atherosclerosis

# *Atherosclerosis*

## Call for original papers on "Influence of sex and gender on biology of atherosclerotic cardiovascular disease"

There are differences in the risk for atherosclerotic cardiovascular diseases (ASCVD) between men and women. These differences stem from various biological aspects related to sex and gender and should be considered when assessing the risk for ASCVD in clinical practice, planning clinical trials, and performing *in vivo* and even *in vitro* experiments in research settings. *Atherosclerosis*, the journal of the European Atherosclerosis Society (EAS), is now **calling for the submission of Original Research Papers for a Special Issue related to the role of sex and gender biology in ASCVD**. These manuscripts will undergo a regular review process and in case of acceptance will go online within the usual time of processing. Submissions are encouraged from all fields related to the topic including clinical, translational, and basic research.

The submitted Original Research Articles will be handled by Elena Osto, Jeanine Roeters van Lennep, and Lale Tokgözoğlu as Guest Editors and Katariina Öörni as Co-Editor of *Atherosclerosis*. They will decide on the peer reviewers of the submitted articles. If a manuscript is accepted for publication, these Original Research Articles will appear printed together in a combined issue of the journal containing roughly a dozen in-depth review articles on the sex and gender biology of ASCVD. The collection aims to provide the most comprehensive, insightful, and current overview of the clinical and translational aspects and basic research related sex and gender differences in ASCVD. The topics and authors for these review articles have already been decided for this project. The publication is planned for spring/summer 2023 and is expected to receive a high visibility. **Accepted papers will be published with promotional open access for a one-year period, free of charge.**

For preparation of the Original Research manuscripts please see the "Guide for authors"

**Deadline for submission of the first draft of Original Research Papers is December 31st, 2022**. This call is only open for Original Research Articles and no review articles are allowed. Please select "Special issue: Gender biology in ASCVD" as article type at submission.

**To submit your paper go to: Editorial Manager®**

# *Atherosclerosis* newsletter

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Human genetics approaches were previously successful in the identification of drug targets for the prevention or treatment of atherosclerotic cardiovascular diseases. Recent examples are PCSK9 and ANGPTL3. The therapeutic interference with these genes led to the identification of unanticipated treatment effects, for example the lowering of Lp(a) levels upon PCSK9 inhibition. Volumes 361 and 362 of *Atherosclerosis* contain several articles which describe research outcomes on the elucidation of pleiotropic effects of PCSK9 and ANGPTL3 or genetic strategies towards the identification and validation of novel target genes.

## Genetic proxies for PCSK9 inhibition associate with lipoprotein(a): Effects on coronary artery disease and ischemic stroke

High lipoprotein(a) (Lp(a)) plasma levels are causally associated with atherosclerotic cardiovascular disease. However, variance in Lp(a) levels is up to 60% determined by genetic variation in the LPA gene, whereas lifestyle modifications, such as diet adjustments, only have modest effects on Lp(a) levels. Furthermore, there are so far no approved specific Lp(a) lowering pharmacological agents shown to reduce cardiovascular events. Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors were originally developed to reduce cardiovascular events by lowering low-density lipoprotein cholesterol (LDL-C) levels. *Post hoc* analyses of clinical trials show that PCSK9 inhibitors might lower Lp(a), but whether this effect contributes to reductions in cardiovascular risk remains unknown. De Marchis et al. aimed to assess whether genetically proxied PCSK9 inhibition influences Lp(a), and whether any such effect could mediate its effects on coronary artery disease (CAD) and ischemic stroke (IS).

To explore associations between the genetic proxies for PCSK9 inhibitors and Lp(a) levels, the UK Biobank data (310,020 individuals) were used. Ten variants in the *PCSK9* gene were associated with lower PCSK9 and LDL-C levels as proxies for PCSK9 inhibition. The effects of genetically proxied PCSK9 inhibition on Lp(a) levels, as well as on odds of CAD and IS were assessed in two-sample Mendelian randomization analyses. In mediation analyses, the effects of genetically proxied PCSK9 inhibition on CAD and IS, mediated through reductions in Lp(a) levels, were determined.

Genetically proxied PCSK9 inhibition was associated with a 4% decrease in log-Lp(a) levels. The authors estimated a 0.8% reduction in the odds for CAD and a 0.5% reduction in the odds for

atherosclerotic IS due to reductions in Lp(a) levels through genetically proxied PCSK9 inhibition, corresponding to 3.8% and 3.2% of the total effects, respectively.

Genetic proxies for PCSK9 inhibition are associated with lower Lp(a) levels. However, Lp(a) lowering explains only a small proportion of the total effects of genetic proxies for PCSK9 inhibitors on risk of CAD and IS.

## Modulation of vascular endothelial inflammatory response by proprotein convertase subtilisin-kexin type 9

In the circulation, lipopolysaccharide (LPS) and other pathogen lipids are incorporated into various serum lipoproteins, including low-density lipoprotein (LDL). LPS within LDL is subsequently cleared from the circulation by hepatocytes through the low-density lipoprotein receptor (LDLR). Proprotein convertase subtilisin-kexin type 9 (PCSK9) impairs this LPS clearance pathway by down-regulating expression of LDLR on hepatocytes. In addition to hepatocytes, vascular endothelial cells also express receptor targets of PCSK9, including LDLR. Leung et al. hypothesized that PCSK9 may regulate vascular endothelial cell uptake of LPS and alter the vascular endothelial cell inflammatory response.

They found that LPS was internalized by human umbilical vein vascular endothelial cells (HUVECs) and LPS uptake dose-dependently increased with increasing LDL concentration. Intracellular LPS co-localized with LDL. PCSK9 and, separately, blocking antibodies against LDLR, dose-dependently decreased the vascular endothelial cell uptake of LPS and inhibition of endocytosis using Dynasore blocked LPS uptake. In contrast, blocking antibodies against TLR4 did not alter LPS uptake. PCSK9 decreased the LPS-induced proinflammatory response in vascular endothelial cells. In addition, a decrease in PCSK9 and increase in LDLR, mediated by triciribine (an AKT serine/threonine kinase inhibitor) or si*PCSK9*, increased LPS uptake and the LPS-induced proinflammatory response. Similar results were also found in aortic vascular tissue from *Pcsk9*$^{-/-}$ mice after LPS injection.

The data suggest that, similar to PCSK9 treatment in hepatocytes, PCSK9 reduces vascular endothelial cell uptake of LPS via LDLR-mediated endocytosis. Consequently, PCSK9 decreases the LPS-induced proinflammatory response in vascular endothelial cells. These results raise the possibility that PCSK9 inhibition may have additional effects on vascular endothelial inflammation via this alternative pathway, beyond the known effects of PCSK9 inhibition on LDL lowering and hepatic endotoxin clearance.

## ANGPTL3 deficiency associates with the expansion of regulatory T cells with reduced lipid content

Angiopoietin-like protein 3 (ANGPTL3) is a protein almost exclusively produced by the liver and acts as an hepatokine involved in the regulation of lipid and glucose metabolism. Loss-of-function

mutations in *ANGPTL3* gene, leading to ANGPTL3 deficiency, cause in humans the familial combined hypolipidemia type 2 (FHBL2) phenotype, characterized by very low concentrations of circulating lipoproteins and reduced risk of atherosclerotic cardiovascular disease. Whether this condition is accompanied by immune dysfunctions is unknown. Regulatory T cells (Tregs) are CD4 T lymphocytes with immune suppressive and atheroprotective functions and sensitive to metabolic signals. By investigating FHBL2, Pinzon Grimaldos et al. explored the hypothesis that Tregs expand in response to extreme hypolipidemia, through a modulation of the Treg-intrinsic lipid metabolism.

Treg frequency, phenotype, and intracellular lipid content were assessed *ex vivo* through multiparameter flow cytometry from FHBL2 subjects and age- and sex-matched controls. The response of CD4 T cells to marked hypolipidemia was tested *in vitro* in low-lipid culture conditions from healthy controls.

The *ex vivo* analysis revealed that FHBL2 subjects showed higher percentages of Tregs with a phenotype undistinguishable from controls and with a lower lipid content, which directly correlated with the concentrations of circulating lipoproteins. *In vitro*, lipid restriction induced the upregulation of genes of the mevalonate pathway, including those involved in isoprenoid biosynthesis, and concurrently increased the expression of the Treg markers FOXP3 and Helios. The latter event was found to be prenylation-dependent, and likely related to increased IL-2 production and signaling.

This study demonstrates that FHBL2 is characterized by high Treg frequencies, a feature which may concur to the reduced atherosclerotic risk in this condition. Mechanistically, hypolipidemia may directly favor Treg expansion, through the induction of the mevalonate pathway and the prenylation of key signaling proteins.

## Gene set analysis of transcriptomics data identifies new biological processes associated with early markers of atherosclerosis but not with those of osteoporosis: Atherosclerosis-osteoporosis co/multimorbidity study in the Young Finns Study

Atherosclerosis and osteoporosis, both complex and multifactorial diseases, are growing public health challenges with a major impact on disease management and health care costs globally. Several studies have suggested that osteoporosis and atherosclerosis are co/multimorbidities as they share common pathophysiological mechanisms, molecular pathways and risk factors such as inflammatory cytokines, lipid oxidation products, vitamin D and K deficiency, bone and vascular mineralization, estrogen deficiency and air pollution. In this study, Mishra et al. aimed at identifying the shared biological processes underlying their co/multimorbidity.

Gene set analysis (GSA) of whole-blood transcriptomic data was performed to identify biological processes shared by the early markers of these two diseases. Early markers of diseases, carotid intima-media thickness (CIMT) for atherosclerosis and trabecular bone mineral density (BMD)

from distal radius and tibia for osteoporosis, were used to categorize the study participants into cases and controls. Participants with high CIMT (>90th percentile) were defined as cases for subclinical atherosclerosis. Study population-based T-scores for BMD were calculated and T-score ≤ −1 was used for the definition of low BMD cases i.e., early indicator of osteoporosis.

No gene sets jointly associated with early markers of atherosclerosis and osteoporosis were identified. Three novel and replicated 234 gene sets were significantly associated with high CIMT with false discovery rate (FDR) ≤ 0.01. Only two genes, both related to the immune system, were identified to be associated with high CIMT by traditional differential gene expression analysis. However, none of the studied gene sets or individual genes were significantly associated with tibial or radial BMD. The three novel CIMT associated gene sets contained genes involved in copper homeostasis, neural crest cell migration and nicotinate and nicotinamide metabolism. The 234 replicated gene sets in this study were related to the immune system, hypoxia and apoptosis, consistent with the existing literature on atherosclerosis.

This study identified novel biological processes associated with high CIMT but not with reduced BMD.

## *Trans*-interaction of risk loci 6p24.1 and 10q11.21 is associated with endothelial damage in coronary artery disease

Cardiovascular disease is the leading cause of mortality globally, with coronary artery disease (CAD) accounting for a majority of cardiovascular deaths  With the heritability of CAD estimated at up to 60%, genetic factors hold an important contribution to the risk of CAD along with other major etiologic determinants such as lifestyle and environmental factors.

Genome-wide association studies (GWAS) have identified several common variants to be risk factors for CAD, but few have been functionally validated. Single nucleotide polymorphism rs6903956 has been identified as one of such genetic risk factors.  However, rs6903956 lies in a non-coding locus on chromosome 6p24.1. Tay et al. investigated the molecular basis of 6p24.1 containing rs6903956 risk alleles in endothelial disease biology.

Induced pluripotent stem cells (iPSCs) from CAD patients (AA risk genotype at rs6903956) and non-CAD subjects (GG non-risk genotype at rs6903956) were generated. CRISPR-Cas9-based deletions (Δ63-89bp) on 6p24.1, including both rs6903956 and a short tandem repeat variant rs140361069 in linkage disequilibrium, were performed to generate isogenic iPSC-derived endothelial cells. Edited CAD endothelial cells, with removal of ‘A’ risk alleles, exhibited a global transcriptional downregulation of pathways relating to abnormal vascular physiology and activated endothelial processes. A CXC chemokine ligand on chromosome 10q11.21, CXCL12, was uncovered as a potential effector gene in CAD endothelial cells. Underlying this effect was the preferential inter-chromosomal interaction of

6p24.1 risk locus to a weak promoter of CXCL12, confirmed by chromatin conformation capture assays on our iPSC-derived endothelial cells. Functionally, risk genotypes AA/AG at rs6903956 were associated significantly with elevated levels of circulating damaged endothelial cells in CAD patients. Circulating endothelial cells isolated from patients with risk genotypes AA/AG were also found to have 10 folds higher CXCL12 transcript copies/cell than those with non-risk genotype GG.

The results reveal the trans-acting impact of 6p24.1 with another CAD locus on 10q11.21 and is associated with increased endothelial injury.