Unfolding and disentangling coronary vascular disease through genome-wide association studies

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This editorial refers to ‘Genome-wide analysis identifies novel susceptibility loci for myocardial infarction’, by J. Hartiala et al., on page 919.

From the day we are born, our arteries deteriorate. As we age, molecular and cellular decay combined with a never-ceasing attack by internal and external factors (think: circulating metabolites, toxins from smoking, elevated blood pressure, unhealthy diet constituents, microbes) weaken the natural endothelial barrier lining of our arteries. Endothelial damage eventually gives way to the formation of a fatty streak combined with changes in the intima and media, leading to a thickening and hardening of the artery that may occur together with a gradual constrictive or expansive remodelling and arterial inflammation: commonly known as atherosclerosis.1 By the time we reach middle age, plaques have formed to varying degrees, some of which progress to an unstable form until a tipping point is reached and a myocardial infarction (MI) ensues.

Atherosclerosis is highly heritable. Findings from large-scale genome-wide association studies (GWAS) and family studies seem to confer additive information: a substantial portion of our lifetime risk of cardiovascular disease (CVD) is explained by variations in our genetics studies proxies of atherosclerotic disease progression (caotsas1o@6a@L).4 However, for roughly half of the loci, the causal variant(s), gene(s), and mechanism(s) are unclear.5 Single-cell RNA sequencing confirms the cellular heterogeneity of atherosclerotic plaques,5 thereby complicating translation to causal gene networks and therapeutic targets.6 These subtle genetic effects act differently depending on the disease stage; at a population level, those having a high polygenic burden develop atherosclerosis sooner and are at higher risk.7 Thus, the processes involved in atherosclerosis and thereby the associated genetic risk loci can be unfolded to an atherosclerotic time scale (Figure 1).

In this respect, Hartiala et al.8 followed the interesting hypothesis that to some degree genetic risk factors might differentially influence risk for atherosclerosis or MI, by affecting plaque stability or thrombotic events. Based on a meta-analysis of GWAS data for MI from the UK Biobank and CARDIoGRAMplusC4D consortium9 and subsequent replication studies, the authors firmly established eight novel genetic risk loci for MI, six of which showed stronger effect sizes for MI than for CAD. Moreover, a locus on chromosome 1p21.3, encompassing choline-like transporter 3 gene (SLC44A3), is significantly associated with MI in patients with CAD, but not with lifetime risk for coronary atherosclerosis itself.

Post-GWAS analyses conducted by Hartiala et al., including association studies with known CAD risk factors, several biomarkers, and plasma levels of metabolites, did not reveal any mechanistic insights. However, by studying gene expression data, the authors showed that gene expression of SLC44A3 is increased in the aorta of risk-allele carriers. Furthermore, the risk-allele is associated with increased expression of SLC44A3 in ischaemic coronary arteries, and in human aortic factors, and atherosclerotic plaque characteristics.4 However, for...
endothelial cells after interleukin-1β treatment. Finally, a functional experiment showed an inverse correlation of SLC44A3 expression with smooth muscle cell migration after stimulation with platelet-derived growth factor BB (PDGF-BB) in vitro, leading to the conclusion that SLC44A3 might be a novel target contributing to risk of thrombosis and plaque rupture through mechanisms at the arterial wall.

While the results of Hartiala et al. are indeed intriguing, especially regarding the consistency of association and functional consequences of the sentinel variant (rs12743267) at the 1p21.3 locus and hence the potential influence on plaque stability and vulnerability, some open questions remain, which might provide additional insights into the role of 1p21.3 in atherosclerosis.

First, for both men and women CVD is still the main cause of death in developed countries. However, men usually develop CVD earlier and with more severe coronary artery plaque formation than women. As a consequence, MI presents up to a decade earlier and is often associated with more widespread plaque formation in men than in women. In men with CAD, high levels of circulating lipids are associated with plaque rupture, whereas smoking is associated with plaque thrombosis. Interestingly, young pre-menopausal women (<50 years) show a high rate of plaque erosion. Given this
known sexual dimorphism, it would have been quite interesting, almost mandatory, to perform a sex-stratified analysis.

Second, based on a GWAS of dozens of quantitative traits in >160,000 Japanese individuals conducted by Kanai et al., the effect allele C of rs12743267, the lead single nucleotide polymorphism (SNP) tagging the SLC44A3 gene, is significantly associated with increased prothrombin time (PT). PT refers to the time needed for plasma coagulation to be transformed into thrombin after adding excessive tissue factor in the plasma without platelets. In general, PT reflects the activity of coagulation factors I, II, V, VII, and X in the plasma, and abnormal PT may reflect liver damage, use of blood thinners, or deficiencies in clotting factors or vitamin K. Here, the significant association of rs12743267 with increased PT, although so far only reported in a Japanese population, may suggest a link to the coagulation pathway representing an alternative molecular mechanism, but hence protective in nature.

Third, cis-expression quantitative loci (eQTLs) exist for any given gene, and eQTL analyses combined with co-localization by no means identify the causal gene for certain; conversely, proximity to a gene offers no guarantees either. In point of fact, just 300 kb from rs12743267 lies F3 encoding tissue factor, and rs12743267 overlaps histone acetylation marks in two endothelial cell lines. This makes 1p21.3 reminiscent of PHACTR1 on 6p24, which is associated with five vascular diseases, but in opposite directions. The sentiment variant is intrinsic to PHACTR1 and overlaps an enhancer element exclusive for aortic tissue that regulates the expression of EDN1 in endothelial cells, almost 600 kb from the sentinel variant at 6p24.

Taking this into account, the results Hartiala et al. present are a bit paradoxical: increased bleeding time would imply a potential beneficial effect and hence reduced risk of MI, whereas the low migration of smooth muscle cells implies an effect on the arterial wall and lesion formation leading to plaque rupture. One explanation could be that individuals carrying the risk allele have expansive remodelling; although their PT will be prolonged, their lesions are prone to be more unstable. Expansive remodelling is also associated with increased matrix protease activity, vascular inflammation, and higher risk of MI, while constrictive remodelling leads to more stenotic arteries, stable plaques, and increased intraplaque bleeding, or through plaque erosion by platelet and endothelial activation.

In conclusion, Hartiala et al. convincingly present six genetic risk loci that show stronger effect sizes for MI than for CAD. Interestingly, the SNP rs12743267 tagging SLC44A3 at 1p21.3 is associated with MI in CAD patients, but not with CAD risk itself. However, the mechanisms at 1p21.3 with respect to MI risk remain somewhat unresolved. This study also makes abundantly clear that translation from GWAS loci to putative biological mechanisms, causal gene networks, and therapeutic targets is difficult to say the least. A one-bioinformatics-pipeline-fits-all solution is unlikely to help us move forward; rather, future studies aimed at precision phenotyping in concert with functional studies will be key to disentangling the underlying pathomechanism of atherosclerotic disease loci. Nevertheless, this study is exhilarating and a firm reminder that in this day and age we have a plethora of data sets at our disposal, and we are confident that through team science we will answer these complex questions about the development of disease for the benefit of patients.

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