GENOME EDITING

Decoding a major CAD risk locus

A study using genome editing to delete large blocks of the genome in human induced pluripotent stem cells (iPSCs) has uncovered new functions of the 9p21.3 risk locus, one of the strongest common genetic risk factors for coronary artery disease (CAD), associated with 10–15% of CAD per year in the USA.

The 9p21.3 risk locus is an ~60 kb block of single-nucleotide polymorphisms (SNPs) that comprises two haplotypes: risk and non-risk. This region is human-specific and lacks known coding genes, which has hampered the identification of its function. To assess the effects of the risk locus, Lo Sardo et al. used iPSCs derived from patients with CAD who were homozygous for the risk haplotype and from healthy individuals homozygous for the non-risk haplotype. Given that it is unclear which SNPs influence the risk of CAD, the investigators deleted the entire haplotype (known as haplotype editing) in the iPSCs using the TALENs genome editing tool. The iPSCs were then differentiated into vascular smooth muscle cells (VSMCs) to assess the effects in a CAD-relevant cell type.

The VSMCs with the risk haplotype had a phenotype consistent with CAD, including abnormal adhesion, contraction and proliferation, as well as a transcriptional profile with alterations in known CAD risk genes and pathways. Deleting the risk haplotype rescued VSMC proliferation, adhesion and contraction; by contrast, deletion of the non-risk region had a minimal effect on the cells. The 9p21.3 region encodes the terminal exons of the long non-coding RNA ANRIL and, interestingly, expressing ANRIL in non-risk VSMCs induced the phenotypes observed in VSMCs with the risk haplotype. Importantly, Lo Sardo et al. were able to identify new risk-dependent gene networks, with ~3,000 differentially regulated genes implicated in the VSMC phenotypes observed with the risk haplotype and including more than one-third of known CAD risk genes. "This study … establishes haplotype-edited iPSCs as powerful tools for functionally annotating the human genome," conclude the investigators.

Irene Fernández-Ruiz

ORIGINAL ARTICLE Lo Sardo, V. et al. Unveiling the role of the most impactful cardiovascular risk locus through haplotype editing. *Cell* **175**, 1796–1810 (2018)
FURTHER READING Strong, A. & Musunuru, K. Genome editing in cardiovascular diseases. *Nat. Rev. Cardiol.* **14**, 11–20 (2017)

GENETICS

ROBO4 variants linked to congenital heart defects

Individuals with bicuspid aortic valve (BAV) frequently develop other cardiovascular complications, such as ascending aortic aneurysm (AscAA). A study provides new insights into the pathogenesis of this congenital heart defect by reporting the identification of *ROBO4* variants associated with both BAV and AscAA.

Whole-exome sequencing of samples from families with BAV and AscAA showed that in one family, eight members who had aortic root aneurysm, including two individuals with associated BAV, had a mutation at the splice acceptor site of *ROBO4* exon 13, resulting in the expression of a shorter form of the ROBO4 protein. Targeted sequencing of 441 additional individual probands with BAV and/or AscAA showed that seven probands were also carrying *ROBO4* missense variants that altered ROBO4 amino acid sequence.

Immunofluorescence staining and histological analysis of aortic tissue from one of the two family members with BAV and AscAA and from a control individual without structural heart disease showed that *ROBO4* mutations led to a disruption of endothelial barrier function, via decreased *ROBO4* expression in endothelial cells, and induction of pathological remodelling in the aortic media. In cultured aortic endothelial cells, *ROBO4* knockdown or overexpression of *ROBO4* variants resulted in a decrease in VE-cadherin levels, a component of the tight junctions between endothelial cells, whereas the expression of aortic smooth muscle actin, a marker of mesenchymal transition, was induced. Knock-in mice with a mutation altering *ROBO4* splicing had a complex cardiovascular phenotype, with aortic valve defect and AscAA. These findings show that altered endothelial function can cause both BAV and AscAA as primary manifestations of the same underlying gene defect.

Alexandra Le Bras

ORIGINAL ARTICLE Gould, R. A. et al. ROBO4 variants predispose individuals to bicuspid aortic valve and thoracic aortic aneurysm. *Nat. Genet.* https://doi.org/10.1038/s41588-018-0265-y (2018)

PREVENTION

Images of subclinical atherosclerosis incentivize cardiovascular risk reduction

Presentation of ultrasonographic pictorial information to individuals about their subclinical carotid atherosclerosis can help to improve their understanding and motivation to adhere to clinical recommendations and reduce their cardiovascular risk. This finding comes from the VIPVIZA trial, part of the Västerbotten Intervention Programme aimed at population-based prevention of cardiovascular disease in northern Sweden.

In the pragmatic, open-label VIPVIZA trial, 3,532 individuals aged 40, 50 or 60 years with one or more traditional cardiovascular risk factors were recruited and underwent clinical examination, blood sampling and ultrasonographic assessment of carotid intima–media thickness and plaque formation. Individuals were then randomly assigned to an intervention group (pictorial representation of the carotid ultrasonograph plus a telephone call from a nurse to confirm understanding, in addition to standard care) or a control group (standard care with no additional information).

After 1 year of follow-up, the Framingham Risk Score had decreased from baseline in the intervention group (–0.58, 95% CI –0.86 to –0.30) and had increased from baseline in the control group (+0.35, 95% CI 0.08–0.63). By contrast, the European Systematic Coronary Risk Evaluation had increased in the intervention group (+0.13, 95% CI 0.09–0.18) and to a greater extent in the control group (+0.27, 95% CI 0.23–0.30).

"This study provides evidence of the contributory role of pictorial presentation of silent atherosclerosis for prevention of cardiovascular disease," conclude the investigators, writing in *The Lancet*. "It is a low-intensity intervention that is valid for clinical practice. This simple intervention could easily be applied in general practice in other similar settings."

Gregory B. Lim

ORIGINAL ARTICLE Näslund, U. et al. Visualization of asymptomatic atherosclerotic disease for optimum cardiovascular prevention (VIPVIZA): a pragmatic, open-label, randomised controlled trial. *Lancet* https://doi.org/10.1016/S0140-6736(18)32818-6 (2018)