HiChIP Assay Links Novel Genetic Markers to Heart Failure

Making Disease Genomics Tractable

Wilson Tan, Chukwuemeka Anene-Nzelu, and colleagues at the Cardiovascular Research Institute in Singapore recently used HiChIP to make significant advances in interpreting the genetics of heart failure.[1] Though heart failure prevalence is high and increasing,[2] knowledge of its genetic and molecular mechanism has remained elusive.

Elucidating molecular mechanisms of disease etiology, in general, faces two imposing challenges. First, traditional genome-wide association studies (GWAS) often require enormous sample sizes as many genes contribute small individual effects.[3] Second, when such studies do identify areas of interest, many of them occur in non-coding DNA, obscuring their role in disease etiology.[4]

Non-coding regions are often assumed to regulate linearly proximal genes. However, endogenously, DNA is organized within the nucleus into a dynamic three-dimensional chromosomal structure. Chromosomal looping can place linearly distal sequences near each other in three-dimensional space, allowing for regulation across quite extensive linear distances.[5]

By analyzing chromosomal structure and its effects on gene expression, HiChIP offers a solution to the challenges of disease genomics. The procedure combines high-throughput chromosome conformation capture (Hi-C) with chromatin immunoprecipitation (ChIP). Hi-C identifies genomic sequences in three-dimensional proximity, allowing researchers to physically link target genes to alleles of interest, such as GWAS loci. ChIP allows researchers to prioritize genetic variants by targeting sequences associated with active chromatin, such as H3K27 acetylation (H3K27ac). Active chromatin marks regulatory sequences and is often involved in gene regulatory interactions and chromosomal loops.[6,7] By combining these data in one workflow, HiChIP can reveal causal relationships between disease-associated alleles and their regulatory effects on target genes.

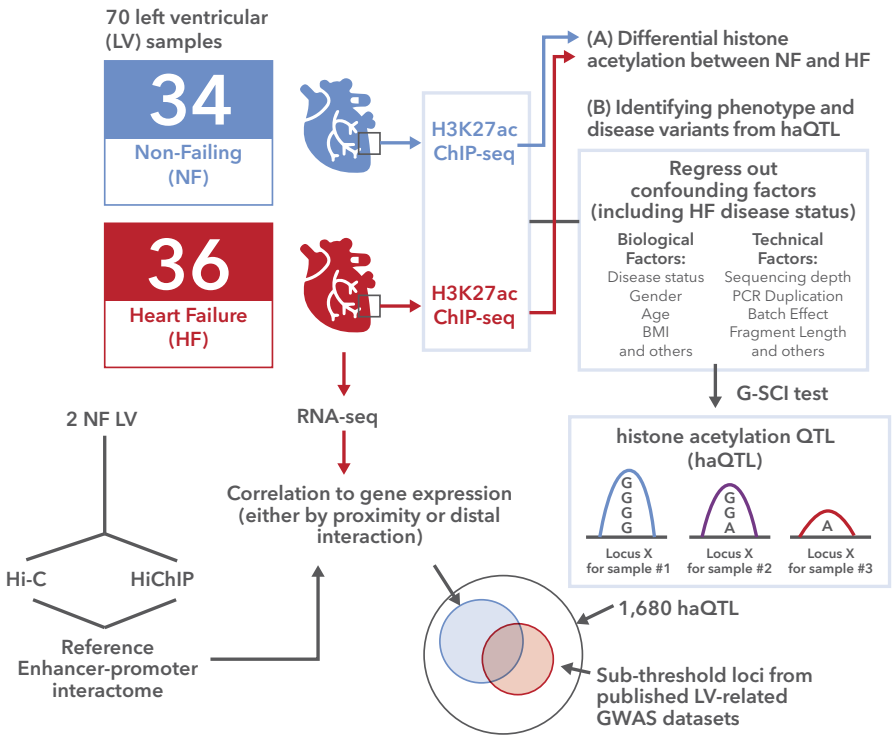

Figure 1. Researchers at the Genome Institute of Singapore used control and diseased human hearts to profile enhancers and long-range chromatin interactions to map the heart epigenome. They associated 70 unique loci with heart disease and heart failure phenotypes by capturing non-coding genetic variants in enhancer elements using H3K27Ac ChIP-seq and disease-associated genes linked to these enhancers using the Arima-HiC kit.

Characterizing Novel Disease-Associated Variants

Histone-acetylation quantitative trait loci (haQTL) reveal variants in histone modification associated with phenotypes at a one-dimensional level, and ChIP-seq reveals disease-relevant variants by associating them with open chromatin. Tan et al. conducted ChIP-seq on both healthy and end-stage failing heart tissue to identify variants and changes in active chromatin that may be associated with disease etiology. The team identified 1,500 haQTL between the disease and control cohort. 161 of these loci were associated with differential expression at either neighboring or distal genes. Furthermore, the team identified 59 new candidate genetic markers. Finally, their data also suggested the function of these alleles: shaping gene expression via effects on H3K27ac chromatin modifications at regulatory elements.

HiChIP is necessary to point to areas of the genome that may be interacting with and regulating the disease-associated variants. HiChIP on control samples revealed 14,500 chromatin loops associated with H3K27ac. The multiomic data set allowed the team to thoroughly characterize these chromatin structures, leading to 613 loops anchored by an haQTL. Fifteen of these haQTLs corresponded to distally regulated loci identified in earlier analysis, and 24 were novel loci with target genes more than 100 kb away. This provided new details about the regulatory mechanisms acting within heart tissue.

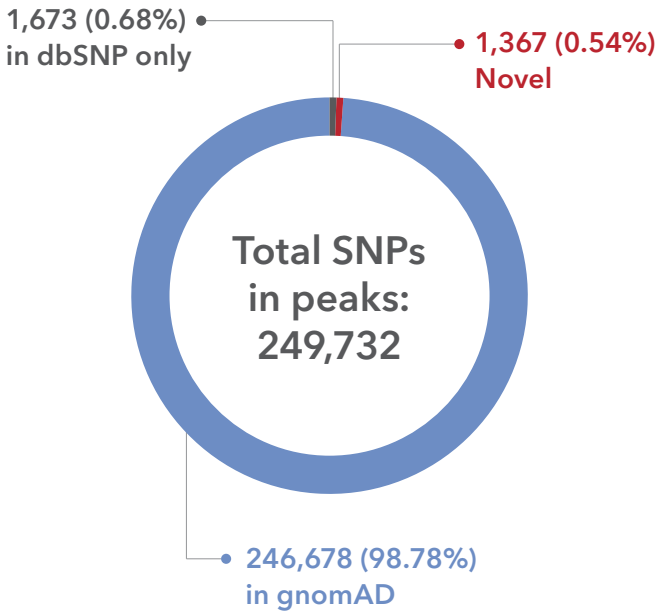

Figure 2. Histone acetylation quantitative trait loci (haQTLs). Pie chart showing 249,732 SNPs underlying all 47,321 H3K27ac loci.

Identifying an Additional Mechanism of Disrupted Gene Regulation

Transcription factors (TF) bind to specific sequences within enhancer and other DNA regulatory regions, known as binding motifs, to either facilitate or impede transcription.[8] Genetic variants within TF binding motifs can affect TF binding, chromatin structure, and gene expression.[9-11]

The researchers identified 963 loci gain or loss of TF motifs. Observed changes to motifs within haQTLs altered H3K27ac, and this effect correlated with gene expression changes. These data create a compelling case that altered TF binding within haQTLs mediates effects of some disease-associated alleles on histone acetylation and gene expression.

The researchers utilized HiChIP's unique features to extensively examine the interplay between chromatin structure and gene regulation at the loci containing genetic variants associated with heart disease. Chromatin is organized into self-interacting regions known as topologically associated domains (TADs).[12] Disruption of these TAD structures and their effects on gene regulation have been implicated in developmental diseases, cancer, and heart disease.[5,13] H3K27ac serves as a ideal target for HiChIP analysis of enhancer-promoter interaction within TADs since H3K27ac is enriched at regions that are associated with chromatin loops.[5,14] By examining the 3D genome landscape in healthy tissue, the researchers were able to examine potential linkages between genetic/epigenetic loci with affected gene expression. Confirmatory experiments in a mouse model were able to provide additional evidence of functional connections.

Providing the What and the How in Disease Research

Tan et al. were able to identify putative genetic markers for heart failure and characterize their regulatory function in one study. They made a strong case for connecting genetic variants, the status of active chromatin, and functionally impacted genes not only in animal models but in human heart tissue as well. HiChIP was uniquely equipped to provide insight into the promoter/enhancer, enhancer/enhancer, and promoter/promoter three-dimensional connections that can have the potential to contribute to disease etiology.

Researchers have struggled to identify what genes are associated with disease while also describing how those genes might impact disease progression in a single study. In the past, technological limits required that Hi-C and ChIP-seq be performed on separate samples in separate workflows. This was expensive and time-consuming. Furthermore, only correlative connections between the data could be examined. Arima Genomic's HiChIP combines these methods in a single, multi-omic workflow, which supports causal investigation of disease-associated alleles and their regulatory roles and gene targets, taking less time and significantly lowering cost.

HiChIP enables important advancement in understanding genetic and molecular bases for disease. Such advances will be foundational to the next steps in disease research and critical in the development of novel therapeutics.

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