Atrial fibrillation is a common cardiac arrhythmia displaying a large heritable component, with twin studies estimating the genetic heritability at ≈60%. Several genome-wide association studies have been performed to identify common DNA variation associated with risk for disease and have to date identified 23 genomic loci with genome-wide significance. Each of these loci contains several common single-nucleotide polymorphisms (SNPs) that are significantly associated with disease risk, lie almost exclusively in nonprotein-coding regions of the human genome, and whose potential effects on surrounding protein-coding genes are largely unknown. The biggest challenge in postgenomic studies therefore lies in the functional annotation of those SNPs and the identification of the downstream target genes.

See Article by Tucker and Dolmatova et al

In this issue of Circulation: Cardiovascular Genetics, Tucker et al elegantly link noncoding variation via enhancer function with a disease phenotype (Figure) at the Paired Related Homeobox 1 (PRRX1) locus. PRRX1 is a mesoderm-specific homeobox transcription factor that upregulates genes, such as muscle creatine kinase. First, the authors used fine-mapping of the PRRX1 genome-wide association study locus to identify the variant(s) most strongly associated with the phenotype. Using publicly available information on mammalian sequence conservation, chromatin accessibility (DNase hypersensitivity) in human cardiac fibroblasts or myocytes, and enhancer (H3K4me1) signals in skeletal myoblasts or neonatal fibroblasts from the Encyclopedia of DNA Elements (ENCODE) project, they then selected 7 putative enhancer regions for functional analysis. They performed in vivo enhanced green fluorescent protein reporter assays in zebrafish that indicated 2 regions upstream of the gene promoter as transcriptional enhancers in cardiac and skeletal muscle tissues. Interestingly, the intronic region harboring the genome-wide association study lead SNP (rs520525) and the fine-mapped SNP (rs10919449) did not display enhancer activity in this model.

To test the effect of variation on the functionality of the 2 identified enhancer regions, the authors performed luciferase reporter assays testing the alleles of 21 common SNPs. This indicated a single functional variant (rs577676), with the risk allele decreasing enhancer activity in agreement with the directionality of an expression quantitative trait locus (genotype–gene expression association) for PRRX1 in human left atrial tissue. Use of chromosome conformation capture showed increased interaction between the upstream enhancers and the PRRX1 promoter in human cardiac fibroblasts compared with embryonic stem cells, proposing that the regulatory element and the functional SNP regulate expression of the PRRX1 gene.

Finally, to elucidate the role of the candidate risk gene in atrial fibrillation, the authors showed that loss of PRRX1 led to a shortening in the action potential without affecting the transcript levels of major ion channels in human embryonic stem cell–derived cardiomyocytes. In parallel, they knocked down prrx1a expression in zebrafish and observed shortening of the atrial action potential.

In summary, this study demonstrates how noncoding variation can affect phenotypes and provides compelling evidence for a role of the PRRX1 gene in cardiomyocyte function—and, by extension, a role in atrial fibrillation, although the latter could not be definitively modeled in human embryonic stem cell–derived cardiomyocytes or zebrafish. The complete body of evidence suggests that there might be 1 independent regulatory unit in the PRRX1 locus, with the here-described upstream enhancer and an intronic region harboring the genome-wide association study lead SNP and the fine-mapped SNP. The latter region did not display enhancer activity in the zebrafish model, perhaps indicating an independent, different functional effect on PRRX1.

Moving forward, productive approaches are needed to identify the causal variants within a genomic locus in a comprehensive manner. This matters greatly because the knowledge of functional SNPs—through the identification of transcription factor binding sites—can suggest upstream signaling pathways involved in disease. The task remains challenging, particularly because the lack of conservation of a substantial fraction of regulatory elements across species limits the informativeness of model organisms for functional screens. However, in vitro cultured human cells often lack the functional characteristics and organ-specific context needed to fully model a disease state. Until the emergence of better models, an integration of both in vitro human cell experiments and in vivo enhancer data may be the best strategy as nicely demonstrated by Tucker et al in their work.

Disclosures

None.
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Figure. Study design for the functional annotation of the PRRX1 locus associated with risk for atrial fibrillation. GWAS indicates genome-wide association study.