Genetic factors in the Y chromosome related to Autism Spectrum Disorder

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Abstract

Autism Spectrum Disorder (ASD) is one of the fastest growing diagnosed disorders in our time. One in 68 children are diagnosed and the number is even greater in males, with one in 42. There is currently no cure nor definitively known cause for autism. Since it is more prominent in males, there has been speculation that ASD can be attributed to a genetic anomaly, but it has yet to be proven. To test this hypothesis, cheek cell samples were taken from two families. In the first family, the two full brothers sampled have been diagnosed with autism; in the second family two full brothers were sampled, one of whom has been diagnosed with autism. Their DNA samples were compared with that of an adult male who is not diagnosed and has never had anyone in his family diagnosed. None of the participants were twins. Their DNA samples were amplified using polymerase chain reaction (PCR). Once their DNA has been sequenced, we can compare the samples to determine whether there are any genetic differences (such as variations in tandem repeats or single nucleotide polymorphisms) between the children diagnosed with Autism and the males who are not..

Keywords: autism, genetics, Y-chromosome

Introduction

Autism Spectrum Disorder (ASD) is one of the fastest growing diagnosed disorders of our time. It is categorized as a spectrum disorder due to the variability of degree of functionality in ASD patients. Some patients are very high functioning while others do not use their voice to communicate. The symptoms are not always as clear cut as in other disorders. This vagueness has caused biologists and psychologists to question what causes the disorder. Clearly, it affects the patients' behavior, but there is a question of whether it is due to something genetic or an external factor in their environment. My hypothesis is that people with Autism Spectrum Disorder have a genetic anomaly that predisposes them to Autism Spectrum Disorder and something in their environment can trigger it. The question to where they lie on the spectrum depends on the degree of genetic predisposition and the degree of their environmental cues.

A genetic anomaly is best described as an abnormality in a person's genetic sequence that can cause a disease or disorder. Our genetic sequences are the basis of who we are. They code for the genes that we carry. If we have an abnormality in an important gene, such as one that codes for brain function, it can cause detrimental effects. I believe that ASD is caused by a genetic anomaly such as either a single nucleotide polymorphism (SNP) or a tandem repeat. Nucleotides are the basic components of our DNA. They are either guanine(G), cytosine(C), thymine(T), or adenine(A). Three nucleotides make up a codon; a codon codes for an amino acid. Amino acids make up proteins-macromolecules that serve multiple important functions in our bodies. Genes can be made up of many nucleotides. Some genes are highly conserved and most of the general population has the same nucleotide sequence for the gene, or at least a very similar sequence. If someone has an adenine where the rest of the general population has a thymine, that is called a single nucleotide polymorphism. It is an abnormality of a single nucleotide in a region of a person's genetic sequence. We also have regions of repeating DNA fragments in our DNA. For example, we can have "GCGCGC" repeated multiple times. The number of times that "GC" repeats could vary person to person. In some cases, the more repeats a person has, the more predisposed they are to a certain disorder.

Cystic fibrosis is a disease in which mucus is produced in a much thicker fashion than normal. The mucus builds up in the lungs of the individual. It has many other symptoms and it decreases the quality of life. This is one of many diseases caused by a single nucleotide polymorphism. Huntington's disease is an inherited disorder that causes degenerative break down of nerve cells. It occurs when a person has more than 36

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repeats of the trinucleotide repeat: CAG. The more repeats they have, the more prone they are to having the disease and the earlier it is likely to manifest in life. These are just two examples of diseases that are caused by genetic anomalies.

Autism Spectrum Disorder occurs in one in 68 children and is more prevalent in males, with the rate increasing up to one in 42. Due to the nature of this huge ratio increase, there has been speculation that ASD could be attributed to something in the Y chromosome, since only males have the Y chromosome. If this is the case, people may wonder how it would occur in females at all since they do not possess the Y chromosome. During prophase one of meiosis, crossing over occurs between homologous chromosomes. It is possible though, for an X and Y chromosome to cross over. It does not always occur, but if it did, it could be possible that a Y chromosome with a genetic anomaly could be crossed over with an X chromosome. There could also be a gene on the X chromosome that is fairly similar to a gene on the Y chromosome. This is how a female would get ASD if it is due to the Y chromosome.

A gene on the Y chromosome, NLGN4Y, has been known to play a role in learning, social behavior, and vocalization. Its protein, Neuroligin-4, belongs to a family of cell adhesion molecules that may be critical for functional neuronal synapses. It is found at the Yq11.221 position and is highly expressed in the brain. I previously hypothesized that if a gene was responsible for ASD, it may be possible that it could cross over from the X chromosome, making it probable to show up in females. This gene, however, is not involved in X-Y crossover events. It does have, however, an X-linked countertype called NLGN4X. NLGN4X is also known as AUTSX2 because of its close association with Autism and Asperger's syndrome. It is also expressed in the brain and affects social behavior, vocalization, and learning. It is believed that its protein plays a role in the formation and reshaping of central nervous system synapses.

Jamain et al (2003) showed a SNP in NLGN3 caused a change from arginine to cysteine in the esterase domain of the protein. This missense mutation was not found in the control group that included 100 females and 100 males. We searched for an arginine to cysteine SNP in the esterase of NLGN4Y and we found one, but it had an extremely low frequency of 0.007%. The SNP with the highest frequency we could find on NLGN4Y caused a frameshift mutation that adds an additional thymine in a region of repeating thymines, causing eight instead of seven. Its frequency was relatively higher at 1.6%.

Polymerase chain reaction, or PCR, is amongst one of the most widely used methods in genetics. It copies a segment of DNA up to the billionth degree so it can be genetically analyzed more efficiently. The first step is denaturation where the temperature increases to roughly 98 degrees Celsius in order for the double stranded DNA to denature, or unbind from its complimentary strand. The genetic sequence that we wish to copy, or amplify, must be known in order to obtain the correct primers. The primers are a short set of nucleotides that will bind to their complimentary pairs on the DNA sample. This occurs at around 68 degrees Celsius. Adenine binds with thymine and guanine binds with cytosine. The primers attach, or anneal, to the split DNA strands so that DNA polymerase has something to adhere to. There are also free nucleotides in the solution. DNA polymerase adds the complimentary nucleotides in the direction of 5 prime to 3 prime. This elongation phase occurs when the temperature is at 72 degrees Celsius. DNA polymerase will detach when the temperature is increased to the denaturation level again. The cycle repeats multiple times and the DNA copies increase exponentially. In the end we can even end up with billions of DNA sample copies.

Methods and Materials

The Y-chromosome SNP database by Tiirikka and Moilanen (2015) and the National Center for Biotechnology Information helped us narrow down the major genes on the Y chromosome that carried the most probable genetic anomalies.

To obtain the DNA, we took saliva samples that contain cheek cells using a saline mouth rinse. We customized primers to create a 300 base product. The forward primer had 24 bases: 5'-ATGAGAGAACATGGGTTCACAAAG-3' and the reverse primer had 21 bases reading: 5'-GTTCCCACCCATGAAAGCATC-3'. After isolating the DNA using chelex beads, we conducted the PCR with a variety of alternating factors such as temp and magnesium concentration. We then ran the PCR product through an agarose gel electrophoresis each time, but this showed the PCR to be unsuccessful on multiple attempts. The samples were run through the PCR machine beginning with 30 cycles, and later altered to 32 cycles. Finally, using a different PCR machine that runs multiple reactions with varying temperatures at once, we were able to obtain a successful PCR product between 56 to 58 degrees Celsius for primer binding. The gel was also modified from the initial 0.75 grams of agarose to 0.80 grams, while keeping the buffer at a constant 50 milliliters. The next step is to extract the DNA from the agarose gel to be sent off for sequencing.

The research focused only on males. DNA will be compared from two families: one with a set of brothers who have both been diagnosed with ASD and the other in which only one of the brothers has ASD. In each case, the brothers are fully genetically related and the samples were only taken from them, not the entire family. Their

samples will be compared to that of an unrelated male who does not have ASD and has no family history of ASD. If we can find a genetic anomaly in the two males who have been diagnosed from the males who have not, then that genetic anomaly may be attributed to Autism Spectrum Disorder.

Results

The DNA samples are being sent out with a primer for sequencing, thus, results are pending at this time.

Discussion

We do not yet have the final results, but we hope to find the frameshift mutation in the target segment of DNA from the boys with ASD, and absent in the males who do not have ASD. If all the males with ASD have the extra thymine in the same spot, but the males who do not have ASD do not carry the extra thymine, it will show up in the genetic sequencing of the PCR product. It only takes one nucleotide to cause a grand change; it is just a matter of how important that gene is. We may not find anything at all, but that means we are saving the trouble of other researchers who may have a similar hypothesis in the future.

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