattern in males, according to CDC (2014). ASD originates in the first years of life and the characteristic symptoms of the disease usually present before 3 years of age, however the trajectory of the disease in each child is not uniform, justifying the fact that the age of diagnosis varies between patients and countries. In most cases, symptoms present consistently between 12 and 24 months of life and may manifest soon after birth, corresponding to Brazilian Society of Pediatrics (2019). Nevertheless, the age of detection usually occurs between 4 and 5 years of age but may be higher in several countries such as Brazil (Mandell *et al*.,

2010), while in the USA it occurs between 3 and 4 years (Chakrabarti *et al*., 2001 and 2005). Additionally, ASD has a wide range of levels of impairment, encompassing even other comorbidities and, therefore, resulting in patients with extremely variable phenotypes. Thus, affected individuals may present severe intellectual disability with impaired behavioral abilities, as well as unchanged intelligence quotient (IQ), resulting in innocuous difficulties in correct and early diagnosis (Griesi-Oliveira *et. al,* 2017). In addition, the ASD is characterized as a pervasive and permanent disorder, as the cure has not yet been established. However, early detection plays a fundamental role in the prognosis, as it allows for early intervention and insertion of therapies that help improve quality of life.

The diagnosis of ASD is clinical and based on protocols where findings such as motor control abnormalities, delay in motor development, decreased sensitivity to social rewards, negative affect, and difficulty in controlling emotion can be observed. Thus, signs such as absence of social smile, low eye contact, low attention to the human face, greater interest in objects than for people, little or no vocalization, low social reciprocity, unusual discomfort to loud sounds and not following objects and nearby people in motion, are examples of findings often found in individuals with ASD (Brazilian Society of Pediatrics, 2019). The etiology of ASD is multifactorial as it involves genetic and environmental predisposition factors, as well as neurobiological components that interrelate during the onset and progression of the disorder making it difficult to investigate its etiology since the causes are not fullyelucidated (Newschaffer *et al*., 2007). In an analysis performed on 2 million individuals, genetic origin was shown to be the prevalent cause, representing 97% to 99% of cases. Of these, 81% were hereditary. The remaining causes seem to be related to environmental exposures and intrauterine agents, which influence about 1% to 3% of cases, according as Bai *et al*. (2019). In this sense, it is observed that, among the described etiologies, the causes comprise a heterogeneous group that is difficult to identify in laboratory diagnostic tests. How genetics plays a fundamental role in the etiology of ASD, genetic tests emerge as fundamental complements in the diagnosis, either for confirmation in cases of lack of typical autistic characteristics or for contesting diagnostic errors. It is suggested that genetic tests can detect the etiology in 10% to 40% of ASD cases in the USA and Canada, increasing rates when ASD is associated with other comorbidities (Yuen, 2017). The genetic basis can be identified by means of methods developed over the years which will be analyzed in the present study, such as conventional cytogenetic studies (karyotyping), microarray-based high-density genomic comparative hybridization (aCGH), broad genome association studies (GWAS), new generation sequencing (NGS), sequencing studies (exome and genome), x-fragile syndrome test, comparative genetic panel creation, and artificial neural networks. It can be observed that, in addition to the multiple existing professional approaches, the component of genetic architecture in ASD has an often-difficult role in diagnostic tests since the disorder also presents different diagnostic approaches in laboratorial terms. Therefore, many genes and associated genetic modifications, such as insertions, dissections, or variations in the number of copies remain unknown, making diagnosis even more difficult in a single test (Sudarshan, 2016). Given the multidimensionality of detection of ASD in genetic tests, the present study asks: what is the diagnostic efficacy of genetic tests in Autism Spectrum Disorder?

The aim of this study was to carry out an integrative literature review, aiming to systematically analyze studies that demonstrate the effectiveness of genetic tests in the diagnosis of ASD.

MATERIALS AND METHODOLOGY

This article consists of an Integrative Literature Review that aims at the synthesis of results obtained in primary research from a theme addressed. In this review, the efficacy of genetic tests used in the diagnosis of Autism Spectrum Disorder (ASD) was addressed. The study was developed according to the following stages: 1) formulation of the problem; 2) data collection; 3) evaluation of the data; 4) data analysis; 5) presentation and interpretation of the results. Data collection was performed at the PubMed and Virtual Health Library (VHL) platforms, using the combination of the following keywords: Genetic tests AND autistic spectrum disorder diagnosis. Articles in the languages of Portuguese, English, and Spanish from journals and magazines worldwide from January 1980 to January 2020 were searched. In addition, articles with repetition in both bases were excluded - counted only once, literature reviews, theses, dissertations, course completion papers, proceedings'summaries, editorials, and abstracts. In this sense, three fundamentals were used in data collection. Firstly, the year of publication, language, title, and article types were evaluated. Next, the study was characterized, including abstract of the article, study information, methodology and primordial results. Finally, the portion of articles in the selected spectrum was elaborated. Thus, the articles were selected from an adaptation of the protocol for systematic reviews PRISMA Flow Diagram (Moher *et al*., 2015). During the analysis, in the evaluation of the full text, 24 articles containing varied diagnostic methods were chosen, including conventional cytogenetic studies (karyotyping), high-density genomic comparative hybridization based on microarray (aCGH), wide genome association studies (GWAS), new generation sequencing (NGS), sequencing studies such as exome and the whole genome, test for fragile X syndrome, creation of comparative genetic panel and artificial neural networks. After this process, there were still full-text articles eligible for exclusion, using the criteria for composition of the study objective and exclusion of remaining literature reviews. Thus, 17 articles present in PubMed were selected from 2006 to 2019 described in English to make up the entire study.

RESULTS

Initially, 633 articles related to the theme were found in the research databases described, which, after analysis, resulted in 17 articles selected of high relevance present in PubMed for the proposed study. Based on the methodology followed, these articles comprise the Integrative Literature Review, according to Figure 1.

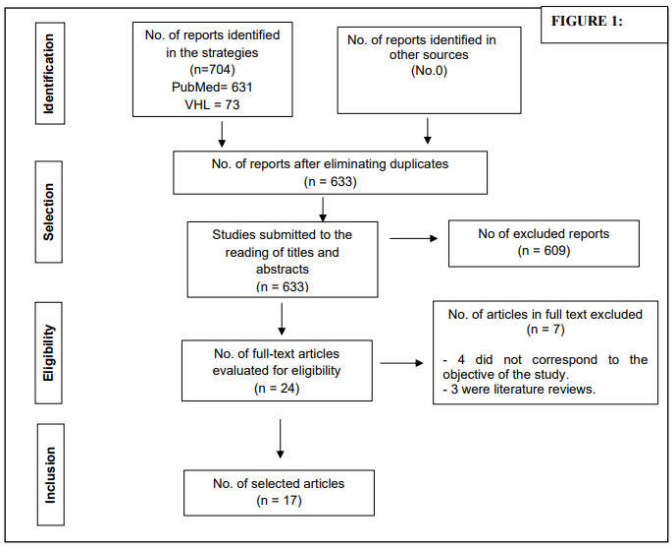

Table 1 represents the specifications of each of the 17 selected articles. All studies were published in English (n=17) and most of them (n=6) originate from the USA, followed by Australia (n=2) and other countries, constituting a variety of nationalities (Iran, China, Korea, Denmark, Slovenia, India, Spain, Italy and France). All selected studies were quantitative (n=17), covering the period from 2006 to 2019, although the initial search in the PubMed database took place from 1980 to January 2020. The content of the analyzed works was initially divided into five groups (G1 to G5), from the perspective of grouping by genetic test described in a systematic way, after the detailed analysis of each full text. G1 (n=2) consists of studies on ANN (Artificial Neural Network); G2 (n=3) consists of studies related to the discovery of candidate genes for ASD; G3 (n=3) is

innovative tests that are not yet routinely applied; the G4 (n=7) comprises the comparative genomic hybridization studies based on microarray (CGH-array); and the G5 (n=2) consists of articles that compare a variety of genetic tests, not focusing on just one of them. Subsequently, the five groups were systematically subdivided into two definitive classes (C1 and C2).

Table 1. Characterization of the selected articles from the Pub Med database scan for the Integrative Literature Review

ID	Group	Class	Origin	Article title	Journal title (vol, nº and p.p.)	Year	Language	Study country
I	G1	C1	PubMed	Application of Single-Nucleotide Polymorphisms in the Diagnosis of Autism Spectrum Disorders: A Preliminary Study with Artificial Neural Networks (Ghafouri-Fard *et al*., 2019).	Journal of Molecular Neuroscience 68(4):515-521.	2019	English	Iran
II	G2	C1	PubMed	Targeted resequencing of 358 candidate genes for autism spectrum disorder in a Chinese cohort reveals diagnostic potential and genotype-phenotype correlations (Zhou *et al*., 2019).	Human Mutation 40(6):801-815.	2019	English	China
III	G4	C2	PubMed	Chromosomal Microarray Analysis as a First-Tier Clinical Diagnostic Test in Patients With Developmental Delay/Intellectual Disability, Autism Spectrum Disorders, and Multiple Congenital Anomalies: A Prospective Multicenter Study in Korea(Jang *et al*., 2019).	Annals of Laboratory Medicine 39(3):299-310.	2019	English	Korea
IV	G3	C1	PubMed	Elevated polygenic burden for autism is associated with differential DNA methylation at birth(Hannon *et al*., 2018).	Genome Medicine 10(1):19.	2018	English	Denmark
V	G4	C2	PubMed	Diagnostic efficacy and new variants in isolated and complex autism spectrum disorder using molecular karyotyping (Lovrecic *et al*. 2018).	Journal of Applied Genetics 59(2):179-185.	2018	English	Slovenia
VI	G5	C2	PubMed	Genetic testing including targeted gene panel in a diverse clinical population of children with autism spectrum disorder: Findings and implications (Kalsner *et al*., 2018).	Molecular Genetics & Genomic Medicine 6(2):171-185.	2018	English	USA
VII	G2	C2	PubMed	Outcomes of Diagnostic Exome Sequencing in Patients With Diagnosed or Suspected Autism Spectrum Disorders (Rossi *et al*., 2017).	Pediatric Neurology 70:34-43.	2017	English	USA
VIII	G4	C2	PubMed	Chromosomal Microarray Analysis of Consecutive Individuals with Autism Spectrum Disorders Using an Ultra-High Resolution Chromosomal Microarray Optimized for Neuro developmental Disorders(Ho *et al*., 2016).	International Journal of Molecular Sciences 17(12):2070.	2016	English	USA
IX	G1	C1	PubMed	Clinical utility of folate pathway genetic polymorphisms in the diagnosis of autism spectrum disorders (Shaik *et al*., 2016).	Psychiatric Genetics 26(6):281-286.	2016	English	India
X	G3	C1	PubMed	Salivary miRNA profiles identify children with autism spectrum disorder, correlate with adaptive behavior, and implicate ASD candidate genes involved in neurodevelopment. (Hicks *et al*., 2016)	BMC Pediatrics 16:52.	2016	English	USA
XI	G2	C1	PubMed	Comprehensive molecular testing in patients with high functioning autism spectrum disorder. (Alvarez-mora, 2016)	Mutation Research 784-785:46-52.	2016	English	Spain
XII	G4	C2	PubMed	Cognitive deficit and autism spectrum disorders: prospective diagnosis by array CGH(Nicholl *et al*., 2014).	Pathology. 46(1):41-45.	2014	English	Australia
XIII	G4	C2	PubMed	Diagnostic yield of array comparative genomic hybridization in adults with autism spectrum disorders(Stobbe *et al*., 2014).	Genetics in Medicine 16(1):70-77.	2014	English	USA
XIV	G4	C2	PubMed	Confirmation of chromosomal microarray as a first-tier clinical diagnostic test for individuals with developmental delay, intellectual disability, autism spectrum disorders and dysmorphic features (Battaglia *et al*., 2013).	European Journal of Paediatric Neurology 17(6):589-599.	2013	English	Italy
XV	G3	C1	PubMed	Predicting the diagnosis of autism spectrum disorder using gene pathway analysis(Skafidas *et al*., 2014).	Molecular Psychiatry 19(4):504-510.	2014	English	Australia
XVI	G5	C2	PubMed	Clinical genetic testing for patients with autism spectrum disorders. (Shen *et al*., 2010).	Pediatrics 125(4):e727-e735.	2010	English	USA
XVII	G4	C2	PubMed	Array-based comparative genomic hybridisation identifies high frequency of cryptic chromosomal rearrangements in patients with syndromic autism spectrum disorders (Jacquemont *et al*., 2006).	Journal of Medical Genetics 43(11):843-849.	2006	English	France

ID = identification

The first class (C1) addresses the question of tests still in studies about its viability, including 2 articles with discoveries of candidate genes for ASD, and was called genetic tests and genes still under analysis: possible discoveries for the diagnosis of ASD (n =7). The second class (C2) corresponds to tests already consolidated in clinical and laboratory practice, including 1 article with discovery of candidate genes for ASD, and was called consolidated genetic tests: efficacy in the diagnosis of ASD (n=10). These classes will be presented and the articles will be described below. Class 1. Genetic tests and genes still under analysis: possible findings for the diagnosis of ASD: Class 1 addresses the main aspects of genetic testing that had not yet been proven against the mission of being accurately used to diagnose the genetic cause of ASD. Considering that many tests already described have a diagnostic performance below expected to prove the clinical diagnosis of the disease the possible findings described below can influence the course of routine tests. In a

medium of difficult standardization of genetic variants for the diagnosis of ASD, the resequencing of genes in panel is a promising method to be added in the discovery of genetic etiology, however it requires a prior clinical diagnosis and is not indicated for screening the general population (II). Based on these observations, the creation of a genetic panel in a cohort study with the main ASD candidate genes, based on a pilot study with comprehensive molecular tests (*FMR1* and *FMR2* molecular analysis, *array-CGH,* geneticpanel and next generation sequencing - NGS), also helped to interpret new genetic variants. Even using molecular tests comprehensively, the diagnostic yield in ASD becomes low, since there is a difficulty in understanding to distinguish the rare variants related or not to the disorder (XI). It is believed that ASD may be related to Single Nucleotide Polymorphisms (SNPs) in several genes, such as SNP rs4774388 in RORα (Alpha Orphan Receptor Related to Retinoic Acid) located in the GABRB3promoter region (I). Therefore, considering the possible relation with the ASD, ASD an Artificial Neural Network was developed based on genomic data of patients with the disease (n=487) versusnon-carrier individuals (n=455) of this SNP. After cross-validation and the training model tested, the method presented approximately 60% to 80% sensitivity and specificity in the diagnosis. The test allows application soon after birth identifying the ASD resulting from this SNP early. (I) In comparison, Artificial Neural Network has also been tested for folate pathway SNPs (*GCPII* C1561T, *SHMT1* C1420T, *MTHFR* C677T, *MTR* A2756G and *MTRR* A66G) revealing, through the test, that they are risk predictors for the development of the TEA and that it obtains 63.8% accuracy in predicting the risk of developing the TEA. Similarly, the meta-analysis also revealed other risk factors intrinsically linked to ASD worldwide, identifying them by *MTHFR* C677T and hyperhomocysteinemia, thus, these SNPs are considered predictors of moderate risk (IX). Based on these considerations, there has been an investigation of SNPs in the Kyoto Encyclopedia of Genes and Genomes (KEGG) in order to validate cellular processes that are affected in ASD and to create an effective diagnostic test. In this sense, it was developed of a genetic diagnostic classifier, containing 237 SNPs in 146 genes that confirmed the diagnosis of ASD correctly in 85.6% of the cases, obtaining an expressive level of accuracy in homogeneous populations. Thus, there is the possibility of early diagnosis of ASD through SNPs, although studies are needed in other populations, since the SNPs differ between ethnicities. However, in the study, it was suggested that there are deficient cellular processes similar between different ethnicgroups, suggesting the use of the test in heterogeneous groups (XV).

Considering the premise that ASD has a range of interrelated genetic and epigenetic components, microRNAs (miRNAs) from epigenetic mechanisms may favor phenotypic characteristics of the disease. After thorough analysis of the expression of miRNAs in the saliva of individuals with ASD *versus*control group, fourteen miRNAs were differentially expressed in the brain of children in the development phase. This fact is impacting to the extent that the identified miRNAs are related to adaptive behavior during neurological development. Therefore, miRNAs have high specificity for the diagnosis of ASD and, if there is cross-validation, they can become a useful tool for early identification of this disorder (X). In another article of the same class, it was observed that, through blood samples of newborns, it is possible to perform the mapping of locus of quantitative characteristics of DNA methylation (mQTL) and can locate loci associated with ASD. This analysis is justified, according to the authors, due to the increase in polygenic load resulting from methylation variations in DNA in individuals with ASD. In addition, the researchers discovered four loci located on chromosome 20 with a higher probability of DNA methylation, with local genes *KIZ*, *XRN2*, and *NKX2-4* that have a potential functional role in ASD. Nevertheless, two CpGislands located on chromosome 8 have a higher methylation risk for this disease, as well as SNPs associated with chromosome 8 have a polygenic effect on DNA methylation in these two CpG islands.Therefore, genetic analysis of the whole blood of newborns minimizes confusion by DNA of the maternal blood when compared to umbilical blood and allows to identify possible candidate genes and methylation variations that influence the development of ASD in children (IV).

Class 2. Consolidated genetic tests: efficacy in the diagnosis of ASD: Class 2 addresses the different genetic tests clinically indicated in cases of suspected ASD. However, the genetic heterogeneity of ASD makes it difficult to establish a diagnostic method with absolute efficacy, revealing the need for studies to compare the tests to prove the diagnostic yield. Currently, copy number variations (CNVs) are possibly related to TEA, so that chromosomal analysis by Microarray (CMA) appears as an alternative for detecting these findings. Compared to conventional karyotyping, for example, it has a diagnostic yield of approximately five to seven times greater according to the prospective multicenter study. However, CMA may not detect polyploidy, balanced translocations, low-level mosaicism, and marker chromosomes. In this sense, aiming to increase the diagnostic accuracy, it is suggested that next-generation sequencing (NGS) be performed after CMA due to the reduction in the detection rate practically by half in the first case (III). Furthermore, studies with high-resolution *microarray* for CNVs detection revealed a detection result of approximately 28% in patients with neurological development disorder and 24.4% for patients with specific ASD. Chromosomal abnormalities commonly found in ASD were duplication of the 15q11-q13 region (maternal origin) and microdissections in the chromosome regions 16p11.2 and 22q. Finally, partial dissections in the *NRXN1* gene were considered essential for the pathogenesis of ASD because there is protein impairment in functional coding with consequent loss of synaptic integrity. Diagnostic yield and detection rate vary between test indications, age, gender and clinical presentation of patients and specialty of the requesting physician. In addition, a study confirmed the relevance of chromosomal *microarray* at the primary level of evaluation ofpatients with ASD so that medical guidelines also considered it as a first-level test in the evaluation of patients with ASD (VIII and XIV).

Comparative genomic hybridization (aCGH) constitutes a diagnostic method with potential efficacy in the detection of ASD (7.3%).In addition, a portion of individuals with ASD have variants of unknown significance (VUS) that can be detected by means of aCGH, affecting, in the study in question, about 10% of individuals. In this bias, four unknown VUS suggested genes involved in TEA, such as SNTG2, PARK2, CADPS2 and NLGN4X. The authors emphasize that aCGH is relevant in the diagnosis of ASD, but still needing studies on its clinical applicability in routine genetic testing (V). Similarly, a standardized aCGH platform was used to identify the frequency of microdeletions and microduplications in CNVs. In the research, it was exposed that there are diagnostic storms in the laboratory routine, mainly due to the use of conventional cytogenetics (XII). In line with another study, aCGH showed 5 to 10 times more resolution when compared to chromosomal analysis by conventional karyotyping (XVII). Thus, as it has greater sensitivity, the authors conclude that aCGH allows for a more accurate determination of molecular defects, optimizing detection and genetic counseling (XII).
In a retrospective review of medical records, the diagnostic yield of GHC was quantified between 5-18% in children (not including the adult population) with developmental disabilities including ASD. Genetic reassessment seems to be crucial, since it can identify presumed pathogenic or pathogenic findings (8.7%) and probable pathogenic variants in the number of copies (8.7%) previously not found and/or not identified through other methods (XIII).In parallel, in another article, it was identified that only about 5% of patients with ASD have visible chromosomal abnormality in cytogenetic tests, most of which are undetectable in routine karyotype. In the current study, fluorescence in situ hybridization (FISH) was performed to confirm the deletions and duplications identified in the microarray analysis. Thus, the research establishes that high-resolution analysis of the entire genome (such as aCGH) appears to be effective in the genetic analysis of patients with ASD. Finally, the benefits of CGH in the genome in the resolution of 1 Mb for the diagnosis of syndromic ASD are notorious, since chromosomal imbalances were three times more detected when compared to the frequency in other tests (XVII).

Another study involving the exome sequencing, presented a diagnostic yield of 25.8% for ASD, so that, by identifying varied genetic etiologies, the article cites this test as being effective in the primary diagnosis of ASD and patients with autistic characteristics. In this study, genes that are candidates for the development of the disease were identified, such as *TRIP12, HDAC1, SETD5, mTOR, HTR2C, RYR3* and *MN1*, so that some of these genes do not corroborate the clinical spectrum of the disease. Finally, the study cites that most mutations in patients with ASD or autistic characteristics result from *new* mutations making explicit the difficulty of standardizing genetic tests in the face of the heterogeneity of thedisease (VII).

In a study that compared several genetic tests, examples such as fragile X test by polymerase chain reaction (PCR) - amplification of the *FMR1* gene, *microarray* and target genetic panel obtained an estimated yield of 12%. In addition, a percentage evolution in the identification of genetic aberrations with pathogenic potential resulted in a yield of about 23%. Another fundamental aspect is this study is that rare variables were discovered in *KIRREL 3* gene with the possibility of association with ASD, demonstrating the use of genetic tests not only for diagnostic purposes, but also for the discovery of new genes that are candidates for the association with ASD. Regarding the yield of complete exome sequencing, there was greater accuracy when compared to the target genetic panel since it obtained rates of 9% to 25% of identified variants. Finally, the study revealed that sequencing of the entire genome has higher yield in the diagnosis of ASD and is likely to be the test of choice during the evaluation of these individuals (VI). Another study compared the diagnostic yield of submicroscopic genomic dissections and duplications of karyotype by G-banding, fragile X test, PCR and southern blot, and CMA. G-banding karyotype for chromosomal abnormalities and fragile X test (PCR and southern blot) have been described as first-level tests for the detection or confirmation of ASD of genetic origin. However, the study mentions that the karyotype does not detect dissections and submicroscopic genomic duplications or copy number variations (CNVs) smaller than ~ 5 megabases (Mb). In another respect, the study points out that sub telomeric hybridization of fluorescence *in situ* (ST-FISH) detected the described CNVs but did not detect other changes in patients already diagnosed with ASD. Alternatively, the CMA achieved an increase in yield when compared to the karyotype, although it did not detect a small number of balanced rearrangements. For this reason, the study does not rule out karyotype by G-banding given this technique detects balanced rearrangements. Additionally, the study points out that genetic tests still have a high value for part of the population, so that when choosing between the two genetic tests before the financial spectrum the chromosomal microarray would be the most viable alternative. Furthermore, the study states that the genome-wide covered chromosomal microarray is more assertive than karyotype and fragile X DNA testing. Finally, the study concludes that, although microarray data are difficult to interpret due to new variants and number of copies of unknown significance (VUS), genetic testing of chromosomal microarray should be considered in the first line of ASD diagnosis (XVI).

DISCUSSION

Given the complex and heterogeneous genetic architecture of ASD, it is essential to recognize that the discovery of all possible causes would be of great value in the medical context. However, it is extremely important to understand that genetic tests have certain limitations to determine the precise and coherent diagnosis of patients, precisely explained by the variability and susceptibility of causal etiology. In this sense, the etiology of ASD is the result of a genetic framework not yet fully elucidated, including rare variants and *new mutations,* related to the influence of environmental aspects, which makes it even more difficult to solve each case. To face these difficulties of diagnosis, the candidate genetic tests were expressive in the studies. Primarily, it is of paramount importance that studies continue theresequencingof genes that are candidates for ASD even if patients require a clinical diagnosis prior to the examination, in order to enable the identification of components related to genetic heterogeneity. The components can be considered rare;however, theyassist in standardizing to compose a genetic panel registered in a database at the international level (Zhou *et al*., 2019 and Alvarez-Mora *et al*., 2016). Cytogenetic studies such as G-banding karyotype and chromosomal microarray analysis (CMA) are tests that investigate variations in genetic material. Variations can be represented by mutations such as substitution, insertions, duplication or deletion which, when the last two are in a block of bases, are called copy number variations (CNVs), according to Shen *et al.* (2010). Nevertheless, CNVs, through genetic tests, have come to be considered relevant in recent years in order to facilitate interpretation (Jang *et al*., 2019). This is justified by the fact that conventional cytogenetics identifies extensive modifications and, generally, does not have the ability to detect submicroscopic structural rearrangements present in chromosomes, such as small CNVs, unlike CMA, which has this ability (Shen *et al*., 2010). In this sense, the CMA, as it allows the detection of CNVs with greater precision, when compared to the conventional karyotype, seems to be a test with greater diagnostic efficacy. Despite this, there are changes that CMA does not detect, such as polyploidy and balanced translocations, requiring subsequent next-generation sequencing (NGS). In this case, there does not seem to be an exclusion relationship, but one of complementarity of genetic tests as a strategy to obtain higher accuracy rates (Jang *et al*., 2019). Different studies have established the CMA as a first-level test in the diagnosis of ASD, a fact that corroborates current medical guidelines, due to the expressive detection rate. However, different method specifications, regarding their resolution, can be used in the detection of CNVs. Thus, it is assumed that there is no absolute standardization or that it is not unanimous regarding the microarray realization method. Therefore, with the standardization of the tests, it would be possible to carry out more studies with fewer variations, optimizing the interpretive analysis (Ho *et al*., 2016 and Battaglia *et al*., 2013).

In these investigations, type SNPs alterations were detected in different candidate genes investigated in patients with ASD and revealed the possibility of developing promising genetic tests in the areas of diagnostic yield and early diagnosis. The Artificial Neural Network (RNA) is one of the methods capable of detecting, soon after birth, SNPs previously described as altered and tested, either through comparative genomic data or from predictive risk of metabolic pathways (folate) (Ghafouri-Fard *et al*., 2019 and Yuen *et al*., 2017). In addition, intracellular changes based on KEGG, through the identification of SNPs were able to verify defective intracellular processes in individuals with ASD from a homogeneous population, which seems to be extremely timely. However, it is worth analyzing that SNPs vary between populations according to different ethnic groups despite the existence ofsimilarly compromised cellular processes between them, which generates a small expectation (Skafidas *et al*., 2014). Thus, although the SNPs proposed for the studies generate a modest diagnostic yield and present a considerable specificity for the disease among the various tests, in-depth studies at the global level are still needed to identify the alteration of specific SNPs for ASD that constitute a detectable group among heterogeneous groups of the population. In this aspect, once again, a standardization that requires resources from various research laboratories to support the findings of very promising original studies is sought. Correlating with the search for an effective method, tests with microRNAs (miRNAs) expressed in the saliva of patients with ASD suggested singular specificity in the diagnosis. The miRNAs constitute a class of non-coding RNAs, conserved throughout evolution, which regulate the expression of various genes by means of RNAm (messenger) in line with the target. For this, some of the identified miRNAs are related to the adaptive development of the neurological system, corroborating for expression in children with ASD. Therefore, if it is validated by cross-methodology with other genetic tests it is extremely valuable to consider its specificity which is well sought in analog tests, corresponding to Hicks *et al*. (2016). In this sense, it is necessary to explain that the recognition of DNA methylation variations (mQTL) in the blood of newborn individuals with ASD seems to be opportune to perform the mapping of altered

loci due to the high polygenic load (Hannon *et al*., 2018). Findings on methylation-susceptible sites, as well as CpG islands and SNPs are part of this analysis that could provide a panel to perform comparative genetic analysis in newborn blood. Moreover, assuming that there are genetic tests considered effective in the diagnosis of ASD, a thorough analysis of each test is essential in a context of multifactorial etiology and variable diagnosis of individuals with ASD. Therefore, the main genetic tests with evidence in the literature were also addressed in this study, especially regarding the percentage of diagnostic yield in each of them. Thus, it is also up to the analysis of the conventional tests explained here, such as chromosomal microarray, comparative genomic hybridization, and exome sequencing, in addition to the comparison of several tests described here.

Thus, a fundamental aspect that has become considered relevant in recent years was the analysis of copies number variations (CNVs), so that there are possibilities for genetic tests to promote interpretations. The microarray chromosomal analysis (CMA) allows the detection of CNVs with greater precision when compared to conventional karyotyping and seems to be a test with greater diagnostic efficacy. Nevertheless, there are cases that are detected by CMA, such as polyploidy and balanced translocations, requiring further new generation sequencing (NGS). In this case, there does not seem to be a relationship of exclusion, but of complementarity of genetic tests as a strategy to obtain higher rates of accuracy (Jang *et al*., 2019). Similarly, other studies have established the chromosomal microarrayas a first-level test in the diagnosis of ASD, a fact that corroborates current medical guidelines due to the expressive detection rate. However, unlike the previous study, there was a specification as to the method that was the use of ultra-high-resolution*microarray* for the detection of CNVs. Thus, it is assumed that there is no absolute standardization or that this is not unanimous regarding the method of performing the microarray. Therefore, with absolute standardization, it would be possible to perform more studies with fewer variations in the characteristics of the tests, possibly resulting in variations automatically related to ASD (Ho *et al*., 2016 and Battaglia *et al*., 2013). However, a preponderant factor of comparative genomic hybridization (aCGH) was the detection of a portion of variants of unknown significance (VUS), which allows us to relate possible genes to the etiology of the disease. Therefore, as the study authors proposed, further studies on aCGH are suggested not only for diagnostic yield, but mainly to relate its clinical applicability in routine genetic testing (Lovrecic *et al*., 2018). Furthermore, considering what was exposed in another study, aCGH at a resolution of 1 Mb seems to be the most effective resolution so far, since it detected three times more the presence of chromosomal imbalances, according to Jacquemont *et al*., 2006). Although the aCGH applicability paradigm and the issues pertaining to the microarray are resolved, it is still emphasized that genetic reassessment is essential, either by identifying previously not found pathogenic findings, or by identifying them in tests that previously did not detect them (Stobbe *et al*., 2014). Assuming that there are genetic tests considered effective in the diagnosis of ASD, a thorough analysis of each test is essential in a context of multifactorial etiology and variable diagnosis of individuals with ASD.

Therefore, the main genetic tests with evidence in the literature were also addressed in this study, especially with regard to the percentage of diagnostic yield for each of them. As for exome sequencing, which is a highly complex genetic test that sequences exons in a large number of genes, today it is known that this test needs to be guided by suspicious clinical characteristics. This peculiarity, added to the characteristic of analyzing small fragments such as exomes, introduces a decisive complexity: as it is a targeted analysis of small fragments, it has a high diagnostic yield, and often superior to aCGH. According to the studies exposed, sequencing can also enable the identification of candidate genes for the development of the disease. However, it is intrinsically dependent on prior and precise knowledge of the etiology of each case – or, at least, on a direction for assertive association with the ASD. Following the line of reasoning of the study presented, this funnel of complexity, combined with the fact that most mutations in patients with ASD or autistic characteristics are de novo mutations, it is difficult to establish a standardization of all exomes related to ASD. In summary, despite being a method considered to have a high diagnostic yield, there is a difficulty in precisely standardizing the fragments that would be related, allowing fluctuations in the percentages of diagnostic indicators used, in consonant with Rossi *et al*. (2017). Concomitantly, another study revealed that the complete sequencing of the exome was more effective when compared to the target genetic panel, allowing the justification of what was exposed in the paragraph above to be legitimized (Kalsner *et al*., 2017).

In another aspect, several comprehensive genetic tests were compared in order to elucidate the specifications of each test and the relationship between them. It was observed that genetic tests do not only serve to diagnose ASD, but also serve to identify candidate genes, which introduces a possible new approach to genetic testing for mass screening and consequent early detection of the disease. In addition, one study has shown that whole-genome sequencing has superior yields in diagnosing ASD, appearing to be a highly effective test. However, there is a high financial barrier that prevents it from being used in the world scenario, making it difficult to implement it as a first-level test (Kalsner *et al*., 2017). In these investigations, SNP-like alterations were detected in different candidate genes investigated in patients with ASD and revealed the possibility of developing promising genetic tests in terms of diagnostic yield and early diagnosis. The Artificial Neural Network (ANN) is one of the methods capable of detecting, soon after birth, SNPs previously described as altered and tested, either through comparative genomic data or through risk predictors of metabolic pathways (folate), in consonant with Ghafouri-Fard *et al*., 2019 and Yuen *et al*. (2017). Furthermore, intracellular modifications based on KEGG, through the identification of SNPs, were able to verify defective intracellular processes in individuals with ASD from a homogeneous population, which seems to be extremely opportune. However, it is valid to analyze that SNPs vary between populations according to different ethnicities, despite the existence of some cellular processes similarly compromised among them, which generates a small expectation (Skafidas *et al*., 2014). Thus, although the SNPs proposed for the studies generate a modest diagnostic yield and present considerable specificity for the disease among the different tests, in-depth studies are still needed to identify specific SNPs for ASD, which constitute a detectable group among different population groups. In this aspect, once again, standardization is sought that requires resources from several research laboratories to support the findings of promising original studies.

Correlating with the search for an effective method, tests with microRNAs (miRNAs) expressed in the saliva of patients with ASD suggested a unique diagnostic specificity. miRNAs constitute a class of non-coding RNAs, conserved throughout evolution, that regulate the expression of several genes through mRNA in line with the target. Therefore, some of the identified miRNAs are related to the adaptive development of the neurological system, supporting their expression in children with ASD. Therefore, if it is validated by cross-methodology with other genetic tests, it is extremely important to consider its specificity, which is so sought after in analogous tests (Hicks *et al*., 2016). Furthermore, the recognition of DNA methylation variations (mQTL) in the blood of newborn individuals with ASD seems to be opportune to perform the mapping of altered loci (Hannon *et al*., 2018). Discoveries about sites susceptible to methylation, such as islands of CpG and SNPs, are part of this analysis that could provide a panel for comparative genetic analysis in newborn blood. Furthermore, given the variety of genetic tests available capable of detecting ASD, it is still essential to consider the possibility of diagnostic failure, as the training and approach of qualified professionals tends to be globally variable, in addition to different perspectives at the time of the exam request. Finally, the use of the Integrative Literature Review as a methodology to analyze the publications on the efficacy of genetic tests in the diagnosis of ASD provided the understanding of the clinical and laboratory applicability of the most diverse tests available. The use of the tests has an etiological complexity of high value, which, by recognizing all the

complicating factors for the detection of ASD in genetic tests in the present study, it was understood that there is an unfavorable influence on the genetic counseling of most families with previously established cases of the disorder. In view of this, treatment is often compromised by refraining from etiological knowledge, both for specialists and for families who remain in constant search for the causal factor. In this review it was evidenced that there are distinct ways to scientifically advance the effectiveness of the tests, either in tests still under analysis or in consolidated tests in medical genetics. Therefore, it was possible to recognize that, for the evolution of the diagnosis, it is necessary to deepen the research in cross-validation of the creation of recent tests and refine the tests proven in the literature through the calculation of the numerous variants. Consequently, the present study gave visibility to the different approaches of diagnostic yield reported by quantitative research from several countries and authors, valuing the mutual sharing of information that can change the course of the disease in question.

REFERENCES

Alvarez-Mora, M.I., Escalona, R.C., Navarro, O.P. *et al*. 2016. Comprehensive molecular testing in patients with high functioning autism spectrum disorder. Mutat Res. 784-785:46-52. Available online at: https://www.sciencedirect.com/science/article/abs/pii/S0027510715300592?via%3Dihub

American Psychiatry Association 2013. Diagnostic and Statistical Manual of Mental disorders - DSM-5. 5th.ed. Washington: American Psychiatric Association.

Bai, D., Yip, B.H.K., Windham, G.C. *et al*. 2019. Association of genetic and environmental factors with autism in a cohort of 5 countries.*JAMA psychiatry.*76 10: 1035-1043. Available online at: https://jamanetwork.com/journals/ jamapsychiatry/ full article/2737582

Battaglia, A., Doccini, V., Bernardini, L. *et al*. 2013. Confirmation of chromosomal microarray as a first-tier clinical diagnostic test for individuals with developmental delay, intellectual disability, autism spectrum disorders and dysmorphic features. Eur J Paediatr Neurol. 176:589-599. Available online at: https://www.ejpn-journal.com/article/S1090-37981300067-6/fulltext

Brazilian Institute of Geography and Statistics 2010. Demographic census 2010. Rio de Janeiro: IBGE.

Brazilian Society of Pediatrics 2019. Scientific Department of Pediatrics of Development and Behavior. Guidance Manual*:* Autism Spectrum Disorder. No. 05, 24 p.

Centers for Disease Control and Prevention CDC, 2014. Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010. MMWR Surveill Summ. 632:1- 21.

Chakrabarti, S., Fombonne, E. 2001. Pervasive developmental disorders in preschool children. Journal of the American Medical Association, 285, 3093-3099. Available online at: https://jamanetwork.com/journals/jama/fullarticle/193955

Chakrabarti, S., Fombonne, E. 2005. Pervasive developmental disorders in preschool children: Confirmation of high prevalence. American Journal of Psychiatry, 126, 133-1141. Available online at: https://ajp.psychiatryonline.org/doi/10.1176/appi.ajp.162.6.1133?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200pubmed

Ghafouri-Fard, S., Taheri, M., Omrani, M.D., Daaee, A., Mohammad-Rahimi, H., Kazazi, H. 2019. Application of Single-Nucleotide Polymorphisms in the Diagnosis of Autism Spectrum Disorders: A Preliminary Study with Artificial Neural Networks. J Mol Neurosci**.** 684:515-521. Available online at: https://link.springer.com/article/10.1007%2Fs12031-019-01311-1

Griesi-Oliveira, K., Sertié, A.L. 2017. Autism spectrum disorders: an updated guide to genetic counseling. Einstein São Paulo.15 2: 233-238. Available online at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5609623/

Hannon, E., Schendel, D., Ladd-Acosta, C. *et al*. 2018. Elevated polygenic burden for autism is associated with differential DNA methylation at birth. Genome Med. 101:19. Available online at: https://genomemedicine.biomedcentral.com/articles/10.1186/s13073-018-0527-4

Hicks, S.D., Ignacio, C., Gentile, K. *et al*. 2016. Salivary miRNA profiles identify children with autism spectrum disorder, correlate with adaptive behavior, and implicate ASD candidate genes involved in neurodevelopment. BMC Pediatr. 2016;16:52. Available online at: https://bmcpediatr.biomedcentral.com/articles/10.1186/s12887-016-0586-x

Ho, K.S., Wassman, E.R., Baxter, A.L. *et al*. 2016. Chromosomal Microarray Analysis of Consecutive Individuals with Autism Spectrum Disorders Using an Ultra-High Resolution Chromosomal Microarray Optimized for Neurodevelopmental Disorders. International journal of molecular sciences. vol. 17,12 2070. Available online at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5187870/

Jacquemont, M.L., Sanlaville, D., Redon, R. *et al*. 2006. Array-based comparative genomic hybridisation identifies high frequency of cryptic chromosomal rearrangements in patients with syndromic autism spectrum disorders. J Med Genet. 4311:843-849. Available online at: https://jmg.bmj.com/content/43/11/843.long

Jang, W., Kim, Y., Han, E., Park, J., Chae, H., Kwon, A., Choi, H., Kim, J., Son, J.O., Lee, S.J., Hong, B.Y., Jang, D.H., Han, J.Y., Lee, J.H., Kim, S.Y., Lee, I.G., Sung, I.K., Moon, Y., Kim, M., Park, J.H. 2019. Chromosomal Microarray Analysis as a First-Tier Clinical Diagnostic Test in Patients With Developmental Delay/Intellectual Disability, Autism Spectrum Disorders, and Multiple Congenital Anomalies: A Prospective Multicenter Study in Korea. Ann Lab Med. 393:299-310. Available online at: https://www.annlabmed.org/journal/view.html?doi=10.3343/alm.2019.39.3.299

Kalsner L, Twachtman-Bassett J, Tokarski K, *et al*. 2017. Genetic testing including targeted gene panel in a diverse clinical population of children with autism spectrum disorder: Findings and implications. Mol Genet Genomic Med. 62:171-185. Available online at: https://onlinelibrary.wiley.com/ doi/full/10.1002/mgg3.354

Lai, Meng-Chuan; Lombardo, V. Michael; Cohen, Simon-Baron 2013. Autism. The Lancet.383 9920: 896-910. Available online at: https://www.thelancet.com/journals/lancet/article/PIIS0140-67361361539-1/fulltext#articleInformation

Lovrečić, L., Rajar, P., Volk, M. *et al*. 2018. Diagnostic efficacy and new variants in isolated and complex autism spectrum disorder using molecular karyotyping. J Appl Genetics. 59, 179–185. Available online at: https://link.springer.com /article/10.1007%2Fs13353-018-0440-y

Mandell, D.S; Morales, K.H; Xie, M., Lawer L.J; Stahmer A.C; Marcus, S.C 2010. Age of Diagnosis Among Medicaid-Enrolled Children With Autism, 2001– 2004. Psychiatr Serv. 618:822–9. Available online at: https://ps.psychiatryonline.org/doi/10.1176/ps.2010.61.8.822?url_ver=Z39.88-2003&rfr_id=ori%3 Arid%3 Acrossref.org &rfr_ dat=cr_pub ++0pubmed&

Mello, Ana Maria S. Ros de; Andrade, Maria America; Ho, Helena; Souza Dias, Inês de 2013. Portraits of autism in Brazil, 1st ed. São Paulo: AMA Available online at: https://www.ama.org.br/site/wp-content/uploads/2017/08/ RetratoDoAutismo.pdf

Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P., Stewart, L.A. *et al*. 2015. Preferred reporting items for systematic review and meta-analysis protocols PRISMA-P 2015 statement. Syst Rev. 1;41:1. Available online at: https://systematicreviewsjournal.biomedcentral.com/articles/10.1186/2046-4053-4-1

Newschaffer, C.J., Croen, L.A., Daniels, J., Giarelli, E., Grether, J.K., Levy, S.E., Mandell, D.S., Miller, L.A., Pinto-Martin, J., Reaven, J., Reynolds, A.M., Rice, C.E., Schendel, D., Windham, G.C. 2007. The epidemiology of autism spectrum disorders. Annu Rev Public Health. 28:235-58. Available online at: https://www.annualreviews.org/doi/10.1146/annurev.

publhealth.28.021406.144007?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed

Nicholl, J., Waters, W., Mulley, J.C. *et al*. 2014. Cognitive deficit and autism spectrum disorders: prospective diagnosis by array CGH. Pathology. 461:41-45. Available online at: https://www.sciencedirect.com/science/article/abs/pii/S0031302516306419

PAHO - Pan American Health Organization 2017. Factsheet - Autism spectrum disorder. Available online at: https://www.paho.org/bra/index.php?Itemid=1098#:~:text=Estima%2Dse%20que%2C%20em%20todo,que%20s%C3%A3o%20significativamente%20mais%20elevados.

Rossi, M., El-Khechen, D., Black, M.H. *et al*. 2017. Outcomes of Diagnostic Exome Sequencing in Patients With Diagnosed or Suspected Autism Spectrum Disorders. Journal Pediatric Neurology. Volume 70, p34-43. E2. Available online at: https://www.pedneur.com/article/S0887-89941630572-0/fulltext

Shaik, M.N., Sai, S.P; Bharathi, V. *et al*. 2016. Clinical utility of folate pathway genetic polymorphisms in the diagnosis of autism spectrum disorders. Psychiatr Genet. 266:281-286. Available online at: https://journals.lww.com/psychgenetics/Abstract/2016/12000/Clinical_utility_of_folate_pathway_genetic.5.aspx

Shen, Y., Dies, K.A., Holm, I.A. *et al*. 2010. Clinical genetic testing for patients with autism spectrum disorders. Pediatrics. 1254:e727-e735. Available online at: https://pediatrics.aappublications.org/ content/125/4/e727.long

Skafidas, E., Testa, R., Zantomio, D. *et al*. 2014. Predicting the diagnosis of autism spectrum disorder using gene pathway analysis. Mol Psychiatry. 194:504-510. Available online at: https://www.nature.com/articles/mp2012126

Stobbe, G., Liu, Y., Wu, R. *et al*. 2014. Diagnostic yield of array comparative genomic hybridization in adults with autism spectrum disorders. *Genet Med*. 161:70-77. Available online at: https://www.nature.com/articles/gim201378

Sudarshan, S., Gupta, N., Kabra, M 2016. Genetic Studies in Autism. Indian J Pediatr83, 1133-1140. Available online at: https://link.springer.com/article/10.1007/s12098-015-1989-7

Yuen, Ryan K.C., Merico, D., Bookman, M. *et al* 2017. The entire genome sequencing feature identifies 18 new genes that are candidates for autism spectrum disorder. Nat Neurosci 20, 602–611. Available online at: https://www.nature.com/articles/nn.4524

Zhou, W.Z., Zhang, J., Li, Z. *et al*. 2019. Targeted resequencing of 358 candidate genes for autism spectrum disorder in a Chinese cohort reveals diagnostic potential and genotype-phenotype correlations. Hum Mutat. 406:801-815. Available online at: https://onlinelibrary.wiley.com/doi/full/10.1002/humu.23724

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