Searching for the Epigenetic Contributions of Addiction

Targeted methylation sequencing using the MiSeq® System and Nextera®XT Library Prep Kit is identifying epigenetic signatures that correlate with brain image scans of substance abusers.

Introduction

Addiction is a chronic disorder—a devastating disease that begins with casual substance use, leading to compulsive use despite the often dire personal and societal consequences. Scientists now understand that addiction is a disease that has biological and environmental components, and that there is no single causal factor. Yet they can't explain definitively why one individual can use a particular substance without long-term effects, while another person develops a lifelong addiction.

Scientists are now looking to the epigenome for some answers. In simplest terms, the epigenome lies above the genome itself. It's a network of chemical compounds surrounding DNA that modifies the genome without altering the DNA sequence. These environmentally induced chemical changes have the power to alter how, where, and when specific genes are expressed. Researchers at the University of Colorado at Boulder Center for Health and Addiction: Neuroscience, Genes, and Environment (CUChange) are hoping to understand how addictions to alcohol and cannabis develop. They are investigating the presence of DNA methylation, a type of epigenetic modification that involves the addition of methyl groups to specific nucleotides, in genes associated with substance use. With luck, their research will shed light on how addiction develops and how we can prevent and treat it.

iCommunity spoke with Marilee Morgan, manager of the genetics laboratory at CUChange, about the challenges of studying a condition as complex as addiction. She provided insight into how epigenetic research and next-generation sequencing (NGS) might help scientists understand the origins of addiction, as well as the etiology of other neurodegenerative diseases.

Q: What is the main focus of the CUChange laboratory?
Marilee Morgan (MM): The CUChange lab is the creation of Drs. Kent Hutchison and Angela Bryan. Dr. Hutchison studies alcohol and cannabis addiction. We're working on studies to understand how addiction affects the brain and the role epigenetics plays. Dr. Bryan is studying the effect of exercise on the brain, if there are positive epigenetic alterations associated with exercise, and if those alterations could help people overcome addictions or substance abuse issues. These studies are ongoing so we don't have any hard and fast answers yet. We're optimistic that we'll find some interesting things.

Marilee Morgan, MS is a Sr. Professional Research Assistant at The University of Colorado, Boulder. She has more than 17 years of experience in the field of genetics and genomics research.

Q: How did you find your way into doing NGS and epigenetic work?
MM: I've been working with Dr. Hutchison for several years. He wanted to move into using NGS to perform epigenetic studies because that's where things in this field are headed. If we have the phenotype and can relate it to what's going on at the cellular and genetic level, we'll be able to understand the origin of the disease. So far, we've been really pleased with the results. We've been able to correlate imaging data—what's going on in the brain *in vivo* as a result of drug abuse—with epigenetic changes in gene expression. While those results are preliminary, they are pretty cool.

Epigenetic changes are potentially reversible. By figuring out the epigenetic changes involved and understanding the mechanism behind addiction, we might be able to develop more effective therapies to turn the addiction or substance abuse switch off. It might not even require a pill. It could involve an environmental change or therapy. It's very satisfying

to play a role in these studies, even if it's just a small role, because there's a potential to help people.

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Q: What are some of the biggest challenges in using genetic methods to study a condition as complex as addiction?
MM: We performed a lot of genotyping initially and found nothing. The studies consisted of hundreds of people, but I think you need a lot more participants than that to get a result. The results of our epigenetic investigations have proven to be much more significant. Those studies correlate well to functional magnetic resonance imaging (fMRI) data that we've collected about how the brain is actually behaving in addicts and substance abusers.

Q: How could epigenetic studies offer a deeper understanding of the etiology of addiction?
MM: Epigenetics allows us to look at the environmental impact of drug use. If you are a substance abuser, it's going to affect your DNA and your cells. It's going to affect your brain and body, and how both function. That's why I think we see more correlations in this avenue of study. We are seeing a difference at the epigenetic level in people that drink versus people who don't, and in people who use illegal drugs versus those who do not. We don't have many concrete answers yet, but we are seeing definite correlations between brain function and changes in methylation status in genes of interest, such as dopamine receptor and serotonin-related genes.

Q: What tools did you use for epigenetic analysis before moving to NGS?
MM: I worked with Drs. Hutchison and Bryan when they were at the Mind Research Network in Albuquerque, New Mexico. I ran Illumina HumanMethylation27K and HumanMethylation450K BeadChip arrays and analyzed them on an iScan array scanner. In fact, we identified our target genes using those arrays.

Arrays are great for whole-genome comparisons, but not ideal when you want to identify methylation sites in specific genes. Arrays can identify only a handful of CpG sites—regions of the gene that can be methylated—that might be informative. We needed a platform that enabled us to look at all the CpG sites in a particular gene of interest. That's why we decided to pursue sequencing.

Q: What are the benefits of targeted methylation sequencing?
MM: Sequencing gives us a big-picture view of what's really going on, and lets us figure out what's significant. Our Illumina sales person was a wealth of information. I discussed with her what the lab was interested in doing, and we started looking into the literature and found a paper that described exactly what we wanted to do[1]. We even adopted the library preparation technique they developed using the Nextera XT Library Prep Kit.

What's nice about sequencing is that you can develop the assay for your particular hypothesis and tailor it to the scientific question you are asking. It allows you to focus in on the part of the genome you're interested in and really interrogate that region vigorously. That's really important for us.

Q: What type of sequencing are you using for the different research studies in the CUChange lab?
MM: We design primers for that converted DNA so that we can interrogate regions of interest. It can be difficult, because converted DNA is not normal and primer design is pretty challenging. We're sequencing smaller amplicons across these regions of interest and, using the Nextera XT Library Prep Kit, we are getting some good sequencing results.

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Q: What are some of the attributes you use to assess a good quality sequencing run?
MM: Because this is bisulfite conversion and sequencing, we need to look at conversion efficiency. If the conversion doesn't work well, you aren't going to obtain amplification of the region you want because your primers are designed for converted DNA, not a regular sequence. We have a bioinformatician on our staff who is writing software scripts to help us deal with that.

Another quality control measure is to look for contamination in the samples. We do that by looking at the genotypes. If we have multiple genotypes at one locus, it's likely we have some contamination in our sample.

Finally, we look at the amount of reads we're getting out of our MiSeq System. We know the file sizes, the amount of data that we should get out of the system, and compare that with the actual system output.

Q: What do you think of the MiSeq System performance for your bisulfite sequencing?
MM: The MiSeq System is very user-friendly, that's for sure. We haven't had any problems with the instrument. The assays are great and the technology is awesome.

Because we're performing bisulfite conversion of the DNA, we don't have a converted genome that we can use as a reference. There just aren't any out there. We're providing the FASTQ files from the MiSeq System to the bioinformatician on our staff. Bioinformatics depends on the specific research question you're trying to answer and this is such a new application that there's no commercial software available. We're developing it on the fly. It's cool to be on the cutting edge.

Q: What attributes do you look for in choosing a library preparation kit?
MM: I want it to work! I will say this about the Nextera XT: it's very fast. We can incorporate adapters and primers easily and it only takes about 30 minutes to perform the PCR. It works really well.

Nextera XT library prep is also flexible. I have a run that's going right now where we've added seven different genes as the genome, multiplexing for those seven different regions. We can put many samples or many genes on one particular MiSeq System flow cell.

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Q: What are some of the challenges of working with neural tissue samples?
MM: Brain tissue is always hard to come by. It's very expensive and you don't get a lot sample when you do find it. What's really nice about Nextera XT Library Prep is that you don't need a whole lot of DNA. We're amplifying using PCR upfront, so we just need nanogram amounts of DNA. That's an advantage compared to the sample size required for an array.

Because brain tissue is in short supply, we also use peripheral tissues. Our research has shown that peripheral tissues appear to reference what's going on in the brain. Many of the methylation patterns track across tissue types, although they might not be in the same amounts.

Q: How many samples do you process, on average, per week?
MM: Currently, it takes us about 1–2 weeks to run 96 samples using the index primers. I'm hoping we can ramp that up and double that amount, at least. We have upcoming studies that have nearly 1000 samples. We're looking to get those done as soon as possible.

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Q: How have Nextera XT and MiSeq sequencing helped the CUChange lab achieve its research goals?
MM: What we've done so far are pilot projects, but the results are looking very good. We're getting ready to start processing hundreds of samples across many different studies. As we're submitting grants to move forward, we think this line of research is going to be very fruitful for us.

Q: What other types of substance abuse will you be studying in the future?
MM: We're starting some cannabis studies. We're really interested in the epigenetic response to chronic cannabis use. We'll have a whole new set of genes to look at, such as cytokines. In addition, we'll be performing ELISAs and collecting serum from study participants so we can look at immune response and inflammation before and after cannabis use.

Q: What can epigenetic studies offer that whole-genome approaches cannot?
MM: We're talking about environmental influences on our genome. Everything we do, everything we're exposed to, has the potential to change how genes are expressed, and that can have adverse effects down the road. The impact of epigenetics goes far beyond understanding addiction. There are many neurological and neurodegenerative diseases that might also be epigenetically induced, such as ALS and Alzheimer's disease. We really need to look at the epigenetic underpinnings of these diseases. If epigenetics is involved, we have the opportunity to figure out how to reverse the devastating changes it causes and help many people. That's something I'm very proud to be a part of.

References

1. Masser DR, Berg AS, and Freeman WM. Focused, high accuracy 5-methylcytosine quantitation with base resolution by benchtop next-generation sequencing. *Epigenetics Chromatin* 2013; 6: 33.

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