NGS and GWAS are Driving Advancements in Psychiatric Genetics Research

Working in mice, rats, and humans, Dr. Abraham Palmer uses sequencing on the HiSeq® 2500 System to identify genes that influence behavior.

Introduction

Impulse control is more than resisting the candy display while standing in the checkout line at the grocery store. Varying degrees of impulse control can lead to serious psychiatric disorders. Too much control can cause obsessive compulsive disorder (OCD). Not enough control can lead to drug, eating, or gambling problems. Associating these behaviors with underlying genetics is the focus of a collaboration between Abraham Palmer, Ph.D.[1,2], Associate Professor in the Department of Human Genetics at the University of Chicago, Harriet de Wit, Ph.D., Professor in the Department of Psychiatry and Behavioral Neuroscience, University of Chicago, and James MacKillop, Ph.D., Professor of the Department of Psychiatry and Behavioural Neurosciences, Michael G. DeGroote School of Medicine, McMaster University, and Director, Peter Boris Centre for Addictions Research at St. Joseph's Healthcare Hamilton.

Working in mice, rats, and humans, Dr. Palmer and his colleagues leverage next-generation sequencing (NGS) on the HiSeq 2500 System to perform genome-wide association studies (GWAS) to identify genes that influence behavioral disorders. "In these studies we don't find the usual suspects, such as a dopamine or a serotonin transporter," said Dr. Palmer. "We almost always find novel and unexpected genes that allow us to go back, using model organisms, and manipulate those genes and see how they influence the phenotype. That's where we obtain fundamental new insights about the biology of the traits that we're interested in."

iCommunity spoke with Dr. Palmer about the potential impact these studies have on the understanding and treatment of psychiatric disorders.

Abraham Palmer, Ph.D., is Associate Professor in the Department of Human Genetics at the University of Chicago with a joint appointment in Psychiatry and Behavioral Neuroscience. He is also Co-Director of an NIMH T32 training grant and Director of an NIDA-funded P50 center grant, which is focused on the genetics of substance abuse-related behaviors in rats.

Q: What model systems are important for your research?
Abraham Palmer (AP): We work in various model systems for a number of reasons. We've worked with mice for years because they are cheap, small, and breed rapidly. We have expanded our use of rats recently in our studies, because they are smarter and exhibit more interesting behaviors. Although rats are more expensive to work with, they have more phenotypes—observable characteristics or traits—to study. In humans, we focus on endophenotypes—traits that have a clear genetic component—that have similar phenotypes in mice and rats. The mouse and rat models offer a stepping stone to understanding human diseases.

Q: What behavioral disorders do you study?
AP: Most of our research is aimed at behavioral phenotypes related to drug abuse, anxiety, post-traumatic stress disorder (PTSD), depression, schizophrenia, and attention deficit hyperactivity disorder (ADHD). We view impulsivity as a possible risk factor for developing drug abuse. It might lead people to experiment with drugs or make choices contributing to the risk for addiction. In a recent human study, we investigated amphetamine sensitivity to understand how much non-drug abusers like the way amphetamine makes them feel. We're also exploring how the polygenic risk for impulsivity maps onto other disease traits such as ADHD and drug abuse.

Q: How do you approach these types of studies?
AP: We conduct discovery projects using GWAS, which examines common genetic variants in different individuals and their association with a variety of traits. The challenge when working in a genetically heterogeneous population, whether in mice or humans, is obtaining a large enough sample size.

We need a large sample size to have enough statistical power to detect the associations, because we don't expect any individual allele to have a large effect on the phenotype. Instead, we expect that there are many alleles contributing only a small fraction of phenotypic variability that we observe. The challenge is balancing the need for sample size without sacrificing the quality of the phenotyping.

Q: How do you recruit and test subjects?
AP: In the human impulsivity study, we screened the subjects to make sure that they weren't heavy drug users and that they didn't suffer from any major psychiatric disorders. They completed questionnaires online, as well as performed behavioral tasks in the lab to measure their degree of impulsivity quantitatively. The subjects provided a blood or saliva sample as a source of genomic DNA. We performed genotyping to assess alleles at hundreds of thousands of sites across the genome. We measured variable sites where both alleles are common in human populations. At each of those alleles, we determined whether or not a variation at that site influences variation in the phenotype that we're interested in.

We take a similar approach in rats and mice. We obtain a quantitative measure for a number of different phenotypes and then isolate genomic DNA from the tail tip or spleen, and used that sample to genotype hundreds of thousands of sites across the genome. One of the added benefits of working in animals is that we can measure genome-wide gene expression in trait-relevant brain regions. We can associate phenotypes with genotypes and gene expression, and determine if a particular locus influences a behavioral trait through its influence on the gene expression trait. We can test this hypothesis by manipulating gene expression in the model organisms, and then assessing how the implicated gene affects behavior.

Q: What Illumina technologies have enabled these studies?
AP: We've been working with Illumina technology for the past decade. In our early mouse genotyping studies, we customized a GoldenGate® array. More recently, we've relied entirely on NGS with the HiSeq 2500 System for mouse and rat studies. We use a genotyping by sequencing (GBS) protocol. Similarly, we used to use microarrays to measure gene expression in mice and rats. In the last three or four years, we've transitioned to using NGS to perform RNA sequencing (RNA-Seq) to obtain measures of gene expression in the brain quantitatively. In human genetic studies, we continue to use single nucleotide polymorphism (SNP) arrays for genotyping. We're really excited about the Illumina PsychArray.

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Q: What are the advantages of GBS over genotyping arrays?
AP: GBS is a good fit for us because there aren't any SNP genotyping array products available for mice and rats with coverage for all the applications we're interested in. GBS is well suited for mice because they have a greater density of polymorphisms, with common alleles occurring at 1 per 100 sites. If you randomly sequenced regions of the mouse genome, you're more likely to come across an informative marker than if you did the same thing in humans.

GBS provides an efficient way to capture polymorphic alleles without having to design an array or select a subset of SNPs in advance. The selection of SNPs carries biases that can confound analysis. GBS gives us a relatively unbiased sampling of SNPs that exist within a particular population. Some SNPs are already known. The ones we're discovering *de novo* are either recent mutations or relatively rare in the mouse strains that have been sequenced to date. Working with Illumina, we've modified GBS to allow us to obtain 100,000 or more SNP genotypes per animal at a low cost.

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Q: How are you using the Illumina Infinium® PsychArray BeadChip?
AP: The PsychArray is perfect for our human studies. It was designed through a collaboration between Illumina and leading scientists in the field and includes customized content informative for psychiatric traits. When we're looking at intermediate phenotypes it's not clear *a priori* whether content relevant to schizophrenia, smoking, or drug abuse will be of interest. We'd much rather have a product that has all the SNPs previously implicated in psychiatric and behavioral traits represented. Then we don't have to impute key SNPs.

Q: What have your studies revealed so far?
AP: We've done a number of GWAS in mice and discovered several genes that were not previously suspected of being involved in physiological and behavioral traits. We found the gene *Csmd1* associated with behavioral traits in mice. That gene was subsequently implicated by the Psychiatric GWAS Consortium (PGC) schizophrenia effort as being a schizophrenia risk allele. We're actively working on mechanisms for the mouse behavioral effects of *Csmd1*.

We're also looking at the gene *Cdh13*, which has been linked with subjectively positive effects of amphetamine in our animal and human studies. It's also implicated in other drug abuse and ADHD traits. We're interested in understanding the mechanism of *Cdh13* in mice and rats.

Q: What is the most surprising finding you've seen so far in your GWAS studies?
AP: When we were looking at the subjectively euphoric effects of amphetamine in healthy human subjects, we looked at how the polygenic influence of that trait maps onto different disease traits. We saw there is a common genetic influence on high amphetamine-liking scores and schizophrenia. We were interested in the direction of that association, so we investigated whether people that like amphetamine were more or less prone to schizophrenia. I was absolutely certain that the high scores for liking amphetamine would increase susceptibility to schizophrenia. The data proved otherwise. It demonstrated clearly that liking the subjective effects of amphetamine was somewhat protective against the development of schizophrenia in subjects. That really surprised me.

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Q: How is your research impacting advancements in understanding behavioral disorders?
AP: We've associated the gene *Glo1* with anxiety-like phenotypes in mice. We've shown that the effect of *Glo1* on anxiety-like behaviors in animals is mediated by a previously unknown system that links glucose, or energy, utilization to the inhibitory tone as mediated by GABAA receptors in the brain. *Glo1* encodes the enzyme that eliminates methylglyoxal, which is a GABAA receptor agonist, something that hadn't been recognized previously. It's through the effect of methylglyoxal at GABAA receptors that the anxiety-like behavior is mediated. We are actively working with medicinal chemists to develop novel inhibitors of *Glo1*. We believe this is a completely novel target to treat anxiety disorders and possibly other disorders that are currently treated with GABAA agonists, particularly anxiety and epilepsy, we've already filed a patent along these lines.

That's just one example of the fundamental insights into basic cellular and systems-level biology that we've gained as a direct consequence of our quantitative genetic studies. We've identified novel candidates and been able to understand their underlying mechanisms. On the day that we saw an association between *Glo1* and anxiety-like behavior, it would not have occurred to us that it was mediated by GABAA. It was only through understanding the underlying mechanism that we were led to think about the possible clinical applications of GLO1 inhibitors.

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Q: What are the next steps in your research?
AP: Illumina technologies are enabling much of our work, including expression quantitative trait loci (eQTL) studies using RNA-Seq. We're exploring parent-of-origin effects: alleles whose expression is biased either towards the maternally or paternally derived allele. Parental origin is often overlooked when using standard GWAS models. Because we know the paternity and maternity, and often the genotypes of the parents in our animal studies, we're able to look at that in controlled conditions. Other projects involve epistatic interactions, or gene–gene interactions, which are easier to study in model organisms. We're interested in the gene expression profiles associated with relevant genotypes, which might underlie the differences in phenotype. We're also considering how epigenetics influences some of the traits that we're been studying, and traits that we hope to study in the future.

References

1. Palmer Lab. Behavioral genetics of mice and men. http://palmerlab.org/. Accessed October 2, 2014.
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