Enduring Mystery of the Chromosome 9p21.3 Locus

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Study Hypothesis

Out of the long list of genetic loci that have been discovered by genome-wide association studies to be associated with a variety of clinical traits and diseases, one of the earliest such discoveries remains one of the most tantalizing. In 2007, 3 groups independently reported a locus on chromosome 9p21.3 to be associated with coronary artery disease (CAD), a finding that has been replicated by dozens of subsequent studies and seems to represent the single most important population-wide genetic risk factor for CAD. Especially noteworthy is that the locus has not been linked to any of the traditional CAD-risk factors, suggesting that the locus represents a novel pathogenic mechanism that could potentially be exploited to develop an entirely new class of therapeutics.

A study by Harismendy et al1 attempted to address this putative mechanism using a combination of genetic and epigenetic datasets to identify potential transcriptional regulatory elements in the locus, focusing on 1 element in which 2 linked index SNPs were predicted to create (non-CAD-risk alleles) versus disrupt (CAD-risk alleles) a canonical STAT1 transcription factor binding site. Experiments in cultured HeLa cells, human umbilical vein endothelial cells (HUVECs), and 4 human lymphoblastoid cell lines (LCLs) with differing genotypes at the SNPs suggested that interferon-γ (IFN-γ) induces STAT1 activity that, depending on genotype, acts via the binding site to increase CDKN2BAS mRNA expression and reduce CDKN2B gene expression. The investigators then used chromatin conformation capture (3C) and other techniques to detect long-range interactions between the putative STAT1 binding site and the regions of the CDKN2A, CDKN2B, MTAP, and IFNA21 genes (the last being almost 1 Mb away). Although they did not provide any additional supporting evidence, the investigators speculated that the 9p21.3 locus influences CAD risk by modulating inflammatory signaling through the STAT1 site and thereby affecting the proliferation of tissues involved in atherosclerosis.

Almontashiri et al2 have now performed an important follow-up study that attempts to validate the hypothesis that IFN-γ influences the expression of CDKN2B via the chromosome 9p21.3 STAT1 binding site.

How Was the Hypothesis Tested?

Almontashiri et al2 used a much more diverse collection of cell lines than in the original study of Harismendy et al1, performing experiments with HeLa cells, HUVECs (albeit a different line than the HUVECs used by Harismendy et al1), aortic smooth muscle cells, HEK293 cells, and 16 human LCLs of varied genotype at one of the index SNPs, rs10757278 (6 LCLs homozygous for the CAD-risk allele, 5 LCLs heterozygous at the single-nucleotide polymorphism, and 5 LCLs homozygous for the non-CAD-risk allele). They treated each of the cell lines with and without IFN-γ, using experimental conditions closely matching those used by Harismendy et al1, and assessed p16, p15, and phosphorylated (activated) STAT1 protein levels. In a subset of the cell lines, they also measured CDKN2A and CDKN2B levels by Northern blot analysis.

Principal Findings

The results obtained by Almontashiri et al2 starkly contrast with those of Harismendy et al1. With the LCLs, they found that IFN-γ invariably increased not only phosphorylated STAT1 levels, as expected, but also p16 and p15 levels regardless of rs10757278 genotype, with similar degrees of induction seen in each genotype group (≈5-fold increase in p15, ≈20-fold increase in p16). Similarly, induction of phosphorylated STAT1, p16, and p15 levels by IFN-γ was observed in HeLa cells, aortic smooth muscle cells, and HEK293 cells. They observed an increase in phosphorylated STAT1 but no changes in p16 and p15 levels in HUVECs—despite these cells being homozygous for the non-CAD-risk allele of rs10757278, and therefore predicted to have intact STAT1 binding sites on both chromosomes 9 and to be the most responsive to the action of IFN-γ/STAT1. Northern blot analysis showed that CDKN2B mRNA levels were unchanged in HUVECs and slightly increased in HeLa cells. The finding in HeLa cells is noteworthy because the same cell line was used by Harismendy et al1, who found that the CDKN2B mRNA level as measured by quantitative reverse transcription polymerase chain reaction was decreased with IFN-γ treatment.

Implications

One limitation of the study of Almontashiri et al2 is that they did not assess the expression of the CDKN2BAS noncoding...
RNA with which Harismendy et al observed some of their more impressive changes in response to IFN-γ treatment. Another caveat is that the data in the study of Almontashiri et al, for the most part, are not directly comparable with the data in the study of Harismendy et al because the former focused on protein levels, the latter on mRNA levels. However, changes in mRNA levels may be misleading as those changes may not be translated to protein levels; protein levels are more likely to reflect bona fide functional consequences. Where both the studies examined mRNA expression, Almontashiri et al used Northern blot analysis and Harismendy et al used quantitative reverse transcription polymerase chain reaction; Northern blot analysis remains the gold standard. Thus, the findings of Almontashiri et al call into question the conclusion of Harismendy et al that IFN-γ stimulation and STAT1 binding have important roles in regulating the expression of CDKN2B and CDKN2BAS and that inflammatory signaling influences CAD via the STAT1 binding site.

Further studies to assess the relevance of the STAT1 binding site to gene expression and, ultimately, CAD are clearly warranted. In the meantime, it would seem that 6 years after its reported discovery the chromosome 9p21.3 locus continues to be a mystery, and definitive studies explaining the mechanistic role of the locus in CAD remain to be done.

**Disclosures**

The author is a member of the Early Career Committee of the American Heart Association Functional Genomics and Translational Biology Council.

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