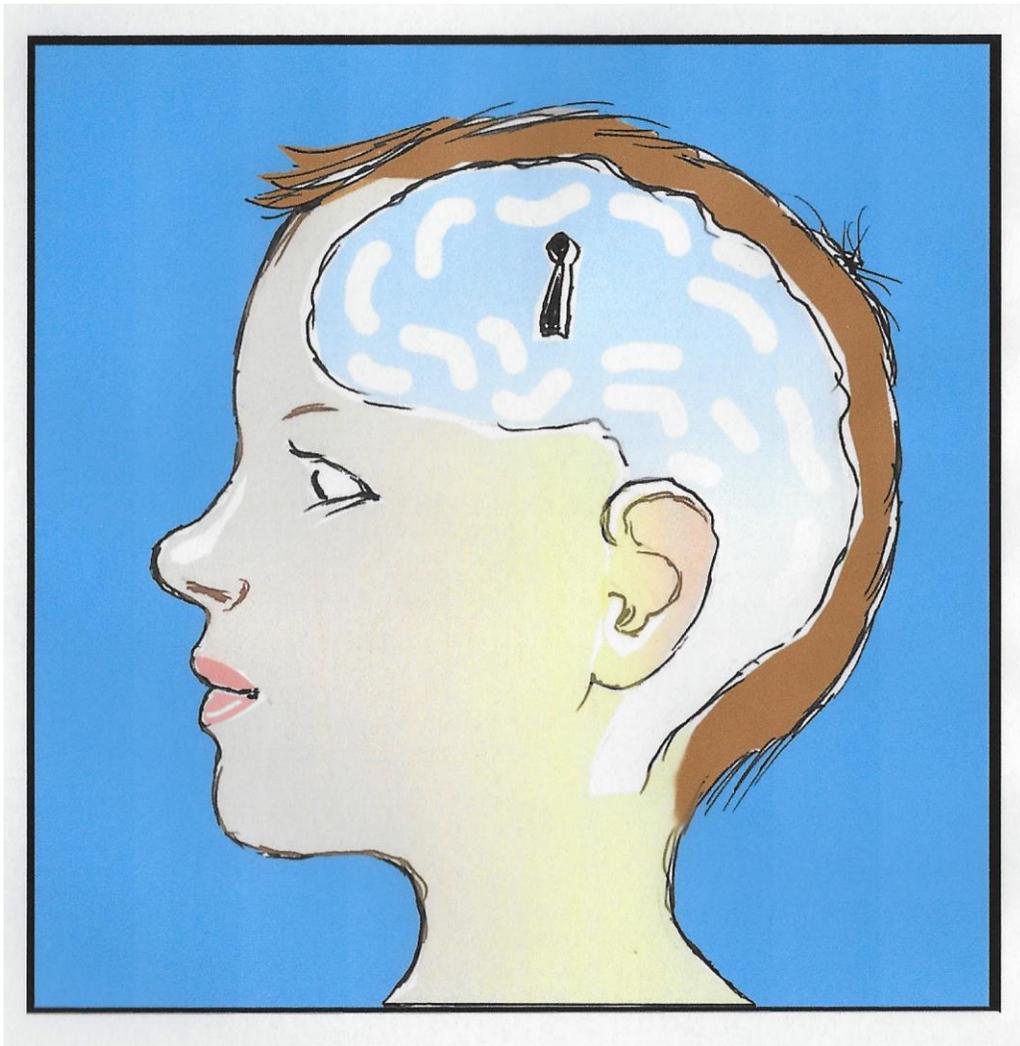# Examining the Causes of Autism

By David G. Amaral, Ph.D.

Illustration/William Hogan

Editor's Note: Autism is a broad, complex, and increasingly important brain disorder. New data from the Center for Disease Control and Prevention indicate that one in sixty-eight children is born with some degree of autism. Autism is also more common in males by a four to one ratio. Making it especially difficult to discuss in finite, conclusive terms is the fact that there is no biological test for autism; diagnosis is based on behavior, and the only verified treatment is intensive behavior therapy. Our author, one of the nation's foremost researchers on autism, examines the prenatal factors that contribute to the disorder.

As an autism researcher, I often try to put myself in the shoes of parents who have just been told that their child has autism. More and more families in the United States and around the world are facing this difficult news. The families that I've seen go through this often respond emotionally at first. Some go through denial; others are sad or furious. But emotions soon give way to questions. What caused my child's autism? Was I to blame? Which treatments will help? And what does the future hold?

Autism research has made tremendous progress over the last 20 years, but yet we still can't provide definitive answers to most of these questions. I find the autism community to be proactive, combative, and opinionated. The complexity and ambiguity of autism has spawned myriad speculations about causes—many of which have little supportive evidence. It seems clear at this point, however, that when all is said and done, we will find that autism has multiple causes that occur in diverse combinations.

To begin with, many people struggle to understand the nature of a condition so wide ranging in its severity. Autism Spectrum Disorder (ASD) or autism is a behaviorally defined neurodevelopmental disorder characterized by 1) persistent deficits in social communication and interaction across multiple contexts, and 2) restricted, repetitive patterns of behavior, interests, or activities. Few would dispute that the causes of ASD include both genetic and environmental factors. Indeed, more than 100 genes are known to confer risk[1,2] and 1,000 or more may ultimately be identified.[3] A wide range of potential environmental challenges have also been associated with autism, although studies in this area lag behind genomics research. A short overview of data supports genetic and environmental contributions to ASD etiology. A focus on prenatal events will hopefully clarify that the cause of autism, in the vast majority of cases, occurs prenatally, even if behavioral signs first appear several years after birth.

Twin Studies

Strong evidence against the unfounded view that autism results from neglectful parenting came in 1977 from Folstein and Rutter and the first systematic, detailed study of twin pairs containing at least

one child with autism.[4] In this study, 11 of the twin pairs were monozygotic (nearly identical genetics) and 10 were dizygotic (shared approximately half of their genome with each other). The major finding was that four of the monozygotic twin pairs were concordant (both had autism), whereas none of the dyzygotic twins were. Beyond autism, nine of the eleven monozygotic pairs were concordant for some form of cognitive impairment, compared to one of ten of the dyzygotic pairs.

The researchers concluded that autism and other neurodevelopmental disorders have a strong genetic component. But environmental factors must also contribute to autism etiology, they pointed out. For the 17 twin pairs that were discordant for autism—one child had a diagnosis and the other did not—the authors speculated that direct damage to the brain might have affected the diagnosed twin. They identified five features known to be associated with brain damage, such as severe hemolytic disease, a delay in breathing of at least five minutes after birth, and neonatal convulsions. In six of the pairs, one twin—always the autistic one—experienced one or more of these insults. Looking further, they found that one of an expanded list of "biological hazards" (e.g., discrepancies in birth weight, a pathologically narrow umbilical cord) occurred in the autistic twin in 6 of the 11 remaining discordant pairs and never in the non-autistic twin. The authors concluded that "some form of biological impairment, usually in the perinatal period, strongly predisposed to the development of autism."

Since the Folstein and Rutter paper cited above, there have been a total of 13 twin studies focused on autism. All find genetic and environmental contributions to autism, although conclusions about the proportions of the two factors and interpretations have varied substantially. One research team,[5] for example, concluded that a large proportion of the variance in liability (55 percent for strictly defined autism and 58 percent under a broader definition) can be explained by shared environmental factors, whereas genetic heritability accounts for 37 percent. This somewhat surprising finding—that environmental factors contribute more substantially than genetics—has been challenged by a more recent, large-scale twin study,[6] which found that the largest contribution to autism liability comes from additive genetic effects. And, a recent meta-analysis[7] concludes that the causes of autism are due to strong genetic effects, and that shared environmental influences are seen only if autism is very narrowly defined. A brief synopsis of the history of autism twin studies[8] finds that concordance for monozygotic twins is roughly 45 percent, versus 16 percent for dizygotic twins.

The reason for this short review of autism twin studies is to emphasize that even the best evidence for both genetic and environmental etiologies of autism leads to inconsistent conclusions about their proportional contributions. Moreover, twin studies do not typically consider that the cause of autism may involve genetic and environmental factors working together (the so-called gene by environment effect); i.e., certain environmental exposures only cause autism in individuals with a particular genetic composition. The second point is that if autism had a completely genetic etiology, we would expect a much higher concordance rate in monozygotic twins; the actual rate may reflect, in part, that even monozygotic twins do not share an identical environment prenatally.[9,10] Therefore, one must seriously search for environmental factors that either alone, or in combination with genetic predisposition, can increase autism risk. What are these factors?

Maternal Infection

If twin studies provide the best evidence for a genetic basis of autism, then naturally occurring pathogen exposures offer the strongest evidence of environmental etiology. The best example is maternal rubella (German measles) infection during pregnancy. Before development and widespread dissemination of effective vaccines, major pandemics occurred every 10 to 30 years.[11] The last of these was from 1963 to 1965 and infected an estimated 10 percent of pregnant women, resulting in more than 13,000 fetal or early infant deaths; 20,000 infants born with major birth defects and 10,000 to 30,000 infants born with moderate to severe neurodevelopmental disorders. Stella Chess, a child psychiatrist at New York University, studied 243 children exposed to rubella during pregnancy[12,13] and found that the largest category of neurodevelopmental disorder was intellectual disability, which affected 37 percent of the sample. Nine of these children were also diagnosed with autism; another, without intellectual disability, had a possible diagnosis; and eight a partial syndrome of autism. These numbers would translate to an autism prevalence of 741 per 10,000 rubella-exposed children, just over seven percent. This is striking in comparison to published prevalence rates, at the time of the study, of two to three per 10,000 in the general population. Fortunately, rubella epidemics have ended due to widespread dissemination of the measles, mumps and rubella vaccines and the association of autism with other viral or bacterial infections is weaker than with rubella.[14]

Collier et al[15] have pointed out that nearly 64 percent of women surveyed in the US have experienced

an infection during their pregnancies. This obviously does not lead to autism or any other neurodevelopmental disorder in most cases.

Examining prenatal environmental factors is best conducted in very large cohorts of subjects that have excellent health care records. This can be done in Scandinavian countries with their nationalized health care systems, and in large health care providers in the US.

One such study, conducted in Denmark, found no association between maternal bacterial or viral infection during pregnancy and diagnosis of ASD in the offspring,[16] although viral infection during the first trimester, or admission to the hospital due to infection during the second trimester were associated with the diagnosis. In a more recent study[17] Atladottir and colleagues found little evidence, overall, that common infectious diseases or fevers (lasting more than seven days) during pregnancy increased the risk of autism—noting, however, that influenza increased the risk of having an autistic child twofold. Use of antibiotics also increased risk. The link between influenza exposure during fetal life and increased risk for autism is in line with a series of animal studies[18, 19] suggesting that the influenza virus activates the maternal immune system, which may be harmful to fetal brain development. But the Danish researchers seem to downplay even their statistically significant findings, suggesting that their results do not indicate that either mild infection or the use of antibiotics represent strong risk factors for autism.

A parallel set of studies has been carried out by Zerbo and colleagues in California. The first,[20] based on 1,122 children, found no association between maternal influenza and ASD but (in contrast to Atladottir et al), the occurrence of maternal fever did increase risk. A second study[21] of 2,482 children (407 with ASD) found that mothers of children with ASD were diagnosed with viral infections during pregnancy no more often than mothers of non-autistic children. Maternal bacterial infections during the second trimester and the third trimester, however, were associated with a twofold increase in ASD risk, and two or more infections diagnosed in the third trimester with even higher risk, again suggesting a link with more severe infection during pregnancy. The most recent study,[22] based on a large cohort of children (196,929) born between 2000 and 2010, found that neither maternal influenza infection during pregnancy nor influenza vaccination were associated with increased risk for ASD.

In conclusion: Some infections during pregnancy, such as German measles, clearly increase the risk of ASD. However, there seems relatively little evidence that today's widely experienced infectious illnesses, such as influenza, during pregnancy substantially increase the risk of ASD. Perhaps the signal is weak because of gene by environment effects [as seems to be the case for different strains of mice[23, 24]]. If so, evidence will need to come from studies that combine large scale epidemiology with sophisticated genomic analyses.

Maternal Antibodies

Autoimmune diseases (in which immune cells erroneously identify cells in the body as foreign and attack them) mediated by circulating antibodies currently affect as much as nine percent of the world's population,[25] and the notion that autoimmunity may be associated with neurological and psychiatric disorders goes back to the 1930s. Reviewing this contentious area of research, Goldsmith and Rogers[26] conclude that the literature, though conflicting, "contains a large amount of circumstantial, but not conclusive, evidence for immune dysfunction in patients with schizophrenia." Interestingly, an auto-immune disorder with antibodies directed at the NMDA receptor causes an encephalopathy, which in its early stages can be indistinguishable from schizophrenia.[27]

Precedents for antibody-related CNS disorders include Rasmussen encephalitis, stiff-person syndrome, neuromyelitis optica, post streptococcal movement disorders (Sydenham's chorea and PANDAS), and systemic lupus erythematosus.[28] Judy Van de Water, of UC Davis, the main proponent of the idea that circulating antibodies may cause some forms of autism, first reported in 2008 that 12 percent of mothers of children with ASD have unusual antibodies directed at fetal brain proteins.[29] Based on more specific assays for these antibodies, she has since proposed that Maternal Antibody-Related (MAR) causes may be associated with as many as 22 percent of autism cases, suggesting that this may be a preventable form of ASD.[30] This area of research is exciting because it suggests potential therapeutic targets. Although many questions remain (e.g., how antibodies would enter the fetal brain, what neurodevelopmental processes they may alter), it is entirely possible that circulating antibodies represent prenatal environmental risk factors for ASD.

Drugs

Efforts to understand the increased prevalence of autism spectrum disorder have led some to wonder whether the use of various drugs during pregnancy might be partly responsible. Historically, a strong case could be made for an association between autism and thalidomide, a potent sedative that was used (for several years around 1960) during pregnancy for the relief of nausea. A study of 100 adult Swedish patients whose mothers had taken thalidomide while pregnant[31] found that at least four had clear autistic characteristics. This was the first evidence that a drug ingested during pregnancy could substantially increase autism risk. More recently, concerns have been raised about valproic acid and serotonin reuptake inhibitors.

Valproic acid, an approved drug since the early 1960s, is primarily prescribed for epilepsy and seizure control, but also used for ailments ranging from migraine headaches to bipolar disorder. Both animal and human epidemiological studies have raised concerns that valproic acid is a teratogen. The largest epidemiological study to date[32] tracked 415 children, 201 of whom were born to mothers who took antiepileptic medication during their pregnancies. Nearly 7.5 percent of the children of the treated women had a neurodevelopmental disorder, primarily some form of autism, versus 1.9 percent in the non-epileptic women.

A recent concern has been the use of serotonin reuptake inhibitors (SSRIs) for the treatment of depression during pregnancy. Serotonin is an important brain neurotransmitter that plays a significant role in functions ranging from sleep to mood to appetite, and whose dysregulation during early fetal life can have serious negative consequences for brain development.[33] As the name implies, SSRIs, which have been in use since the late 1980s, delay the reuptake of serotonin from the synaptic cleft into the presynaptic terminal and thus enhances its effect on the postsynaptic receptors. A recent review and meta-analysis of six case-control studies and four cohort studies concluded that SSRI use during pregnancy[34] was significantly associated with increased risk of ASD in offspring.

The effect was most prominent with use of the drugs during the first and second trimesters of pregnancy. Interestingly, the researchers found that preconceptual exposure to SSRIs was also associated with increased ASD risk—as was the use of non-SSRI antidepressants. They note that a

large cohort study found that, while ASD rates in the SSRI-exposed group were significantly higher than in the unexposed group, the rates in the SSRI-exposed group did not significantly differ from those among mothers with unmedicated psychiatric disorder and those who had discontinued SSRIs. It currently appears impossible to disentangle the deleterious effect of SSRIs from the fact of a maternal condition that necessitates the drug. Many authors also comment on the potentially worse effect on pregnancies of untreated maternal depression.

In sum, a brief review of the literature indicates that ingesting some drugs during pregnancy increases the risk of ASD, suggesting the need for more careful evaluation of drug safety during fetal development prior to widespread medical use.

Environmental Toxicants

Beyond viral and bacterial pathogens and medically prescribed drugs, researchers have begun investigating environmental toxicants. These range from automobile-produced air pollution to cigarette smoke to heavy metals and pesticides.[35,36] Small increases in autism risk have been reported if, for example, a family lives closer to a freeway or to an agricultural area during pregnancy. The field of autism environmental epidemiology is still in its infancy and techniques to comprehensively establish a prenatal "exposome" (i.e., all environmental factors affecting a fetus during pregnancy) are still under development. That said, given the unlikelihood that all autism will be explained by genetic factors, the determination of environmental causes, some of which might be avoided or minimized, may have far greater translational impact than the much better funded genetic studies. Strategies for exploring gene-by-environment interactions need to be enhanced with haste.

Postnatal Factors

Since autism is a neurological disorder that undoubtedly reflects altered brain function, it is possible that the insult to the brain occurs after birth. There is currently very little evidence for this. One historical concern was that vaccines, such as the measles, mumps, and rubella (MMR) vaccine, administered initially when the child is about one-year old, might transform a healthy child into one with autism. This fear was fueled by regressive onset in some cases—a child seems fine for the first year or so, then loses social and language function and regresses into a classical autistic syndrome. But we have found that even in children who demonstrate this regressive form of autism, brain

changes begin by four to six months, long before behavior changes.[37] Moreover, many large-scale epidemiologic studies have unequivocally demonstrated no link between MMR administration and the risk of ASD (summarized in 38), the same conclusion that the US National Academy of Sciences reached in a thorough review carried out in 2011.[39]

The only other postnatal experience that has been linked to the onset of ASD is profound social isolation in institution-reared children, such as those in the Romanian orphanage system.[40] Rutter and colleagues[41] found that nearly 10 percent of children raised in Romanian orphanages and adopted by British families showed some features of autism. These children were very poorly treated in the orphanage (most were underweight and had intellectual disability and various medical problems). While fully qualifying for an autism diagnosis at age 4, they showed substantial improvement and less severe autism symptoms by age 6. Is this truly autism? The authors conclude: "The characteristics of these children with autistic features, although phenomenologically similar in some respects to those found in "ordinary" autism, differed sharply in the marked improvement evident between 4 and 6 years of age and in the degree of social interest... The quasi-autistic pattern seemed to be associated with a prolonged experience of perceptual and experiential privation, with a lack of opportunity to develop attachment relationships, and with cognitive impairment."

This sad epoch demonstrates both the potential for severely abnormal rearing practices to influence brain regions that are affected by typical causes of autism, and the resilience of the brain in compensating and restoring once the individual is placed in a more normal environment. But it does not provide evidence for the postnatal genesis of autism.

The research picture regarding the causes for Autism Spectrum Disorder remains complex, although there is certainly a very strong genetic component. While there are some genes, such as CHD8, the mutation of which almost always cause autism in a very low percentage of cases[42] most mutations seem to confer small increases in risk. Similarly, while some environmental factors, such as rubella infection or fetal exposure to valproic acid, have been highly associated with autism risk, the increase in risk associated with others, such as living close to a highway, is small. It is very likely that the answer to what causes autism will not reside solely in genetics or in environment but in a combination of the two. Whatever factors go into the mix, they most likely have their effect during fetal life: a person

with autism is born with autism.

Bio

David G. Amaral, Ph.D., is a Distinguished Professor in the Department of Psychiatry and Behavioral Sciences at UC Davis. He is also the Beneto Foundation Chair and Research Director of the MIND Institute, which is dedicated to studying autism and other neurodevelopmental disorders. As research director, he coordinates a multidisciplinary analysis of children with autism called the Autism Phenome Project to define clinically significant subtypes of autism. More recently, Amaral has become Director of Autism BrainNet, a collaborative effort to solicit postmortem brain tissue to facilitate autism research. In April of 2015, Amaral became editor-in-chief of *Autism Research*, the journal of the International Society for Autism Research. In 2016, he was appointed to the Interagency Autism Coordinating Committee by the Secretary of Health and Human Services. Amaral received a joint Ph.D. in neuroscience and psychology from the University of Rochester and conducted postdoctoral research at the Department of Anatomy and Neurobiology at Washington University. He also conducted research at the Salk Institute for Biological Studies and served as an adjunct professor in the Department of Psychiatry at UC San Diego.

View Financial Disclosure

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