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EDITORIAL

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 DNA sequence, while gene–gene and gene–environment interactions also contribute to our cardiovascular heritage.[2] Over 160 genetic loci are associated with risk of coronary artery disease (CAD) and MI, together explaining ~25% of the heritability.[3] Cardiovascular genetics studies proxies of atherosclerotic disease progression (carotid intima-media thickness and coronary calcification), relevant traits, risk factors (circulating lipid levels, blood pressure, coagulation factors), and atherosclerotic plaque characteristics.[4] However, for roughly half of the loci, the causal variant(s), gene(s), and mechanism(s) are unclear.[3] 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 consortium[9] 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 of 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

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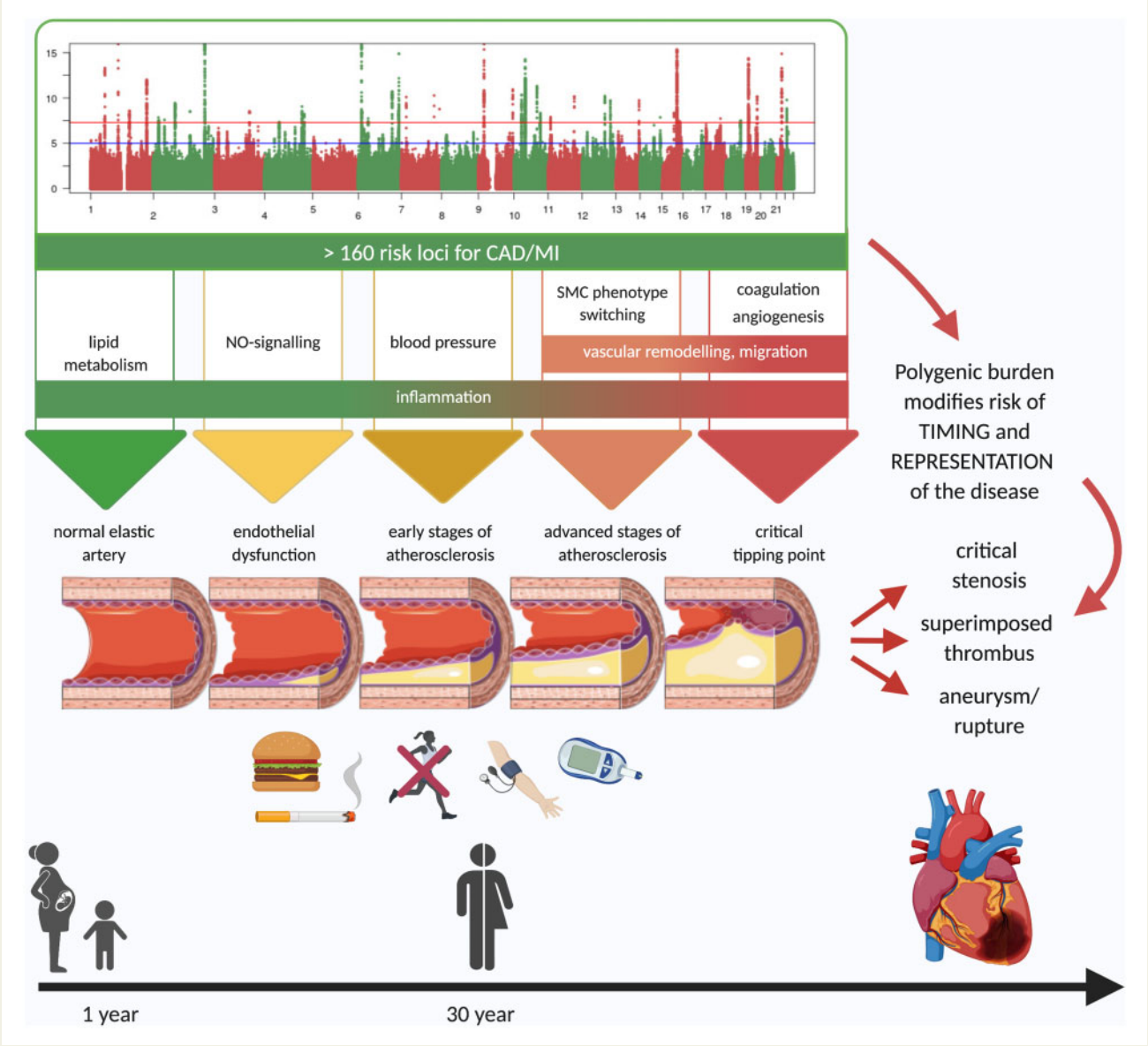

Figure 1 Over 160 risk loci for CAD and MI have been identified by GWAS. Roughly half of them are functionally known to act in pathways involved in the process of atherosclerosis and its sequela MI. Atherosclerosis is a progressive disease starting early in life, and genetic variants as well as risk factors, such as high cholesterol, smoking, no sports, hypertension, and diabetes, affect throughout a person's lifetime the susceptibility to the initiation, progression, and the final outcome of atherosclerosis. Created with BioRender.com.

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.[10] In men with CAD, high levels of circulating lipids are associated with plaque rupture, whereas smoking is associated with plaque thrombosis.[11] Interestingly, young pre-menopausal women (<50 years) show a high rate of plaque erosion.[12] 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).[13] 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.[14] The sentinel variant is intronic 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 lesions, and stable angina.[1] This could be an alternative explanation for the low migration rate of smooth muscle cells associated with *SLC44A3* expression, and at the same time the prolonged bleeding time; more platelets will be activated until a thrombus is formed and platelets in turn cause vascular inflammation and plaque instability. Interestingly, a family member of *SLC44A3, SLC44A2*, is linked to thrombosis, and *in vivo* experiments showed that *SLC44A2* plays a vital role in choline transport to platelet mitochondria.[15] Choline is also of critical importance to a properly functional cellular membrane. The overlap with histone acetylation marks in endothelial cells potentially points towards a role for the endothelium and plaque erosion. Intraplaque bleeding is associated with increased plaque vulnerability, and unstable lesions are characterized by increased intraplaque neovessel formation.[16] These vessels are leaky, and a prolonged bleeding time might exacerbate the intraplaque bleeding as well as add to the deposition of lipids from the erythrocytes, enlargement of the necrotic core, and infiltration of inflammatory cells.[17] Thus, it is unclear whether this locus leads to plaque vulnerability through arterial remodelling by smooth muscle cells, vascular inflammation by platelet activation and *SLC44A3*-induced matrix turnover, 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.[6] 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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