

# The Challenges and Difficulty of Determining the Genetic Factors Associated with Autism

KELLY BROTTO

Honors 291B (Scientific Literature)

Mentor: Dr. David Haymer

---

*The body of this research essay stems from academic literature, as well as other organizations involved in the field of research for autism spectrum disorder (ASD). Geneticists have found a correlation in the mutation or the deletion of genes in patients with ASD, predominantly on the X-chromosome. This has led researchers to delve deeper into trying to understand the roles of certain genes and how the alteration of these genes, both hereditarily and spontaneously, affect the ASD population. Thus far, research has signaled that ASD is not a one-gene-fits-all-cases type of disorder. Rather, some researchers are now trying to narrow their research to particular genes and a limited subgroup in lieu of analyzing the disorder as a whole. From the research that I have gathered, though there are many challenges that researchers face in trying to gain a fuller understanding of this field, many remain optimistic about the future.*

---

## What is Autism?

Autism Spectrum Disorder (ASD), a behavioral disorder, is the category which encompasses the group of genetically heterogeneous conditions that are clinically characterized by three main features. According to a study published by the International Journal of Molecular Sciences, the features are described as the following:

(1) marked impairments in verbal and non-verbal communications; (2) impairments in social

interactions; and (3) restricted repetitive behaviors and interests (Butler et al., 2015)

Statistically, ASD affects approximately 1.5% of children diagnosed by the age of eight years old. However, there is a discrepancy between males to females affected. Approximately, four males are affected for every female affected by this condition (Butler, et al., 2015). This has led researchers to postulate that perhaps this disorder is correlated with X-chromosome factors. In about 30% of observed cases, there is a clear regression of skills in children with ASD; of those, 60% demonstrate intellectual disability. Differences in phenotype expression are often



I am a sophomore student, originally from Montreal (Quebec), pursuing a Bachelor's of Science in Biology. My highest academic goal is to pursue an MD. This is one of the first pieces of research that I have written thus far in my undergraduate career. I was given the challenge of crafting a research paper based upon scientific literature on work related to the field of genetics. I decided to take this opportunity to learn about a neurodevelopmental disorder that affects many people today.

what researchers find in their studies. In about 30% of observed cases, small or large head size related to a mutation of the PTEN tumor suppressor gene is present in patients (Butler, et al., 2015). The challenge researchers face now though is how do ASD and genetic mutations commonly viewed in autism cases correlate and/or possible present a causation to one another? Researchers have already identified that genetic factors have a contribution as high as 90% to autism; hence, heritability is a key issue (Butler, et al., 2015). In fact, in a family where there is one child with ASD, the likelihood of having another is significantly increased (National Fragile X Foundation, 2017). The biggest difficulty now is isolating and identifying the gene locations on chromosomes that trigger the onset of autism.

## Neuropathology and Autism

Studies conducted in neuropathology hint towards prenatal and postnatal abnormalities in multiple regions of the brain such as the amygdala, the brain stem, the cerebellum, and the cerebral cortex in cases of ASD. Thus far, studies have concluded that in the majority of ASD cases, there are “reductions in cell number and cell size in the cerebellum, limbic system, brainstem, cortex, amygdala, and hippocampus” (Butler, et al., 2015). Furthermore, structural neuroimaging investigations have demonstrated a decrease of gray and white matter in the cerebellum. In a recent study of a 6:4 ASD to control-case analysis, researchers found that Purkinje cell number reduction occurred in 50% of the ASD brains, meaning in three of the six ASD cases. In older studies this percentage had been both lower in some research and higher in other research, demonstrating variability in the data (Gadad, et al., 2013). Hence, it is clear that this is a challenge for even the neuropathological approach to assessing autism.

Additionally, it should be noted that the research done in this area of study is rather limited; “since 1980, only 120 postmortem from people with autism have been studied” (Gadad, et al., 2013). Moreover, many parts of the brain are still, as of yet, to be analyzed in ASD brain function. Therefore, the research is still open to further investigation.

## X-Y Correlation

Currently, there is a 4:1 ratio of ASD in boys to girls. It is postulated that this may be attributable to X-Y configuration in males and the X-X configuration in fe-

males. Therefore, males have only one expressible X-chromosome, making them more susceptible to the effects of X-chromosome-related genetic disorders. As a result, many suspected ASD-causing inactivation of genes on the X-chromosome cannot be compensated for in males. In fact, a search on a range of publications since 2008 in the autism research database, found only three clinically relevant genes for ASD on the Y-chromosome and sixty-eight on the X-chromosome (Butler, et al., 2015).

Furthermore, a team of geneticists in the United States and Switzerland proposed that it all comes down to “the female protective model.” Basically, what this suggests is that females have a higher tolerance for “harmful” genetic mutations than males and require a larger number of them in order to show any physical effects that can be diagnosed. Therefore, under these parameters, it is possible that a male and female have the same genetic mutation on a particular gene, but that only the male will show any physical and/or behavioral impairment. This also raises the question whether it is once again sex-linked or if it is even possible that it may also be hormone linked (ex. estrogen versus testosterone). To this question, researchers have no clear answer as of yet. However, what has been found in relation to this is that a study published in the American Journal of Human Genetics found that as this “female threshold” for genetic mutation resilience is higher, when girls are diagnosed with ASD, they tend to fall on the more severe side of the spectrum, whereas males are more spread out on the spectrum. In fact, the same researchers analyzed 16,000 samples from both males and females with neurodevelopment disorders and found that for females diagnosed with ASD, they have 1.3 to 3 times more severe genetic alterations than the males diagnosed with ASD (Jacquemont, et al., 2014).

## Fragile X Syndrome

Fragile X Syndrome (FXS) is a known single gene correlation for behavioral disorders, including but not limited to ASD. Hence, it is only a related syndrome. The gene that causes FXS is the “FMR1 gene,” located at Xq27.3 on the X chromosome (National Fragile X Foundation, 2017). Usually, the mutation with this is that the cells do not make enough of the FMR protein. In a certain way, this solidifies researchers’ suspicions of X-related mutations as ASD particularly affect males (XY) over females (XX). The gene at Xq27.3 is responsible in helping the communication between dendrites and the central nervous

system (National Fragile X Foundation, 2017). Hence, reduced protein production of this gene often leads to difficulty in intellectual communication as neuronal activity in the central nervous system, encompassing complex thought, is hindered.

Nonetheless, FXS only accounts for 10% of ASD cases in children, leading researchers to dig deeper and suspect that ASD is a multiple-gene disorder. Additionally, it is possible to have FXS and not autism, but rather other behavioral disorders such as ADHD or ADD. Therefore, this postulation is not a genetic variant that solely accounts for ASD, but also other behavioral disorders. This is a challenge for researchers as it only obscures that possible correlation, leaving even more and leaves holes in other portions of solving this mystery.

### PTEN Gene

The PTEN gene is located on the q-arm of chromosome 10 at position 23.31. Its primary function is to block the activity of the PI3K pathway, which regulates cell growth—sometimes called the tumor-suppressor gene. Researchers used MRI to scan the brains of 17 with autism and the PTEN mutation, 16 with autism and macrocephaly and normal copies of PTEN, 38 with autism and average head size, and 14 normally developing controls. They found that those with PTEN mutations have larger brains overall than in all other groups.

The study suggests that PTEN mutations trigger an overgrowth of white matter in the brain. This has helped researchers to understand why macrocephaly, or head enlargement, sometimes is present in those with autism. Additionally, they found that of those with autism and macrocephaly, an estimated 7% have a mutation in the PTEN gene. Additionally, other impairments include a deficiency in working memory and slower processing of new information. However, unlike typically observed increased cortex thickness in people with autism, those with PTEN mutations have normal cortex thickness. Moreover, what makes this condition unique is the outcome of bigger brains, but no increase in cortical thickness. Additionally, though researchers have yet to find out what it means, those with autism and PTEN mutations have more abnormal patches of white matter. What's interesting is that the next step for researchers is to compare people with autism and PTEN mutations to those with PTEN mutations but not autism. This would help to further explain whether it is the white matter abnormalities

that are the leading cause of autism from the perspective of this pathway, the PTEN gene (Zeliadt, 2014).

### CHD8 Gene

The CHD8 gene is located on the q-arm of chromosome 14 at position 11.2. Its primary function is to regulate the structure of DNA. Mutations in this gene generally lead to the same set of symptoms: large head (macrocephaly), constipation, distinct facial features (wide-set eyes, large ears, broad foreheads and noses), and sleeping issues. As with the previous case study, researchers have found that it is more effective to try a reverse approach—start with the genetic mutations and then classify the symptoms, rather than finding a simple gene for all symptoms. Evan Eichler, the professor at the forefront of this study, argues “I think the most important realization is that not all autisms are created equal.” Again, the idea of creating subgroups is becoming increasingly prominent among researchers (Wright, 2014).

Researchers found that with a sample size of nine autistic patients with the CDH8 gene mutation, no family members had the mutation; hence, this mutation of this gene is a spontaneous mutation. In this case, inheritance does not play a role. Researchers took this study a step even further by blocking the expression of this gene in a sample size of zebrafish embryos. The result of this study was that the fish born from this experiment had large eyes and took longer to move a fluorescent pellet along their digestive tract. This further advanced the argument made by researchers on the specific effects of a malfunction in the CDH8 gene as the results from the zebrafish experiment showed changes in the facial features and presented digestive issues to the affected sample size (Biagioli, et al., 2014).

### Multiple Genes: No Clear Answer as of Yet

The number of genes that have been found to correlate to autism, among other behavioral disorders surpass six hundred. Researchers use a multitude of techniques to study affected genes, such as microarray analysis. Chromosomal microarrays (CMA) are becoming increasingly popular in the field of researchers working on studying disorders associated with developmental delay; this study is a form of cytogenetic testing (Miller, et al., 2010). This type of analysis helps to compared changes in DNA be-

tween samples, indicating is any sample has a possible mutation or anomaly.

Standard routine chromosome studies in individuals with ASD have shown abnormalities of over one dozen chromosomes. Various cytogenetic findings are reported including deletions, duplications, translocations, and inversions often involving the chromosome 15q11-q13 region or the 22q11.2 band. More advanced chromosome microarray studies are powerful in finding cytogenetic small submicroscopic deletions or duplications in individuals with ASD indicating the presence of hundreds of candidate and/or known ASD genes localized to each human chromosome [...] In a recent review of genetic linkage data, candidate genes and genome-wide association studies along with further advances in genetic technology including high resolution DNA microarray and next generation sequencing have led to a compilation of 629 clinically relevant candidate and known genes for ASD (National Fragile X Foundation, 2017).

For example, mutations in the X-linked genes of neuroligin 3 and neuroligin 4 has been linked to cases of autism and other neurological-debilitating disorders. Another example is the link between the CNTNAP2 gene and an increased risk of autism (Gadad, et al., 2013). There is possibly over a few hundred different genes suspected of having an effect. In the opinion of Gadad, Hewitson, Young, and German, "it is clear that there is no "autism gene" (2013). As of now, at least, there is no single answer.

## Conclusion

The challenge is made even harder as ASD is such a broad category as it is only diagnosed based on behavioral trait exhibition. However, if a clear genetic cause could be identified, then autism can be more clearly diagnosed and better treated in addition to creating more narrow categories for specific needs in ASD children. Nevertheless, research thus far has indicated that autism, like many other behavioral disorders, is not a single gene cause. Moreover, researchers are now realizing that whilst looking at particular gene mutations at a specific location on the X-chromosome, those with that mutation or inactivation tend to express the same phenotypic facial characteristics.

This has led researchers to reexamine their method of study as they are now coming to realize that perhaps the key to understanding autism is to understand that autism is a very diversified disorder with the possible need for subgroups. As one study put it, one form of autism does not equal all forms of autism. By isolating and seeking to understand smaller subgroups, developing better treatments and knowledge in this field becomes more tangible.

## Works Cited

- Biagioli, M., Blumenthal, I., Brand, H., Erdin, S., Golzio, C., Gusella, J. F., et al. (2014). CHD8 regulates neurodevelopmental pathways associated with autism spectrum disorder in neural progenitors. (P. K. Donahoe, Ed.) *Proceedings of the National Academy of Science of the United States of America*, 111 (42), E4469–E4477.
- Butler, M. G., Rafi, S. K., Hossain, W., Stephan, D. A., & Manzardo, A. M. (2015). Whole Exome Sequencing in Females with Autism Implicates Novel and Candidate Genes. (W. Chi-shing Cho, Ed.) *International Journal of Molecular Sciences* (16), 1312–1314.
- Gadad, B. S., Hewitson, L., Young, K. A., & German, D. C. (2013, August 9). Neuropathology and Animal Models of Autism: Genetic and Environmental Factors. (M. V. Pletnikov, Ed.) 1–12.
- Jacquemont, S., Coe, B. P., Hersch, M., Duyzend, M. H., Krumm, N., Bergmann, S., Beckmann, J. S., Rosenfield, J. A., Eichler, E. E. (2014). A Higher Mutational Burden in Females Supports a "Female Protective Model" in Neurodevelopmental Disorders. *American Journal of Human Genetics*, 94(3), 415–425. <http://dx.doi.org/10.1016/j.ajhg.2014.02.001>
- Miller, D. T., Adam, M. P., Aradhya, S., Biesecker, L. G., Brothman, A. R., Carter, N. P., Ledbetter, D. H., et al. (2010). Consensus Statement: Chromosomal Microarray Is a First-Tier Clinical Diagnostic Test for Individuals with Developmental Disabilities or Congenital Anomalies. *American Journal of Human Genetics*, 86(5), 749–764. <http://doi.org/10.1016/j.ajhg.2010.04.006>
- National Fragile X Foundation. (2017). *Autism Spectrum Disorder and Fragile X Syndrome*. Retrieved February 11, 2017, from National Fragile X Foundation Web site: [fragilex.org](http://fragilex.org).
- Wright, Jessica. (2014). Cluster of Symptoms Reveals Gene's Link to Autism Subtype. Web site: [spectrumnews.org](http://spectrumnews.org).
- Zeliadt, Nicholette. (2014). Scientists Catalog Effects of PTEN Mutations in Autism. Web site: [spectrumnews.org](http://spectrumnews.org).