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DNA Methylation and Human Heart Failure: Mechanisms or Prognostics

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Heart failure is a growing public health concern, affecting 20% of persons at some point in their lifetime and contributing to 11% of deaths, with an incidence that is expected to rise by 25% over the next 15 years.[1] As a condition that can develop asymptomatically for years—after which the efficacies of most interventions are modest and palliative—heart failure is a prime target for the development of novel biomarkers that could be used in a clinical setting for stratification or as prognostic markers detectable prior to clinical presentation (the ultimate prize).

Are the tools of high-throughput biology, so-called "'omics" investigations, up to the task? Discovery technologies and their analytical platforms, especially for genomics, epigenomics and transcriptomics, are comprehensive and, if not fully mature, rapidly approaching that point, while other 'omics techniques like proteomics, metabolomics and lipidomics are increasingly quantitative, reproducible and amenable to application outside of specialized laboratories. These approaches have been widely applied to lower organisms and, at an increasing rate, the clinical setting. Get on with it then, one might argue, and use these methods to improve human cardiovascular health on a population scale.

If only it were so easy. Genetic variability is a massive confounder in the study of common disease in large human populations: many of the most successful and largest cohort studies are characterized by modest ethnic and genetic diversity. Syndromes like heart failure are polygenic and GWAS has identified only a few causal variants, each of which explains only a small fraction of the genetic basis of the syndrome. Add to this the contribution of

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environmental factors and it is easy to see why current efforts to bring discovery technologies into the clinic have had limited success in heart failure.

It might seem counterintuitive that one possible way forward is to add another layer of complexity in the form of epigenetic variability—that is, modifications to the genome that are persistent and maintained through cell division but do not involve sequence changes—notably DNA methylation. Although stable, DNA methylation is not immutable: environmental factors can change methylation patterns, making correlation with phenotype a moving target, but also meaning that DNA methylation is an appealing molecular integrator of genetics and exposure.

The paper[2] by Meder *et al.* in this issue combined multiple 'omics techniques to investigate human dilated cardiomyopathy (DCM), defined in this study by the exclusion of coronary artery disease by angiography. An epigenomics investigation of a common disease with known risk factors can proceed either by identifying new risk factors or by investigating how the epigenome contributes to known risk factors. Meder *et al.* take the latter approach: as shown in Figure 1 of their paper, RNA-seq and DNA methylation (via Illumina 450k methylation chip, allowing comparison to preceding human studies) were performed on cardiac biopsies from DCM patients (experimental) and heart transplant recipients (control), allowing the investigators to determine: (i) global changes in gene expression, (ii) global changes in DNA methylation and (iii) the relationship between these two biological layers and phenotype using so called epigenome wide association studies (EWAS) to identify DNA methylation events which associate with disease status and then (iv) link these events to changes in gene expression. Previous studies had identified epigenomic risk loci[3] and global changes in methylation with DCM,[4] but this investigation is the first to couple DNA methylation with gene expression in humans in a sizable cohort of heart failure patients.

Biopsying the heart is not a reasonable way of surveying for biomarkers in otherwise healthy people. Thus, the investigators carried out transcriptome, methylome and EWAS in the blood from DCM and control patients. Furthermore, the studies were repeated in multiple replication cohorts, increasing the likelihood of relevance in larger populations and diminishing concerns of bias due to batch effects, sample collection (e.g. biopsies in live patients versus road accident victims devoid of cardiovascular disease) and over-fitting of models in data analysis. One last methodological note: whole genome sequencing was used to rule out sequence variation as the cause of epigenetic changes, an important step towards definitive demonstration of links between DNA methylation and complex phenotypes.

The paper identifies 59 differentially methylated CpGs between control and DCM hearts, 3 of which reached epigenome-wide significance. Obvious patterns of DNA methylation difference across the genome could distinguish the epigenomes of control from DCM patients, and bioinformatic analyses of differentially modified regions showed enrichment for modification around binding sites for transcription factors involved in cardiac phenotypes. The authors next examined the relationship between epigenotype and mRNA expression by methylation-expression quantitative trait locus analysis, or met_eQTL. Examining all CpGs within 10kb of each gene is a tradeoff which maximizes their ability to recover significant associations, but precludes exploration of the effects of DNA methylation

on gene expression beyond 10kb of the gene body and limits the ability to correct for inherent biases in the data such as population substructure. Several methylation sites exhibited strong effects on gene expression, although the mechanisms for how this regulation occurs remain unknown (Regulating protein binding or chromatin structure?). It also remains unclear if altered DNA methylation is a driving factor in disease onset and/or progression or instead merely an indicator, in a genome-wide sense, of specific forms of transcriptional reprogramming (*nota bene*: this is hardly a criticism of the findings presented by Meder *et al.*—rather it is a statement highlighting what remains, to us at least, an interesting question in the field of DNA methylation). The answer to this question may not matter if DNA methylation can be proven to be clinically actionable, which remains on the horizon at least in cardiovascular disease, although DNA methylation is already used clinically for cancer diagnosis.

One of the obvious goals of such studies is to identify epigenomic risk factors for heart failure, which requires a consideration of study power. Tsai and Bell[5] recently used simulated EWAS data to estimate power calculations for a number of different effect sizes and significance thresholds, coming to the conclusion that a single-cohort study would require 211 cases and 211 controls to obtain 80% power of seeing an effect size of 7% at a genome-wide significance level. Multi-cohort studies such as Meder *et al.* are able to achieve similar levels of significance with fewer individuals, having roughly the same power using only 41 cases and 31 controls in their first layer, when compared to the hypothetical single-cohort study. Despite this, both studies would have only a 1% likelihood to pick up an effect size of 5%. Using the same methodology as Meder *et al.*, a 100 case/100 control discovery cohort would give 100% power at a 5% effect size. An alternative solution is to improve data sharing among the community, especially of control samples, as 50 cases and 200 controls has equivalent power to the balanced 100/100 discovery cohort.

Meder *et al.* look towards a diagnostic future in their study, examining the methylomes of peripheral blood cells in addition to cardiac cells. They identify 217 methylation sites whose altered methylation between control and DCM patients are conserved between these two tissues, including sites located near the disease-associated genes *NPPA* and *NPPB* (whose protein forms are clinical biomarkers). Using these sites, the investigators were able to demonstrate that methylation in peripheral blood may have the potential to act as a diagnostic biomarker of DCM without the need to access the heart itself. Strikingly, these methylation marks hold across blood and heart cells from transplant patients, which is intriguing given that these cells have distinct genomes, and the heart had a different epigenome when it was transplanted.

Important challenges remain. The heart failure-associated methylomes revealed in this paper will need to be rigorously examined across genetically distinct human cohorts. Other factors such as patient medications, co-morbidities and differences in blood cell composition (including inflammation, which correlates with DNA methylation in the setting of heart disease[6]) will have to be understood to enable clinical application. Meder *et al.* and many human studies make use of the Illumina 450k methylation chip, which queries a fixed set of methylation sites across the genome, whereas other investigators have examined animal models (where DNA methylation has been shown to correlate with complex phenotypes

independent of genetics[7]) and humans with the non-biased approaches of whole genome bisulfite sequencing or its cheaper alternative, reduced representational bisulfite sequencing.

It is increasingly apparent that epigenomic diagnosis of complex diseases in humans may be possible. The next step, then, is prognosis, or the identification of epigenomic risk factors and their implementation to stratify and manage patients longitudinally. Initial studies in animal models of heart failure[8] and human studies of all cause mortality[9] suggest this is not unrealistic. Discovery of basic principles in human populations is exceedingly difficult given the inability to control for the myriad factors that can influence ‘omics measurements. If prognosis is the goal, combining longitudinal ‘omics data from individual patients with deep phenotyping in the electronic medical record and sharing of this data across large human cohorts is a realistic path toward clinical implementation.

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Abbreviations

DCM	dilated cardiomyopathy
CpG	cytosine-phosphoguanine
GWAS	genome-wide association analysis
EWAS	epigenome-wide association analysis

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