

Solving the Puzzle of Autism:
To find reliable treatments, we first need to untangle its myriad causes

By Alan Packer, Ph.D.

Editor's note: Desperate to understand and to cure autism, many activists argue that the disorder can be traced to a single source. But in order to understand autism, writes Alan Packer of the Simons Foundation, we first need to determine the genetic, neuronal, and behavioral elements at play. These elements, argues Packer, are more complex than those involved in cancer. Researchers will then need to translate their understanding of autism into treatments, an undertaking that will require a long-term, interdisciplinary approach.

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It is much more difficult to be convincing about ignorance concerning disease mechanisms than it is to make claims for full comprehension, especially when the comprehension leads, logically or not, to some sort of action. When it comes to serious illness, the public tends, understandably, to be more skeptical about the skeptics, more willing to believe the true believers. It is medicine's oldest dilemma, not to be settled by candor or by any kind of rhetoric; what it needs is a lot of time and patience, waiting for science to come in, as it has in the past, with the solid facts.¹

—Lewis Thomas

In the 67 years since Leo Kanner's pioneering work on what he called a deficit in "biologically provided affective contact with people,"² the diagnosis of autism has become a feared and all too common one. Physicians typically base the diagnosis of autism on three core features: deficits in language development, abnormal social interactions, and repetitive behaviors.³ Recent studies estimate a strikingly high prevalence of 1 child affected in 110. The Centers for Disease Control reports a 57 percent average increase in prevalence of autism across all sites examined in the United States from 2002 to 2006. While epidemiologists debate the validity of these statistics, it is difficult to overestimate the toll this disease takes on children, families, and society at large. The recent reports have led to a surge in both public and private funding for research on the causes of autism, as well as new efforts to provide families with better support services. Spurred on by activist parents and advocacy groups, the increased attention to autism has been largely for the good.

The possibility of a genuine increase in the prevalence of autism has led, reasonably, to heightened concerns about environmental risk factors. At the same time, the understandable desire for an "answer," coupled with an easy willingness to believe the worst about pharmaceutical companies and government agencies, has led some people to conclude that this complex disorder can be traced to a single source, whether it be a particular vaccine, the mercury-based preservatives used in vaccines or other environmental toxins. The evidence for these claims of a single source is unconvincing, however, even as the scientific community agrees that the search for environmental risk factors must continue. My main focus here, rather, will be on the scope and complexity of autism as a neuropsychiatric disorder, and the likelihood that a deep understanding of its causes will require a long-term, multidisciplinary approach.

This is not to say that the outlook is bleak. To the contrary, I will also emphasize the power of new technologies in genetics and neuroscience to uncover important clues about autism, some of which may soon have a real-world impact. But the most salient fact about autism is that it is a disorder of the developing social brain, the understanding of which may be the

greatest challenge facing life scientists today. The history of biomedical research shows that the development of successful treatments for arguably less complex diseases has been far from simple.

Lost in Translation

A recent issue of the *New England Journal of Medicine* features an opinion piece by geneticist Joel Hirschhorn, who points out how difficult it can be to translate basic discoveries into clinical practice. Using the example of heart disease caused by a buildup of cholesterol in the arteries, Hirschhorn writes, “nearly a century and three Nobel Prizes separate the determination of the chemical composition of cholesterol from the development of statins.”⁴ Picking up on this thread, science writer Carl Zimmer noted that if a journalist writing in 1913 had published a story describing the experiments in which Nikolai Anichkov discovered that rabbits fed a cholesterol-rich diet develop atherosclerosis, the most accurate headline would have been “RUSSIAN SCIENTIST DISCOVERS LINK BETWEEN MOLECULE AND HEART DISEASE. WILL LEAD TO POWERFUL NEW MEDICINE IN EIGHTY YEARS.”⁵

From the vantage point of today’s healthcare consumer, it doesn’t necessarily seem unjust or unusual that we benefit from basic research that had no impact on the lives of those who first read about it in the morning paper. That Anichkov’s posterity benefited is enough for us. Perhaps the more important lesson is to note how long it took for a relatively simple problem to lead to an effective intervention. There is a biochemical pathway that leads to the biosynthesis of cholesterol, and statins are drugs that interfere with the action of a key enzyme in this pathway. In other words, there is a target and an end point. If we compare the development of an inhibitor of a single enzyme with the search for what Leo Kanner called the “constitutional components of emotional reactivity,” we have some idea of the quantum leap in complexity that autism likely presents.

But perhaps this stacks the deck. Perhaps an early 20th-century discovery like Anichkov’s would necessarily be a premature one, given that it predates the flowering of biochemistry and modern methods of drug development. Perhaps. And so another, perhaps fairer, comparison is warranted.

The Thirty Years' War

The War on Cancer started in the early 1970s. Cancer geneticists speak enthusiastically about the revolution in our understanding of the mutations that drive the aberrant cell behaviors underlying the disease. On the other hand, clinical oncologists, as well as the general public, may have a less sanguine view, given the modest reductions in cancer deaths since cancer research became a priority.

Cancer research has had its triumphs, of course, and one in particular is instructive. In the 1960s, Peter Nowell and David Hungerford identified a shortened version of chromosome 22, the so-called Philadelphia chromosome, in cells from patients with chronic myelogenous leukemia. In 1973 Janet Rowley showed that this odd piece of DNA is the result of a translocation, or exchange of material, between chromosomes 9 and 22. In the 1980s, other scientists showed that this translocation results in the inappropriate fusion of two genes called BCR and ABL, and that the protein produced by the fusion of these genes promotes excessive cell growth. It wasn't until 1998 that scientists developed Gleevec, an inhibitor of the BCR-ABL fusion protein, and tested it in a spectacularly successful clinical trial.

As was the case with cholesterol and atherosclerosis, we again had a target and an end point, and it again took decades (though not eight of them) for Gleevec to become the standard of care for patients with chronic myelogenous leukemia. The BCR-ABL fusion protein sends signals to orchestrate the production of two cells from one, which, after all, is the *raison d'être* of a cell. The route from the mutated gene to the aberrant cellular behavior is complex, to be sure, but not so complex as to make it impossible to draw a simplified version of it on a laboratory whiteboard. On the other hand, consider the autistic brain, with its 100 billion neurons, each with several thousand connections to other neurons. The task of understanding the way in which typical social behavior emerges (or doesn't emerge) from this developing neuronal network, in concert with environmental cues, is likely to require coming to grips with a completely different scale of complexity. This is not to say that a full mechanistic understanding of the neuropathology of autism will necessarily be required to devise an effective treatment, only that the kind of fully rational, targeted therapy that such an understanding allows will require large advances in brain science.

Gene by Gene

Despite the size of the challenge of providing the “answer” to autism, there is considerable hope for the future given several remarkable new technologies in the fields of genetics and neuroscience. Autism is a multifactorial disorder, and as mentioned earlier its most prominent feature is a disruption of normal social interactions, such that personal relationships with others are profoundly affected. Another cardinal feature of autism is an insistence on sameness. We still do not know why males are affected by autism significantly more often than females, nor do we know why autism is frequently accompanied by epileptic seizures. While a diagnosis of autism does not necessarily imply low IQ (some people with autism may have savant-like qualities), many individuals with the disorder have moderate-to-severe intellectual disability.

Although genetic variation does not explain all of the differential susceptibility to autism, studies of twins point to a remarkably strong, if complex, genetic component. In studies of identical twins, who share all of their genes, researchers have found that if one sibling has autism, the chances that the other also has autism are at least 60 percent, and in at least one report this figure approaches 90 percent.⁶ For fraternal twins, who share only half their genes, the concordance rate is substantially lower, strongly implicating genetic influences on autism risk. And while we are only now getting a glimpse of the genetic variants involved in autism, we do know the identities of certain genes underlying a number of syndromes that are frequently accompanied by autistic features. These syndromes include fragile X syndrome, tuberous sclerosis, Rett syndrome, Angelman syndrome, Timothy syndrome, and others. As such, to say that genetic mutations are a critical component of autism is no longer merely a hypothesis, even if we know only the identities of genes involved in less common, syndromic forms of the disorder.

Whether the genetic variants contributing to complex diseases are common or rare—common variants being defined as those present in more than 5 percent of the population—has been a subject of considerable debate. For neuropsychiatric disease, it seems likely that most of the variants that have a substantial effect on one’s risk of autism are quite rare. Fortunately, the methods required to detect these rare variants are available and gaining power by the month. Researchers are carrying out scans of whole genomes to detect large deletions and duplications of genes. They recently identified one such deletion on chromosome 16, encompassing

approximately 30 genes, and this deletion appears to be a strong risk factor for up to 1 percent of cases of autism.⁷ Several others will likely be identified in the next two years.

The final genetic frontier, which researchers have applied to a few individuals, is the sequencing of the whole genome. The cost of such sequencing has fallen rapidly, and in early 2010 it stood at less than \$50,000 per genome. As the price continues to fall, it should become possible within the next five years to sequence the genomes of large numbers of individuals with and without autism. There is a tremendous analytic challenge in interpreting such data, but in principle such an effort will reveal the genes—perhaps numbering in the hundreds—whose variation affects autism risk to some degree.

A Fine Balance

The power of genetic insights is perhaps most clearly apparent in the case of fragile X syndrome, which is the most common inherited form of intellectual disability and, in 15 to 30 percent of patients, is accompanied by autism. Stephen Warren, Mark Bear, and their colleagues have shown that fragile X is caused by a mutation in a gene called FMR1, whose normal role is to suppress the production of new proteins in neurons. One usual trigger for new protein synthesis is the excitatory neurotransmitter glutamate, a chemical that promotes neuronal firing and the development of new connections between neurons. In the absence of FMR1, new neuronal proteins are overproduced; this results in an exaggerated response to glutamate signaling and, eventually, the sort of cognitive abnormalities that are observed in people with fragile X syndrome.⁸

Recent exciting work from the Bear lab has shown that deletion of a key glutamate receptor can prevent most of the effects of loss of FMR1 function in a mouse, in much the same manner as taking one's foot off the gas pedal is a good idea when driving a car with malfunctioning brakes.⁹ Moreover, MPEP, a specific inhibitor of this glutamate receptor, has the same effect in mice, and researchers are now testing it in human clinical trials as a potential therapy for fragile X syndrome.

The genetics of autism are probably more complex than those of fragile X syndrome, but the idea that inappropriate levels of neuronal firing and connectivity (either too much or too little) may be one underlying cause is a promising one. While molecules such as glutamate promote neuronal firing, other molecules trigger the inhibition of neuronal firing by activating a distinct class of inhibitory neurons that dampen the firing of the glutamate-responsive excitatory

neurons. As epilepsy is characterized by uncontrolled excitatory signaling, its frequent association with autism suggests that the latter may also be driven in part by imbalances in excitatory and inhibitory neuronal signaling. In 2009, Takao Hensch and colleagues showed that there is a nearly identical “signature” in the brains of a variety of mouse models of autism, even though the disorder in each model is driven by a different genetic mutation.¹⁰ Specifically, a certain class of inhibitory neuron is dramatically reduced in number. Here again, the car’s brakes have malfunctioned. The hope is that these convergent lines of evidence will reveal a common pathway that can make sense out of a variety of clues.

In the end, of course, autism is defined neither as a single molecule nor as a neuronal circuit, but as a set of behaviors that must be explained. A closer look at one such behavior is particularly revealing in showing what we must eventually understand about how the development of the brain is affected when a person has autism.

Theory of Mind

Picture the following scenario: Two children, John and Robert, are playing together. John puts a ball in a blue box and then leaves the room. While John is gone, Robert moves the ball from the blue box to a red box. John then comes back into the room. If you present this scenario to a typically developing five-year-old and ask her where John will look for the ball, she will answer that John will look in the blue box. She understands that there is such a thing as another person’s state of mind, which cognitive neuroscientists usually refer to as having a theory of mind. In this case, the five-year-old deduces that John has what neuroscientists would call a false belief. A typically developing three-year-old, however, will answer that John will look in the red box, as she has not yet developed the ability to attribute other people’s actions to their view of the world.

What about children with autism? In a classic 1985 study, Simon Baron-Cohen, Alan Leslie and Uta Frith showed that many children with autism fail the false belief test at an age when most typically developing children pass it.¹¹ Remarkably, Rebecca Saxe, Nancy Kanwisher, and others have shown that the ability to take account of other people’s states of mind develops in specific brain regions.¹² Some researchers have referred to these regions as a theory of mind network. One major task ahead is to understand how deficiencies in the theory of mind network’s development relate to the many other behavioral deficits observed in people with autism.

Putting It Together

What, then, is the most useful way to think about prospects for autism research in the coming years? Returning to the initial comparisons, recall that cholesterol synthesis is a linear pathway with a clear intervention point. Cancer involves a more complex network of biochemical pathways and interactions between cells and their environment. Autism, however, has additional, higher-order elements of neural circuits and behavior that have to be explained. These elements do not apply to the study of cholesterol metabolism, and they probably apply only partly to research on cancer. In the case of autism, however, they are the essence of the disorder. As such, the ultimate challenge in autism research is to fully integrate our understanding of the disorder at these different levels.

Over the next few years, as scientists identify new genetic risk variants, researchers will ask how their identities can be integrated into pathways such as the one regulating protein production in neurons. They'll want to know whether these genes also affect the balance between excitatory and inhibitory neuronal signaling, and whether they function preferentially in certain classes of neurons and at certain times during brain development. Finally, they'll want to know if any of these insights can explain the ability of a five-year-old to figure out what someone else is thinking.

This is a tall order. And it should be noted that space constraints have prevented me from discussing many other clues at the genetic, neuronal and behavioral levels, but we must account for them as we assemble the pieces of this puzzle. Progress will require geneticists, cell biologists, and cognitive neuroscientists to talk to one another, despite the fact that by and large they speak different languages.

That said, there is real reason for hope. Perhaps the most promising recent news is that the remarkable plasticity of the developing brain may allow for the reversibility—or at least the amelioration—of many of the most difficult features of autism. In 2007, Adrian Bird and colleagues showed that the neurological defects in mice carrying the genetic mutation causing Rett syndrome—a developmental disorder with many similarities to autism—can be reversed by restoring the gene's function even after onset of the disease.¹³ Of more immediate relevance, last year scientists showed that intensive and early behavioral intervention significantly improves the language and behavioral skills of some children with autism, and larger studies of this approach are in progress.¹⁴ Finally, tantalizing results from the study of so-called baby sibs—infants at high risk of developing autism because they have an older sibling with the diagnosis—show that

at 54 months of age there are no significant group differences between a group of children who had cognitive or language delays at 14 or 24 months, and a group of high-risk infants who had no early delays.¹⁵ These data are consistent with another study showing that up to 19 percent of children receiving a diagnosis of autism around age two can move off the spectrum as they age.¹⁶ Together, these findings suggest that, despite the complexity of autism, there will be ways to intervene if the appropriate targets can be found.

How are we to know if we're making progress? Editor Neil Patterson has written, "We live in polities captured by *belief*. Belief is a golden virtue; merely by asserting beliefs one can win huge support. . . . But believing is easy, and knowing is hard, and it's knowing that matters most."¹⁷ The kind of skepticism that informs the best science will help nonscientists distinguish what we know from what we merely believe. This skepticism keeps us open to new evidence but requires us to ask whether a finding has been reported by more than one laboratory; whether the finding is derived from a large study or a small one; whether the result is statistically significant; and whether the finding is consistent with what we already know about the epidemiology of autism, or the functioning of neurons, or the architecture of the brain (revolutions happen, but not that often). If such a finding passes these tests, and if it has the kind of explanatory power that ties together a number of different observations about the course of autism, then we can say with some confidence that we are moving in the right direction. With this stance, and with continued support for all facets of an interdisciplinary research agenda, we can give ourselves the greatest possible chance to improve the lives of children and families who are living with autism.

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