Epigenetics Studies Uncover Obesity-Driven Methylation Signatures

Researchers use the Infinium® HumanMethylation450 BeadChip to identify cell signaling disruption that potentially contributes to the negative downstream effects of high BMI.

Introduction

As he was finishing his research degree at the University of Leicester, Nilesh Samani made an important decision. While his goal was to become a cardiologist, he had enjoyed conducting research into the molecular biology of hypertension. He decided to chart a new course, combining his clinical practice with research into the genetic underpinnings of cardiovascular disease. Over the next 30 years, he produced groundbreaking research, publishing more than 400 scientific papers including many of the key genome-wide association studies (GWAS) in coronary artery disease.

Professor Samani and his team took this work a step further in 2013, conducting an epigenetic-wide association study (EWAS) to see if DNA methylation changes could be linked to cardiovascular traits. The study leveraged blood and tissue samples from a number of well-known cardiovascular studies, for assessment with the HumanMethylation450 BeadChip. GWAS and EWAS data analysis identified a DNA methylation change in the hypoxia inducible transcription factor (HIF) in patients with high body mass index (BMI).[1]

HIF is best known for its key role in hypoxia-sensing. However, emerging evidence has also demonstrated its involvement in metabolism, specifically the cellular response to glucose and insulin, and as an accelerator of adipocyte differentiation. Previously, the HIF system had been linked with obesity in animal models, but this was the first time that HIF signaling disruption was shown to potentially contribute to the deleterious downstream effects of high BMI in humans.

iCommunity spoke with Professor Samani to learn more about this research and the role EWAS studies will play in his future cardiovascular research.

Nilesh Samani, M.D., is British Heart Foundation Professor of Cardiology and Head of the Department of Cardiovascular Sciences at the University of Leicester, and Director of the NIHR Leicester Biomedical Research Unit in Cardiovascular Disease.

Q: How has your research into the genetics of cardiovascular disease evolved over the years?

Nilesh Samani (NS): I used experimental models in most of my early hypertension research, including research performed with spontaneously hypertensive rats. Once I completed my clinical training, I began focusing on the genetics of hypertension and coronary disease in humans, which both have a strong genetic determination. I started with candidate gene studies and linkage analysis using microsatellite markers, moving into GWAS studies as part of the initial Wellcome Trust Case Control Consortium (WTCCC). I've been privileged to be involved in many of the GWAS studies performed in the cardiovascular disease area.

Q: What caused you to look for a link between epigenetic changes and cardiovascular diseases?

NS: We became interested in epigenetic changes as they could reflect the most proximate change to explain some of the GWAS signals we had identified. Techniques to look systematically for epigenetic changes have trailed behind those for GWAS and gene expression, but the HumanMethylation450 BeadChip has changed the picture.

Our initial hypothesis was that we would find methylation changes linked to genotype. We thought that the process would flow forward, with the genotype causing a methylation change that in turn caused the disease in some way. As it turned out, it is much more complex.

Q: What epigenetic and phenotype associations did you investigate?

NS: We initially focused on BMI as this is an important risk factor for cardiovascular disease. The association between specific epigenetic signals and BMI appeared to be quite

striking in our discovery cohort so we decided to see if we could replicate those signals in other cohorts.

Q: What samples did you use for this study?
NS: The discovery cohort was made up of healthy blood donors and individuals with a history of myocardial infarction that were recruited for the Cardiogenics Consortium. Blood samples from the MARseille Thrombosis Association (MARTHA) and Cooperative Health Research in the Region of Augsburg (KORA) studies made up our primary and secondary replication cohorts, respectively. We also analyzed EWAS in skin and adipose tissues from the Multiple Tissue Human Expression Resource (MuTHER) study.

Q: What did the EWAS results show in these cohorts?
NS: The methylation values of the discovery cohort were adjusted for age, sex, smoking, and case-control status. We identified five probes, three of which were in *HIF3A* on chromosome 19. The three *HIF3A* probes—cg22891070, cg27146050, and cg16672562—reside alongside each other in intron 1 of the gene encoding HIF.

We took the three *HIF3A* probes forward for analysis in the primary replication cohort (MARTHA), and found all three significantly associated with BMI. The same was true in our secondary replication cohort (KORA), although the BMI association was weaker.

Q: What is HIF and what does it regulate?
NS: HIF is an oxygen sensing system consisting of a number of different proteins that mediate the cellular response to oxygen deprivation. Researchers have investigated the role of HIF signaling in ischemia in general. In the past few years, they've begun to study the role HIF plays in cancer, because many cancers are ischemic.

Only recently have we begun to consider that this transcription factor may modulate other genes as well. Its link with obesity may not actually be driven by the hypoxia signal. There are studies that show that knocking out certain HIF signaling proteins can cause changes in metabolic parameters. We're just beginning to understand the multiple roles HIF plays in the body.

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Q: Were you concerned that the epigenetic signature you were seeing might represent variations in the proportion of different blood cells in the whole blood samples you assayed?
NS: Yes, we recognized that the DNA used in our methylation analysis was derived from a mixture of different white blood cell types. Each of those cell types might have slightly different epigenetic levels, causing us to mistake differences in epigenetic signature for disease changes rather than differences in cell type proportion.

To clarify what we were seeing, we decided to determine if there were epigenetic differences between each cell type. We used one of the probes and examined the association of methylation and cell type, adjusting for lymphocyte, monocyte, and neutrophil counts. We found that the association between probe methylation and BMI was not attenuated after this adjustment.

> "In this case, blood DNA acted as a reasonable surrogate for epigenetic changes present in a biologically relevant tissue."

Q: What was the association between the *HIF3A* probes and BMI?
NS: Using the cg22891070 as an exemplar, every 0.1 increase in methylation ß value for the probe was associated with a 3.6% higher BMI in the discovery cohort. The increase in BMI was greater in participants who had a myocardial infarction than in healthy blood donors. We also investigated the association of DNA methylation at *HIF3A* with two components of BMI, height and weight. We found the methylation signature significantly associated with weight, but not with height.

Q: How did the blood sample *HIF3A* methylation signatures compare with those you found in skin and adipose tissue?
NS: We wondered if the epigenetic signature that we saw in blood was indicative of changes in relevant tissues. In the MuTHER cohort, we assessed the methylation level at the three *HIF3A* sites in skin and adipose tissue. We were pleased and surprised to see that the data showed a very specific epigenetic change in adipose tissue that was strongly associated with BMI. We did not see a similar association in skin. In this case, blood DNA acted as a reasonable surrogate for epigenetic changes present in a biologically relevant tissue. The results provide the epigenetic community with a measure of confidence that assessing epigenetic signatures in blood has value.

Q: Is the methylation at *HIF3A* responsible for high BMI?
NS: GWAS signals by definition are causal. One cannot make the same interpretation for EWAS signals as they could be altered by environmental factors. Indeed when we probed into the association of methylation at *HIF3A* with BMI further, we found evidence that indeed it may be BMI that is driving the change in methylation at *HIF3A*. Specifically, we found some methylation QTLs for *HIF3A* (ie genetic variants affecting methylation at this locus) but these did not associate with BMI directly. That suggests that the epigenetic present at the HIF locus represented obesity driving methylation.

Obesity causes all sorts of deleterious things downstream and researchers have long wondered how obesity causes these changes. I think the epigenetic signatures we uncovered may provide important clues in this area. When we started this study, we hadn't appreciated that it could provide us with important biological information about the downstream mechanisms of obesity.

Q: Do your research results suggest a new approach to reducing the health impact of obesity?
NS: Preventing obesity is the focus of most therapeutic approaches. There's been much less emphasis on the downstream impact of obesity, including its links with cancer and cardiovascular disease. There are few studies focused on the biology behind why obesity increases the risk of these diseases.

What our study highlights is that we may have the opportunity to influence the relationship between obesity and downstream diseases. Methylation signatures in *HIF3A* may act as one mediator of that link, providing us with therapeutic targets.

Q: Why did you choose the HumanMethylation450 BeadChip for this study?
NS: I think the HumanMethylation450 array is ideal for EWAS studies. It is the best and most comprehensive array out there.

Q: How do you foresee EWAS studies contributing to our understanding of complex disease?
NS: One of the things that our paper shows is that the interpretation of EWAS studies is going to be much more complicated. Apart from issues of tissue and cellular specificity, the main challenge is to determine directionality. With GWAS, the findings indicate causality: this gene caused this effect. EWAS results can be bidirectional, with epigenetics being either the cause or the consequence. We need to be aware of that. Provided the findings are interpreted carefully, they can nonetheless inform biology.

I think over the next two to three years we're going to see a fairly large increase in EWAS studies and more definitive EWAS studies being published. I think we'll see EWAS studies evolving, maybe not quite to the scale of GWAS studies, but becoming substantially larger. I suspect that the sort of epigenetic changes we'll be looking for in EWAS studies are not going to require 100,000 sample cohorts to generate data. Data analysis will be more complicated because of the complexity of merging EWAS data sets due to cell type, etc. I think EWAS is going to require a higher degree of sophistication that hasn't been necessary for GWAS studies.

> "Interpretation of EWAS studies is going to be much more complicated than GWAS studies."

Q: Do you see array-based methods like the HumanMethylation450 BeadChip and sequencing complementing each other moving forward?
NS: I think we will see methylation array and sequencing used in tandem, but it may require new sequencing approaches to be developed. We saw half a dozen or more epigenetic signals linked with BMI in our study. To study those signals, we'd need a sequencing approach that doesn't differentially amplify methylated or non-methylated alleles.

Q: What are the next steps in your research?
NS: The *HIF3A* study was a valuable one and I think it highlighted the opportunity to use the HumanMethylation450 array and EWAS approach to pursue the role of epigenetics in cardiovascular disease. We'll continue to use GWAS and EWAS to pursue our study of coronary and other cardiovascular diseases.

References

1. Dick KJ, Nelson CP, Tsaprouni L, et al. DNA Methylation and Body-Mass Index: a Genome-Wide Analysis. *Lancet* 2014: 383: 1990-1998.

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