Schizophrenia Genetics: 2015

By Michele Solis

Editor's note: In Schizophrenia Research Forum's five-part 2015 schizophrenia genetics update, reporter Michele Solis surveys leaders in the field about milestones, challenges, and current research. It is an interesting exercise to compare it to Patricia McCaffrey's original 2010 series. A few things stand out: The first series began with a look back at linkage studies that had identified regions of the genome that likely harbored risk genes, setting the stage for the subsequent boom of candidate gene studies. The SchizophreniaGene database was considered by many the state of the art: the meta-analyses of more than 1,500 candidate gene studies had resulted in the publication of a Nature Genetics article (Allen et al., 2008).

In 2015, linkage is ancient history, and candidate genes are now in the bust portion of their gene-mining cycle, supplanted mainly by genomewide association studies (GWAS). Two developments that were heralded in the 2010 series—rare variants, especially copy number variants, and whole-genome sequencing—have yet to exert a large impact on biological research. Indeed, the "special" rare genetic abnormality of mental illness research—the DISC1 disruption—has lost its ingenue status, though it remains a consistent performer in biological studies. Finally, a new actor has entered the stage: Patient-derived induced pluripotent stem cells, which were not mentioned even once in 2010, are seen as the best hope to make sense of risk variants for schizophrenia.

With all that said, the final articles of each series do not make significantly different points. Pending the identification of the source of the more than 100 GWAS signals, we still await clear targets for biologists and drug designers, we still wonder whether endophenotypes or clinical subgrouping will provide better clues, and we still hope there are some low-hanging fruit in pharmacogenomics. We wish the geneticists the best of luck in their daunting task and look forward to surveying a new landscape in 2020.—Hakon Heimer.

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Part 1, Renaissance

August 7, 2015. With a steady stream of papers in high-profile journals, front page headlines, and proliferating collaborations, schizophrenia genetics has arrived. It even has its own T-shirt. And a lucrative sign of the field's heightened status came last summer with the $650 million pledge to the Broad Institute by philanthropist Ted Stanley for research on psychiatric disorders. This definitive vote of confidence came at a time when drug companies had all but given up on psychiatric research.

Once a scientific backwater beset by false leads, the field now claims over 100 regions of the genome firmly associated with schizophrenia, a severe mental illness that has stymied researchers for over a century. The explosive transformation has been wrought by international collaboration and ever more economical tools to explore the genome in an unbiased way.

"Honestly, schizophrenia genetics is a completely different field now," said Patrick Sullivan of the University of North Carolina at Chapel Hill. "It has shifted to a highly critical, highly empirical, enormously data-driven enterprise, which has been pretty neat to see." Sullivan is a leader of the Psychiatric Genomics Consortium (PGC), which convinced researchers around the world to share their data to get genomewide association studies (GWAS) off the ground. The most recent GWAS, published in 2014, zoomed in on 108 regions of the genome linked to the disorder.

"I think there's been enormous progress in schizophrenia genetics; that's the bottom line," said Daniel Weinberger of the Lieber Institute for Brain Development in Baltimore, Maryland. Weinberger has been on the hunt for genes in schizophrenia for decades.

"Genes related to risk have been identified with a level of statistical evidence that is above the fray of controversy—and this kind of evidence is attracting a new generation of scientists to understand psychiatric illness," he said.

Despite the good news, researchers may still find themselves bewildered. In very short order, they've gone from having no firm leads to an embarrassment of riches, casting suspicion on hundreds of genes. Turning these insights into an understanding of schizophrenia's biology or potential therapeutic targets will be a long haul.

"Realistically, until we start to understand and interpret the clues that genetics is giving us, we're not actually accomplishing that much," said Mark Daly of the Broad Institute, Cambridge, Massachusetts, who chairs the PGC's analysis group.

The lack of biological understanding looms large. "I think it would be fair to say that we haven't made any quantum leaps in understanding causal pathways and mechanisms," says David Porteous of the University of Edinburgh, Scotland. "But perhaps we now have a better feel for just how big the task is that lies ahead."

This five-part series, an update to SRF's first schizophrenia genetics series published in 2010, covers the developments in the past five years and surveys researchers on the best ways forward. Read on to find answers to these questions:

- How will the disease-influencing genes in the 100-plus loci be found?
- Do we need more GWAS?
- What is the status of the old candidate genes from the pre-GWAS era?
- Are there any new copy number variants (CNVs) on the horizon?
- When will definitive rare variants finally turn up in sequencing studies?
- Will paying attention to schizophrenia's different features, including endophenotypes, help find genes?
- How do genetic signals that confer risk for multiple disorders eventually contribute to a specific disease?

Growing GWAS. The complicated genetic story mirrors the complexities of the disorder itself. Appearing in late adolescence or early adulthood, schizophrenia is a mix of frightening symptoms consisting of psychosis—hearing voices, having fixed delusions usually of a paranoid nature, and experiencing disordered thinking—as well as negative symptoms, which comprise the paralyzing lack of motivation and emotional expression that cut a person off from the rest of the world. Cognitive deficits, such as impaired attention and memory, also plague most people with schizophrenia.

People differ in their symptom profiles. One person may have little psychosis but all the motivation-crushing negative symptoms, whereas another may have a sampling of all. To complicate matters, the psychotic symptoms may wax and wane. Outcomes differ, too: some return to full lives with work and satisfying relationships, usually with the help of antipsychotic drugs, whereas others struggle with addiction, end up homeless, or even commit suicide.

One might worry that this hodgepodge would resist genetic analysis. But epidemiological studies established that schizophrenia runs in families, with an estimated heritability of 65-80 percent, meaning that a majority of the liability for the disease in the population can be attributed to genetic factors.

But how best to identify the genes involved depended on people's hunches about where risk might lie. Human genomes teem with different kinds of variation. Some are single base changes commonly found in 5 percent or more of the population, akin to a misspelled word in the book that is our genome. Others are rare, consisting of single base changes, or insertions or deletions of a few bases; in the protein-coding part of the genome book, these might scramble a word beyond recognition. Another kind of rare variant, called a copy number variant (CNV), deletes or duplicates long stretches of DNA, similar to dropping or repeating a sentence or paragraph.

Common variants, because they are widespread in the population, confer only subtle effects on risk, nudging a person toward disease. Rare variants consist of genetic glitches that natural selection has not yet weeded out, and so they can potently escalate risk.

To detect common variants, researchers use microarrays to screen millions of single nucleotide polymorphisms (SNPs) commonly found in humans. These not only tally the sort of allele a person has at each SNP location, but can also detect rare CNVs encompassing these locations. Finding the rare variants, however, requires more expensive and time-consuming sequencing, which reads out DNA letter by letter in order.

Though most geneticists agree that both common and rare variants have a hand in schizophrenia, their relative importance is still debated. In one way, the discussion boils down to the general versus the particular: should we seek genetic clues that, though weak, are generally applicable to all people with schizophrenia or go after the powerful ones that apply to a rare few, betting these would lead to key biological processes relevant to many with the disorder?

"The common variants are where the money is," said Patricio O'Donnell, head of psychiatry at Pfizer in Cambridge, Massachusetts, which is also a member of the PGC. "We want to find a target that eventually might have an impact in a relatively significant proportion of people with schizophrenia."

By contrast, rare variants might produce more personalized insights.

"Ultimately, treatment is based on the individual person who is sitting in front of you," said Jack McClellan of the University of Washington in Seattle. McClellan and colleague Mary-Claire King, who have been searching for rare variants in schizophrenia, have proposed that most people with schizophrenia have a unique or "private" genetic cause.

Five years ago, the field was divided about the best course to follow. At the time, the returns from the common variant approach, which uses GWAS to see if certain SNPs are overrepresented in disease compared to controls, fell short of what many had hoped for (see SRF Genetics Series 2010). Worried that lumping heterogeneous cases together diluted genetic signals, some felt the common variant approach should be abandoned. Proponents, on the other hand, argued that larger samples were needed.

The PGC pressed on, increasing its sample size to 150,000 in the latest GWAS—five times the number in a combined analysis of the first studies in 2009 (see SRF related news report). While some samples came from people whose symptoms had been comprehensively documented, others had only a schizophrenia diagnosis. Still others didn't even have that, coming anonymously from clozapine clinics, which regularly take blood from people on the antipsychotic to check for deadly side effects.

This approach couldn't be more different from what came before it. In the past, a single researcher might have canvassed the countryside to carefully interview and assemble people with an artisanal level of detail. Compared to that, GWAS can seem like a trip to a big-box store.

"We have to face up to the fact that the PGC has largely been focused on large numbers. With the money, I could study more people more superficially or fewer people deeply," said Kenneth Kendler of Virginia Commonwealth University in Richmond, who has participated in both styles of inquiry. "The field still doesn't know which is better."

Parts list. So far, the big-box model has prevailed. The PGC's latest schizophrenia GWAS found 128 SNPs that occurred more frequently in people with schizophrenia than in controls, with a high enough level of statistical significance to sidestep many of the concerns about false positives that had dogged the field before.

The findings also clearly endorse the PGC's data-sharing experiment and its lumping approach.

"I must say that the team science approach has been really successful," said Thomas Lehner, director of the Office of Genomic Research Coordination at the National Institute of Mental Health (NIMH) in Bethesda, Maryland. "It's delightful to see how these competitive groups can actually put aside the competitiveness and work together on problems. I think that's the future."

"I'm encouraged that we can find real associations with risk of schizophrenia, which suggests that the category is a meaningful category for genetic investigation," said David Goldstein of Columbia University in New York City, who has been a critic of GWAS.

But, he adds, "We don't yet have any genetics that provide enough biological insight to provide pointers to new treatment opportunities. I think that's what we'd like to see."

Of the hundreds of genes that have fallen under suspicion thanks to the landmark GWAS, some will turn out to be innocent bystanders, while others will take a place in the parts list for schizophrenia risk.

Most of the loci are new but implicate processes already suspected of malfunctioning in schizophrenia, including glutamatergic signaling, dopamine signaling, calcium channels, and immune signaling (see SRF related news report).

Drug companies are taking note. "In my career it's the one paper that has truly resonated within the industry," said Nick Brandon, head of discovery at AstraZeneca's neuroscience program in Cambridge, Massachusetts, who has been working on psychiatric disease in industry for 14 years. "This dataset has clearly reinvigorated industry's interest in going after schizophrenia again."

Though the exact length of the parts list remains unclear, the fact that it comprises many entries fits with the idea, first suggested by Irv Gottesman and James Shields in the 1960s, that schizophrenia is a "polygenic" disorder. This means one person's schizophrenia stems from disruptions to multiple genes.

"I am very pleased with these signs of progress after waiting all my years in the field," said Gottesman of the University of Minnesota in Minneapolis. "But we have yet to make the bridge between those SNPs and the biology of the nervous system."

The challenge is that each SNP has a tiny—some say uniformative—effect, increasing risk ever so slightly. This means that none of the SNPs by themselves explain very much about an individual's liability to develop schizophrenia.

But the effects may not be too small to matter. To wit: one of the SNPs points to DRD2, the gene encoding the dopamine 2 receptor, which is the main target of antipsychotic drugs.

Combined, these small effects could pack a punch. A polygenic risk score (PRS) adds up all of the schizophrenia-associated SNPs carried by people to give a sense of their overall risk (ISC, 2009). In the new GWAS, the highest scores add up to an effect size about 10 times that of a single SNP, and more people with schizophrenia score high compared to controls. The score does not take into account specific genes, however, so one person with a high score could have a different combination of risk SNPs than another with the same score. This suggests that different people may carry different constellations of these risk factors.

Still, the 128 SNPs combined strain to explain schizophrenia's liability, accounting for only 3.4 percent (65-80 percent would explain all heritability). Analyses of patterns of all SNPs in schizophrenia and controls—regardless of their association status—suggest that common variants will eventually explain 30-50 percent of schizophrenia's heritability (see SRF related news report; SRF news report).

The unaccounted-for heritability leaves room for risk factors not probed by GWAS. Sullivan suggests a pie metaphor, with slices of as yet unknown size due to common variation, CNVs, protein-coding variation, environment, and interactions between genes and environment.

The PGC is interested in all of these slices. "Our view is that the genome is going to tell us what the answer is, and we simply don't care if it's a common variant or a rare variant," Sullivan said. "If it's important, we want to find it."

Wait, there's more. The PGC has a much larger GWAS already in the works, called PGC3, with 60,000 cases. Some of the PGC3 samples will be genotyped with the Psych Chip, a low-cost array specifically designed to detect common and some rare variants under suspicion for a variety of psychiatric disorders, including schizophrenia. Given the rate of genomewide-significant hits per sample so far, hundreds of more genomewide-significant hits are expected.

"There's nothing magic about it," Sullivan said. "In the end we have a carefully considered, mature, and relatively inexpensive way to actually learn more."

One expectation of the PGC is that getting as complete a catalog as possible will help delineate the operative biological processes impacted by these genes. But others worry that GWAS can become a numbers game, unmoored from biology.

"With each turn of the GWAS wheel, things that were previously highly significant hits don't necessarily survive into the next round," Porteous said, referring to five SNPs from previous GWAS that were not among the PGC's latest hits. Though this may reflect weeding out of false positives, SNPs with such small effect sizes may just shuffle in and out of statistical significance as sample sizes grow.

Others praise the PGC results to date but don't see the point of much more GWAS. "The published GWAS are a landmark. What now?" said Francis McMahon of the NIMH and an SRF advisor. "I'd be more excited to see someone making sense of one of these genes than I would about another paper with another hundred genes."

"We will keep looking for more genes, because it's like going to the moon—because you can go to the moon, you have to go the moon," Weinberger said. "Frankly, we have plenty of genes. The question now becomes, What do we do with them?"

The difference between finding a genomewide-significant SNP and finding a bona fide risk gene is a chasm that will take different approaches to cross. But, as will be discussed in the second installment of this story, "From discovery to understanding," bridging this gap is an important step for turning GWAS findings into actionable biological insights about schizophrenia.

Part 2, From Discovery to Understanding

August 11, 2015. Last year's prized genomewide association study (GWAS) of schizophrenia identified 128 single nucleotide polymorphisms, or SNPs, that were found more often in people with schizophrenia than in controls (see SRF related news report). These identify regions of the genome that harbor common DNA changes that increase risk for the disorder, and still more are expected to emerge in a larger GWAS currently being conducted by the Psychiatric Genomics Consortium (PGC).

But scientists are not waiting for the complete gene catalog before exploring what the findings might mean. Drug companies are already drawing up plans for finding drug targets; analysts are looking for themes among the implicated genes; and researchers are turning to postmortem brains or neurons derived from stem cells for functional insights.

"The results are seeding all sorts of biomedical research here at Cardiff, ranging from brain imaging to animals to cellular studies," said Michael O'Donovan of Cardiff University in Wales, who chairs the PGC's schizophrenia group. "For the first time, people are in a position to probe the biological consequences of genetic clues that you can pretty much guarantee are related to psychiatric disorders."

But if getting GWAS off the ground was strenuous, interpreting their findings will be an even longer haul. Most of the hits lie in relatively uncharted non-coding "regulatory" regions that control gene expression, leaving researchers in the dark about which genes are influenced. The tiny effect sizes of these SNPs may also make it hard to identify their effects in biological assays. And even when a true risk variant is pinpointed within the GWAS-implicated heap, understanding its function and dysregulation in schizophrenia is hampered by the inaccessibility of brain tissue.

"The genetics of schizophrenia aren't particularly different from the genetics of other complex human diseases," said Mark Daly of the Broad Institute in Cambridge, Massachusetts. "What sets schizophrenia apart is lack of access to the tissue that matters."

To get around this, researchers will draw from multiple types of data: snapshots of gene expression during brain development, as put together from postmortem brain mapping; human cell models of neurons derived from stem cells that can, with new genome editing techniques, systematically test the effects of risk variants on cell function; network analysis to generate ideas about the biology that is derailed in schizophrenia.

Even with all these data, making final inferences will be tricky.

"The genetics is difficult, but it's going to work. Making lines of cells and differentiating them is going to be hard, but that's going to work. But what we really have to figure out is how to get from cells to people," said Steven Hyman, director of the Stanley Center for Psychiatric Research at the Broad Institute in Cambridge, Massachusetts.

Finding culprits among nominees. Though GWAS findings narrow in on the sectors of the three billion base pairs of the human genome that contain risk variants, they don't actually pinpoint these variants. That's because the 128 genomewide significant SNPs flag stretches of DNA that tend to stick together during recombination—that is, they are in "linkage

disequilibrium" with each other. These tight stretches may contain many genes, which constitute nominees for schizophrenia's risk factors.

Getting to the actual culprits will require fine mapping of these regions to find the causal variant driving the genomewide-significant signal. Doing this, however, has been harder than expected. Of thousands of associations found by GWAS for other diseases, only a handful have been tracked to a causal variant.

This may be because common variants in a locus have such subtle effects that they're hard to recognize. Another possibility is that rare variants in a locus may be driving GWAS signals, a situation referred to as a synthetic association (Dickson et al., 2010). However, some researchers argue this does not explain the bulk of the schizophrenia GWAS results (Wray et al., 2011).

"Synthetic association has not proved a fruitful resolution to fine mapping efforts," Daly said, noting that more comprehensive scans of less common alleles find that they don't account for GWAS signals as well as common ones do. This doesn't rule out the possibility that independent rare variants in the same genes implicated by GWAS will turn up, however.

New statistical methods are making headway in fine-mapping the thorniest region, the major histocompatibility complex (MHC) region on chromosome 6. This region contains 200 genes, yet has resisted analysis because it is replete with linkage disequilibrium. But recent conditional analyses of the region that take into account correlations between different SNPs, or different haplotype structures, have narrowed in on several independent signals (for details, see SRF related conference report).

Another way to fine-map GWAS loci looks to the genomes of non-European ethnicities, which have not so far contributed much to genetic studies. For example, Sub-Saharan Africans have the "oldest" genomes, with smaller haplotype blocks. Finding a GWAS-fingered SNP within one of these smaller blocks would constrain the risk variant to a smaller region. The Stanley Center has begun a series of collaborations to collect DNA from diverse populations around the world, including Africa, Japan, and Mexico.

These approaches won't deliver an unequivocal causal variant for each and every locus, however, which means researchers will have to take indirect paths toward understanding the GWAS results. One option is to do targeted sequencing of the genes influenced by these variants to provide a sense of naturally occurring variation that would likely include risk factors for human diseases.

But much of the trouble in interpreting GWAS stems from an incomplete grasp of the human genome and its component parts. Most of the GWAS clues for schizophrenia lie outside of genes, in non-coding DNA that is thought to control how, when, and where different genes are turned on or off. This casts schizophrenia as a disorder of perturbed gene expression rather than one of broken proteins.

The genome's "control panel" is just starting to come to light through projects such as the Encyclopedia of DNA Elements (ENCODE) project, which identified regulatory elements that control gene expression such as promoters that provide landing pads for transcription machinery (see SRF related news report). More recently, the Roadmap Epigenome Mapping Consortium identified the DNA modifications in many cell types that flagged enhancers,

short stretches of DNA that can increase gene transcription, even from a distance. A more brain-focused initiative called PsychENCODE began in 2013 to examine the epigenomic landscape of neurons taken from postmortem brain samples, including those from people with schizophrenia. Together, these efforts will create a picture of how transcription unfolds in different cell types at different times, which will help researchers pick out any disease-related irregularities.

Mapping the brain's molecular landscape. With risk for schizophrenia embodied in the ups and downs of gene expression, the brain's profile of transcripts will be a critical touchstone for interpreting GWAS results.

"If the change is not in the amino acid sequence, it has to be in the regulation of gene expression by whatever mechanism—epigenetic, microRNA, non-coding RNA, promoters, enhancers—pick your mechanism; it doesn't matter " said Daniel Weinberger of the Lieber Institute for Brain Development in Baltimore, Maryland. "It's in the transcript—it has to be in the transcript."

If schizophrenia has its roots in early brain development, as is generally accepted, then the critical, risk-related perturbations to gene expression might only be glimpsed in a subset of neurons in fetal brain tissue. Databases of gene expression in the human brain are being generated across the lifespan for different brain regions (e.g., BrainSpan; BrainCloud), composed of data from either microarrays that probe for specific transcripts, or from RNA sequencing, which captures all transcripts, many new.

These data can be mined to find SNPs associated with expression, known as eQTLs (expression quantitative trait loci). Finding overlaps between human brain-specific eQTLs, regulatory elements such as enhancers or promoters, and schizophrenia-associated SNPs can help prioritize which genetic lead to follow up. This strategy showed researchers that a GWAS-implicated SNP in an intron of CACNA1C, a calcium channel subunit, flagged a nearby enhancer. The enhancer interacted with a promoter some distance away, and the risk variant resulted in decreased expression of CACNA1C in vitro (Roussos et al., 2014).

Transcriptome profiling in postmortem brains may help find genes hiding in GWAS signals, too. The Lieber Institute Pharma RNA-seq Consortium, a collaboration between the Lieber Institute and multiple pharmaceutical companies to sequence the transcriptomes of 1,600 brains, recently reported that genomewide-significant SNPs in a region of chromosome 10 all regulated expression of one gene nearby (for details, see SRF related conference report).

Eventually, postmortem brain studies may also implicate specific circuits or cell types involved. Studies of gene expression networks in autism have pointed to perturbations in excitatory neurons in the deep layers of cortex during mid-fetal development (Willsey et al., 2013). Getting ever more granular, a technology called drop-sequencing will allow high-throughput single-cell transcription profiling, which could lead scientists precisely to the altered circuits in mental disorders (Macosko et al., 2015; Klein et al., 2015).

Stem cells and networks. With hundreds of variants of interest expected for schizophrenia, the field also needs a high-throughput scheme to analyze the biological effects of each variant. To do this, stem cells—not knockout animals—are in the front lines. Currently, the Stanley Center is constructing libraries of well-characterized embryonic stem cell and

induced pluripotent stem cell (iPSC) lines that carry individual, or combinations of, risk alleles introduced through genome editing technology.

These cell lines can be made into neurons, and researchers plan to systematically compare neurons with a risk allele to those without it, but otherwise genetically identical. Because synapses are thought to play a role in schizophrenia risk, these neurons can also be coaxed to form brain "organoids" that recapitulate some aspects of neural organization, including synaptic connections between neurons (see SRF related news report).

"Undoubtedly, we will find cellular phenotypes, but the hard, hard problem in all of this is knowing what these cellular phenotypes mean for humans," Hyman said.

The emphasis on human cell models not only comes from recognition that regulatory, transcription-controlling elements are poorly conserved across species, but also from past disappointments in which insights based on rodent models did not apply to humans.

This "translational valley of death" puts a premium on human data when selecting potential drug targets, said Nick Brandon of AstraZeneca in Cambridge, Massachusetts, which is collaborating with the Lieber Institute to develop human stem cells for drug discovery. He sees human cell models taking a leading role in probing compounds for drug responses or efficacy, leaving animal models for testing drug safety and specific mechanisms of action.

"We absolutely still need animal models," he said. "But we just have to take the animals for what they are and not overinterpret them. We need to focus on what they are telling you about particular mechanistic aspects of your target or your compounds."

Another strategy to probe function relies upon bioinformatics, which offers tools to search for common themes among the genes nominated by GWAS. Called pathway or network analysis, this approach asks whether these genes are enriched within specific biological processes, or pathways.

Although the field has not yet settled on standard methodologies, the approach could highlight how variants of small effect flag problems in critical biological processes, which as a whole may be better therapeutic targets than any individual risk variant.

A pathway analysis of the PGC's earlier round of hits highlighted histone methylation, which controls gene expression, synapses, and immune signaling (see SRF related news report). These are very general processes that impinge upon all of development, however, leaving any therapeutic insights unclear. Adding more genomewide-significant hits to the analysis might lead to more specific components of these pathways, or schizophrenia's heterogeneity could stymie that hope.

"It's my own personal prejudice that there are lots of ways the brain can go wrong to lead to the disorders we currently call psychosis. If true, then it's quite likely there will be a lot of fairly general pathways," said Cardiff's O'Donovan.

Where do the old candidates stand? As the new, GWAS-sanctioned genes capture everyone's attention, what to make of those old, pre-GWAS era candidates? Genes such as neuregulin (NRG1), dysbindin, and catechol-O-methyltransferase (COMT) were identified by linkage in smaller family studies or by candidate gene studies that explored a gene based on

ideas about its role in schizophrenia biology. Except for a hit near the DRD2 gene, which encodes the D2 subtype of the dopamine receptor, the primary target of antipsychotic drugs, these old candidates don't turn up in the PGC's GWAS. Though future GWAS iterations may eventually bring to light some of these old favorites, opinions vary on how to reconcile them with the new data.

"The way to reconcile them is that they don't reconcile," said Thomas Lehner of the National Institute of Mental Health (NIMH) in Bethesda, Maryland, noting the exception of the MHC region, which was immediately recognized in early linkage studies. A recent survey shares this sentiment (Farrell et al., 2015).

Part 3, Rare Allure

August 17, 2015. Beyond the common genetic variants that all people carry, rare variants are also expected to contribute to schizophrenia risk. To geneticists, rare means occurring in fewer than one out of 100 people, and finding them requires reading out genomes letter by DNA letter in thousands of people. But this fine-combing may be rewarded by findings that are simpler to interpret.

"Rare contributions are incontrovertible because we have the mutations in our hands," said David Goldstein of Columbia University in New York City, who leads several sequencing projects for different human diseases.

Unlike SNPs in genomewide association studies (GWAS), which only flag a region that contains a risk variant, rare variants themselves are the nucleotides of interest. They are also more likely to have a large effect on risk, given that their rarity reflects a relatively recent change to the human genome, with little time for suppression by natural selection.

Yet so far, the dividends remain elusive. Rare variants are indeed found in people with schizophrenia, but they are rarer than expected and scattered across the genome, only occasionally hitting the same gene in different people. This, combined with an unexpected amount of rare variation in everyone, has made it hard to statistically peg any one genetic mishap to schizophrenia.

"The large sequencing studies published to date have relatively modest results—not so dissimilar from the results in the first rounds of GWAS," said Mark Daly of the Broad Institute in Cambridge, Massachusetts.

This leaves rare variant hunters with the same mantra as their GWAS counterparts—increase sample size.

"I think there's just a heck of a lot of loci for schizophrenia," Goldstein said. "So if a lot of different genes can confer risk, you need really large sample sizes in order to implicate any of them."

As the search for rare variants intensifies, more traction has come from contributions made by another type of genetic rarity called copy number variants (CNVs). CNVs involve the loss

or gain of DNA segments containing multiple genes and elevate risk for diverse psychiatric disorders (Malhotra and Sebat, 2012).

Yet even these potent rare variants, CNVs or otherwise, may not lead exclusively to schizophrenia. Instead, certain combinations of factors—genetic and environmental—could put someone on the path for a specific disorder.

"Risk for a disorder is rare variant plus common variant plus environment, and under environment you can put in chance," said Michael O'Donovan of Cardiff University in Wales.

Sequencing sense. Sequencing data for schizophrenia has begun to surge in the past five years, with results reported from several groups. So far, all studies have restricted themselves to the exome, the protein-coding parts that comprise 1 percent of the genome. Exome mutations may damage the protein building blocks of a cell and lead directly to insights about the biology of schizophrenia.

But even for these, interpretation can get tricky. We all carry rare, probably protein-damaging, mutations, with no obvious untoward consequences. This complicates making a connection between a certain rare variant and disease. For this reason, studies have focused on "de novo" mutations—non-inherited mutations that spontaneously occur in sperm or egg cells. If found in people with schizophrenia, but not in their unaffected parents, then chances would seem to be better that they have something to do with the illness. Plus, de novo mutations are more likely to include damaging variants because natural selection hasn't had a chance to temper their actions.

This approach has successfully identified risk variants for autism (see SRF related news report), but the jury is still out on whether they contribute similarly to a disorder like schizophrenia, which typically starts during late adolescence and early adulthood. De novo variants do turn up in schizophrenia, often at a rate higher than in controls, but these are distributed across the genome. Two studies published last year hint that some of these might pile up on the same genes: one reported deleterious mutations in SET1DA in two different people (see SRF related news report), and another reported 18 different genes hit twice by de novo mutations, including TAF13 (see SRF related news report). Both SET1DA and TAF13 have roles in transcription, but more mutations hitting them need to be found to turn them into statistically definitive risk factors.

Another approach forgoes de novo finding and instead sequences lots of cases and controls, with the idea that a true risk variant would turn up more often in schizophrenia. The largest effort of this kind, led by Shaun Purcell of Mount Sinai School of Medicine in New York City, sequenced nearly 5,000 people, yet found only a small enrichment of rare variants in schizophrenia, and no pileups of mutations hitting the same gene (see SRF related news report).

At the very least, sequencing efforts have been large enough to rule out contributions by moderately rare mutations, occurring in 0.5 to 1 percent of people. Thought to inhabit a genetic sweet spot in that they are not so rare as to be hard to find, yet not so common as to have weak effects, these "medium rares" are not enriched in schizophrenia, according to the Purcell paper and an earlier paper published by Goldstein's group (see SRF related news report).

Yet the ragtag crew of rare variants found so far in schizophrenia may act on some of the same biological processes, including early brain development (see SRF related news report), chromatin modification (see SRF related news report and SRF news report), or synaptic machinery (see SRF related news report). Researchers also point to the overlap with GWAS-implicated genes, including those involved in glutamate and calcium signaling.

"I think the main message from the rare variant stuff is that, while we've seen convergence on certain biological pathways, identifying individual genes in significant numbers, much less individual mutations, will require much larger samples than currently published," O'Donovan said. "And that's an expensive endeavor. It will take a bit of time."

Sequencing families enriched for mental illness might expedite the discovery of repeat instances of the same mutation.

"I think that the degrees of heterogeneity we now all agree exist tell you that you've got to do something differently. Included in that would be a return to family-based studies," said David Porteous of University of Edinburgh in Scotland. Porteous and colleagues discovered the gene disrupted-in-schizophrenia-1 (DISC1) in a large Scottish family beset by mental illness, including schizophrenia (see SRF related news report).

Targeted sequencing of DISC1 is now underway to probe its role in mental illness beyond the original family. This has revealed a huge amount of variation in the gene. So far, these variants have been tied more to major depressive disorder than to schizophrenia (Thomson et al., 2014).

Though DISC1's status as a schizophrenia gene is contentious (Sullivan, 2013; Porteous et al., 2014), it remains a favorite among biologists because many are convinced that it could reveal biological mechanisms that are subverted in psychiatric disorders beyond the Scottish family. Last year, a high-profile paper reported that stem cell-derived neurons from two people with DISC1 mutations and psychiatric disorders had weakened synapses; this deficit could be rescued in vitro by correcting the DISC1 mutation through genome editing (see SRF related news report).

"There's little doubt in my mind that DISC1 is a real risk gene that has probably taught us more about the biology of schizophrenia than anything we've found in GWAS so far," said Francis McMahon of the National Institute of Mental Health (NIMH) in Bethesda, Maryland.

Despite the murky picture of rare variant contributions to schizophrenia, researchers are already bringing exome sequencing to the clinic. For example, Anna Need of Imperial College London is sequencing the exomes of children with a variety of psychiatric illnesses, including schizophrenia, in order to find some genetic explanation for their disorders. In some cases, the children carry mutations in genes associated with a Mendelian disorder that doesn't match their symptoms. This kind of genetic diagnosis could inform treatment plans and help chart the connections between a disrupted gene and its resulting phenotypes.

"We thought we had a reasonable idea of what the genes causing known Mendelian disorders did. But they're actually turning out to be linked to a much more heterogeneous spectrum of conditions and symptoms," said Need, who co-authored a recent survey of the diverse outcomes that can stem from disruptions to the same gene (Zhu et al., 2014).

We are the 99 percent. The bulk of the risk conferred by rare variants may well reside in non-coding regions, which comprise 99 percent of the genome. Whole genome sequencing (WGS) to probe these stretches of DNA has begun for schizophrenia, but researchers know that they are unprepared for interpreting the onslaught of data.

"Going out and trying to find the risk factors anywhere in the genome for schizophrenia now is an intimidating proposition, since our ability to interpret mutations outside of the exome is currently limited," Goldstein said. "Our hope is to help build up the science of interpreting regulatory variation by integrating whole genome sequence data with comprehensive transcriptomic data."

The Whole Genome Sequencing for Psychiatric Disorders (WGSPD) consortium has been formed among those collecting WGS data for schizophrenia and other disorders, with one aim being to come up with interpretation aids. Goldstein, Daly, and Matthew State of the University of California, San Francisco, are building a framework for recognizing the most important non-coding variants through analysis of transcriptome data from blood cells and neurons derived from stem cells. For example, a variant of interest could be identified when changes in how a gene is spliced or expressed occur in tandem with a variant in a splice site or regulatory region for that gene.

CNV signposts. In contrast to the incipient returns from sequencing, CNVs are more established as risk factors. Although rare, CNVs in certain locations of the genome turn up in multiple cases of schizophrenia, and significantly less often in controls. For example, 25 percent of people carrying a large 3 Mb deletion on chromosome 22—a CNV first identified in 1995—will develop psychosis. Since then, new technologies, including SNP arrays, have detected other CNVs in either case-control or de novo studies (see SRF related news report). CNVs are found in a minority of schizophrenia cases, but they attract interest because they are associated with big effects on risk, with odds ratios about 10 and beyond.

The Psychiatric Genomics Consortium (PGC) has combined some of the array data used for its GWAS efforts to look for schizophrenia-associated CNVs. Ongoing analyses of 41,000 people suggest that researchers have, for the most part, found the big ones.

"The CNVs we know and love—they stand out like a sore thumb," said Jonathan Sebat of the University of California, San Diego, who co-leads the analysis. "But there are some additional genetic factors in there that we haven't yet identified."

But even with such strong associations, CNVs evade straightforward interpretation. Most involve numerous genes, making it hard to pin down exactly which contribute to schizophrenia. Analyses of the long list of potential culprits have at least highlighted post-synaptic machinery (see SRF related news report).

"Knowing that the synapse is involved in schizophrenia—that's not really earth-shattering news," Sebat said. "But I think it's more interesting to see that we're now starting to get down to components of the synapse that appear to be driving that signal."

Another wrinkle is that CNVs are not terribly specific. For example, a duplication at chromosome 16p11.2 is associated with increased risk for schizophrenia, bipolar disorder, and intellectual disability. Interestingly, other CNVs associated with psychiatric disorders can be carried with apparently no ill effects, though closer examination has revealed subtle effects

on cognition and brain structure (see SRF related news report). This suggests that CNVs must work with other factors to produce a particular phenotype.

Psychosis may be an extreme outcome of a CNV, Sebat suggested.

"The typical presentation of the CNV may not be schizophrenia but may be the unfortunate kid in the back of the classroom who is struggling with reading comprehension and having difficulties in mathematics, and maybe not socializing to the extent the other kids are socializing. Whether that kid goes on to develop psychosis later in life involves a number of factors, both nature and nurture," he said.

Such non-specificity seems to be a rule even for rare point mutations and common variants. Time and again, genetics declines to follow the diagnostic categories set forth by psychiatrists. There is much speculation about what else is going on to lead to specific mental disorder phenotypes. We will explore this in story 4 of the series, "Rethinking diagnoses."

Others say that the GWAS design only rules things in rather than out. "What we can say is that the data and the types of analyses we've done don't particularly favor any of the old candidates," O'Donovan said. "But that's a very different statement from saying the old candidates aren't involved."

Citing NRG1 as an example, O'Donovan suggested the old candidates may reside in complicated haplotypes that SNP arrays used in GWAS do not capture well.

Another idea is that the old candidates may be context dependent, so that a given gene doesn't produce a signal unless it combines with a particular environment or genomic background—effects of which would be lost in the giant samples needed for GWAS. The PGC's latest GWAS did not find evidence for gene-gene interactions (called epistasis) between pairs of risk SNPs, but this does not rule out the existence of interactions among three or more genes.

"We're going to find that the reason some of these candidate genes didn't stay significant was because they were critically dependent on other factors that varied tremendously across different samples," Weinberger said.

Brandon, who has pursued many of these previous candidates, feels they still have a lot of mileage left in them. "We would be foolish to throw these away," he said, adding that AstraZeneca is keeping them in mind as the new data emerge. "We are certainly trying to be inclusive of these prior findings because you never know when things interact."

Our next story in the Schizophrenia Genetics 2015 series—"Rare Allure"—will detail the hunt for another category of possible contributors to liability for schizophrenia: the rare and elusive genetic glitches that could have robust effects on risk.

Part 4, Rethinking Diagnoses

August 21, 2015. As the genetic risk factors underlying schizophrenia come into focus, the findings blur the categories of mental illness. All types of genetic risk factors for schizophrenia—from common single nucleotide polymorphisms (SNPs) to rare copy number variants—include variants that boost risk for other disorders, too. These shared genetic roots do not surprise most researchers.

"Genes don't know about psychiatric diagnoses; they know about development and function of the brain," says Daniel Weinberger of the Lieber Institute for Brain Development in Baltimore, Maryland. "The more we can understand our categories of mental illness in terms of how a brain develops, the more we'll understand what these genes mean to mental illness."

In order to explain how to get from a gene-induced vulnerability for mental illness in general to a specific diagnosis such as schizophrenia, researchers will need to consider how different genetic variants combine to build risk. To complicate things, they will need to consider the environment, a sector of risk that teems with so many unknowns that most are content to wrestle with purely genetic puzzles. But the shared genetics undoubtedly point to shared biology and so argue for conceptualizing psychiatric disorders in terms of their disrupted brain processes rather than their observable symptoms. This means grappling again with the nature of schizophrenia by using tools such as brain imaging or in-depth cognitive testing. Any core phenotypes that come to light, in turn, can be marshaled in the hunt for more genes.

"We need to start honing down and refining our phenotypes to really find the remainder of the genes, or even to understand the genes that have been found," said Anil Malhotra of Zucker Hillside Hospital in Glen Oaks, New York.

Carefully phenotyping thousands of people is expensive and time consuming but worth the trouble to some.

"I think with 5,000 subjects who are exquisitely well characterized, you can find out as much or more than other platforms where you have many more subjects but you really don't know much about them," said David Braff of the University of California, San Diego.

Although lumping cases of schizophrenia together without special consideration of their heterogeneous features has dominated the genetics field, Braff sees the two approaches as complementary. "Both strategies are valid. If one thing worked, we'd only be doing one thing, and we'd have resolved this."

Getting to schizophrenia. Overlaps occur among all flavors of genetic variation. For example, both chromosome 16p11.2 duplications and 15q13.3 deletions increase risk for schizophrenia, autism, and intellectual disability. Damaging point mutations found in schizophrenia, for example, in SCN2A and POGZ, are also linked to autism and intellectual disability (Fromer et al., 2014).

Common variants also predispose people to a range of psychiatric disorders. In 2013, the Psychiatric Genomics Consortium (PGC) published a cross-disorder genomewide association study (GWAS) which combined samples with schizophrenia, bipolar disorder, major depressive disorder, autism, and attention deficit-hyperactivity disorder (ADHD) (SRF related news report). Comparing this melting pot to controls revealed four regions containing

generic vulnerability factors for mental illness, including genes important for calcium signaling.

Studying genetic signals across disorders can give a kind of taxonomy of mental illness. The greatest shared risk arose between schizophrenia and adult-onset disorders, in particular, bipolar disorder and major depressive disorder (see also SRF related news report).

"How much are these really the same disorders, schizophrenia and bipolar? We've been debating this for a hundred years, swinging back and forth," Kendler said. "This is an area that will need more clarification."

Variants shared by multiple disorders may set a common stage upon which disease-specific risk factors act. In the brain, this stage may take the form of a brain circuit sensitized to stress, for example, which could produce a cascade of ill effects on cognition and emotion regulation. Additional genetic or environmental risk factors may tilt a person toward a specific disorder. Alternatively, how well a brain can compensate for these risk factors may determine the resulting outcome. Another scenario includes modifiers, which by themselves do not impact risk for a disorder but instead influence the form that a risk factor takes.

Yet no one understands how these combine, especially with the unwieldy environment. Perinatal complications, urban birth, season of birth, and famine are among established risk factors for schizophrenia, but these have not yet been integrated into the genetic results. They may leave a signature on the genome in the form of methylation (see SRF related news report), which controls which genes are turned on or off, or somehow subtly alter brain development. Though researchers agree that taking environment into account will be important, studying the environment, and funding those studies, is a challenge.

"In my view, it's going to be important to put genes and environment together," Kendler said. "The trouble is, there are very few good environmental risk factors for schizophrenia you can measure when you talk to an affected person."

A new Danish project, called the Initiative for Integrative Psychiatric Research (iPSYCH), is poised to systematically explore gene-environment interactions. The national registries in Denmark track information on every resident's education, health, and other demographics, as well as genetic information obtained from blood samples collected at birth. One project will monitor the children of people with schizophrenia or bipolar disorder, pulling together measures of cognition, brain imaging, environment, and genetics to find the mix that leads toward or away from particular disorders.

Refining phenotypes. Recognizing that the road from a risk variant to schizophrenia is a long and winding one, some researchers have gravitated toward understanding intermediate phenotypes that are presumed to lie closer to genes. These reflect concrete features of brain function measured, typically, with cognitive testing, electroencephalography, or magnetic resonance imaging (MRI). Dubbed "endophenotypes" by Irving Gottesman (Shields and Gottesman, 1972; see SRF online discussion), they are by definition heritable, and so might yield clearer genetic signals than the diagnostic category of schizophrenia, as well as extract the malfunctioning brain circuits.

A similar perspective was adopted five years ago by the National Institute of Mental Health (NIMH) with its Research Domain Criteria (RDoC). The initiative offers a framework for

researchers to move beyond the traditional, symptom-based classifications of psychiatric disorders to focus on questions oriented around their underlying biology. For example, instead of studying schizophrenia, a project may study the brain circuits underlying social cognition or auditory hallucinations (see SRF webinar).

"It certainly is reinforcing what I've been selling," said Gottesman of RDoC, though he worries that its implementation in the NIMH's grant structure is premature.

For the past 10 years, Braff has led the Consortium on the Genetics of Schizophrenia (COGS), which has pursued cognitive and electrophysiological endophenotypes. From 100 original candidates, the group has homed in on 12 they consider the most important (see SRF related news report; SRF related conference report).

But collecting this fine-grained phenotypic data is time consuming, requiring participants to spend two days in the lab and ultimately taking 10 years to characterize 5,000 people. A GWAS of the sample is expected to be finished in the next year, which could point to the genetic contributors to endophenotypes such as working memory. Sequencing of the COGS samples is also underway.

Braff fully expected it would take this long. "These are really long-term projects. We have to have a lot of patience and diligence," he said.

The most direct readout of the brain may come from brain scans. Measuring the sizes of brain regions with structural MRI, or the flow of activity between regions with functional MRI, may illuminate brain-based endophenotypes that could build bridges between genes and brains. The field of imaging genetics demands sample sizes larger than typically seen for straight imaging studies. Pooling data from many sites, such as is done by the ENIGMA Network will be essential.

"Brain imaging is still a wide open game, there are lots of techniques out there, and you're still inferring a lot," Malhotra said. "But as we're better able to visualize brain structure and function, I think phenotype will matter more and more to schizophrenia genetics."

Beyond their application to genetics, some endophenotypes may be useful biomarkers that could carve out new classifications of disorders. For example, at the 2015 International Congress on Schizophrenia Research, the Bipolar Schizophrenia Network on Intermediate Phenotypes (B-SNIP) reported three new "biotypes" based on measures of cognitive function, eye movement control, and responses to auditory stimulation (see SRF related news report). Alternatively, some biomarkers might delineate subsets of people with schizophrenia. Still others may predict treatment response: Malhotra and colleagues recently reported that changes in functional connectivity between the striatum and the cortex upon taking antipsychotics were greater in people who got more relief from the medicine (see SRF related news report).

Ultimately, endophenotypes may prove more useful in helping researchers understand the biology of risk variants identified by standard case-control GWAS than in identifying schizophrenia's genes.

"Everyone genuflects to say these are really complex disorders, but to date we haven't had the stunning advance by dividing these disorders into something that was meaningful," Kendler said.

With so many possible genes and phenotypes, biologists may be left scratching their heads on which leads are best to follow. The next and final story—"Part 5, Plan of Action"—explores opinions about how to prioritize these leads, how to use them to tailor treatments to individuals, and how the landscape of schizophrenia genetics research will change as team science takes hold.

Part 5, Plan of Action

August 25, 2015. Between schizophrenia's landmark genomewide association study (GWAS) last year and reports of rare genomic variants that increase risk for the disorder, there is no shortage of genetic clues to follow up. As the gene discovery phase continues apace, it will continue to add to this line-up of hundreds of suspect genes. Researchers and drug companies must wrestle with how to prioritize these clues to find therapeutic targets efficiently, a decision that should not be taken lightly.

"We've seen examples where labs have launched major efforts on genes where the evidence simply wasn't unequivocal," said Patrick Sullivan of the University of North Carolina in Chapel Hill and a leader of the Psychiatric Genomics Consortium (PGC). "I think neuroscientists need to be far more cautious than they sometimes are. We want people to work on the best clues."

Along with the choice of genes comes a choice of directions. One path is an attempt to relate the genetic findings to how schizophrenia takes hold in the brain, which potentially leads to new therapeutic targets. Another path looks for subtypes of the disorder, which may involve a combination of genetic information and other biomarkers. This may eventually prove useful in the clinic to predict risk or outcomes. Matching a person's genetic and/or biomarker profile to a customized treatment is the hope of precision medicine, a fledgling idea for psychiatric disorders (Insel et al., 2015).

Moving toward personalized medicine will depend on the efforts of many. The deluge of genetic data, and the scale of the follow up it requires, is reshaping the way science is done both in academia and industry. With a collaborative framework becoming the norm, researchers will have to tackle questions about how to assign credit, how to rigorously evaluate their work when every known expert is a collaborator, and how to maintain individual research programs.

"It's a big-data science now, which changes the whole experience pretty significantly," said Jonathan Sebat of the University of California, San Diego. "But we haven't abandoned those really rewarding solo projects that we love doing."

He added, "We could easily devote all of our time to big science projects because there's that much richness in the datasets. But of course we need to maintain a balance between contemplation and conference calls."

The expectation is that giant collaborations will set the stage for productive smaller-scale research. "Anyone who wants to solve the problem has to work in large groups, both the genetics and the stem cell biology must occur at scale, and then the useful transfer of information requires interdisciplinarity," said Steven Hyman, director of the Stanley Center for Psychiatric Research at the Broad Institute in Cambridge, Massachusetts. "Once all these initial observations are made, there will be many hypotheses for small labs to test."

Finding targets. Despite the tiny effects of common variants found by GWAS, drug companies are sifting through them for new drug target gems. Pfizer, a member of the PGC, has developed a pipeline to sort through the latest GWAS hits to come up with targets that may generalize to the schizophrenia population as a whole (described in Schubert et al., 2014). This approach banks on the fact that while the small but significant GWAS hits may say little about biological effect size, they do have an unequivocal relationship to disease.

For Pfizer, high-priority targets include calcium channels, glutamatergic signaling, and intracellular signaling molecules along the Akt/mTOR pathway, which may be selectively perturbed in the service of dopamine signaling.

For prioritizing other genes, the company sees gene expression data from the brain as critical, prompting Pfizer to collaborate with the Lieber Institute for Brain Development's RNA-Seq Consortium (see SRF Genetics Series, Part 2). Integrating other veins of information will also delineate worthy targets; for example, knowing how druggable a molecule is, or with what molecules it interacts, could lead to targets that don't belong to the GWAS hit pantheon.

"We are trying to escape from this perception that a clear GWAS hit has to be your target," said Patricio O'Donnell of Pfizer in Cambridge, Massachusetts. "I think we can use the current information to point to a network of genes, ask what are the biological processes that these genes are serving, and then find the way to intervene there."

AstraZeneca in Cambridge, Massachusetts, has made collaboration their mainstay. In 2012 the company downsized drastically, closing its labs but preserving a very small drug development team that formed partnerships with academics or other companies. They also are working with the Lieber Institute to integrate data from genetics, brain transcriptomes, and induced pluripotent stem cells (iPSCs).

"With so much information breaking in neuroscience in the last five to 10 years, we saw that we had to be more flexible and work externally in a much more complete way," said Nick Brandon of AstraZeneca. "Now, if things don't work out for a drug candidate, we can change very quickly to another."

Brandon expects that this approach will deliver drug candidates for schizophrenia in five years.

Subdividing schizophrenia. Both company research leaders stressed the importance of identifying subgroups of people with schizophrenia who may respond differently to treatment. Drawing from biomarkers and genetics alike, researchers might be able to verify that a particular target is indeed dysregulated in a subgroup before advancing to clinical trials.

Pfizer is involved in analyzing a well-phenotyped cohort collected at the University of Maryland to search for subgroups. With genotype, brain scans, brain electrophysiology,

cognitive testing, and blood samples collected from 1,100 people, 400 with schizophrenia, the team hopes that combining the information will reveal distinct subgroups of people with different causes of their illness.

"This would give us a more personalized medicine," O'Donnell said. "It's going to take a few years to get there, but I think we now have the tools to do it."

One of the first subtypes to be delineated may be treatment-resistant schizophrenia (TRS). About one-third of people with schizophrenia have TRS, meaning that symptoms are not alleviated by antipsychotic drugs that block dopamine signaling. Brain imaging finds that those with TRS do not have the excess dopamine signals found in others with schizophrenia (see SRF related news report), and genetic differences are also falling out of GWAS that compare those with TRS to others with schizophrenia (see SRF related conference report). If a gene test or series of biomarkers could be developed for TRS, this would spare patients the lengthy antipsychotic trial and error. It might also explain why clinical trials of glutamate pharmacology, which did not select patients for glutamate disturbances, failed (see SRF related news report).

Although people develop schizophrenia for different genetic and environmental reasons, whether genes alone can extract subtypes remains unclear. In 2014, a controversial study purported to identify eight different subtypes by analyzing the patterns of SNPs loosely associated with schizophrenia by early GWAS. The study was pilloried, with critics pointing to more mundane reasons for the segmentation (see SRF related news report).

Yet purely genetic information can be put together to estimate a person's risk for schizophrenia, such as the polygenic risk score (see SRF Genetics Series, Part 1). This index can partition people into groups with different levels of risk, which may be useful clinically, though it lacks the selectivity and specificity needed for an actual predictive test for an individual.

"You're never going to get an accurate estimate of risk for an individual from genetic data alone," said Naomi Wray of the University of Queensland in Brisbane, Australia. Wray and colleagues have been investigating ways of combining genetic data to improve risk measures (e.g., Maier et al., 2015).

This type of approach might also guide treatment for those in the earliest stages of mental illness. For example, of young adults reporting mild delusions and hallucinations, between one-quarter and one-third later develop full-blown psychosis (Fusar-Poli et al., 2013). A genetic tool could identify a sector of people with these symptoms who are at high genetic risk, and that group might be enriched for those who eventually transition to schizophrenia, Wray said.

Pharmacogenomics. As in the rest of medicine, pharmacogenomics is a hope in psychiatry. It is assumed that people's genetic makeup will influence how they respond to different drugs, and so far the only traction for schizophrenia has been in side effects. A GWAS exploring antipsychotic-induced weight gain found a common variant near the MC4R gene, which encodes a melanocortin receptor with links to obesity (see SRF related news report).

MC4R is not among the PGC's hits, and probably has nothing to do with schizophrenia's origins. This means that genetic variants important for treatment response, such as those controlling drug absorption or metabolism, need not be among the disorder-related hits.

"Our position on this is really that the genes for the disorder may not necessarily have any pharmacogenetic impact," said Anil Malhotra of Zucker Hillside Hospital in Glen Oaks, New York, who led the weight gain study, and who founded and organizes the Pharmacogenetics in Psychiatry conference.

Another study last year linked two rare variants in HLA that confer high risk for agranulocytosis, a life-threatening side effect of the antipsychotic clozapine. Half the cases of agranulocytosis did not carry either variant, however, limiting the findings' clinical utility (Goldstein et al., 2014).

"Drug response may not be as complex as schizophrenia, but it's still pretty complex," Malhotra said, noting that ideally a study would treat subjects with the same drug at the same dose for the same duration. "So I think we need larger sample sizes with these parameters held pretty steady to start to be able to discern those genes."

But this complexity leaves funding agencies skittish.

"Pharmacogenomics is a tricky and difficult field," said Thomas Lehner, branch chief of genomics research at the National Institute of Mental Health (NIMH) in Bethesda, Maryland. Noting that drug metabolism can be a slippery phenotype, with multiple interacting and sometimes opposing variants involved, he added, "We should only make limited investments in it at the moment."

Dawn of the team science era. Lehner stressed the need to push research in all directions, with an eye toward collaboration. For example, the NIMH started a partnership with the Stanley Foundation to form the Whole Genome Sequencing for Psychiatric Disorders (WGSPD) Consortium to comprehensively address the function of the genome's mysterious regulatory regions (see SRF Genetics Series, Part 3). He also called for work geared toward understanding the biology behind the genetic clues rolling in.

"Would I say that the PGC has done its duty and should be disbanded? I don't think so. The PGC serves an important function," Lehner said, noting the PGC's Psych Chip experiments, which will allow more gene discovery and replication at a fraction of the cost of previous GWAS.

As consortia continue to mushroom, the field seems to grow more comfortable with collaboration as a necessary reality, though some worry the drive for large datasets will create a gap between knowledge and understanding.

"I see the field of schizophrenia genetics, and more generally the origins of mental illness, to be in a state of chaos induced by too much information," said Irv Gottesman of the University of Minnesota. "It may be generating too many data for our current comprehension to go forward to biology." The shift toward collaboration will also reshape how funding agencies and academic departments judge an individual's work. While doing science in a big group might seem to make it hard for an individual researcher to make a mark, there are also benefits.

"It's a great opportunity to people starting out in the field," Francis McMahon of NIMH said. "There's always a role to play in these consortia. Just get involved, learn a lot, and get access to datasets you couldn't dream of getting on your own."

Even the Stanley Center, with its recent windfall of hundreds of millions of dollars, sees collaboration as critical. "I think that our good fortune really demands of us that we support not only our own efforts, but other peoples' efforts, to understand schizophrenia and to facilitate a path to new treatments," said Hyman.

"Team science is never going to go away," he added. "But it will coexist with small science as well."