Regulatory Elements in Noncoding DNA in the Chromosome 9p21 Locus

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How Was the Hypothesis Tested?

The authors integrated multiple data sets to identify potential transcriptional regulatory elements in the 9p21 CAD locus. They searched in various types of human cells for chromatin modification “marks” of 3 types—promoters marked by enrichment of histone H3 trimethylated at lysine 4 (H3K4me3); insulators marked by CTCF-binding sites; and enhancers marked by enrichment of histone H3 monomethylated at lysine 4 (H3K4me1), or p300- and MED1-binding sites, or presence of DNase hypersensitivity sites. They found that the locus is particularly rich in predicted enhancers, with a total of 9; many of these enhancers were confirmed to cause transcriptional activation in luciferase reporter assays. The authors sequenced the entirety of the 9p21 CAD locus in 50 individuals of European descent to identify all common DNA variants present in the locus and determine the linkage disequilibrium among the variants. Of several hundred DNA variants, 41 were found to be in perfect linkage disequilibrium with CAD-associated single-nucleotide polymorphisms (SNPs) identified in genome-wide association studies. Of these 41 DNA variants, 33 were located in the 9 predicted enhancers in the locus and were therefore thought to be most likely to have functional effects. The authors then used computational algorithms to predict which of the 33 DNA variants alter consensus transcription factor–binding sites. On identifying candidate functional DNA variants, the authors chose to exhaustively study 2 of the variants—2 SNPs that lie within the same transcription factor-binding site. They examined whether the transcription factor predicted to interact with this binding site differentially bound to versions of the site with either the CAD-risk haplotype or the non–CAD-risk haplotype by using chromatin immunoprecipitation in cells with the different haplotypes. They also used short interfering RNAs targeting the transcription factor gene to assess whether the factor differentially altered the expression of the nearest RNA transcripts in cells with the different haplotypes, with the goal of connecting the transcription factor to gene expression through the binding site altered by the SNPs. Finally, they used chromatin conformation capture (3C) combined with DNA capture and high-throughput sequencing to identify the sites on chromosome 9p21 with which the transcription factor–binding site engaged in long-range interactions in the presence of the transcription factor. To complement this approach, they used fluorescence in situ hybridization to confirm that the transcription factor–binding site and long-range interacting sites colocalized in cells more often in the presence than in the absence of the transcription factor.

Principal Findings

Of the 9 predicted enhancers harboring DNA variants in perfect linkage disequilibrium with index SNPs from genome-wide association studies of CAD, the 9th enhancer (termed ECAD9) harbored the plurality of DNA variants, 11 of 33. Two of these DNA variants, the SNPs rs10811656 and rs10757278, lie 4 nucleotides apart in a consensus transcription factor–binding site for the STAT1 protein, which is induced by a variety of interferons, including interferon-γ (IFN-γ). The haplotype with the alleles of the SNPs that disrupt the predicted STAT1-binding site is associated with CAD risk, whereas the haplotype that preserves the STAT1-binding site is protective against CAD risk. Chromatin immunoprecipitation experiments in cells homozygous for the nonrisk haplotype showed STAT1 binding at ECAD9,
whereas STAT1 did not bind in cells homozygous for the risk haplotype.

Consistent with the idea that STAT1 modulates RNA transcription through the ECAD9 site, treatment of heterozygous cells (HeLa and HUVEC cells) with IFN-\(\gamma\) repressed the expression of CDKN2B, the nearest gene, and induced the expression of CDKN2BAS (also known as ANRIL), a long noncoding RNA of unclear function partly contained in the 9p21 CAD locus. In a different cell type (lymphoblastoid cell lines), knockdown of the STAT1 gene with short interfering RNAs robustly increased CDKN2BAS expression in cells homozygous for the nonrisk haplotype (ie, with intact STAT1-binding sites) but not in cells homozygous for the risk haplotype (ie, with disrupted STAT1-binding sites). With 3C and fluorescence in situ hybridization, ECAD9 was shown to physically interact and colocalize with a variety of sites in chromosome 9p21 genes as far away as 946 kb, including CDKN2A/B, MTAP, and IFNA21. These long-range interactions were strengthened when cells were treated with IFN-\(\gamma\), suggesting that STAT1 may be driving the interactions.

**Implications**

This study is noteworthy in that it successfully demonstrates an approach that can be used to identify transcriptional regulatory elements in noncoding regions of the genome. This will undoubtedly prove to be useful in interrogating the many noncoding regions that have been implicated by genome-wide association studies of various clinical traits and diseases. Indeed, the authors provide compelling evidence that STAT1 can alter gene expression at a distance through a regulatory element in the chromosome 9p21 CAD locus and that CAD-associated DNA variants in the regulatory element modify its activity. However, the study stops well short of demonstrating that these DNA variants are causal for CAD or even that IFN-\(\gamma\)-induced expression of STAT1 influences the pathogenesis of CAD, attractive as the notion might be that this pathway provides a link between inflammation and heart disease. It is plausible that DNA variants in 1 or more of the many other potential 9p21 transcription factor–binding sites identified in this study are more directly related to the disease process. Future work on the locus will undoubtedly investigate the rich variety of transcriptional regulatory elements found therein and seek to connect DNA variation to atherosclerosis in cell-based or animal models.

**Disclosures**

None.

**Reference**

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