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Genetic Variation in Autism Spectrum Disorder (ASD) Diagnostic Yield of Current Testing for ASD through Commercial Laboratories in an Academic Medical Genetics Center

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Abstract

We conducted a retrospective analytical study in order to determine the yield of currently available diagnostic testing for ASD at our academic medical genetics center (State University), where all genetic tests are send-out, and no institutional funding is available to cover the cost. The diagnostic laboratories were selected based on acceptance and authorization by the patient's insurance plan. Therefore, the diagnostic studies were not universal, and not every patient had the same set of diagnostic testing. Still the overall diagnostic yield was 36.8% (39/106), which is greater than or equal to that of published studies. A total of 106 patients with autism spectrum disorder (ASD) from Appalachian region of Northeast Tennessee, and Southwest Virginia were retrospectively studied. The clinical presentations (phenotypes) were categorized to ASD-Simplex (no dysmorphic features), ASD-Complex (with dysmorphic features), Syndromic (confirmed by genetic tests), and Autistic behavior (not diagnosed as ASD by a psychologist). The male to female ratio in patients with abnormal test result was 3:1 for ASD-simplex, 2:1 for ASD- complex, and 2.83:1 for combined, not including syndromic group, as the ratio depends on the mode of inheritance of the identified syndrome. The primary diagnostic test was chromosome microarray (CMA) with pathogenic/Likely pathogenic results in 23% of patients (22 out of 95 patients who had CMA analysis) showed a copy number variant (CNV) of microdeletion/microduplication with the most frequent CNVs involved 7q11.22 and 16p11.2 chromosomes. Point mutations were identified in 14% (15), through ASD panels (4); whole exome sequencing (5); and syndromes' single gene/panel (6) with each mutation found in a single patient except for PTEN mutations which was identified in 2 patients. Chromosome aneuploidy was identified in 2 patients (T21 and XXY).

Keywords: Autism Spectrum Disorder; Chromosome Aneuploidy; ASD panels; Copy Number Variant; Chromosome Microarray

Abbreviations: ASD: Autism Spectrum Disorder; CMA: Chromosome Microarray; CNV: Copy Number Variant; NGS: Next Generation Sequencing; WES: Whole Exome Sequencing; WGS: Whole Genome Sequencing; CDC: Centers for Disease Control and Prevention; ADDM: Autism and Developmental Disabilities Monitoring; NIH: National Institute of Health; NIMH: National Institute of Mental Health.

Introduction

Autism comprises a clinically heterogeneous group of neurodevelopmental and behavioral disorders - collectively referred to as "autism spectrum disorders" (ASD) - that share common features of impaired social relationships, impaired language and communication, and repetitive behaviors or

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a narrow range of interests [1]. ASD is usually presented in childhood. Approximately 1-2% of the populations are affected, with a male to female ratio of about 4:1[2].

For most children with autism, symptoms develop gradually, although approximately 30% have a "regressive" onset usually between ages 18 and 24 months. About 50%-70% of children with autism are identified as intellectually disabled by nonverbal IQ testing and approximately 25% develop seizures. Some patients may have structural brain malformation, typically identified by brain MRI. Significant dysmorphology present in 25%, indicating an insult in early development. Microcephaly (head size <2nd centile for age) occurs in 5% to 15% of children with autism and Macrocephaly (head size >98th centile for age), in approximately 30% with no outcome correlation [3].

Autism can be considered complex (presence of dysmorphic features and/or microcephaly), simplex or essential (absence of physical abnormalities and microcephaly), or syndromic (autism is part of a defined genetic syndrome) [2]. About 25% of children who fit the diagnostic criteria for ASD at age two to three years subsequently begin to talk and communicate, and by age six to seven years blend to varying degrees into the regular school population. The remaining 75% have lifelong disability requiring intensive parental, school, and social support [2]. For individuals with autism in whom the etiology is known, genetic counseling and risk assessment are based on the mode of inheritance of that specific diagnosis. For autism of unknown cause, the empiric aggregate risk to sibs is 5%-10% for autism and 10%-15% for milder conditions, including language, social, and psychiatric disorders. For families with two or more affected children, the recurrence risk approaches 35% [4].

Because of its prevalence in the general population worldwide and remarkable advances in the knowledge of genetic aspects of autism based on improved clinical diagnosis and advanced molecular diagnostic tools we have a good understanding of the disorder, but still not complete. Currently, using available diagnostic tools, the potential underlying genetic cause can be identified in approximately 25% of patients. The diagnostic yield may reach 30-40% when it is combined with clinical assessment, prenatal history, family history, and disease presentation [5]. Current diagnostic genetic testing for ASD includes chromosomal microarray (CMA) analysis which can identify small copy number variants (CNV) of chromosomal microdeletion and microduplication. Some of the CNVs detected by CMA have been associated with autism. If learning disability/ intellectual disability is present, molecular studies such as ASD/ID panel (usually by next generation sequencing: NGS), or even whole exome sequencing (WES) or whole genome sequencing (WGS) should be considered [5].

Accurate diagnosis will contribute to a better genetic counseling regarding the patient's everyday life, management of the disease, recurrence risk, prenatal diagnosis and reproductive options available to the family. Therefore, molecular genetic diagnosis has become increasingly important. However, diagnostic genetic testing is not universally available to the patients. Depending on the area that the family resides, availability of a genetic diagnostic laboratory offering specialized molecular studies for ASD, and the number of genes tested which are known to be associated with ASD; the diagnostic outcome would be variable.

Another major preventive factor to perform a molecular testing is the cost to the patient, and coverage of the cost by the patient's health insurance. Some genetic testing, such as Whole Exome Sequencing/ Whole Genome Sequencing, may not be covered at all by some health insurance companies, specially, the government-sponsored programs, such as Medicaid/ Medicare. Most patients with a birth defect and / or genetic condition do have government-sponsored health insurance.

We conducted an analytical study to determine the yield of currently available diagnostic testing for ASD at our academic medical genetics center (State University), where all genetic tests are send-out, and no institutional funding is available to cover the cost. The diagnostic laboratory was selected based on accepting patient's insurance plan, and if the authorization for testing is granted by the patient's insurance plan. A couple of genetic diagnostic laboratory may offer a major discount for some of the tests which can be afforded by the patient. Still the diagnostic studies were not universal for the reasons stated above, meaning not every patient had the same set of diagnostic testing.

Methods

This is a retrospective study of 106 patients with a clinical diagnosis of ASD who were seen at the ETSU Medical Genetics Clinic between January 2016 and June 2019. All patients were examined by the clinical geneticist at the campus. Testing included chromosomal microarray (CMA) analysis, single gene or gene panel molecular studies (mostly through Next Generation Sequencing: NGS), and Whole Exome Sequencing (WES). Not every patient had all the above-mentioned studies.

Demographic Data

REGION: All patients were from the Appalachian region, 79% of whom are currently living in Northeast Tennessee.

ASD Categories:

a. ASD-Simplex (no dysmorphic features)
b. ASD-Complex (with dysmorphic features)
c. Syndromic (confirmed by genetic tests)
d. Autistic behavior (not diagnosed as ASD by a psychologist)

Age Range: 1.5 - 36 years. Median age: 7.5 years.
Male to Female Ratio: 2.83
(For simplex and complex groups combined – Not including syndromic ASD)

Pre-term delivery: 24%
(For patients with available ETSU birth records).

Diagnostic Tests

Overall diagnostic yield: 36.8% (39/106)

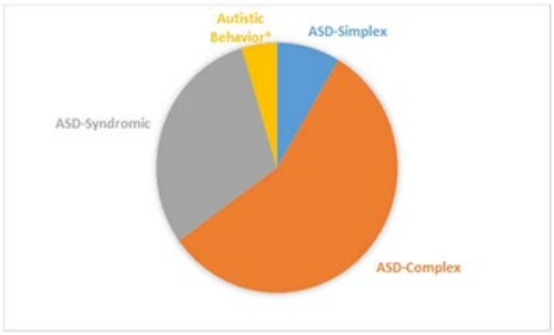
- CMA (Primary diagnostic tool): 23%: 22 out of 95 of patients who had CMA analysis showed a Pathogenic/ Likely Pathogenic copy number variant (CNV) with the most frequent CNVs being 7q11.22 deletion: 3 patients (a mother and her two children), and 16p11.2: 2 (1 deletion, 1 duplication)
- Point mutations: 14% (15) with each mutation in a single patient except for PTEN mutations in two patients.
- Chromosome aneuploidy: 2% (2) (Trisomy 21 Down syndrome and Klinefelter syndrome: 46,XXY).

Results

This research project was approved by the ETSU-IRB.
A total of 106 patients with autism/ ASD from Appalachian region were retrospectively studied. M/F ratio: 3:1 for ASD-simplex, 2:1 for ASD-complex, and **2.83:1** for combined, not including syndromic group, as the ratio depends on the syndrome's mode of inheritance.
This is lower than the CDC reported ratio of 4:1 in USA, probably due to our small sample size, and/or excluding the syndromic ASD (~30 patients).

The intra-familial overlapping mutation supports the genetic heterogeneity of ASD.

The genetic causes of ASD were identified in 36.8% of our patients, greater than or equal to that of published studies.

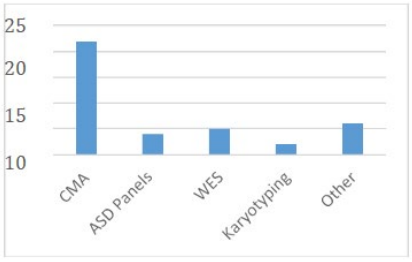


ASD-Simplex: **8.5%**

ASD-Complex: **56.6%**

ASD-Syndromic: **30.2%**

Figure 1: Autism/ ASD Categories



CMA analysis: **22**

ASD panels (48 genes or Expanded ASD panel of 2500 genes): **4**

Whole Exome Sequencing (WES): **5**

Figure 2**:** Positive Test Results in ASD

Discussion

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that affects communication and behavior. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) [1], it is characterized by (a) Ongoing social problems that includes difficulty in communicating and interacting with others; (b) Repetitive behaviors, as well as

limited interests or activities; (c) Symptoms that typically are recognized in the first two years of life; and (d) Symptoms that hurt the individual's ability to function socially, at school or work, or other areas of life.

Additional information can be found on the NIMH Topics page on Autism Spectrum Disorder, and on the Centers for Disease Control and Prevention (CDC) Autism Spectrum Disorder web page. Prevalence data for ASD based on the CDC's Autism and Developmental Disabilities Monitoring (ADDM) Network (most recently published March, 2020).

- Across the CDC surveillance sites, averages of 1 in every 54 (1.9%) 8-year-old children were identified as having ASD in 2016.
- ASD is 4.3 times more common in boys (3.0%) than in girls (0.7%).
- Although autism can be diagnosed at any age, it is said to be a "developmental disorder" because symptoms generally appear in the first two years of life. It can usually be reliably diagnosed by the age of 2 years.
- ASD occurs in all ethnic, racial, and economic groups.

Autism is known as a "spectrum" disorder because there is a wide variation in the type and severity of symptoms in affected individuals. Although ASD can be a lifelong disorder, treatments and services can improve a person's symptoms and ability to function.

The American Academy of Pediatrics recommends that every child should receive well-child check-ups with a pediatrician or an early childhood health care provider, and all children should be screened for autism. The evaluation may assess Cognitive level or thinking skills; Language abilities; and Age-appropriate skills needed to complete daily activities independently, such as eating, dressing, and toileting. The comprehensive evaluation may include, hearing test, genetic evaluation, and molecular studies.

Researchers suggest that multiple genes (multigenic) together with environment factors (multifactorial) may be the cause of ASD. It is not known yet why some people develop ASD and others don't. Some risk factors include, having a sibling with ASD, having older parents; having certain genetic conditions (e.g. Down syndrome, fragile X syndrome, Rett syndrome, etc.); and very low birth weight [5]. Currently, a number of specific genetic variant are known to be associated with ASD, while sets of common low-risk variants that would lead to polygenic or multifactorial forms of ASD are still not well recognizable.

Therefore, parents should understand the molecular testing may identify the molecular basis of the disease only in a limited number of patients [6].

Altogether, for the majority of ASD cases, no specific genetic alteration can be identified. However, ASD can be part of a monogenic syndrome and/or metabolic disorders with a known mode of inheritance (autosomal dominant, autosomal recessive, X-linked, etc.).

The chromosomal copy number variants (CNV), such as microdeletions and microduplication could be associated with ASD in about 10% of patients [7]. The rate is even higher in those with microcephaly (or macrocephaly), dysmorphic features, congenital anomalies, seizures, or those with a family history of neurodevelopmental and psychiatric disorders. Therefore, all patients with ASD should be screened for CNV by CMA analysis [8]. The most common CNV found in ASD patients are located at 15q11-13, 16p11 and 22q11-13, with an overall incidence of around 3 to 5% [9].

Examples of chromosomal microdeletions/microduplication syndrome (copy number variants: CNV), include velocardiofacial/DiGeorge syndrome (22q11.2 deletion), and Phelan-McDermid syndrome (22q13 deletion). The list of CNVs associated with ASD is constantly expanding since the advent of CMA and other molecular studies.

Constitutional chromosome analysis with karyotyping are indicated only when there is a suspicion of aneuploidy or a history of repeated abortions suggestive of chromosomal rearrangements.

The monogenic syndromes associated with ASD include Fragile-X syndrome (FMR1 gene), which has a high prevalence among patients with ASD [10] (and therefore, all affected males should be tested for FRAX syndrome with or without related clinical features) [8], Rett syndrome (MECP2 gen), which is seen in 4% of females with ASD and severe ID (All females with ASD and severe ID should be tested for MECP2 gene mutation), and Cowden syndrome (PTEN gene)/ hamartoma tumor syndrome, presenting with macrosomia/ macrocephaly [8]. Some other monogenic syndromes associated with ASD include Noonan syndrome (PTPN11), Angelman syndrome (UBE3A), and Smith-Magenis/Potocki-Lupski syndrome (RAI1), among others.

ASD can also be associated with metabolic disorders in a relatively small number of patients, which have mostly an autosomal recessive mode of inheritance, and characteristic clinical presentation, such as seizures, neurological regression and multisystem involvement. Screening for metabolic disorders should be considered in all patients with ASD [5,8].

The whole exome sequencing (WES) and whole genome sequencing (WGS) may show *de novo* disruptive single nucleotide variants (SNP) in about 8-20% of individuals with

ASD [9,11] specially in those with moderate to severe ID [12]. However, the rate of variants with uncertain significance (VUS) is also high in these studies. Therefore, the WES is not considered as a first-tier diagnostic tool in ASD. However, development of new analytical approaches and affordable cost of WES and WGS (the WGS is not yet available as much as WES) will eventually make them the gold standard molecular test for ASD [5].

Currently no mutation-specific treatment is available for ASD. Though, some metabolic disorders or monogenic syndromes, and those associated with tumorigenesis and some other comorbidities (e.g. hamartoma tumor syndromes, neurofibromatosis type I, and tuberous sclerosis) may benefit from an accurate diagnosis for both treatment and prevention.

The estimation of recurrence risk might be hampered by incomplete penetrance of some of the ASD- related variants, and association of many well-known ASD-related variants with susceptibility to other psychiatric phenotypes.

The chromosomal microarray analysis and next generation sequencing technologies can currently identify around 25% of etiological factors in ASD [12]. Combined with clinical phenotype and family history, and biochemical and molecular testing for known metabolic and monogenic ASD-related syndromes, the etiology of ASD can be determined for approximately 30 to 40% of the cases [8]. Thus, a careful clinical evaluation of the patient by a clinical geneticist, and assessment of family history, which can give some insights on the pattern of inheritance, can improve the diagnostic yield and the choice of appropriated molecular testing in each case.

The medical genetics specialists are available in some of the academic centers, but not all. The genetic testing including chromosome microarray (CMA) analysis, molecular studies (single gene or panel), whole exome sequencing (and whole genome sequencing) are usually offered by specialized laboratories, and any, or some of these tests may not be authorized by the patient's health plan. Therefore, at the present there is no consistency in coverage for clinical evaluation, and genetic testing nationwide. However, we are in the right direction and eventually, these services could be offered to all patients with some consistency and at an affordable cost.

Conclusion

- All patients with ASD should have a psychological evaluation for autism to verify the clinical diagnosis and to determine the level of autism in the patient.
- All patients diagnosed with ASD should have evaluation by a clinical geneticist to identify the syndromic type of ASD, usually a single gene disorder, to be confirmed by molecular studies.
- Children with a clinical diagnosis of ASD will benefit from specialized genetic tests. The genetic diagnosis will help improve patient care and identify other at-risk family members, depending on the type of genetic abnormality/ condition identified in the patient, and the carrier status of the parents.
- If a genetic syndrome is not identified in the patient, he or she should have a **chromosome microarray (CMA)** analysis, as the first tier diagnostic test (as recommended by the American Academy of Pediatrics [AAP], and American College of Medical Genetics [ACMG]). If the CMA is normal, other diagnostic molecular studies should be considered (based on availability of the test): **ASD panel** (40-50 genes or more, depending on the laboratory offering the test), reflex to **ASD/ID panel** (~2,500 genes or more), reflex to **whole exome sequencing** (~20,000 genes).
- If the above studies are Negative/Inconclusive, consider **whole genome sequencing**, if available, and if covered by the patient's insurance plan (and if the laboratory accepts [or contracted with] the patient's health insurance to get authorization for testing).

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