

What are the Possible Causes for Autism Spectrum Disorder?

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Abstract

Ever since the mid 1980's when Autism Spectrum Disorder (ASD) started to become increasingly prevalent, researchers have been trying to find a possible cause for it. Autism Spectrum Disorder is a developmental disorder that manifests itself in children who are between 18-30 months old. People with autism have reduced social skills, and they have a difficult time communicating verbally and non-verbally. Autism is diagnosed through a questionnaire and other instruments that allow psychologists, psychiatrists, speech therapists, neurologists and many other doctors to determine if a child has a form of ASD. There are many theories about what causes autism. It has been widely believed that vaccines, specifically the MMR vaccine causes autism, but that theory has been disproven. Another possibility is the role genetics plays in ASD. Many studies have been done to determine which genes might cause autism. Because of the heterogeneity of the symptoms and behaviors of people with autism and general genetic complexity it has been hard to find a specific genetic cause of autism. While a confirmed gene has not been determined to cause autism many studies have been done, some using whole-exome sequencing (WES) to see if mutations in parent's genes caused their children to have autism. It is also possible that autism can be caused by hereditary or parental factors. There have also been theories that environmental factors play a role in autism such as exposure to tobacco, pollutants, and metals, and including maternal conditions that could affect the fetus. While many of the studies were inconclusive, and a confirmed cause of autism has not been found, the studies do show that genetics does play a major role in ASD, though more studies will have to be done to determine how and which genes cause it.

Introduction

Autism Spectrum Disorder (ASD) is a neurological impairment where the child has a problem interacting with others, has trouble communicating verbally and non-verbally, and exhibits odd behaviors such as repeating certain actions over and over again and acting out if something does not go according to routine (Block et. al., 2006). A child with ASD will have trouble making eye contact with those interacting with him and will be unable to understand what others are thinking or feeling because they have trouble picking up on social cues ("What is Autism", 2009) ASD is predicted to occur in three to six children for every 1000 who are born. It is 5 times more likely for a male to get autism than a female, however if a female does have autism her symptoms are more severe. Autism Spectrum Disorder is categorized as a pervasive developmental disorder because there are many different symptoms and behaviors for people with autism. Someone who has severe symptoms of autism can be placed on one end of the spectrum, while someone with a milder case can be on the opposite end of the spectrum. Different forms of ASD include Rett's disorder, childhood disintegrative disorder, and Asperger's syndrome. (Block et. al., 2006).

Methods

The information that was collected for this research paper was obtained through Proquest, Ebsco, Pubmed, and the NIH website. These resources were provided from the Touro College Library. Key words included autism, ASD, vaccinations, genetics, and environmental factors.

Discussion: Diagnosing Autism

There is no specific test done to determine if a child has ASD because the symptoms and behaviors are so broad and can vary

significantly between each child. Although children can start showing symptoms between 15-18 months of age, the average age of diagnosing is four to five years old. Diagnosis for ASD should be done by gathering developmental history of the child, and by direct observation of the behaviors of the child. There should be a team of different professionals who can assess symptoms and behaviors of the child. The team should include a pediatrician, a child psychiatrist, and a speech and language therapist, and a child psychologist. Behavior of the child should be observed in different settings such as in his home and in his school. The team should be looking for different behaviors that are prevalent in children with ASD. Prognosis of each case is done on an individual basis because the behaviors of the child can vary greatly, and the behaviors can even change over time. Some people with autism can lead productive lives, such as those with high functioning Asperger's syndrome, while others will need assistance all through their lives (Yates et. al., 2013).

MMR Vaccine and Autism

There has been much talk over the years about a possible link between the Mumps, Measles and Rubella (MMR) vaccine and autism. The supposed link caused major panic because vaccines are needed to prevent, in this case, mumps, measles and rubella from spreading. Vaccines were a vital discovery which prevented many infectious diseases from recurring over and over again around the world.

The link between the vaccine and autism was introduced by Andrew Wakefield in 1998 (cited in Richards, 2011), when he conducted an experiment using twelve children to prove the link. It came out later, while his experiment was under

investigation, that he altered many of the details of the children's previous symptoms and behaviors. Therefore his study had no scientific basis to prove a link. Many studies have been done since then to disprove the supposed link between the MMR vaccine and autism (Richards, 2011).

One reason why people may think there is a link between the MMR vaccine and ASD is because children start showing signs of autism right around the time that they get the vaccine. The explanation for this is that the vaccine is given to children only after they are a year old, which is when children start showing symptoms and unusual behavior. Just because these events happen around the same time does not mean that there is any basis for a connection. Many studies were done to try to prove a link between the vaccine and autism but none of them have been successful ('Autism and the MMR Vaccine', 2001).

Investigations done to disprove the link

In 2000, the Institute of Medicine at the National Academy of Science, under the request of the CDC and NIH conducted a review of all the evidence linking the MMR vaccine to ASD. They took under consideration all the studies and concluded that the evidence did not support an association between the vaccine and autism. Furthermore in 2000, the American Academy of Pediatrics held a conference to review the information and found no evidence in support of a linkage ('Autism and the MMR Vaccine', 2001).

In 1999, Taylor and colleagues published a study to determine a connection between the MMR vaccine and autism ('Autism and the MMR Vaccine', 2001). He took all the known cases of ASD in children living in certain districts in London who were born in 1979 or later and matched the ASD patients with an independent registry of vaccinations. His results were: 1. the number of ASD cases had increased steadily since 1979 but there was no steep incline in cases after doctors started using the MMR vaccine. 2. Children showed symptoms and were diagnosed with ASD all at around the same age, regardless if they got the vaccine before or after 18 months of age. This is important because if the children were vaccinated they would have shown signs of ASD earlier than those children who were not vaccinated. 3. Lastly, by age two the number of children who got the vaccine and showed signs for ASD was almost the same amount as children who got the vaccine but did not show any symptoms. If there was a connection between the MMR vaccine and autism more children should have shown symptoms for ASD (NICHD, 2001). Although the MMR vaccine was a popular theory concerning the causes of autism, many later studies failed to show a connection. This still remains controversial and continues to be studied.

Genetic factors that may cause ASD possible genetic mutations that cause autism

Another possible cause of autism is genetic mutations in the genome of the affected person. Mutations in genes that are responsible for encoding proteins which are involved in molecular machinery regulating synaptic protein synthesis is strongly connected to autism. Autism possibly can be caused due to mutations known as Fragile X Syndrome and tuberous sclerosis (TS). Both of these syndromes are caused by abnormal mRNA translation, resulting in excess protein synthesis.

Fragile X Syndrome (FXS) is caused by an unstable expansion of the CGG repeat in the FMR1 gene. The prevalence of autism in the FXS mutation is 30%. This gene mutation causes abnormal methylation production, FMR1 transcription silencing, and decreased FMRP protein levels in the brain. ASD in FXS is mainly characterized by deficits in peer interaction. Tuberous sclerosis is an autosomal dominant disease that is caused by a mutation on either the TSC1 or TSC2 gene. Clinical manifestations of tuberous sclerosis included epilepsy, learning difficulties, and behavioral problems. There are a variety of mutations that cause tuberous sclerosis including nonsense, missense, insertion, and deletion mutations. Autism is significantly more frequent among TS patients than in the general population. Because there is a large phenotypic variability in FXS and TS with patients who have autism, research is being done to make a clear correlation (Perisco et. al., 2013).

Using Whole-Exome Sequencing (WES) to determine possible genetic links to ASD

Whole-Exome Sequencing (WES) is a technique which captures and sequences the exons in the genome to reveal all de novo and low frequenting alleles that contribute to genetic risk for disease. This method is cheaper than Whole-Genome Sequencing, because instead of searching through the entire genome, it only goes through the exons in the genome, which only takes up 2-3% of the genome. This technique is better than the previously used technique of Genome Wide Association Studies (GWAS) because GWAS only reveals risk factors that are common in the population (Icahn School of Medicine, 2012). WES is particularly good for finding the cause of ASD because ASD can be caused from different genes in many different locations with a weak genotype and phenotype correlation (Perisco et. al., 2013).

A study was done applying WES on families where the spouses were related and on families where cousins shared the same disease. This way it is easier to identify specific mutations that cause ASD. The mutations that were identified on the genes were hypomorphic mutations, meaning that the altered gene product has a reduced level of activity, which can explain the wide spectrum of ASD. This analysis was done on three families. In this paper the first family will be discussed.

The first family had three children with ASD and two unaffected children. The parents were first cousins. WES was performed and it showed a single linkage peak in a large homozygous interval, and suggested a 900:1 likelihood that the responsible mutation was found in this homozygous interval. Then WES was performed on one of the affected children. The linked interval showed only a single rare change that was absent in the known databases and population matched controls.

This mutation was on a gene that is responsible for the production of the enzyme AMT, aminomethyltransferase. This enzyme is needed for the breakdown of glycine. Sanger Validation confirmed that the mutation was heterozygous in both parents, homozygous in the affected children, and absent or heterozygous in the unaffected children.

Mutations in AMT cause nonketotic hyperglycemia (NKH), which is a neonatal syndrome that causes lethargy, hypotonia, several seizures and death within the first year. Rarer, atypical forms of NKH have been described in association with hypomorphic missense AMT mutations. This manifests at a later age and causes delay in expressive language, behavioral problems and seizures. While individually non diagnostic, when all three children were tested they exhibited a range of neurological symptoms that were strongly suggestive of NKH (Yu et. al., 2013).

The amygdala theory of autism

There is a network of neural regions that comprise the “social brain”: the orbit-frontal cortex, the superior frontal sulcus, and the amygdala. This region of the brain is responsible for social intelligence, a quality which ASD patients are known to be lacking. The Neuroscience and Behavioral Reviews has come up with The Amygdala Theory of Autism, where they hypothesize that patients with ASD have an amygdala deficit which causes the deficit in social skills.

An experiment was done on rhesus monkeys which showed that ibotenic acid lesions of the amygdala affect the social behavior of the monkeys and they also became socially isolated. When the amygdala-lesioned monkeys were sent into the wild, they were unresponsive to group members, failed to display appropriate social signals and they withdrew from other animals. When another study was done, where lesions were made to the monkeys' anterior temporal lobe, which included the amygdala, the monkeys showed specific symptoms, for example they had a tendency to overreact to all objects, they were hyperemotional, they had a loss of fear, and they started to investigate objects with their mouth instead of their hands.

Then a study conducted with humans was done. They used single photon emission computed tomography that showed that

patients with ASD show significant reductions in the temporal lobe blood flow. The study was done in fuller depth where an fMRI (functional MRI) was done on adults with ASD that showed significantly less amygdala activation during a mentalizing task called “Judging the Mind in the Eye” task. In this experiment, six people with autism were matched for mean age, handedness, IQ, socioeconomic status and educational level with twelve people in the normal group.

In the fMRI scanner a blocked periodic ABA design was used. There were two tasks. The first task, Task A, was supposed to induce periodic MR signal changes, with signal maximum in brain regions relatively specialized from gender recognition from facial stimuli. In this task, the subjects were visually presented with pictures of eyes and were asked to indicate by right button pressing if the picture was a male or female.

The second task, Task B, was checking the periodic MR signal change with signal maximum in brain regions relatively specialized in mental state recognition from facial stimuli. In this task, a response involved choosing between two words that described the mental state of the photographed person.

The key difference between the two tasks was seeing the subject's judgement between the two pictures. Social intelligence is about picking up social cues, such as the expression of people's faces to understand what the person is thinking.

The subjects who had ASD had a hard time doing task B. The fMRI data was analyzed in two stages. First, generic brain activation maps were constructed separately for the control and autistic groups. By doing so, they were able to determine which voxels were activated in each group by each of the two tasks. Secondly, ANOVA was used to determine the voxels that demonstrated a significant difference between groups in mean power of response to each task.

The autistic group activated the frontal components less extensively than the control group, and they did not activate the amygdala at all. The control group demonstrated significantly greater power of response in their bilateral superior temporal gyrus. The autistic group seems not to use the amygdala for the task, and compensated by using a greater processing load on temporal lobe structures, which specializes in verbally labeling complex visual stimuli. This may be because of compensation for an amygdala abnormality (Baron-Cohen et. al., 2000).

Maternal diabetes and autism

A study was done to investigate if Gestational Diabetes Mellitus (GDM) in the mother may result in the child having ASD. Specifically, researchers were determining if the timing of when

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the mother developed GDM had any significance whether the child would develop ASD.

This study included children who were born at 28-44 weeks gestation. Women who had type 1 diabetes and congenital anomalies were excluded from the study. The primary variable was maternal type 2 diabetes or maternal GDM during pregnancy. Exposures were broken up into three categories- 1. No exposure to maternal diabetes, 2. Exposure to maternal type 2 diabetes, and 3. Exposure to maternal GDM. Other variables that were included were maternal age at delivery, education, self-reported maternal race/ethnicity, and the gender of the child. For the GDM exposed group, the different methods that were used to diagnose were also recorded. The gestational weeks were also split up into three categories- 1. Diagnosed 26 weeks or earlier, 2. Diagnosed after 26 weeks but before 30 weeks, 3. Diagnosed at 30 weeks or later.

The primary data analysis was collected from 322,323 children. The children were checked up on approximately five and a half years after birth. During this time, 3,388 children were diagnosed with ASD. A large amount of mothers who got GDM during the first 26 weeks of pregnancy gave birth to a child with ASD. It is also interesting to note that among the children who had ASD, 121 of them had older siblings who also had ASD.

To summarize, mothers with pre-existing type 2 diabetes were not significantly associated with risk of ASD, but mothers who got GDM earlier than 26 weeks of pregnancy were significantly associated with risk of ASD (Xiang et. al., 2015).

De Novo Mutations

De novo mutation is an alteration of a gene that was present for the first time in one family member as a result of a mutation in a germ cell (egg or sperm) of one of the parents or in the fertilized egg itself (Genetics Home Reference, 2015). De novo mutations are important in finding the cause of ASD.

Studies show that the majority of de novo mutations do not cause the disease, they only increase the risk of getting the disease. There may be several genes in which a high risk of de novo mutations can occur from. Through these studies many de novo mutations were found that predicted that these mutations would disrupt the gene function in a child with ASD, however these mutation are not necessarily what causes the disease. Two studies also found that de novo mutations are caused from the paternal side and are age dependent. There is a detectable increase in autism risk with children who were born to older fathers (Perisco et. al., 2013).

Advancing parental age and autism

There are a number of studies which are trying to determine if the age at which the parents conceived the child increases the risk of the child being born with ASD. Whole-Exome Sequencing links older fathers to de novo mutations and increased risk of having a child with ASD. The linkage of older mothers having a child with ASD follows different pathways.

Prevalence of ASD has increased from 5 cases per 10,000 people in the 1980's to the latest CDC's estimate of 1 case in every 68 people. This may be due to a variety of reasons, including changes in diagnostic practice, heightened awareness, or even an actual increase in the disorder. One factor that might cause ASD is parental age, since there is a tendency in recent years for people to become parents later in life. In Spain and England the proportion of mothers who had children after 35 years old in 1980 was 14% and 25% respectively. In 2007, the proportion of mothers who had children after 35 years old increased to 25% and 40% respectively.

There are more than forty studies being done trying to link age of parents when they had kids to ASD. While individual studies are not consistent with their findings, this can be because of a number of factors. The discrepancies can be caused by inconsistent sample sizes and characteristics, or missing data. The studies did conclude an increased risk of ASD from both older parents, an older father but not older mother, and older mother but not older father, and neither an old mother or old father. Inconsistencies could have also been affected by the fact that these studies did not take into account socioeconomic status of the families or co-parental age.

The general consensus is that advancing paternal age (APA) and advancing maternal age (AMA) are independent factors that can increase the risk of having children with ASD.

In 2012 meta-analysis focused on advancing maternal age and a link to autism, using data from over 25,000 ASD patients, and eight million controls aggregated from 10 studies. The results were that mothers who were 35 years or older when they had the child had a 1.5 fold increased likelihood of having a child with ASD compared to mothers who had children at 25-29 years of age. In 2010, a study was done to test the theory of advancing paternal age and its link to autism. Meta-analysis was done on eleven studies and found that fathers who were 40-49 years old when they had children, had a 1.8 fold increased likelihood of having a child with ASD, compared to fathers who were 29 years old or younger.

APA effects on ASD

At this point the question is how APA and AMA are associated

with a child being born with ASD. One possibility of why age affects ASD is age-related mutagenesis in the male germ cell. While a female germ cell undergoes 22 mitotic cell divisions in utero, male germ cells undergo 30 mitotic cell divisions in embryogenesis, and then divide every 16 days from puberty and on. Because the male germ cell undergoes many more cell divisions during his reproductive age, there is more of a possibility of gene copying errors, which can lead to de novo mutations in the male's child, which can cause ASD.

Multiple studies that are using Whole-Exome or Genome Sequencing found that higher numbers of de novo loss-of-function single nucleotide variants are seen in fathers of increasing age. While it is fine for a new born to have between 30-100 de novo point mutations, there is an increase of about one to two mutations for each increasing year of a father's age. If the mother is also older, it increases the likelihood of more de novo mutations, because AMA and APA can be linked together.

Two studies were conducted to disentangle APA effects from AMA effects on the child. The studies used multiple regression which was adjusted so that both parental ages were entered as predictors. It was found that APA remained a significant predictor in de novo mutations while AMA effects were negligible. Two thousand, five hundred families who had one child with ASD were analyzed and found that a majority of the de novo mutations originated from the father. It should be pointed out that although de novo mutations can affect any biological system, there is reason to believe that de novo mutations differentially impact brain development because the brain may have fewer redundancy mechanisms that are needed to inhibit mutations (Lee et. al., 2015).

They hypothesized that if mutations in germ cells result in a slightly faster rate of cell division, then cells with these mutations will expand within the testes and logically they will contribute to the larger proportion of sperm. This process is known as "selfish spermatogonial selection". This mechanism can favor the propagation of germ cells which are carrying mutations that prefer to impact certain cellular signal pathways. (Lee et. al., 2015).

AMA effects on ASD

One possible correlation between AMA and ASD would be assisted reproductive technologies which are used by couples more commonly with increasing age. A Swedish study was done which involved 2.5 million infants. The results were that in vitro fertilization procedures had no association with risk of ASD, while a specific procedure called "Intracytoplasmic Sperm Injection (ICSI)" has a slightly increased risk of having

children with ASD. ICSI is done when there is paternal infertility. The reason why ICSI can increase risk of ASD is because the injection process can damage the egg which can increase the risk of adverse outcomes.

Another possible correlation can be the fact that a person accumulates more and more environmental toxins with increasing age. Environmental factors such as pesticides, heavy metal, and organic pollutants have been linked with increased risk of ASD. Human brain samples were examined and found that exposure to polychlorinated biphenyl 95 strongly predicted maternal 15q11-q13 duplication, which is one of the most common CNV findings in ASD cases (Lee et. al., 2015).

Other factors that effect advancing parental age and autism

A study was done using 1,251 ASD cases occurring in over 250,000 births throughout the United States. This study found a connection between parental age and birth order with increasing risk of ASD. Younger parents (mother younger than 35, father younger than 40), on their first birth, had a 1.7 fold increased odds of having a child with ASD compared to other young parents on their third birth and later. Regardless of the age of the parents, the risk of ASD is increased in first born children. Older parents (mother older than 35, father older than 40) on their third child or later had a 1.8 fold increase on the likelihood of having a child with ASD. Older parents on their first child had a 3.1 fold increase on the odds of having a child with ASD. To conclude, the effect of parental age on ASD is amplified if it is their first birth (Lee et. al., 2015).

Environmental chemical exposures and autism spectrum disorder

Autism can be caused by a mixture of genetic and environmental influences, and studies are being done to understand the interplay of both contributions. These contributions can be different from one person to the next. A study in Sweden was done which included 14,000 children with autism. The study showed that heritability contributed to 50% of a possible cause of autism while environmental factors contributed an equally strong role. Genetic and environmental factors may work together to disrupt the normal process of nervous system development, interfering with neuron formation and migration, synapse formation, or neurological connectivity which can cause ASD.

In this study, the chemical exposures that were included are ones that can be reduced in exposure, which opens up possibility for prevention of autism. Of course it can only be reduced because not all exposure can be controlled, such as air pollution and any toxic chemical that are unknown to the patients.

Maternal smoking in pregnancy and ASD diagnosis

Seven studies were included which were done in the United States, Canada, Sweden, Norway, Finland, and England/Wales. These studies showed no association between maternal smoking during pregnancy and ASD diagnosis. Some of the studies focused on the spectrum of autism. There was an elevation in two of the studies between maternal smoking during pregnancy and higher functioning autism, such as Asperger's syndrome, compared to lower functioning autism. Overall, there is no conclusive evidence that maternal smoking or tobacco exposure to a fetus has any effect on a child being born with autism (Kalkbrenner et al., 2014).

Regulated and traffic related air pollutants exposure and ASD

Air pollutants include hundreds of chemicals which can be categorized into three categories: 1. Point pollutants arising from spatially separated large buildings, for example factories and power plants. 2. Area pollutants associated with population density, for example gas stations and dry cleaners. 3. Mobile pollutants associated with vehicles. In this paper we will be discussing the third category. When these pollutants enter the atmosphere they undergo reactions that create new pollutants. Specific chemicals that arise from traffic that will be discussed here are particulate matter (PM), NO₂; nitrogen dioxide, and O₃; ozone.

Air pollutants enter the body through inhalation and direct transportation from the nose to the brain via the olfactory bulb. Some chemical pollutants are known to induce oxidative stress and cause a systemic inflammatory reaction, which can alter normal neurodevelopment. Fetuses itself can come in contact with chemicals from air pollutants or they can be exposed to them from the elevated levels of inflammatory cytokines in the maternal circulation.

Seven studies were done in the United States with adequate sample sizes ranging from 284 to 7,594 children with autism. The studies also had controls under consideration such as maternal age and the parent's level of education. Season of birth is also important to take under consideration because occurrence of autism varies by season of conception for unknown reasons. It is also important because certain air pollutants have a stronger concentration in some seasons over other season, such as influenza infection and vitamin D levels.

Results for this study concluded that exposure to pollutants such as PM_{2.5}, PM₁₀, and NO₂, were associated with the risk of getting autism. For almost every pollutant in every study, associations were stronger for exposures in the third trimester

of pregnancy and the first year of life compared to earlier on in the pregnancy. All of these studies estimated exposure to outside levels of these air pollutants using historical data that could be related geographically to the home residence of the pregnant woman or infant, because retroactive direct person-based air sampling was not possible (Kalkbrenner et al 2014).

Metal (Ethylmercury) from vaccinations and ASD

Connecting this discussion back to vaccinations, thimerosal was thought to be a toxic compound that increased the risk of autism. Thimerosal is a vaccine preservative that contains ethylmercury which is considered a less toxic form of mercury compared to methylmercury.

Although the Institute of Medicine already stated that there is no concrete evidence that the MMR vaccine causes autism, people are still skeptical. No one suspected thimerosal to be toxic, seeing as it was found in such small doses in vaccines. In the 1970's it was known that large doses of mercury were harmful. It was only in the late 1990's that scientists suggested that even relatively low exposure to organic mercury could be dangerous to fetuses and young infants (Baker, 2008). The U.S. Food and Drug Administration suggested removing thimerosal from vaccines in 2001, because of the biological plausibility that thimerosal may pose as a neurotoxic harm. However, trace amounts of thimerosal are present in influenza vaccines which infants and pregnant women may be exposed to. Six studies were included, testing for a link between thimerosal-containing vaccines and autism. The results of the studies suggested that there is no connection between thimerosal and increased risk of autism (Kalkbrenner et al., 2014).

Conclusion

The causes of autism are still vague. Because it is characterized as a spectrum disorder there can be many different variables that can play a role in causing different levels of it. The theory that vaccinations cause autism has become a popular idea, therefore much research has been done to make a correct conclusion. Until the causes of autism are confirmed research is still being done to see what role vaccinations have on autism, however the research that has been done until now has concluded that vaccinations do not have a link to ASD. Another highly researched cause of autism is a genetic cause, be it a genetic mutation or a number of other different genetic variables. In this paper the idea that different genetic mutations that can cause autism has been discussed, and while some studies have brought optimistic results, further studies have to be conducted. Other genetic factors that have been discussed are the effect of maternal diabetes on the fetus, and parental age

when the child was conceived. While further research is needed to confirm these factors, the studies that have been done have given additional insight into what causes autism. Another possible cause for ASD that was discussed was a mixture of genetic and environmental factors that the child was exposed to either as a fetus or as an infant. While some environmental factors that have been tested had no correlation to ASD, other environmental factors did show a correlation, although more research is required.

Many studies are constantly being done, and researchers are discovering more factors that might be a cause for ASD. The closer they come to finding a cause, the closer they get to trying to find a way to prevent autism from becoming more prevalent, and maybe even closer to finding a possible cure for Autism Spectrum Disorder.

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