The Psychiatrist's Jigsaw

Researchers are piecing together the devilishly complex sets of genetic alterations underlying schizophrenia and bipolar disorder.

By Megan Scudellari | November 1, 2013

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When Anne Bassett told colleagues in the late 1980s that she was planning to study the genetics of schizophrenia, they winced. "People thought I was going into a field without a lot of promise," she says.

To some degree those people were right. Schizophrenia seemed to have an inherited component, because it was known to run in families, yet no one seemed to be able to find its genetic roots. Even as DNA sequencing technologies advanced, and genes associated with Huntington's disease, cystic fibrosis, and other inherited disorders were identified, schizophrenia researchers were coming up empty-handed.

It was an early hint at the now notorious complexity of schizophrenia genetics. The diversity of symptoms in patients was similarly inscrutable. "Anybody who thought schizophrenia was easy has never seen a patient with the disorder," says Patrick Sullivan, who studies the genomics of psychiatric disease at the University of North Carolina School of Medicine. "It's called a complex trait for a damned good reason."

A person diagnosed with schizophrenia can fall victim to an array of ills: delusions, hallucinations, social withdrawal, depression, cognitive disabilities, and more. Today, geneticists are finding that same heterogeneity in the DNA of patients with the disorder. "What we're talking about is a serious brain disease that can involve all regions of the brain," says Bassett. "It's going to be every bit as complex as cancer."

In fact, according to recent genome-wide association studies (GWAS), schizophrenia and the related bipolar disorder may actually be the most genetically complex neurological disorders plaguing humans. Over the last five years, large-scale GWAS have identified a handful of alleles associated with a risk of developing schizophrenia and/or bipolar disorder, but each accounts for only a very small percentage of the risk. For every risk allele identified, there may be 1,000 more pieces of the genetic puzzle that are still missing, not to mention the environmental factors thought to significantly contribute to each disorder.

But even a little knowledge is better than nothing,researchers argue. "We still have a lot of work to do, but we are at a different place than we were even five years ago," says Jordan Smoller, a psychiatrist at Harvard Medical School. "We've gone from very little to at least something."

"Now we're moving," agrees Bassett, currently director of clinical genetics at the Centre for Addiction and Mental Health in Toronto. Despite the dire predictions of her naysayers, she has identified numerous novel schizophrenia risk alleles—and other researchers are confirming the findings. "For the first time, we're starting to see serious, compelling replication of results within our field," she says.

Genes at fault

In 2005, University of California, San Diego, researcher Jonathan Sebat was eager to start using a new genome-wide screening method called a microarray. It employs tiny spots of DNA attached to a solid surface as probes to detect genetic sequences. At the time, sequencing of individual mutations, or single nucleotide polymorphisms (SNPs), was very difficult to do with a microarray, so Sebat decided to search for copy number variants (CNV), or genome "hiccups," in which a chunk of DNA is copied numerous times. Though CNVs had not been explicitly linked to disease at the time, Sebat suspected they might underlie disease susceptibility in schizophrenia. "You can't escape the heterogeneity, but you can grapple with it," says Sebat.

In 2008, Sebat's team found that CNVs occur three times as often in people with schizophrenia as in their healthy counterparts, and four times as often in people whose schizophrenia had been diagnosed before age 18. But

schizophrenic patients did not share CNVs at the same locations in the genome; each had his own distinctive genome alterations.1

There have long been two theories of the genetic cause of schizophrenia. Some, like Sebat, have argued that the disorder is caused by a few mutations, each with a strong effect, that are unique to each patient. Other researchers have suggested that the disorder results from a crowd of common variants shared among patients, each with a subtle effect. Today, the data are pointing toward the latter.

The most common of the CNVs associated withschizophrenia is a 1.5- to 3-megabase deletion onchromosome 22 in a region of the genome thatnormally contains 30 to 40 genes, many of whichare not well characterized. Approximately 25percent of people with this mutation exhibitpsychosis, but the deletion, called 22q11.2, is found in only 1 percent of schizophrenia cases. Despite this low proportion, however, no other genetic mutation has been found that accounts for a greater share of the risk than this particular deletion. Most mutations that are associated with schizophrenia are found in fewer than 1 in 1,000 patients, and those are still the low-hanging fruit, says Sebat. There are probably many more mutations that contribute to the disease, but they're found at far lower frequencies—on the order of 1 in every 30,000 cases.

"There's a famous line from Tolstoy: 'All happy families are alike; each unhappy family is unhappy in its own way,' " says Sebat. "That applies directly to schizophrenia: there are a multitude of ways in which you can disrupt cognition and produce psychosis."

Now that technology allows easier scrutiny of SNPs, Patrick Sullivan and colleagues at the University of North Carolina this year performed the largest schizophrenia GWAS to date, scanning the genomes of more than 59,000 people, including 7,500 schizophrenia patients. The team identified 22 risk alleles for schizophrenia, 13 of which were identified for the first time. Based on the number of patients studied and the low risk contribution of each identified allele, Sullivan extrapolates there may be between 6,300 and 10,200 SNPs associated with schizophrenia. "We finally have an idea as to how many puzzle pieces there are. We've got 22 pieces, maybe of the corners and edges," he says. "Now we need to get the rest of the edges together. We need to find, say, 2,000 of these 6,000 loci. If we do that, we'll actually have a good fix on the pathways involved."

And that is the ultimate goal—to use schizophrenia-associated genes flagged by GWAS to identify pathways underlying the disease. Some of the SNPs recently identified affect molecules involved in synaptic function, such as neurexin, a presynaptic protein. Other SNPs alter components of glutamate signaling and genes that regulate protein synthesis and cell growth. Of Sullivan's 22 variants, one plays a role in a microRNA pathway and two are components of a calcium channel signaling pathway. (See illustration below.)

The old notion that a schizophrenic simply has a chemical imbalance in the brain is outdated, says Sebat. "I suspect that this universe of rare mutations will start to condense into constellations of genes that are involved in similar pathways," says Sebat. "There won't be one schizophrenia pathway, but we have the very beginnings of a molecular understanding now."

No working theory

In spite of the vast unknowns still hanging over the field, theories about the molecular cause of schizophrenia do exist—though the evidence for each is weak and conflicting. One, the glutamate hypothesis, first proposed in 1980, posits that schizophrenia is caused by dysfunctional glutamate receptors, yet agonists of the receptors have repeatedly failed to treat the disorder. Another is the dopamine theory, which holds that the disease is caused by disturbed dopamine signaling. There is evidence both for and against this idea, but most researchers agree that it is an incomplete explanation at best.

More recently, two other possibilities have arisen from genetic studies. One, championed by Ronald Yeo and Steven Gangestad at the University of New Mexico, is the idea that schizophrenia is partly caused by developmental instability—that individuals with schizophrenia lack the repair mechanisms that typically jump into action when something perturbs the brain during development.

Numerous studies support this theory. In a study the duo published last year, for example, 79 individuals with schizophrenia and 110 controls had similar numbers—about 10 to 12—of randomly distributed, large deletions in their genomes. Strangely, that deletion burden was correlated with lower cognitive ability in people with schizophrenia but not in controls.2 The finding implies that healthy individuals can withstand certain deletion mutations without symptoms, but individuals with schizophrenia cannot.

Another compelling theory that has arisen from genetic studies is that schizophrenia is caused by dysfunctional oligodendrocytes—non-neuron brain cells that produce myelin sheaths to protect neurons and speed transmission of action potentials. In 2001, Joseph Buxbaum and colleagues at Mount Sinai Hospital in New York City ran gene expression microarrays of brain samples from patients with schizophrenia and healthy, matched controls from the hospital's brain bank. When the results came in, "there was only one thing you could point to that was glaringly obvious: there were coordinated groups of genes [whose expression levels in schizophrenic patients] were all going down, and they were all directly tied to oligodendrocytes," says Buxbaum.

Intriguingly, myelin continues to form in the developing brain until around 16 to 19 years of age—the same window as the typical onset of schizophrenia. In numerous brain-imaging experiments, individuals with schizophrenia almost all demonstrate disorganization of myelin, and two of the main genes linked to schizophrenia risk, neuregulin 1 and its associated receptor ErbB4, regulate oligodendrocyte survival. And in August, researchers at VU University in Amsterdam analyzed GWAS data from more than 31,000 individuals, including schizophrenia patients and healthy controls, and found additional evidence that alterations to genes expressed by oligodendrocytes are associated with an increased risk for schizophrenia.4

But no single causal theory fully explains schizophrenia. With better technologies, however, scientists hope to delve into the "dark matter" of SNPs, says Yeo—those estimated 6,000 yet-to-be-identified SNPs that would require impractically large GWAS to identify. "There is much more variation in [the] SNPs we can't measure than [in] those we can," he says. "Within the next five years, we'll have a much clearer picture of schizophrenia."

Manic mice

Kafui Dzirasa awkwardly balances a thin slip of metal between his pointer finger and thumb. Delicate hairlike fibers protrude from one side of the microchip, and Dzirasa, a psychiatrist and neuroscientist at Duke University in Durham, North Carolina, strokes these with his other hand. An anxious young technician stands next to Dzirasa, his eyes glued to the fragile piece of technology. "Everyone's super nervous I'm going to break something," says Dzirasa with a laugh, as he flips the chip upside down to show it off.

A room away, two small black mice scurry around in a cage. The mice wear similar microchips as hats, but now the hair-like probes aren't visible. Instead, they are embedded in the mice's pea-sized brains. The probes are measuring, in real time, electrical signals from 16 areas, attempting to detect electrical irregularities caused by genetic mutations engineered into the rodents to mimic symptoms of bipolar disorder.

Bipolar disorder, also known as manic-depressive illness, is a psychiatric disease characterized by bouts of mania and depression. But, as with schizophrenia, different patients seldom present the same suite of symptoms: some show only mania and no depression; some have both; the mood alterations can be accompanied by signs of psychosis, or not. As in schizophrenia, genetic heterogeneity appears to be the rule, rather than the exception.

Researchers have yet to identify a single "bipolar gene." During the 1990s, numerous genome screens of families affected with bipolar disorder, including an international collaboration examining 972 families, turned up no positive findings. Even initial GWAS failed to yield convincing results.

More recent work has finally started to shed some light on the disorder's complex genetics. Some of the first genes linked to bipolar disorder were those involved in the circadian clock. Since episodes of mania and depression in bipolar disorder generally develop a regular periodicity, often linked to the seasons of the year, and since sleep disturbances are a common feature of the disorder, scientists had suspected circadian rhythms might be involved, and in 2003 scientists linked two mutations in *Clock,* a gene that encodes a transcription factor critical to normal circadian rhythms, with sleep-related symptoms and recurrence rates of bipolar disorder.5,6 In 2007, when

researchers induced *Clock* mutations in mice, the animals showed symptoms very similar to those of the manic phase of human bipolar disease: hyperactivity, decreased sleep, reduced anxiety, and increased response to cocaine.7

Currently, about 10 genes have been robustly associated with bipolar risk, says Nick Craddock, director of the National Centre for Mental Health at Cardiff University in the United Kingdom. Most of these were found in the last five years. And it appears that in bipolar disorder, as in schizophrenia, much of the evidence implicates many common alleles, each with a small effect.

In contrast to schizophrenia, however, there are no major structural changes in the genomes of bipolar patients, such as large deletions. In 2011, the Bipolar Disorder Working Group of the Psychiatric Genomics Consortium performed the largest GWAS of bipolar disorder to date, which highlighted *ODZ4,* a gene encoding a cell-surface protein that may be involved in signaling, and NCAN, the gene for a brain- expressed extracellular matrix protein, as risk variants. The study also identified *CACNA1C,* a gene that has also been implicated in schizophrenia and possibly the single strongest allele associated with bipolar risk. (See "Common Changes," below.) But even the effect of *CACNA1C* is small, contributing about an 18 percent risk for bipolar disorder in carriers.

With so few risk alleles identified, theories of what causes bipolar disorder are even sparser than those for schizophrenia. Schizophrenia is the poster child for psychiatric illnesses, and as such, it tends to receive more research dollars and attention than bipolar disorder. Additionally, patients with bipolar have episodic symptoms, so are not typically permanent residents in mental hospitals, where researchers tend to have easy access to patients with schizophrenia.

As an alternative, many researchers, like Dzirasa, are applying genetic findings to mice, rather than people, to determine how these mutations lead to a psychotic phenotype. Dzirasa implants his microchips into mice with mutations in *Clock* to measure how the animals' brains look different from those of healthy control animals. From that genetic basis, he has already uncovered an electrical biomarker by which the mutation may lead to psychiatric symptoms: in the nucleus accumbens, a part of the brain that plays a role in reward, two electrical signatures—including one linked to the amygdala, which processes fear and anxiety—are out of sync in bipolar animals. When the timing of these electrical impulses is disjointed in mice, so too is the balance between reward and anxiety.

Treatment track

In June, Bassett and colleagues performed one of the first efforts to assess the potential role the scant genetic information known about schizophrenia might play in a clinical setting. Her team used microarrays that lit up with rare CNVs, excluding the already known 22q11.2 deletion, for 459 people with schizophrenia and matched controls. When two independent clinical cytogenetic laboratory directors reviewed the microarrays, the researchers reported numerous CNVs "highly enriched" in schizophrenia, in contrast to controls, that they considered to be clinically significant.8

In a follow-up study, Bassett's graduate student, Gregory Costain, used the results to counsel patients and their families. He found that sitting with patients and discussing what is and isn't known about the genetics of schizophrenia "reduces stigma, concern, and anxiety in families, especially among mothers who feel guilt," says Costain. This was true even for patients in whom molecular testing revealed no known genetic risk variants.9

A schizophrenia microarray available in a doctor's clinic is a far cry from Bassett's early days in genetic research, when her colleagues thought there was nothing to be gained from studying the genetics of psychosis. "To actually be able to give an individual genetic result to a patient is astonishing, to have something actually to say to them," says Bassett.

But beyond sharing genetic results, there has been little effort to move genetic findings on schizophrenia and bipolar disorder into the clinic. Although drugs such as lithium for bipolar disorder and antipsychotics for schizophrenia have been the go-to treatments for decades, few doctors are happy with them. These nonspecific compounds can help relieve symptoms, but they also cause many undesirable side effects that cause patients to stop taking the drugs. With new genetic data, it's time to reframe discussions and ideas for treating psychiatric illnesses, says Dzirasa.

“The genetic findings are popping up,” agrees Sebat; “now the translational work has to take place.”

Common Changes

In 1970, Scottish researchers identified a young criminal with a psychiatric disorder and a unique translocation on chromosome 1. Soon, they found the translocation was rampant in his family, as was severe mental illness. Within the family, geneticists identified a gene they named Disrupted in Schizophrenia-1 (DISC1) that appeared to be associated with risk of developing the disorder.

DISC1 encodes a protein with numerous functions, including key roles in neuronal development. But despite the name given to the gene, family members did not just suffer from schizophrenia, but also from bipolar disorder and depression. It was one of the first hints that psychiatric diseases, which share many symptoms and often overlap in patients, might also share underlying genetic mutations.

Indeed, one of the defining features of psychiatric disorders is the co-occurrence of symptoms: one of the common features of bipolar disorder is depression; patients with schizophrenia show manic and depressive episodes; patients with bipolar disorder may have psychotic delusions and hallucinations; and so on. This April, the dramatic results of a five-year international study may have found the genetic roots of that overlap. With funding from the National Institutes of Health, the multisite Psychiatric Genomics Consortium (PGC) looked at genetic data from more than 60,000 people worldwide, including healthy controls and psychiatric patients. The authors found that five psychiatric illnesses—schizophrenia, bipolar disorder, autism, depression, and attention deficit hyperactivity disorder—share a range of disease risk alleles.10

Two of the shared SNPs that popped out were variations in genes that encode subunits of calcium channels, which are crucial for neuronal communication, synapse function, and the release of neurotransmitters. A pathway analysis also showed that calcium-channel signaling genes were significantly involved in all five disorders. It could be that disrupted neuronal communication is the foundation for the development of these psychiatric illnesses.

A second paper from the PGC, published in August, quantified the shared genetic components. The overlap in heritability was the greatest between schizophrenia and bipolar disorder—with about 15 percent of each disease’s total heritability caused by the same alleles. There was about a 10 percent overlap between bipolar and depression, and 9 percent between schizophrenia and depression.11

The common alleles “probably represent a subset of the genetic component of each disease,” says Jordan Smoller, a psychiatrist at Harvard Medical School who led the study. “The puzzle, then, is what is it that leads someone with this shared component to develop one or another of the disorders.”