Bentzon, J. F. (2019). Tapping Into the Strengths of Diversity Among Atherosclerotic Pigs. *Arteriosclerosis, Thrombosis, and Vascular Biology, 39*(11), 2203-2204. doi:10.1161/ATVBAHA.119.313404

Tapping into the strengths of diversity among atherosclerotic pigs

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Word count: 858 excluding figure and references.

Experimenters who study the mechanisms of atherosclerosis or develop therapies and diagnostic tools to combat its consequences, often require an in vivo representation of lesion development. These are offered by over a century's worth of development of feeding regimens and genetic techniques that can force hypercholesterolemia and atherosclerotic lesions in mice, rats, hamsters, rabbits, pigs, and non-human primates. Today, the mouse is by far the preferred model, both because of the relative ease by which complicated questions can be addressed using genetic tools, and because it is feasible to study sufficient numbers to achieve statistical power.

Lesions in mice and other animals develop for the same principal reason that human atherosclerosis does: because of a plasma level of apoB-containing lipoproteins that is too high. Furthermore, they also contain the same major cell subsets (macrophages, smooth muscle cells) and plaque components (necrosis, fibrosis). This gives important validity to the approach. Yet it is equally clear that very important aspects of human atherosclerosis, foremost the thrombotic complications, are lacking in murine models, and while general mechanisms, such as the role of lipoprotein retention or inflammation, are well conserved between animal and humans atherosclerosis, the exact genes that are the most important regulators in these pathways may not be.[1,2] At least, this is one way of reconciling the modest number of successful therapeutic targets that thorough exploration of gene function in atherosclerotic mice has to date brought forward.

Minipigs are unique among model animals by providing hearts and arterial system at a size comparable to our own. Studies in pigs, therefore, have their clearest merit where size matters, such as in atherosclerosis imaging and for studies of the interplay between biomechanical forces and lesion development. But also for studies of other atherosclerosis mechanisms and drug testing, pigs have an advantage over mice in that their genetic kinship with us is higher,[3] and therefore more conservation on the molecular level could be expected. More research in atherosclerosis mechanisms in pigs is needed before we will know if that prediction proves true.

There are also clear practical challenges involved in studying atherosclerosis in pigs. An important one is the variability in the rate of atherogenesis, which generally exceeds the level in mice. The variable outcome may be caused by the genetic diversity that exists in pig strains, and possibly also the less controlled environment that pigs are typically housed in. It is possible to argue that variability is a human-like quality, and one may even think that some patients with coronary artery disease are akin to the "outliers" in experiments that scientists look at accusingly for sabotaging statistical power. The result of the variability, however, means that intervention studies in pigs must be dimensioned with larger group sizes than comparable mouse studies.

But where some see difficulty, others see possibilities. In the present issue, Hoogendoorn et al. explore the source of variation in atherosclerosis in a group of Familial Hypercholesterolemia Bretoncelles Meishan (FBM) pigs.[4] These pigs are a down-sized line of the Rapacz farm pigs, in which a natural loss-of-function mutation in the low density lipoprotein (LDL) receptor was identified, and they develop severe, reproducible hypercholesterolemia on a high-fat diet.[5] The resulting atherosclerosis development is, however, variable. Hoogendoorn et al. found that the atherosclerosis phenotype in 10 castrated adult male pigs fell equally into two categories. Half developed advanced coronary fibroatheromas after 12 months of feeding with a high-fat diet; the others developed much smaller lesions. In their search for a cause, they carefully analyzed apoB-lipoproteins for size, density, and lipid composition. Consistent with previous analysis in FBM and other pigs with reduced LDL clearance,[5,6] they found lipoproteins of LDL density with diverse sizes, with some being considerably larger than regular LDL. Interestingly, the pigs that early after the onset of high-fat feeding showed more regular-sized LDLs were the pigs that eventually developed aggressive atherosclerosis. The differences in LDL size waned over time, but the

authors suggest that early exposure may have kick-started atherogenesis and eventually led to the more aggressive atherosclerosis phenotype.

While the associations of the study will need to be reproduced and tested for causality before conclusions can be made, the ideas are in keeping with observations in other reports. Among D374Y-PCSK9 transgenic Yucatan minipigs, we also noted some pigs that developed many-fold more coronary atherosclerosis than others,[6] and while these pigs were not analyzed individually for lipoprotein size and density, they did stand out by having a more rapid onset of hypercholesterolemia compared with the rest (unpublished). Furthermore, we found evidence that the type of hypercholesterolemia of APOE knockout minipigs, indeed characterized by large cholesterol-rich lipoproteins of LDL density, was not very efficient in inducing early lesions.[7] The findings also align with previous demonstrations in mice that a load of plasma cholesterol is more atherogenic if carried in many small lipoproteins rather than few larger ones.[8]

Altogether, the work of Hoogendoorn et al. provides new hypotheses for the determinants of atherosclerosis susceptibility in pigs, and it lays out a path by which pigs with more and less variable coronary atherosclerosis may be selectively bred (if the cause is genetic) or selected (if the cause is non-genetic) by using the short-term response to high-fat feeding as a susceptibility marker.

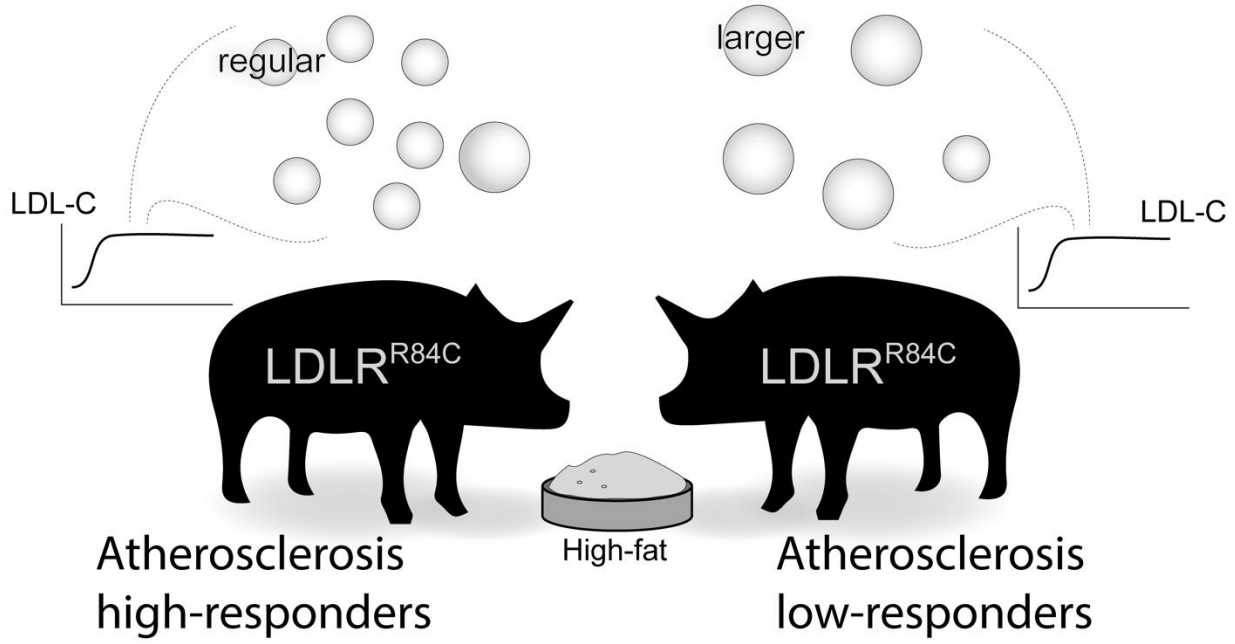

Figure. Despite similar LDL cholesterol, some FBM pigs develop much more atherosclerosis than others. Hoogendoorn et al. find that high-responders are characterized by developing hypercholesterolemia characterized initially by regular-sized LDLs, whereas low-responders have more larger-sized LDLs.

Acknowledgments

a) Acknowledgments: None.
b) Sources of Funding: The CNIC is supported by the Instituto de Salud Carlos III (ISCIII), the Ministerio de Ciencia, Innovación y Universidades (MCNU) and the Pro CNIC Foundation, and is a Severo Ochoa Center of Excellence (SEV-2015-0505)
c) Disclosures: JFB is an inventor on patents on gene modified minipigs owned by Aarhus University.

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