Fishing Expeditions and Autism: A Big Catch for Genetic Research?

by John J. Medina, PhD

I am a fan of the television show *Deadliest Catch*—a documentary series that follows the travails of deep-sea fishermen in the Bering Sea. (Actually, it is mostly about deep *crab* fishing.) Living in Seattle, I have actually seen some of the boats filmed on the show.

The variety of equipment the fishermen use to capture sea life is extraordinary. Trawlers and purse seiners—boats that use long-line nets and gill nets—make it possible to catch thousands of fish at a time. I am constantly struck by the comparison between these large, industrial efforts and the "weekend" fishermen that Seattle also has by the thousands. The amateurs use simple fishing poles to catch one fish at a time. Where the *Deadliest Catch* boats are based, you can often see both styles side by side.

I mention these 2 contrasting styles of fish harvesting because there is a comparison that I would like to make in this month's column and in the next.

It is not much of a stretch to say that isolating the genes responsible for complex behavioral disorders can seem like fishing expeditions (complete with analogous net comparisons). There are giant efforts that deploy the molecular equivalent of purse seiners designed to snag large groups of genes that share a potential involvement in whichever presenting behavior is under study. These efforts can be contrasted with technologies that use the equivalent of small fishing poles, the goal of which is not to catch large, glittering groups of nucleotides but single genes, one at a time.

In this column and the next, we will tackle one of the most slippery issues in the behavioral sciences: the genetic basis of autism. We will closely examine 2 sets of genetic "fishing" techniques that each attempt to isolate sequences associated with the disorder. This month's column will describe the success of the genetic equivalent of *Deadliest Catch* nets—large genetic screens that are capable of isolating many genes at one time. Next month, I will focus on research that is more reminiscent of our weekend fishermen with fishing pole–like techniques that can isolate single sequences.

My description of one of these larger fishing techniques for genes will begin with some comments on the diagnostic categories of autism. I will show just how hard it is to come up with behavioral profiles that are sufficiently robust to withstand the cold mathematical scrutiny of the behavioral genetics laboratory. I will then briefly describe some of the details of a technique called homozygous mapping and some of the surprising recent success using the technique with Eurasian and Middle Eastern families.

Part 1

Diagnostic difficulties

One of the biggest difficulties in characterizing autism at the molecular level is its complexity: autism is impossible to characterize in monolithic, overarching diagnostic terms. Symptoms can include social deficits, communication problems, and obsessive-compulsive and repetitive behaviors. Many patients with autism cannot detect changes in the affective state of another person or predict a person's interior motivational states based on specific visual cues (canonical Theory of Mind tests). And many of these behavioral symptoms are accompanied by GI complaints, seizures, epilepsy, and sleep disorders. Do these variations in symptoms describe specific disease states, each with their own unique genetic etiologies?

We do not currently know. Autism is usually classified as a severe form of 1 of the 5 so-called pervasive developmental disorders (PDDs). Children who display milder symptoms may have an autism spectrum disorder (ASD). Asperger syndrome is often separated from classic autism because there is usually no delay in language development. These terms are frequently used interchangeably, unfortunately, which reflects the fluid nature of the diagnostic observations. One of my favorite categories decries a form of diagnostic surrender: PDD, not otherwise specified.

(Just so you know, terms like these drive behavioral geneticists nuts!)

But there are genes

All this has not stopped us from researching the phenomenon, of course. And the results after years of looking are clear: there is a tantalizing and substantial genetic component to the disorder regardless of how it is classified.

Initial studies that confirmed a genetic role came from the traditional family and twin heritability studies, some of which are now decades old. Some of the best recent work comes from assessing sibling recurrence risk. Usually described as a percentage, sibling recurrence risk is the formal probability that a younger sibling of a child with autism will also have the disorder. When autism is defined narrowly, the normal rate in unrelated populations is about 1 affected child per 500 (0.002%). When you look at sibling recurrence risk, the rate rises to about 1 in 6 (15%). Thus, there is ample reason to pursue genetic research in this area.

But that is where the easy stuff ends. Although many genes over the years have been nominated as the source of the behavioral anomalies, few studies have been successfully replicated. Specific chromosomal inversions, large deletions, chromosomal translocations, and changes in copy number of individual genes (**Figure**) have been observed as risk factors for autism. In fact, you can practically name any type of mutation and find that it has been associated in the past decade, at least to some extent, with some part of the autism spectrum.

It is now clear that multiple genes expressed in specific combinations are involved differently in creating specific autistic behavioral profiles. It is also clear that wide nucleotide variations within these candidate genes exist that are undoubtedly more capable of predicting discrete autistic behaviors than others.

Looking at first cousins

Given the large number of potential genes in autism, one might expect that the research method of choice would include the deployment of large, *Deadliest Catch*–like gene fishing protocols. Recent progress has indeed been made using one of these larger screening technologies, a technique called homozygous mapping. What follows is a brief description of the technique.

Human genetic disorders that have complex, multigene origins have 2 overall causes. The first arises from the random roll of the meiotic dice—presenting cases that show no pattern of previous inheritance. In rare cases, heritable forms of what appears to be the same disease also exist. Mutations in these patients clearly show a pattern that can be transferred from one generation to the next.

Homozygous mapping is capable of identifying these rare, heritable (invariably recessive) disease forms. The technology takes advantage of the presence of consanguineous families . . . which should probably be explained before we go further.

For decades, molecular biologists have known about the great power of studying persons whose parental lineages share a close, common ancestor. The probability of their offspring exhibiting an autosomal recessive trait is much greater than in the general population. (Recall that autosomal recessive conditions are traits that are expressed when the subject has 2 identical copies of a particular gene in a nonsex chromosomal background. Homozygous mapping employs such populations and can be divided into 2 steps:

1. Subjects who carry a specific, well-defined disorder are identified. Accomplishing this first step, which requires the researchers to decide on a specific set of diagnostic criteria, is one of the hardest parts of the entire procedure.
2. Once identified, subjects are screened for nucleotide sequences that they share in common and are homozygous for both chromosomes. The assumption is that these regions are donated from both paternal and maternal lineages who themselves shared a recent common ancestor. That is a reasonable supposition if you are studying closely related persons, such as first cousins.

Although admittedly a tough technique to execute properly, homozygous mapping has proved to be successful in isolating gene sequences that mediate rare diseases related to neural development. Until very recently, however, it had not been tried

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on such complex challenges as autism. When it was, it proved to be invaluable in the autism-screening procedures.

The data

Researchers first had to find consanguineous families with autistic children. They established a collaborative network called the Homozygosity Mapping Collaborative for Autism (HMCA) in the Middle East and throughout Eurasia.[1] The reason for this geographic localization has to do with statistical access. It is quite common in the Middle East for cousins to marry each other. Since the families tend to be large, the researchers reasoned they would most likely find persons that met both their genetic and behavioral criteria. They hit pay dirt. The researchers were able to find 88 consanguineous families with autistic children.

The investigators next scanned the genomes of all participants at high resolution. They were looking for a wide variety of chromosomal aberrations, such as inversions, deletions, duplications, and something called copy-number variations.

After exhaustive screening, the researchers found that 6 chromosomal regions in the HMCA sample had inherited, homozygous deletions. These deletions varied in size from a low of 18 kilobases to more than 880 kilobases.

Exactly what genes were on these important chromosomal regions, and how might their characterization increase our understanding of autism? To discover what happened next, we need to switch fishing protocols. We are going to tie up our large fishing trawlers, which is what homozygous mapping is, and inspect the catch. Once inspected, the next steps will then involve breaking out our much smaller fishing poles, putting some bait on the end, and casting our lines back into the genomic waters.

As you see in the **Figure**, a large number of genes were netted in this experiment. I will describe exactly what was in the catch and how this increases our understanding of autism next month.

Dr Medina is a developmental molecular biologist and private consultant, with research interests in the genetics of psychiatric disorders.

Reference

1. Fliesler N. Middle Eastern families yield intriguing clues to autism. *Harvard Science: Medicine + Health.* July 10, 2008. http://www.harvardscience.harvard.edu/medicine-health/articles/middle-eastern-families-yield-intriguing-clues-autism. Accessed December 9, 2008. ❒

Figure

The genetic basis of autism

Shown below are some of the genes whose mutations result in autism or autism-related disorders. This list is limited to copy-number variations or specific chromosomal abnormalities. There are many other mutation categories involved in autism, ranging from point mutations and deletion mutations to chromosomal inversions.

There does not appear to be any consistent story regarding biological functionality that emerges from the examination of these sequences. Some of the genes listed below are involved in endosomal trafficking, others in neuronal cell adhesion. Some are clearly ion channels. The genes determined to be involved in autism by the homozygous mapping technique described in the text are red.

Allele	Name of protein
A2BP-1	*Ataxin 2-binding protein 1*
CNTNAP-2	*Contactin-associated protein-like 2*
CNTN3	*Contactin-3*
D1A1	*Deleted in autism 1*
MECP-2	*Methyl-CpG-binding protein 2*
NHE9	*Na^+/H^+ exchanger isoform 9*
NRXN-1	*Neurexin 1*
PCDH10	*Protocadherin 10*
RNF-8	*Ring finger 8*
SCN7A	*Na^+ channel, voltage-gated, type VII*
SHANK-3	*SH3 and multiple ankyrin repeat domains 3*
UBE3A	*Ubiquitin protein ligase E3A*