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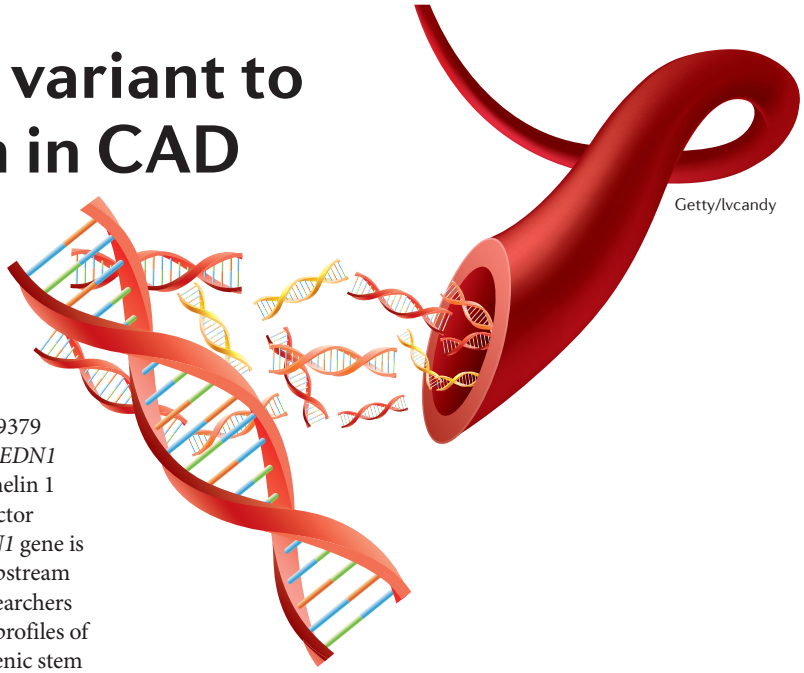
GENETICS

From non-coding risk variant to biological mechanism in CAD



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Analyses of the genetic architecture of complex diseases such as coronary artery disease (CAD) have revealed that most genetic variants are located in non-coding regions of the genome. As regulatory variants can act over long genomic distances, the causal gene and specific mechanism through which a variant affects disease risk often remain elusive. Gupta *et al.* now combine genetic fine mapping, epigenomic profiling and CRISPR–Cas9 genome editing to determine the effect of a common non-coding variant in the 6p24 locus on gene expression and the risk of five vascular diseases.

Several genome-wide association studies for vascular traits have identified an association between disease risk and the single nucleotide polymorphism (SNP) rs9349379, a common SNP in the third intron of the *PHACTR1* gene at the 6p24 locus. The minor allele of this variant (G allele) is associated with an increased risk of CAD and coronary calcification, as well as a decreased risk of migraine headache, cervical artery dissection, fibromuscular dysplasia, and hypertension. Now, a phenome-wide association study for rs9349379 using UK Biobank data from 112,338 individuals of European ancestry confirmed the published associations for CAD and migraine headache.

Epigenomic profiling revealed the presence of histone 3 Lys27 acetylation (H3K27ac), a histone mark associated with enhancer activity, in aortic artery samples, whereas no histone marks were detected in data sets from other tissue types. This finding suggests that rs9349379 resides in a vascular-specific regulatory element.

CRISPR–Cas9 genome editing in pluripotent stem cells that were subsequently differentiated into endothelial cells or vascular smooth muscle cells showed that loss of an 88 bp region flanking rs9349379 increased expression of the *EDN1* gene and its product endothelin 1 (ET1), a potent vasoconstrictor peptide. Strikingly, the *EDN1* gene is located more than 600 kb upstream of the variant. Next, the researchers compared gene expression profiles of genetically engineered isogenic stem cell lines that were homozygous for either the major allele (A/A) or the minor allele (G/G). *EDN1* expression was significantly increased in G/G embryonic stem cell-derived endothelial cells, the predominant cell type source of ET1, compared with A/A control cells.

Circularized chromosome conformation capture (4C) assays in human coronary artery endothelial cells did not identify a high number of contacts or a loop between rs9349379 and the *EDN1* promoter. However, the researchers report the presence of a common contact site approximately 300 kb from both the *EDN1* promoter and rs9349379. Thus, the distal *cis*-regulatory effect of rs9349379 on *EDN1* expression might be mediated by a mechanism other than direct enhancer–promoter contact.

Analysis of plasma samples from 99 healthy individuals of all genotypes at rs9349379 (A/A, A/G, and G/G) showed that the G genotype was associated with higher levels of Big ET1, the protein precursor of the 21-amino acid ET1 peptide. Overall, the study suggests that increased endothelial production of ET1 promotes atherosclerosis, for example, through its effects on vascular tone and on the proliferation of vascular smooth muscle cells. Moreover, migraine headache has been associated with intracranial vasodilatation, so an increase in vasoconstriction mediated by higher ET1 levels could explain the reduced risk of this disorder in individuals with the rs9349379 G allele.

Taken together, this study nicely illustrates the value of an integrative approach to link causal non-coding variants to remote genes and disease risk.

> “the *EDN1* gene is located more than 600 kb upstream of the variant”

Linda Koch, Chief Editor, *Nature Reviews Genetics*

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ORIGINAL ARTICLE Gupta, R. M. *et al.* A genetic variant associated with five vascular diseases is a distal regulator of endothelin-1 gene expression. *Cell* http://dx.doi.org/10.1016/j.cell.2017.06.049 (2017)

FURTHER READING Khera, A. V. & Kathiresan, S. Genetics of coronary artery disease: discovery, biology and clinical translation. *Nat. Rev. Genet.* **18**, 331–344 (2017)