Scouring The Epigenome For The Origins Of Dementia and Mental Illness

The Infinium® HumanMethylation450 BeadChip gives researchers a peek into epigenetic changes that might contribute to neurodegenerative and cognitive disorders.

Introduction

Neurodegenerative and psychiatric disorders are tricky to study. They can develop at several stages during a person's life and are associated with a range of cognitive and physical symptoms. In Alzheimer's disease (AD), a progressive neurodegenerative disorder, symptoms often begin to appear around age 65 and can be subtle at first. Early stages begin with memory lapses, confusion, mood, and personality changes. As the disease progresses, sufferers are unable to recognize family and friends; experience hallucinations, paranoia, and impulsivity; and eventually lose their ability to communicate and complete daily tasks. AD is estimated to afflict more than 26 million people worldwide[1]. We still don't fully understand its cause and have yet to develop a test to diagnose the disorder definitively while the patient is still alive.

Burgeoning research demonstrates that more than our inherited genes play an important role in dementia and psychiatric disorders. Environmental factors such as cigarette smoke or alcohol exposure also have the potential to alter our DNA and initiate the onset of disease pathology. Scientists at the University of Exeter Medical School and the Institute of Psychiatry, Psychology and Neuroscience (IoPPN) at King's College London are using genomic technologies to peer into the epigenome, the suite of DNA chemical modifications that alter the way certain genes are expressed. The Infinium HumanMethylation450 BeadChip enables them to scan for methylation signatures and other common epigenetic changes that can influence the onset of neurodegenerative and cognitive disorders. Researchers hope these studies will lead them to the mechanisms responsible for disease onset, while offering targets for the development of therapeutics and diagnostics.

iCommunity spoke with Jonathan Mill, PhD, Professor of Epigenetics at the University of Exeter and King's College London, about the challenges and advances in understanding neurodegenerative and cognitive diseases. He and his collaborators use array and next-generation sequencing (NGS) technologies to probe how our genome and epigenome contribute to maladies of the mind.

Q: What motivated you to combine genetic and epigenetic analyses to study complex diseases like AD?
Jon Mill (JM): Cognitive and neurodegenerative disorders are some of the foremost problems in the world, contributing greatly to the global burden of disease. At some point, many of us will be affected by mental illness or dementia, resulting in a growing public health burden as population demographics shift towards older ages. These aren't diseases that can be studied easily, in part because they are difficult to categorically and definitively diagnose. It's not like high blood pressure or obesity where you can measure the trait using a standard blood test or routine observation. What's going on inside the brain is much harder to assess in living people.

Q: What inspired you to study psychiatric genetics?
JM: I'm very interested in understanding the molecular processes in the brain that make us all different, and uncovering the reasons why some people are more susceptible than others to diseases like schizophrenia, autism, and dementia. It's clear that genetic variation and the environment act together; we are not just the result of our genetic code, but also of life experiences and exposures.

I was lucky to join a PhD program at the Institute of Psychiatry at King's College London that tackled mental illness and mental health from an integrated genetic and an environmental angle to explore why some people are more susceptible to diseases and disorders over their life than others. At the time, the focus was on identifying associations between specific genetic risk variants and environmental exposures and disease, but we didn't know anything about the underlying mechanisms. That moved me into thinking about the specific molecular processes involved in mediating these interactions.

Q: Which diseases are the focus of your epigenetic studies?
JM: We focus on a broad range of brain diseases, but we're also interested in understanding the "normal" genomic processes involved in brain function and development. We study diseases of childhood, like autism, ADHD (Attention Deficit Hyperactivity Disorder), and

Jonathan Mill, PhD, is a Professor of Epigenetics at the University of Exeter and King's College London.

childhood psychosis. We are also interested in schizophrenia, bipolar disorder, and neurodegenerative diseases of old age such as AD and other types of dementia.

Q: Why would DNA methylation changes be associated with these diseases?
JM: Certain features of these diseases suggest that factors additional to DNA sequence variation are important in their etiology. In identical twins, for instance, it's sometimes often the case that despite sharing the same genetic code 1 twin will develop a disease like schizophrenia and the other will be unaffected. All the disorders we study are highly heritable, so we know that there is a strong genetic component. However, epidemiological work is now identifying strong environmental risk factors for these diseases too. How these come together at a mechanistic level is not understood. We're looking into epigenetic variation in specific regions of the brain as a possible mechanism.

Q: What do you see when you're studying DNA methylation that you wouldn't see with gene expression or genotyping studies?
JM: We're actually trying to integrate all 3 approaches because they're all intricately related, although the relationships between them are highly complex. We're still not sure how epigenetic variation directly links to gene expression, for example. The traditional dogma has been that elevated DNA methylation leads to gene silencing. While that could be true for classical CpG islands in gene promoters, there are many instances where epigenetic variation doesn't have a direct or predictable effect on gene expression. While DNA methylation might not alter the absolute level of an RNA transcript, for example, it might bring about alternative forms of that transcript by directing alternative splicing or isoform expression. To see the whole picture, it will be important to integrate multiple layers of genomic data.

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Q: What environmental factors cause epigenetic changes in our DNA?
JM: Although this type of work is still in its infancy, there's now clear evidence that you can pick up striking hallmarks of certain environmental exposures, for example smoking, in an epigenetic profile. We're exploring how other lifestyle exposures that might be related to the diseases we study—such as drug and alcohol abuse, and stress—affect the genome.

Q: How did you perform methylation analysis when you first began studying epigenetics in complex diseases?
JM: The field of epigenetic epidemiology has changed dramatically, driven by new technologies. Most of our early work was done on small numbers of samples. We used to do a lot of targeted clonal bisulfite sequencing to study DNA methylation at discrete regions of the genome, which was very low throughput and required intense, time-consuming lab work. We targeted specific regions of candidate genes that we hypothesized might play a role in a disease. The problem was that even within a gene, we didn't necessarily know where to look; it was like looking for a needle in a haystack.

Q: What new methylation analysis technologies have had the greatest affect on your research?
JM: The HumanMethylation450 BeadChip has certainly moved the field of epigenetic epidemiology forward. It's enabled researchers to screen DNA methylation at multiple CpG sites across the genome and driven the adoption of a common strategy. Previously, different labs would use a range of fairly bespoke methods. Now researchers can collaborate, work on much bigger projects, and compare data across studies. Novel sequencing-based approaches give you much higher resolution and coverage, but they're still too expensive for large-scale epidemiological studies. That's where the HumanMethylation450 BeadChip is currently really powerful and important. People can profile large numbers of samples, which is required to give us the power to detect what are often very small effects.

> "[In our AD study] we identified several loci at which altered DNA methylation was associated with AD neuropathology. We were particularly interested in the methylation signature we found in a region of the *ANK1* gene."

Q: What prompted you to examine the methylation state of brain tissues in people with and without AD?
JM: We know that there is a strong inherited genetic component in AD, but we don't know how these genetic factors operate mechanistically, and how they neuropathology across different regions of the brain.

We conducted the study in multiple brain regions from affected and unaffected donors from 3 independent cohorts.[2] One goal of the study was to understand why different regions of the brain experience different levels of neurodegeneration during AD. Some regions, such as the entorhinal cortex, become affected very early on in the disease. Other regions, such as the cerebellum, are relatively protected and show little neuropathology. We were interested in comparing the epigenetic profiles of brain regions that are and aren't affected in the same individual.

We also compared matched blood samples from some of the study participants. There's no definitive diagnosis of AD during life and the early stages of the disease are asymptomatic. One of the goals of our work is to identify blood-based biomarkers that might signal early stages of neurodegeneration or other neurological and neuropsychiatric diseases occurring in the brain.

Q: What methylation signatures did you find and what genes were they in?

JM: We identified several loci at which altered DNA methylation was associated with AD neuropathology. We were particularly interested in the methylation signature we found in a region of the *ANK1* gene. This region was differentially methylated in the cortical regions that are affected in AD such as the entorhinal cortex, superior temporal gyrus, and prefrontal cortex. In regions of the brain not affected in AD, and also blood, we found no differences at this locus. Not much is known about the functions of this gene beyond its role in cell mobility and structure. Of note, genetic variation in *ANK1* has been associated with type 2 diabetes. This is interesting because individuals with type 2 diabetes are at increased risk of developing AD, so this might suggest some common pathway.

We were able to replicate these effects in several independent brain sample cohorts, making this quite a robust finding. To my knowledge, it's one of the first epigenome-wide association studies to identify methylomic variation associated with a complex disorder across disease-relevant tissues in several independent sample cohorts.

This study was only the first step in understanding mechanistically what's going on. We're now studying this region in more depth, looking at the function of *ANK1*, what it controls, and how the epigenetic variation we identified alters gene expression. We're also studying the of the identified DNA methylation changes on regulating the expression of different isoforms or different splicing variants of the gene itself. It's still early days in that regard.

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Q: Were the epigenetic signatures also present in premortem blood samples?

JM: We naively hoped that the epigenetic changes we identified in the brain would be detectable in the blood, and could therefore be used as potential biomarkers of the disease. It turns out that wasn't the case. Many of the changes we saw were specific to the brain regions involved in AD. However, we were able to show that there were epigenetic differences in blood from individuals with the disease compared to those that didn't have the disease. Despite these epigenetic signatures in blood being different to those observed in the brain, they might have some future utility as biomarkers. It's plausible that what we're picking up isn't directly related to the onset of AD. For example, it is likely that there are functional genomic changes occurring in response to neuropathology in the brain or the lifestyle differences caused by the disease. It's something that needs to be studied more.

Q: What do the results of this study mean for AD research in general?

JM: It potentially identifies new mechanistic pathways involved in disease pathology. It might even highlight useful targets for developing novel therapeutic strategies. It could also tell us something about the tissue-specific molecular changes occurring in the context of the disease and how genetic factors are influencing neuropathology, but that's something that we'll need to explore further. Our future studies will focus on purifying specific cell populations from the brain tissue so we can see the changes occurring in specific neuronal cell populations within the cortex.

> "A key trend is integrating epigenetic data with the underlying DNA sequence so that we can look at how genetics, epigenetics, and the environment all come together."

Q: How has the Infinium HumanMethylation450 BeadChip Kit enabled this research?

JM: It has enabled us to perform the study economically and systematically. There was no other affordable way to profile the hundreds of samples we assessed in one experiment. It also enabled us to perform subsequent meta-analyses and comparisons with other groups. Because we were all using a common array platform, it allowed us to combine our data together, highlighting how reproducible these changes are across different studies.

Using the HumanMethylation450 BeadChip has been productive, and the data appear to be reproducible and reliable. In our Alzheimer's work, we've shown that we can confirm the changes we observe using other methods such as bisulfite pyrosequencing. Rather than looking at 5, 10, maybe 100 samples, it's now feasible to perform studies across thousands of samples. This sort of sample size may be required to pick up the often small signals present in the context of disease.

Q: Do you process your arrays in your lab?

JM: We perform all our own processing. We have developed our own pipeline for analyzing and normalizing data, and for quality control. We currently use the HumanMethylation450 BeadChip in our large, epidemiological studies, but are increasingly using sequencing-based approaches as well.

Q: What's the difference between the HumanMethylation450 BeadChip and other array platforms?
JM: The HumanMethylation450 BeadChip is really the only commercial product widely adopted by the community for use in large numbers of samples. Other array platforms are based on more enrichment type approaches where you're using an antibody for methylated DNA or some enzyme digest before array hybridization. In terms of sensitivity, I think they are much less reliable and sensitive.

Q: When do you use sequencing in your studies?
JM: We're increasingly using the HiSeq® System for reduced representation bisulfite sequencing and targeted bisulfite sequencing. We also use it to perform RNA-Seq for gene expression studies to understand the of DNA methylation differences on gene function.

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Q: Are you considering whole-genome methylation sequencing for discovery projects?
JM: The HumanMethylation450 BeadChip targets only a small percentage of the potentially methylated sites in the genome and they're not necessarily distributed in the regions that are biologically relevant. We'd love to do more whole-genome bisulfite sequencing, but it's not economically feasible to use it for hundreds or thousands of samples. We're hoping costs will come down and methods will be developed that enable higher throughput bisulfite sequencing.

Q: How will the NIH Epigenome Roadmap benefit your research?
JM: The NIH Epigenome Roadmap has transformed our understanding about the molecular processes involved in mediating differences between various cell types and tissues. The data from this project have told us about the regions of the genome that could be important for shaping the function and expression of genes. with this added layer of annotation to the genome, we now understand the potential functional consequences of variation in specific regions of the genome. That's essential if you want to perform targeted analysis of certain regions, or if you want to explore what a genetic or epigenetic change at a certain locus means.

Q: What trends in neurodegenerative research are having the greatest on your work?
JM: It's becoming increasingly clear that we need to look beyond DNA methylation and assess the whole suite of epigenetic modifications, including other modifications to DNA, histone modifications, and small noncoding RNA. A key trend is integrating epigenetic data with the underlying DNA sequence so that we can look at how genetics, epigenetics, and the environment all come together to regulate gene function. It will be important to develop tools for single cell genomics, particularly at the level of transcriptomics and epigenomics. We want to look at individual cells at a genomic level to understand what might be going on in very small populations of cells.

References

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2. Lunnon K, Smith R, De Jager PL, et al. Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease. Nat Neurosci. 2014; 19(9): 1164-1170.

Learn more about the Illumina products and systems mentioned in this article:

- Infinium HumanMethylation450 BeadChip, www.illumina.com/products/methylation_450_beadchip_kits.html
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