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EDITORIAL

Statins for *ACTA2* mutation-driven atherosclerosis?

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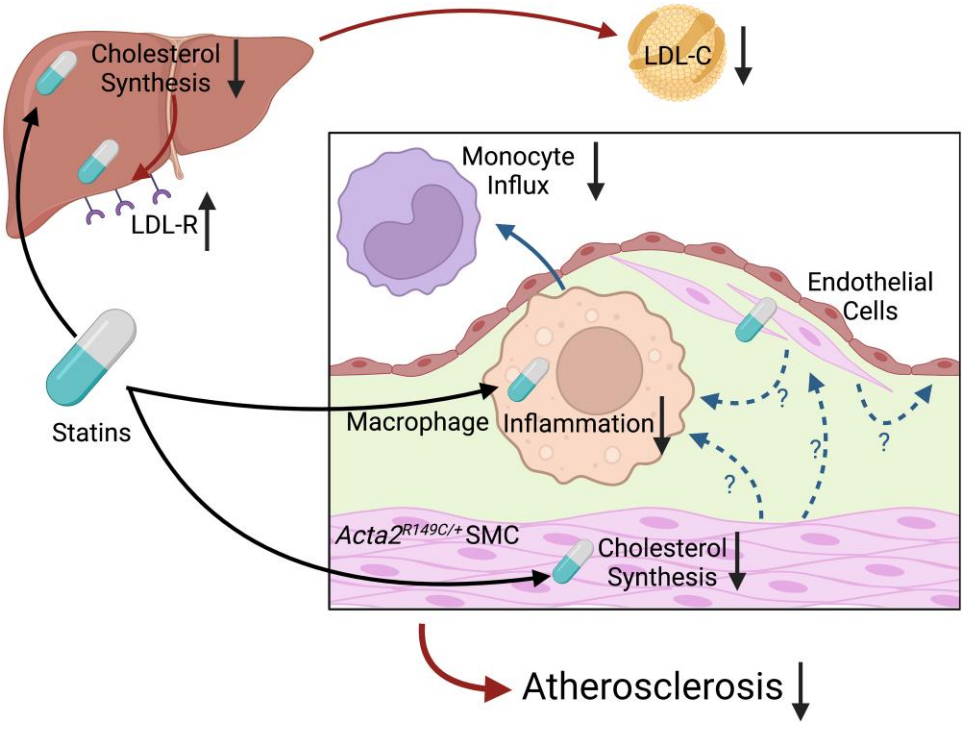
This editorial refers to 'Smooth muscle α-actin missense variant promotes atherosclerosis through modulation of intracellular cholesterol in smooth muscle cells', by K. Kaw *et al.*, https://doi.org/10.1093/eurheartj/ehad373.

Graphical Abstract



Statins suppress atherosclerosis via multiple mechanisms. Statins decrease plasma LDL-cholesterol (LDL-C) by suppressing cholesterol synthesis in the liver and increasing the expression of the LDL receptor (LDL-R).[1] Previous studies have shown that statins, targeted to the arterial wall in nanoparticles, decrease macrophage inflammation and monocyte influx (cross-talk indicated by the blue arrow).[2] Black arrows indicate delivery of statins to the liver or atherosclerotic plaques. The study by Kaw *et al.*[3] shows that statins decrease atherosclerosis in mice carrying the *Acta2*$^{R149C/+}$ variant, mainly by suppressing smooth muscle cell (SMC) cholesterol synthesis. The athero-protective effects of statins in *Acta2*$^{R149C/+}$*Apoe*$^{-/-}$ mice may support the cross-talk between SMCs and other cell types in atherosclerotic plaques (indicated by dashed blue arrows). The figure has been created with Biorender.com.

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Statins decrease cardiovascular events by ~30%, mainly by lowering plasma LDL-cholesterol.[1] However, more than half of cardiovascular events occur in apparently healthy men and women with average or low levels of plasma LDL-cholesterol.[4] Interestingly, statins also reduce cardiovascular events in non-hyperlipidaemic subjects, broadening their therapeutic application. The JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial has shown that statins reduce plasma levels of C-reactive protein (CRP) and the rate of a first major cardiovascular event in non-hyperlipidaemic subjects with high CRP, presumably independent of lowering plasma LDL-cholesterol.[5] In this issue of the *European Heart Journal*, Kaw *et al.*[3] provide evidence that statins may decrease cardiovascular risk in heterozygous carriers of variant p.Arg149Cys in *ACTA2* (p.R149C) independent of lipid lowering. Heterozygous pathogenic variants in *ACTA2*, which encodes the smooth muscle cell (SMC)-specific isoform of α-actin (SMA), predispose patients to a variety of diffuse and diverse vascular diseases, including thoracic aortic aneurysms and dissections (TAAD), premature coronary artery disease (CAD), ischaemic strokes, and Moyamoya disease (MMD).[6,7] The p.R149C variant predisposes to premature onset of CAD in the absence of hypercholesterolaemia or other cardiovascular risk factors.[7] As the variant is highly conserved, Kaw *et al.*[3] generated a mouse model by CRISPR (clustered regularly interspaced palindromic repeats) gene editing, i.e. *Acta2*$^{R149C/+}$ mice. Employing this model, Kaw *et al.*[3] provide evidence that statins may decrease atherosclerosis in carriers of the p.R149C variant by suppressing vascular SMC cholesterol synthesis. These findings are especially important since they may broaden the therapeutic applications of statins (*Graphical Abstract*).

Statins reverse the effects of the p.R149c variant in SMCs

Kaw *et al.*[3] show that *Acta2*$^{R149C/+}$ mice on the *Apoe*$^{-/-}$ background have a 2.5-fold increase in atherosclerotic lesion size in the whole aorta compared with *Apoe*$^{-/-}$ mice. Mechanistically, the p.R149C variant induces misfolding of the α-actin protein in SMCs, activation of heat shock factor 1 (HSF1), endogenous cholesterol biosynthesis, endoplasmic reticulum stress, and activation of the PERK–ATF4–Krüppel-like factor (KLF4) axis. This resulted in SMC dedifferentiation *in vitro*, which was reversed by statin treatment, indicating that this mechanism is crucially dependent on cholesterol synthesis. The mechanism was replicated in SMCs explanted from human aortas, even though the amino acid change was different (p.R149H).

SMC cholesterol accumulation and atherosclerosis

It has been well established that cholesterol loading of murine and human SMCs induces SMC dedifferentiation, characterized by decreases in SMC contractile markers, including *ACTA2*, and increases in markers of macrophages and osteogenic cells *in vitro*.[8–10] The study by Kaw *et al.*[3] corroborates the crucial role of cholesterol accumulation in SMC phenotypic switching. Previous studies have shown that cholesterol loading induces the transition of SMCs to macrophage- and osteogenic-like cells by down-regulating myocardin and up-regulating KLF4 expression.[8–10] *Klf4* deficiency in SMCs decreases macrophage-like and osteogenic-like cells in atherosclerotic plaques in *Apoe*$^{-/-}$ mice, and increases SMC and collagen content of the fibrous cap,[9] substantiating that pathways downstream of SMC cholesterol accumulation regulate SMC dedifferentiation *in vivo*, which compromises plaque stability.

Acta2$^{R149C/+}$ and atherosclerotic plaque SMCs

It is unclear whether increased SMC cholesterol synthesis affects plaque stability in the study by Kaw *et al.*[3] While the *in vitro* data clearly show that *Acta2*$^{R149C/+}$ induces SMC dedifferentiation, features of plaque stability, such as SMC or collagen content, as well as necrotic core area, were not assessed in mice *in vivo*. Regardless, single-cell RNA sequencing (scRNA-seq) analysis of aortas collected from the root to the arch of *Acta2*$^{R149C/+}$*Apoe*$^{-/-}$ and *Apoe*$^{-/-}$ mice fed a high-fat cholesterol-rich diet (HFD) for 12 weeks did yield further insights into SMC characteristics. A total of five clusters were identified in fate-mapped SMC-derived cells, including one contractile SMC cluster that represents the majority of the SMC-derived cells (~50%), two modulated SMC clusters, and two minor clusters (~10%). *Acta2*$^{R149C/+}$ did not affect the proportion of these subpopulations. Among the five clusters, the contractile SMC cluster showed the most significant change in transcriptomic signature, while modulated SMC clusters, presumably present in the intima, had only a modest number of differentially expressed genes. These results imply that *Acta2*$^{R149C/+}$ is unlikely to affect atherosclerosis by driving SMC dedifferentiation in the intima. Kaw *et al.*[3] explain this by high levels of exogenous cholesterol in the intima that promote SMC cholesterol accumulation, suppressing cholesterol synthesis, and as such abolishing the effect of *Acta2*$^{R149C/+}$. Cholesterol accumulation in intimal SMCs may be aggravated by low expression of the ATP Binding Cassette A1 (ABCA1) cholesterol transporter compared with media SMCs, and low lysosomal acid lipase (LAL) relative to macrophages, as demonstrated in both mouse and human atherosclerotic plaques.[11–13]

Acta2$^{R149C/+}$, contractile SMCs, *Klf4*, and atherosclerosis

In line with the *in vitro* data, *Acta2*$^{R149C/+}$ increased *Klf4* expression in contractile SMCs. Of interest, KLF4 is among the genetic loci associated with CAD in humans.[14] The study by Kaw *et al.*[3] suggests that increased *Klf4* expression downstream of increased cholesterol synthesis in contractile *Acta2*$^{R149C/+}$ SMCs contributes to atherogenesis. This is further supported by their striking finding that at similar plasma cholesterol levels, pravastatin decreased atherosclerotic lesion size in *Acta2*$^{R149C/+}$*Apoe*$^{-/-}$ mice to the levels of *Apoe*$^{-/-}$ mice. Cross-talk between contractile SMCs and other plaque cells may account for this effect (*Graphical Abstract*).

Remaining questions

Mutations in *ACTA2* are inherited in a Mendelian manner yet lead to adult-onset vascular diseases with reduced penetrance.[6,7] Among a handful of these mutations, p.R149C and p.R118Q lead primarily to increased genetic predisposition to premature CAD.[7] The current study demonstrates, using *Acta2*$^{R149C/+}$ mice, that the variant itself suffices to drive atherosclerosis in the absence of hypercholesterolaemia. Uncovering the mechanisms for this finding will be key in determining how the human p.R149C variant is associated with premature CAD

in the absence of cardiovascular risk factors. The *Acta2*$^{R149C/+}$ mice, however, do not resemble the reduced penetrance of p.R149C in humans. This reduced penetrance for p.R149C and other *ACTA2* mutations may result from a combination of genetic, environmental, and lifestyle factors. The developmental origin of SMCs, a major determinant for regional susceptibility to vascular diseases,[15] may be key to the diversity of disease manifestations of *ACTA2* mutations. In that regard, although coronary arteries in mice are in general less susceptible to plaque build-up, plaque burden at anatomical positions with distinct developmental origins was differentially affected by the p.R149C variant in mice. For example, increased plaque burden was seen in thoracic and abdominal aortas, but not in the ascending aortas in *Acta2*$^{R149C/+}$ *Apoe*$^{-/-}$ vs. *Apoe*$^{-/-}$ mice after 12 weeks of a HFD.[3] Human induced pluripotent stem cell-based disease modelling that leverages lineage-specific SMCs and recapitulates *in vivo* environmental conditions may represent tools to address the reduced penetrance and regional susceptibility to vascular diseases due to *ACTA2* mutations.[15]

How the molecular mechanisms discovered in the current study may be generalized towards the pathogenesis of CAD will be subject of future study. The differentially expressed genes in the contractile SMCs of *Acta2*$^{R149C/+}$*Apoe*$^{-/-}$ vs. *Apoe*$^{-/-}$ mice showed some overlap with candidate causal genes in CAD genome-wide association studies,[14] including KLF4 and TCF21. Both are up-regulated in *Acta2*$^{R149C/+}$*Apoe*$^{-/-}$ compared with *Apoe*$^{-/-}$ contractile SMCs, yet KLF4 promotes[9] while TCF21 inhibits[16] SMC dedifferentiation. Nonetheless, the overlapping genes represent important candidates in SMC biology and function.

In summary, Kaw *et al.*[3] discovered that expression of the *ACTA2* p.R149C variant drives atherosclerosis by increasing SMC cholesterol synthesis, even in the absence of hypercholesterolaemia, probably explaining the premature CAD in carriers of the p.R149C variant in the absence of cardiovascular risk factors. Statins decreased atherosclerosis in mice expressing the p.R149C variant, suggesting additional therapeutic benefits. Further, employing nanoparticles to target statins to the atherosclerotic plaque reduces atherosclerosis to a larger extent than oral administration of statins in *Apoe*$^{-/-}$ mice.[2] Future studies will be needed to explore whether suppressing cholesterol synthesis in SMCs may contribute to this effect.

Declarations

Disclosure of Interest

None declared.

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