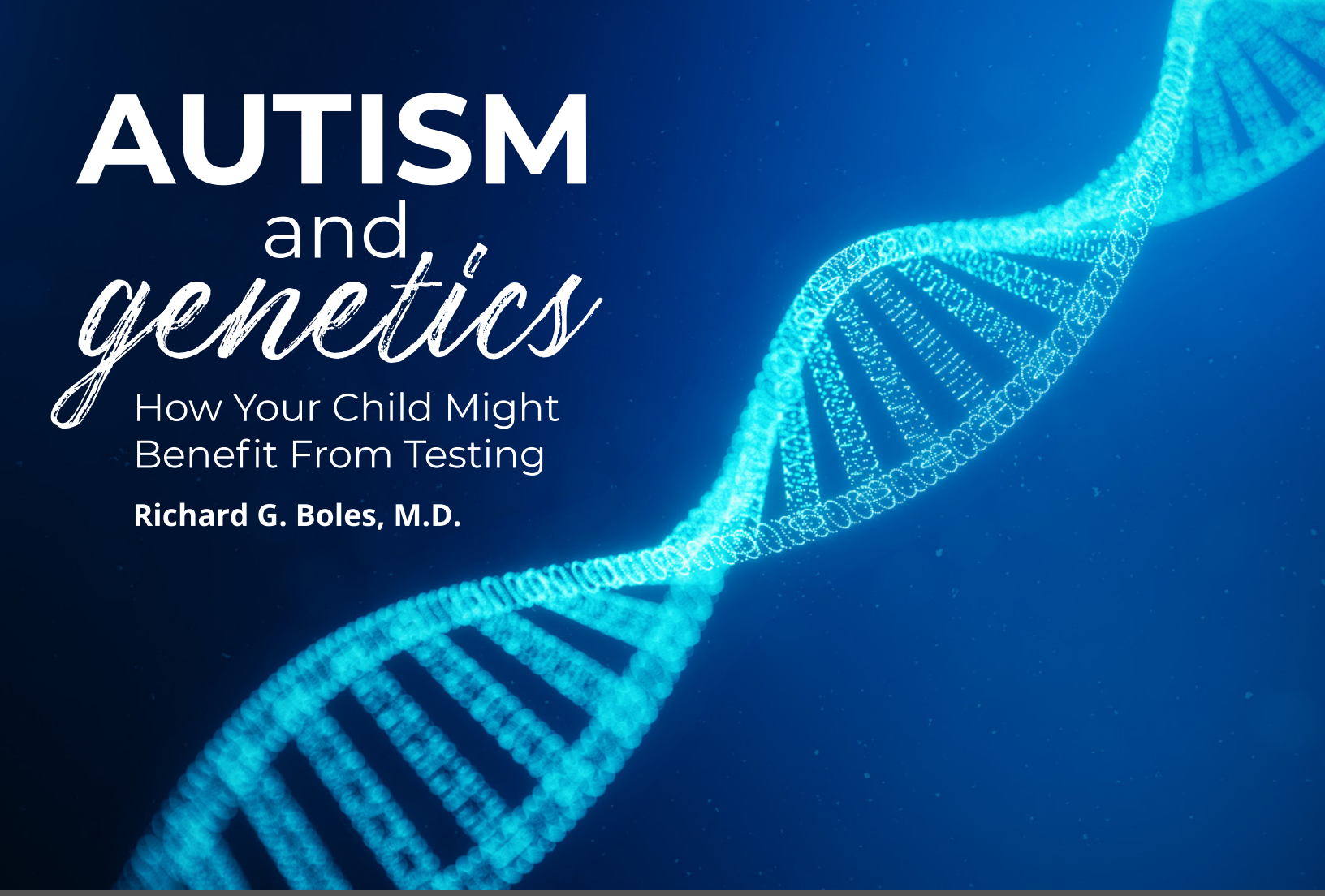
AUTISM and genetics

How Your Child Might Benefit From Testing

Richard G. Boles, M.D.



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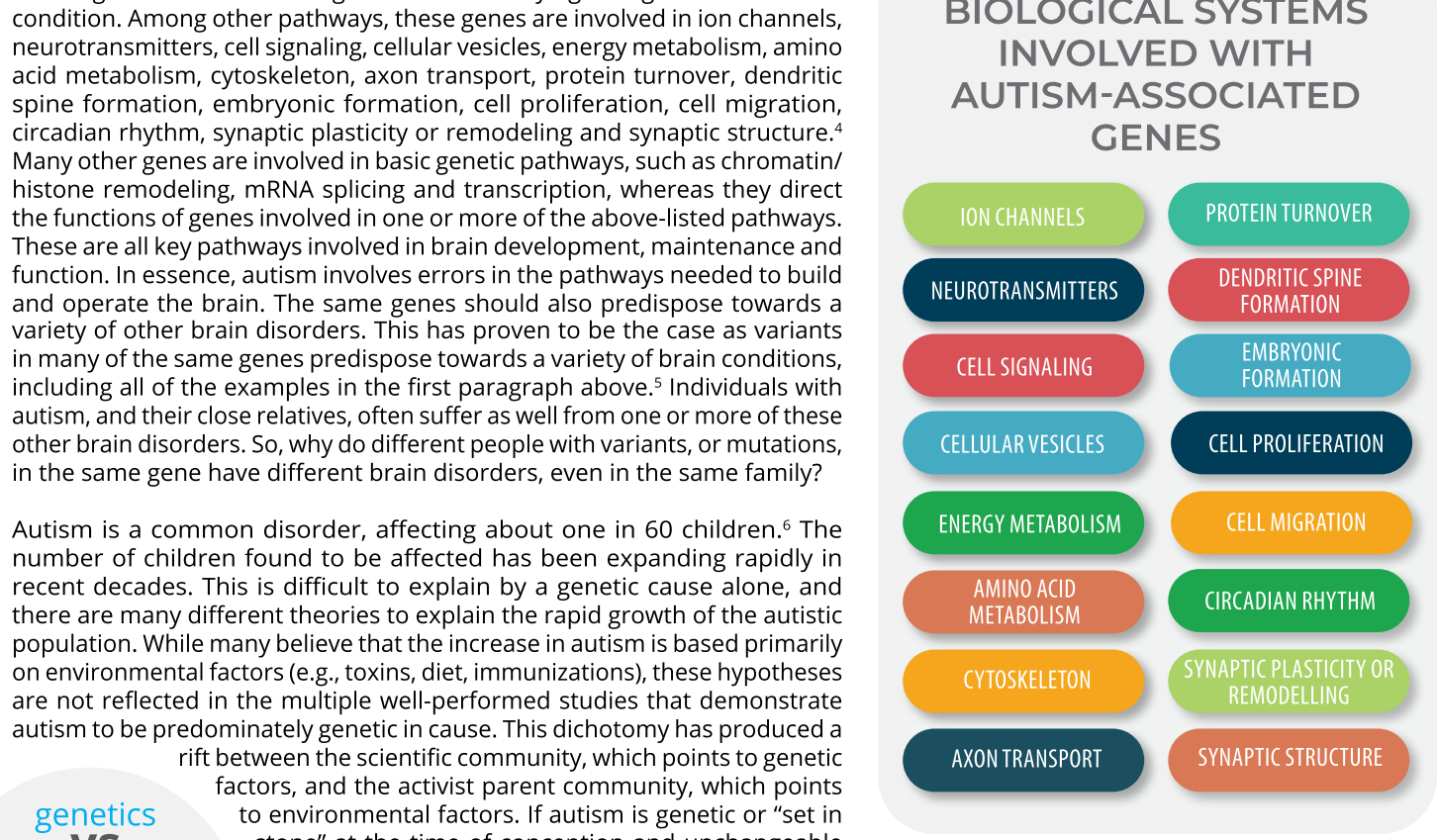
Autism Spectrum Disorder and Genetics

Autism is a common condition that is defined based on behavioral observation. In essence, the term "autism" is applied when there is substantial impairment in social communication with onset in early childhood.[1] Intelligence can be low, normal or high, as long as social communication skills fall far below that of other cognitive, or thinking skills. Specific behavioral criteria are used to determine the presence or absence of the diagnosis, regardless of the underlying biology. Thus defined, and given the incredible complexity of the human brain, it is not surprising that hundreds of known factors can result in autism. In this regard, autism is not an exception since many other common brain disorders, including migraine, epilepsy, schizophrenia, intellectual disability and depression, are also defined based on specific diagnostic criteria based primarily on simple observation. Indeed, multiple factors can lead to each of these conditions.

All of the conditions listed above are "multifactorial," in that there are multiple factors, or components, both genetic and environmental, that influence whether any person will develop the disorder. While autism has both genetic and environmental factors, autism is believed to have the highest genetic component of all common disorders.[2] The heritability of autism has been tested through multiple twin and familial studies to be about 80 percent. This figure means that, on average, 80 percent of the reason why a person has autism is based on genetics. In the 1990s, the first evidence for the genetic etiology of autism was suggested through the identification of gene mutations in "syndromic autism," which is autism as part of a specific identifiable condition, such as Fragile X and Rett syndrome.[3] At the time of writing, hundreds of genes have been identified as being involved in autism pathogenesis.[4]

The nature of the genes that predispose towards, or confer risk to develop, autism gives tremendous insight into the underlying biological nature of this condition. Among other pathways, these genes are involved in ion channels, neurotransmitters, cell signaling, cellular vesicles, energy metabolism, amino acid metabolism, cytoskeleton, axon transport, protein turnover, dendritic spine formation, embryonic formation, cell proliferation, cell migration, circadian rhythm, synaptic plasticity or remodeling and synaptic structure.[4] Many other genes are involved in basic genetic pathways, such as chromatin/histone remodeling, mRNA splicing and transcription, whereas they direct the functions of genes involved in one or more of the above-listed pathways. These are all key pathways involved in brain development, maintenance and function. In essence, autism involves errors in the pathways needed to build and operate the brain. The same genes should also predispose towards a variety of other brain disorders. This has proven to be the case as variants in many of the same genes predispose towards a variety of brain conditions, including all of the examples in the first paragraph above.[5] Individuals with autism, and their close relatives, often suffer as well from one or more of these other brain disorders. So, why do different people with variants, or mutations, in the same gene have different brain disorders, even in the same family?

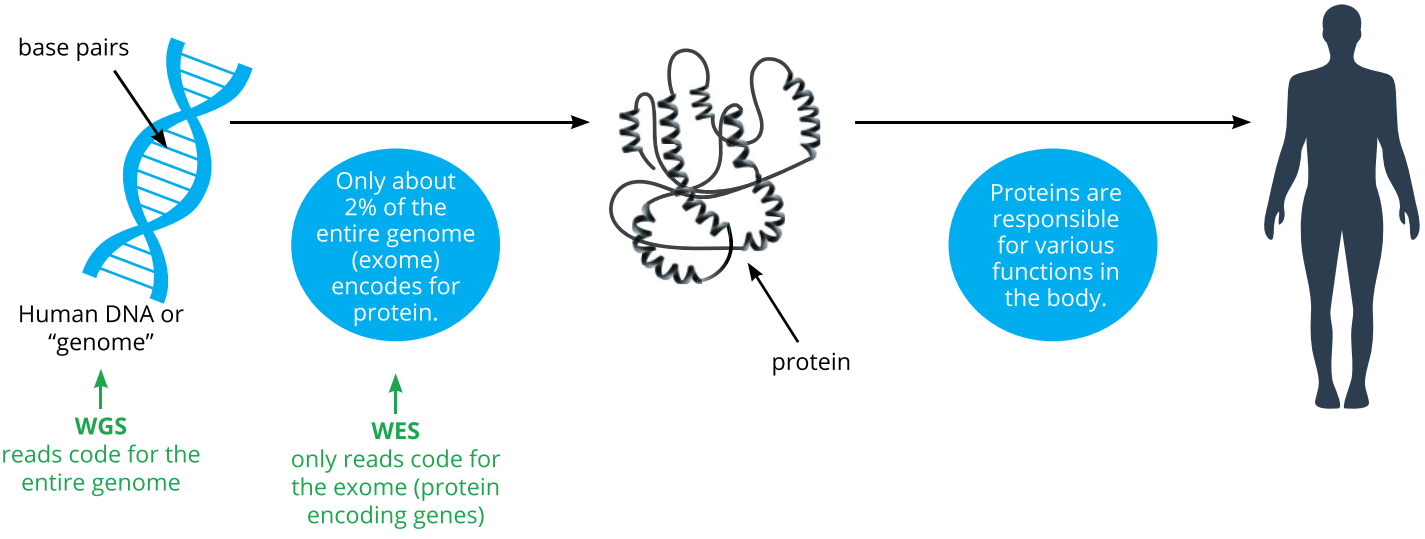


Autism is a common disorder, affecting about one in 60 children.[6] The number of children found to be affected has been expanding rapidly in recent decades. This is difficult to explain by a genetic cause alone, and there are many different theories to explain the rapid growth of the autistic population. While many believe that the increase in autism is based primarily on environmental factors (e.g., toxins, diet, immunizations), these hypotheses are not reflected in the multiple well-performed studies that demonstrate autism to be predominately genetic in cause. This dichotomy has produced a rift between the scientific community, which points to genetic factors, and the activist parent community, which points to environmental factors. If autism is genetic or "set in stone" at the time of conception and unchangeable thereafter, does that mean that any attempts at treatment are doomed to fail? This suggestion goes against the direct observations of many families in which autistic behaviors started abruptly with an environmental stimulus, and later improved with specific treatments, which are also environmental factors. Can both the researchers and the families be correct? Yes! The solution to the dichotomy may lie in the fact that both parties underestimate, or fail to communicate, the extent to which genes are under environmental control. **Autism is, by and large, a genetic condition resulting from variants in a very large number of genes. In general, however, environmental factors often act to trigger the development of disease, while genetics determines the predisposed population at risk.**

Autism – Genetic Testing

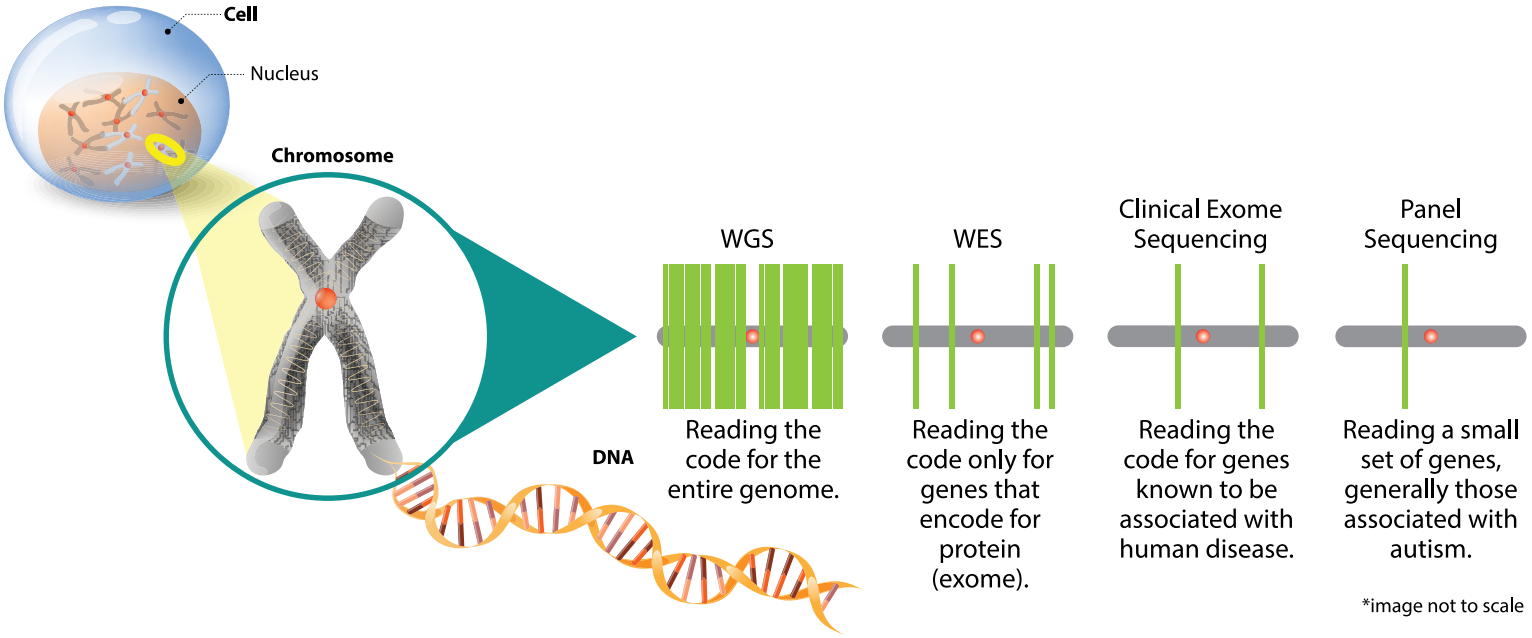
Genetic testing has improved dramatically since the human genome was first sequenced almost 20 years ago and as technology and knowledge have expanded rapidly. In particular, as the cost of this testing has dropped dramatically, accessible DNA sequencing has entered into medical practice and, in many places, become routine.

Human DNA, or the "genome," contains three billion base-pairs of DNA, and there are roughly two copies of this genome, one derived from each parent. Reading the code of essentially all of this DNA is termed "whole genome sequencing" (WGS). DNA generally



operates by encoding proteins, which do most of the work within the body. The region of DNA that is devoted to each protein is called a gene, and there are about 23,000 different genes in the genome. About two percent of the genome encodes for the amino acid sequence of proteins. These encoding sequences, collectively termed the "exome," are read by "whole exome sequencing" (WES). Note that "clinical exome sequencing" is not the same as WES. Rather, it involves the sequencing of a much smaller subset of genes known to be associated with human disease. The exact genes included in each test will vary among laboratories.

Sequencing a much smaller subset of genes, generally those associated with a specific condition, is called "panel sequencing." Many laboratories offer panel sequencing for autism. While hundreds of genes are associated with autism, and many more are likely involved, panels typically are far smaller and vary greatly from laboratory to laboratory.



Which test is best for autism? In the United States, most academic programs, such as those associated with university teaching hospitals, at present order WES or clinical exomes. Most private offices, on the other hand, order panels if they order any sequencing at all. Insurance companies often demand that panels be run first, and that exome testing be performed if the results from panels are negative. However, I strongly recommend WES over clinical exome or panels as WES is more likely to identify changes in genes, called "variants," that are related to disease. The additional information provided is frequently useful in making diagnoses and determining treatment options. Furthermore, WES is more economical in the long run (Table 1).

TABLE 1. Advantages of Whole Exome Sequencing (WES) over Clinical Exome and Panel Sequencing

SENSITIVITY	POLYGENIC	ECONOMIC
WES is more likely to find disease-related variants. This is especially true of most panels, which include only a small minority of autism-related genes.	Most people have multiple variants in different genes that predispose towards disease, instead of a single variant in one gene that causes disease by itself. If a panel is positive and testing stops there, other disease-related variants would be missed, and those additional variants might have been treatable.	**Costs are comparable**: Since the costs are largely involved in marketing and reimbursement, the price of WES is often no higher than that of panels or clinical exomes. **Rapid expansion of knowledge**: Additional genes related to autism are published rapidly, and all panels and clinical exomes are out-of-date even before they are marketed. It is far costlier to reorder testing every other year or so as an undiagnosed patient is re-evaluated. **Specificity**: Insurance companies are often reluctant to cover WES in the belief that more sequencing will identify more "variants of uncertain significance" (VUS), which will require additional costs in added testing and referrals. While finding more VUS is certain, in my experience additional costs related to these VUS are moderate and only occur occasionally.

What about WGS? Since insurance companies often consider WGS to be "investigational," the lack of coverage leads to this test being performed infrequently outside of academic environments. Even in academia, it is not common. The majority of the 98 percent of the DNA beyond the exome that does not code for proteins is **not** "junk DNA," but has many purposes. Most of it is involved in regulating which genes are operating at what time in each tissue. Many non-exomic variants are known to be associated with disease, and the list is growing rapidly as WGS becomes more common. As costs decrease and the clinical benefits of the added sequencing coverage become more evident, the use of WGS is expanding. While WES is workable in many cases, I strongly recommend WGS over WES for the same reasons that I recommend WES over clinical exome and panel sequencing: the higher likelihood of diagnoses and treatment options, and lower costs. (Table 2) While the latter seems counterintuitive, the lower costs stem mainly from the fact that WGS includes many tests usually ordered separately for a higher total cost.

In my opinion, whole genome sequencing (WGS) is clearly the preferred test to order in the case of a patient with autism. The denial of WGS services not only adversely affects patients and families by denial of diagnoses and treatment, but also increases costs due to individual ordering of testing, repeat testing, and by continuing the "diagnostic odyssey."

TABLE 2. Advantages of Whole Genome Sequencing (WGS) over Whole Exome Sequencing (WES)

SENSITIVITY

WGS is more likely to find disease-related variants. While the added benefit relative to WES is modest at present, this advantage is growing rapidly.

Pharmacogenetics: These variants are generally outside of the exome, and thus detectable by WGS but not by WES. These variants oftentimes are important for determining both which drug to choose and the appropriate dosage level.

POLYGENIC

While WES may identify a disease-related variant, additional disease-related variants outside the exome that may be easier to treat could be present.

CONVENIENCE

WGS from some laboratories provides much information that must be ordered as separate tests if WES is ordered, but are important in autism. This includes:

Structural data: Disease-related variants can be small (< about 50 base-pairs and detected by sequencing), medium (no separate tests available), or large (> about 500 base-pairs and detectable by chromosomal microarray (CMA)). WGS detects all variants, regardless of size, and thus a CMA does not need to be ordered separately. In fact, WGS detects more large variants than CMA does.

mtDNA: Mitochondrial DNA is often important in autism, in particular as variants therein are generally treatable.

Trinucleotide repeats (TNRs): Several such TNRs are important in neurological and neurodevelopmental conditions, including Fragile X syndrome.

ECONOMIC

Costs are less: While the costs of WGS are higher than that of WES alone, they are generally lower than that of WES + CMA + mtDNA + TNRs. All of these tests are generally indicated in autism.
Rapid expansion of knowledge: Additional genes related to autism are published rapidly outside of the exome as WGS catches on. Any patient in which WES is ordered today will likely be retested by WGS within one to a few years. This is wasteful.
Specificity: Again, in my experience, additional costs related to VUS found on WGS are both moderate and uncommon.
Diagnostic odyssey: Families will demand — and physicians will order — testing until the "cause" of disease is found. While this is appropriate, it does continue to increase costs for as long as a diagnosis remains elusive. In addition, many of these tests are expensive (e.g., brain MRI) and have risks (e.g., MRI, if sedation is needed). With its high rate of diagnoses, WGS can be quite cost-effective at ending the diagnostic odyssey.

ETHICS

WGS is sometimes criticized on the basis of ethics as it would provide information that families are not prepared to hear and do not want to know. However, these concerns are not reflected in my own experiences in offering WES/WGS to hundreds of my patients. For more information on this topic, see the section below on risks, limitations, and costs.

NON-INVESTIGATIONAL

In most settings, the use of WGS in autism is based on the physician's desire to obtain diagnoses and to alter clinical management, including to identify treatment options, and thus is not investigational.

Autism and Genetic Variation

An important minority of individuals with autism appear to have monogenic "cause" of disease related to a single variant (or pair of variants) in a single gene. These disease-causing variants used to be called "mutations," but this word is now considered pejorative. There are many models to show how a gene can result in autism, and some of the more important ways are summarized here. Most commonly, monogenic autism-related variants are "heterozygous," meaning that a single variant is sufficient to result in disease. Since it is uncommon that either parent has a full case of autism, these variants are generally not present in either parent, but the result of new genetic changes that only affect that one individual. New genetic variants (referred to as "*de novo*") are quite common in autism.[7] They are identified as likely the primary cause of disease in approximately one-third of all cases, in my experience. In order to detect most *de novo* variants, "trio" sequencing of the patient and both parents is required.

Monogenic autism can also be the result of a single disease-related variant on the X-chromosome in males, who have only one X-chromosome inherited from the mother and one Y-chromosome inherited from the father. This X-chromosome variant can be new, inherited from a mother who is mildly affected or from an asymptomatic mother as females have two X-chromosomes, one from each parent. Another form of monogenic autism is autosomal recessive inheritance, in which the child inherited a disease-causing variant in a gene, which can be the same or different variant(s), from both parents. The parents, with one disease-causing variant each, are generally asymptomatic "carriers."

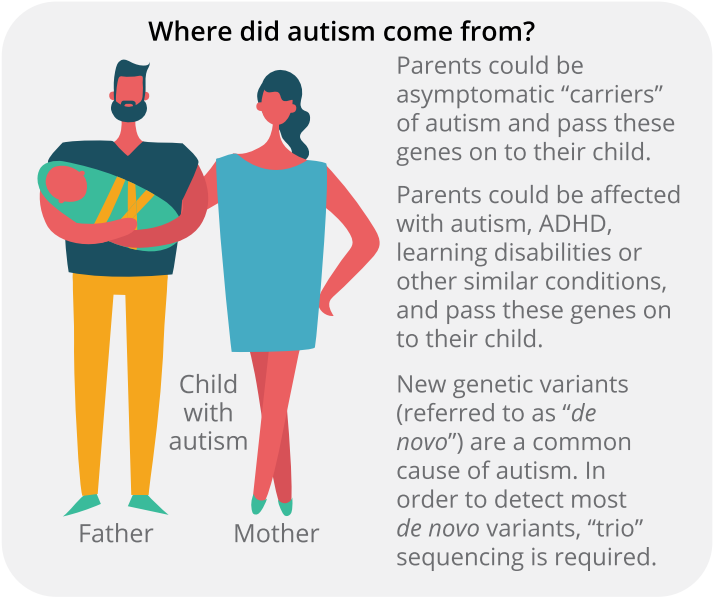
The most common model for how genes lead to autism is polygenic, with the child inheriting autism risk variants from both parents. The parents, as well as siblings and other relatives, obviously carry at least some of these genes, and may also be affected with autism, mildly affected with social issues, ADHD or a learning disability, or completely unaffected and neurotypical.

The Benefits of Genetic Testing

The scientific literature reveals that WES, with all of the different testing components discussed above (CMA, mtDNA, TNR), identifies a diagnosis in about 50 percent of cases.[8] It is worth noting that the studies that this number is based on are dated. In my informal review of my own patients, the figure is closer to 75 to 80 percent with WGS. Like neurodevelopmental disorders in general, autism is a wide spectrum that can range from isolated mild social awkwardness or ADHD to severe intellectual disability, absent speech and social engagement, and multiple additional features of brain dysfunction, such as epilepsy. As expected, genetic diagnoses are more likely on the severe end of the spectrum. In addition, monogenic and *de novo* findings are more common as one moves towards the severe end. However, diagnoses and successful treatment options are not uncommon even on the mild side of the spectrum. In my own practice, I recommend DNA sequencing in all autism cases.

In many cases with autism, multiple genes are involved. Thus, a singular "cause" cannot be identified as one does not exist. Genetic testing in this setting is aimed at identifying risk factors for disease, with the hope that at least one will be treatable.

Inheritance risk quantifies the likelihood that a child not yet born/conceived will inherit a specific trait. Considering all cases of autism, sibling inheritance risks are high: about 25 percent in boys, and about 6 percent in girls. Genetic testing can help determine the recurrence risk in an individual family, being 25 percent in X-linked and autosomal recessive cases, and likely higher than 25 percent in polygenic cases. It is important to understand that these risks are for autism, and additional children are expected to develop other neurodevelopmental disorders, such as ADHD or learning disabilities. In contrast, recurrence risks for autism are very low, less than one percent, in *de novo* cases, which is another reason why trio sequencing is important. Prenatal diagnosis is possible for monogenic cases, but highly problematic for polygenic cases due to a lack of understanding of the risks assigned to each variant identified.



"Trio" sequencing of the patient and both parents is the preferred test in all cases of autism given the high likelihood of new occurrences of *de novo* disease-related DNA variants. However, in cases towards the mild end of the severity spectrum, testing of the patient alone is a good alternative.

Some examples of how genetic testing changes patient management and improves outcomes may be seen in the following clinical vignettes from my private practice (Table 3). Note that in these cases, the finding of a variant of uncertain significance (VUS) that appears to be related to the patient's disease does not indicate that this variant is indeed related to disease. Rather, it provides a treatment option for the physician to discuss with the family.

TABLE 3. Brief Clinical Vignettes Demonstrating How DNA Sequencing Can Lead to Improved Outcomes

Carter has "classical" manifestations of autism, with severe behavioral issues and moderate intellectual disability, without other issues. DNA sequencing revealed a variant found in about one percent of all people, p.Ile253Val in the TRAP1 gene. This gene encodes a protein which protects mitochondrial proteins from adverse environments, such as oxidative stress. Computer analyses at Georgia Tech suggested that the drug granisetron might specifically inhibit the TRAP1 protein. At age eight, he received this treatment and, within days, was more talkative, able to express himself more effectively, and was more tolerant of situations that had previously been challenging, including restaurants. School personnel noted that he was more focused, less aggressive, and had fewer sensory integration issues. The drug was stopped five times in the last four years, and his behavior each time deteriorated and again improved upon restarting granisetron treatment.

Kelly presented to my clinic as a teenager with continuous migraine (24/7/365). Additional issues included nausea, vertigo, fatigue, bowel symptoms, fainting, depression and anxiety. Kelly also has high-functioning autism, and avoided all social contact except with her mother.

Biochemical laboratory testing revealed the presence of a degree of mitochondrial dysfunction. Upon treatment with a mitochondrial cocktail, her physical manifestations improved. DNA sequencing at age 29 identified two probable disease-causing variants in the glutaminase 2 (GLS2) gene, involved in amino acid metabolism. Since loss of this enzyme could regulate cellular energy metabolism as glutamate is converted into alpha-ketoglutarate (aKG), an intermediate of the citric acid (Krebs) cycle in the mitochondria, Kelly was tried on aKG. Significant improvement followed in terms of greatly reduced pain and fatigue, as well as somewhat improved social interaction.

Zach lost all acquired language skills at age 18 months and was diagnosed with autism at age two years. At age six, he developed cyclic vomiting syndrome, a condition defined by distinct stereotypical episodes of nausea and vomiting. At age 12, Zach developed complex regional pain syndrome and was unable to bear weight. Despite high doses of opiates, he was completely wheelchair-bound and in severe chronic pain. Vomiting and pain resolved on treatment with amitriptyline, coenzyme Q10 and L-carnitine, and opiates were withdrawn. However, Zach continued to suffer from severe autism, and his speech was limited to echolalia (repeating word-for-word what he heard). At age 19, DNA sequencing revealed an uncommon variant in the CHAT gene that encodes for the enzyme that makes acetylcholine, an important neurotransmitter. Zach was given Donepezil to block the enzyme that breaks down acetylcholine, thus prolonging its time in brain synapses. Upon treatment, Zach progressed from echolalia to speaking in full sentences, and had fewer autistic outbursts.

Randy already had speech delay when, at age 18 months, he lost all communication skills and parental bonding, leading to a diagnosis of autism. At evaluation at the age of five years, he had severe autistic behaviors, severe intellectual disability and absent speech. Additional problems included chronic constipation, gastrointestinal reflux disease, and a periodic sleep disorder. Regarding the latter, every three weeks, he had an episode in which for four days he would remain awake between 2:00 and 5:00 am, which was highly disruptive for his parents. At age six, DNA sequencing revealed a variant of interest in the AANAT gene, which encodes for a protein involved in melatonin production. Randy's sleep normalized on low-dose (1 mg) melatonin. Additional candidate variants were identified that may be disease-related, including in the HERC2 gene that is in a protein turnover pathway known to be associated with autism, but this additional information did not translate into an improved clinical outcome.

Payam was diagnosed with autism at age 18 months, and also suffered obsessive compulsive disorder, anxiety, irritability and aggression. At age 18 years, DNA sequencing revealed a potential disease-related variant in the SLC6A8 gene, which is on the X-chromosome and encodes for a creatine transporter. Cyclocreatine, which crosses into the brain barrier separate from the creatine transporter, was trialed. Independent observers noted the following changes in Payam after treatment with cyclocreatine: decreased anxiety, more expressive language output, fewer behavioral manifestations of autism, increased ability to handle change and frustrations, greater environmental awareness, improvements in visual attentiveness, decreased hypotonia and a more erect posture.

DNA sequencing does not always find an answer or treatable variants. In my experience, however, about half of the patients do see moderate improvement or more following a treatment that was informed by the results of testing.

The Risks, Limitations and Costs of DNA Sequencing

"Off-target" or "incidental" findings not related to the main purpose of testing (e.g., autism) do occur on WES/WGS, but they usually provide helpful information and rarely pose a burden to families, with expert interpretation. Almost without exception, families want all credible information shared with them. In 2019, this author reviewed 100 of the consent forms signed by the patient/parent(s), and 99 families marked and signed the box that they wanted to know all credible information provided. The sole exception subsequently asked for all of the information when results were discussed during a follow-up visit.

In nearly 30 years of clinical practice as a geneticist, no families informed me that they wished they had not heard the genetic information provided.

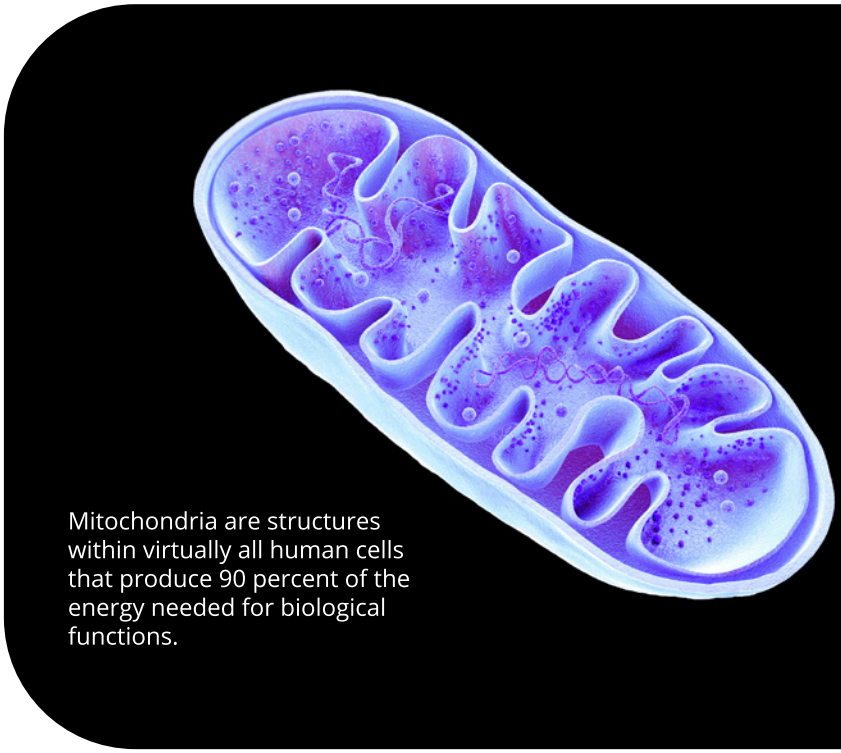
A problem may occur when non-experts attempt to read sequence data and reports. For example, variants that are excellent fits for the patient may not be attended to or be inappropriately excluded. Conversely, variants of uncertain significance (VUS) that are unlikely to be disease-related might not be excluded and confuse the family. This simply underscores the fact that DNA sequence interpretation should only be attempted by medical experts. The main obstacle to widespread DNA sequencing, in my opinion, is the small number of experts that understand both genomics (DNA) and autism. To help address this gap, I partnered with neurologists to found a NeuroGenomics Program which emphasizes the genetics of autism and related disorders.

Costs are another limitation, as well as a justice issue in the USA. DNA sequence interpretation and discussion with the physician and/or family can take several hours of an expert's time, which is not adequately reimbursed by insurance or government. Thus, families often must pay for this service themselves. While costs for the DNA sequencing itself have dropped dramatically, self-pay costs still range from $1,000 for a single WES alone without autism-related interpretation, to about $7,000 for a trio WGS with special interpretation. If ordered by an informed geneticist, insurance or government will agree to cover the sequencing costs in most cases, at least after denial and appeal(s) with a good Letter of Medical Necessity written by the physician.

Not all testing and interpretation are the same. Find an expert who understands both genetics and autism, or who at least works with someone who complements his/her skills. A good team will have both a geneticist and a neurologist, at least one of whom focuses on autism.

Treatment Approaches in Autism

Oftentimes, WES/WGS provides specific genetic information regarding the genetic component(s) in an individual patient that allows for personalized medical therapy (Table 3). However, there are pathways leading to disease that are common to many patients with autism that can be utilized while genetic testing is pending, or if such testing is not feasible.

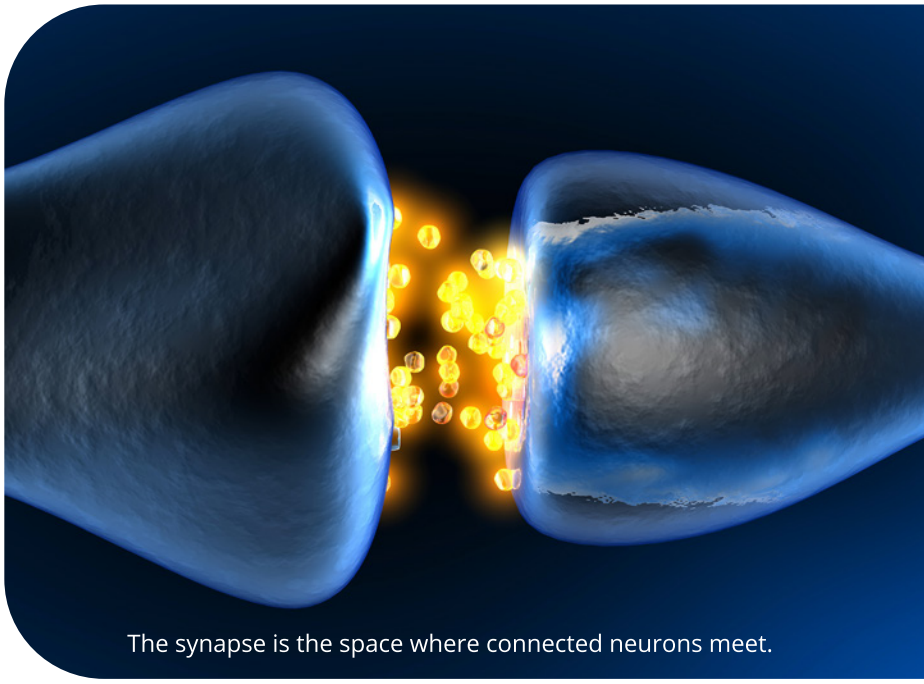


Mitochondria are structures within virtually all human cells that produce 90 percent of the energy needed for biological functions.

Multiple studies have demonstrated that there is a component of mitochondrial dysfunction (abnormal energy metabolism) in many, if not most, cases of autism (Frye and Rossignol). Mitochondria are structures within virtually all human cells that produce 90 percent of the energy needed for biological functions. I have found that most people with autism benefit from mitochondrial-targeted therapies. These are aimed at increasing cellular energy and at detoxifying mitochondria that are dysfunctional through measures such as reducing reactive oxygen species.

"Mitochondrial cocktail" refers to a combined nutritional support, including vitamins, minerals and cofactors, to achieve those aims, and generally consists of about 10 to 30 different components. The data supporting nutritional support in autism are strongest for the following nutrients: carnitine, coenzyme Q10, magnesium, pyridoxine (vitamin B6), folate (vitamin B9), cobalamin (vitamin B12), and vitamin D3.[9] These nutrients are common constituents of a mitochondrial cocktail. A positive response to a mitochondrial cocktail appears to be somewhat independent of the genetic findings in an individual patient since mitochondrial dysfunction appears to be a common phenomenon for autism in general.

In addition, numerous studies have shown that dietary supplementation can have a significant impact on autism in general. In one important study, improvements were seen in hyperactivity, tantruming and receptive language, as well as perceived overall wellness.[10] Proactive physicians who focus on the treatment of autism often emphasize proper nutrition, including diet and the use of dietary supplements. My clinical experience agrees strongly with this emphasis. It is worth noting that all five of the cases in Table 3 saw benefits from mitochondrial cocktail.



The synapse is the space where connected neurons meet.

I generally recommend a combination product such as SpectrumNeeds® or EnergyNeeds® or Autism Nutrition Research Center Essentials in people with any neurodevelopmental disorder, including autism and ADHD, as well as additional coenzyme Q10 in the form of ubiquinol. These products combine mitochondrial support with excellent broad-based nutrition.

The synapse is the space where connected neurons meet, and is an important location where genetic variants predispose towards neurodevelopmental disorders, including autism. The balance between excitatory and inhibitory neurotransmission is of particular importance. One key inhibitory neurotransmitter is known as GABA. GABA signaling is related to energy metabolism in that brain GABA interneurons are particularly sensitive to mitochondrial dysfunction.[11] Decreased inhibitory signaling within the brain can flip the balance into excessive excitatory signaling, predisposing towards the excitatory phenomenon in autistic features, ADHD, anxiety, migraine, and/or seizures. Dietary supplementation aimed at restoring the balance can include GABA itself, theanine, magnesium, zinc, and 5-hydroxy-tryptophan (5-HTP).

For those individuals with troublesome over-excitation, supplements aimed at restoring the excitatory-inhibitory balance are recommended. There are several such products on the market.

Conclusion

It is clear that children with autism spectrum disorder often can benefit from genetic testing. Such testing may lead knowledgeable medical professionals to treatment options that improve behavior, social and communication issues, as well as associated problems such as pain, gastrointestinal symptoms and fatigue. As I have indicated, the preferred test is whole genome sequencing (WGS) for a patient with autism and both parents, referred to as a "trio." Dietary supplements can also have a significant impact on autism. The bottom line is there are steps you can take to help your child and work towards real improvement.

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Disclosure: Dr. Boles is the Chief Medical & Scientific Officer for NeuroNeeds LLC, the start-up company that makes SpectrumNeeds®, EnergyNeeds®, QNeeds®, and CalmNeeds®. As such, he may receive financial compensation based upon his efforts and/or the success of the company. As always, it is recommended that you contact your physician regarding these products and all other changes to disease management.