Circulation Research

ORIGINAL RESEARCH

Female-Biased VSMC GRNs Predict MYH9 as Regulator of Fibrous Plaque Phenotype

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BACKGROUND: Atherosclerosis, an inflammatory driver of coronary artery disease, manifests as unstable atheromatous plaques and stable fibrous plaques. Although atheromatous plaques have been extensively studied, fibrous plaques, particularly in women aged <50 years, where erosion contributes significantly to coronary thrombosis, remain less understood. The molecular mechanisms underlying sex differences in plaque biology, including vascular smooth muscle cell contributions, are incompletely defined.

METHODS: Sex-specific gene regulatory networks (GRNs) were constructed from RNA-sequencing data of cultured human vascular smooth muscle cells isolated from 119 male and 32 female heart transplant donors. Network preservation analyses identified female-biased GRNs, which were evaluated in single-cell RNA-sequencing data sets from human carotid atherosclerotic plaques. Bayesian network modeling and proteomic analyses were used to identify and validate regulatory drivers.

RESULTS: Two female-biased vascular smooth muscle cell networks, $GRN_{floralwhite}$ and $GRN_{yellowgreen}$, were enriched for inflammatory and actin remodeling pathways, respectively. Single-cell RNA-sequencing confirmed sex-specific network activity in plaque vascular smooth muscle cells. Subcellular phenotyping identified a sex-specific gene expression program within $GRN_{yellowgreen}$ enriched for contractile and vascular development pathways. Bayesian network modeling identified *MYH9* (myosin heavy chain 9) as a key driver gene. Elevated MYH9 abundance was associated with increased smooth muscle cell content and reduced lipid content in female carotid plaques compared with males, consistent with fibrous plaque features. Proteomic analyses confirmed MYH9 upregulation in female fibrous plaques and association with stable plaque characteristics.

CONCLUSIONS: These findings identify MYH9 as a regulator of female-biased fibrous plaque biology and highlight the importance of sex-specific network regulation in atherosclerosis.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

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Atherosclerosis, the underlying cause of coronary artery disease (CAD), is a chronic inflammatory condition characterized by the accumulation of lipids, inflammatory cells, and ECM (extracellular matrix) components within the arterial wall.[1] Pathology studies described 2 dominant plaque morphologies with unique pathological mechanisms. Atheromatous plaques, unstable and prone to rupture, are characterized by thin fibrous caps and large necrotic cores consisting of lipids and inflammatory cells. Fibrous plaques, stable and prone to erosion, exhibit a thick fibrous cap rich in smooth muscle cells (SMCs) and ECM.[2] Most attention has been focused on understanding plaque rupture hallmarked by large atheroma and plaque hemorrhage[3]; however, 40% of coronary thrombosis in sudden cardiac death cases involving CAD is associated with plaque erosion. In

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Novelty and Significance

What Is Known?

- Atherosclerosis exhibits sex-specific plaque phenotypes, with females more likely to develop stable fibrous plaques prone to erosion and males more likely to develop rupture-prone atheromatous plaques.
- Prior transcriptomic studies of sex differences in atherosclerosis have primarily relied on bulk plaque tissue, which is confounded by cellular composition.
- Vascular smooth muscle cells (VSMCs) are key regulators of fibrous cap thickness and plaque stability, yet sex-specific molecular mechanisms in VSMCs remain poorly defined.

What New Information Does This Article Contribute?

- This study identifies female-biased gene regulatory networks in human VSMCs that are not preserved in males and are associated with fibrous plaque–relevant phenotypes.
- Integration of bulk, single-cell, proteomic, and network-based analyses implicates MYH9 (myosin heavy chain 9) as a key sex-specific regulator of fibrous plaque formation in females.
- These findings provide a mechanistic framework linking sex, VSMC gene regulation, and fibrous plaque stability.

Sex differences in atherosclerotic plaque biology are well recognized, yet the molecular mechanisms driving these differences remain poorly understood. This study applies a systems genetics framework to human VSMCs to uncover sex-specific gene regulatory networks that are not evident from differential expression or bulk tissue analyses alone. By focusing on VSMCs, a critical cell type governing fibrous cap formation, this work overcomes the confounding effects of plaque cellular heterogeneity and directly interrogates sex-dependent regulatory programs. We identify 2 female-biased VSMC gene regulatory networks associated with calcification and TGFβ (transforming growth factor beta)–driven proliferation and demonstrate their relevance in human atherosclerotic plaques using single-cell transcriptomics. Through Bayesian network modeling and proteomic validation, we further implicate MYH9 as a central regulator linking actin remodeling, extracellular matrix signaling, and fibrous plaque formation specifically in females. These findings advance understanding of sex-specific mechanisms underlying plaque stability and erosion and highlight the importance of cell type–specific network biology in cardiovascular disease. Ultimately, this work provides a foundation for sex-informed therapeutic strategies aimed at stabilizing atherosclerotic plaques and reducing coronary thrombosis risk.

Nonstandard Abbreviations and Acronyms

ACTA2	α-actin 2
BN	Bayesian network
CAD	coronary artery disease
ECM	extracellular matrix
ERK	extracellular signal–regulated kinase
FDR	false discovery rate
GRN	genetic regulatory network
GSEA	Gene Set Enrichment Analysis
KD	key driver
MYH9	myosin heavy chain 9
Ov	ovaries
RNA-seq	RNA-sequencing
SMC	smooth muscle cell
STARNET	Stockholm-Tartu Atherosclerosis Reverse Network Engineering Task
TGFβ1	transforming growth factor beta 1
TPO	thrombopoietin
Ts	testes
WGCNA	Weighted Gene Coexpression Network Analysis

women under the age of 50, this percentage increases to ≈75% to 80%,[2,4] highlighting the importance of understanding sex differences in atherosclerosis.

Gene regulatory networks (GRNs), groups of genes that interact with each other to regulate biological processes, are an effective tool in studying the complexity of common disorders, such as CAD.[5] Highly connected key driver (KD) genes are essential for the activity and impact of GRNs on CAD.[6] GRNs of atherosclerotic tissue between males and females have been constructed.[7,8] However, these GRNs used expression profiles from a heterogeneous population of cells, and did not capture cell type–specific differences.[9] SMC content and phenotypic state play a critical role in defining the characteristics of fibrous versus atheromatous plaques. SMCs migrate from the medial layer into the intima to form the fibrous cap, but their phenotypic plasticity enables a range of behaviors with different impacts on disease.[10] In addition to known cellular composition differences between male and female plaques,[11] sex hormone studies highlight critical roles for both estrogen and testosterone in SMC phenotypic transitions.[12,13] Understanding SMC-specific GRNs may, therefore, better pinpoint individual KD genes contributing to sex differences associated with plaque erosion.

We generated sex-specific GRNs from gene expression data of vascular SMCs (VSMCs) isolated from 119 male and 32 female heart transplant donors from distinct genetic ancestries.[14] We used network-based preservation statistics to identify intranetwork topology differences across male and female GRNs.[15] We prioritized GRNs with female-biased connectivity as well as genetic and biologic signatures associated with VSMC pathology in atherosclerosis. This led to the selection of 2 female-specific GRNs with sex-biased genetic regulation enriched for genes involved in inflammatory signaling ($GRN_{floralwhite}$) and vascular and actin remodeling ($GRN_{yellowgreen}$)—each relevant to known characteristics that differ between plaque erosion and rupture phenotypes.[16,17] Utilizing single-cell RNA-sequencing data from patients undergoing carotid endarterectomy,[18] we validated the female-biased nature of both networks but only found additional evidence supporting the rewiring of actin cytoskeleton remodeling processes between males and females ($GRN_{yellowgreen}$). We then used a Bayesian network (BN) approach and KD analysis and identified myosin heavy chain 9 (*MYH9*) as the KD gene of $GRN_{yellowgreen}$. Finally, we incorporated both in vitro and in vivo phenotypic data specific to atherosclerosis phenotypes. This provided evidence that MYH9 regulates a female-specific, fibrous-associated VSMC signaling process and is directly associated with the high prevalence of fibrous plaques in females.

METHODS

Data Availability

The data that support the findings of this study are available from the corresponding author on request. All processed data sets relevant to the results are included in the article and its Supplemental Material. Data repositories can be accessed in the Major Resources Table in the Supplemental Material.

A detailed description of the methods and the experimental procedures is provided in the Supplemental Material. Please see the Major Resources Table in the Supplemental Material.

Study Populations

Human aortic VSMCs were isolated from 151 donors and cultured under quiescent (serum-free) and proliferative (5% fetal bovine serum) conditions to model phenotypic state transitions.[14,19] Total RNA was extracted and sequenced to ≈100 million paired-end reads per sample. Reads were quality-filtered, aligned to the hg38 reference genome using STAR,[20] and gene expression was quantified as transcripts per million using RNA-SeQC[21] with GENCODE v32 annotations. Genes were considered expressed if they exceeded 6 read counts and 0.1 transcripts per million in at least 20% of samples.

The Athero-Express Biobank consists of patients undergoing carotid endarterectomy, with linked transcriptomic, proteomic, and histopathologic characterization of atherosclerotic plaques.[18] GRNs derived from plaque tissue of age-matched male and female patients were used for comparison with VSMC-derived networks.[8] Additional plaque samples were used for single-cell RNA-sequencing and proteomic analyses to assess sex-specific molecular features in advanced lesions.[22] Detailed proteomic and histopathologic methods are provided in the Supplemental Methods.

The STARNET (Stockholm-Tartu Atherosclerosis Reverse Network Engineering Task) is a multitissue transcriptomic study of patients undergoing coronary artery bypass grafting.[23] Previously published GRNs derived from atherosclerotic aortic root tissue of age-matched female and male patients were used for cross-cohort validation of sex-specific regulatory programs.[7]

The collection of patient samples for this study was approved by the Institutional Review Boards of the University of California, Los Angeles and the University of Virginia, the Local Medical Ethical Committees at the University Medical Center Utrecht and St. Antonius Hospital Nieuwegein, and the Research Ethics Committee at Tartu University Hospital. Source data and additional details can be found in the Major Resources Table.

Construction of Sex-Specific Genetic Regulatory Networks

Sex- and condition-specific gene coexpression networks were constructed using iterative Weighted Gene Coexpression Network Analysis[24,25] applied to 11 300 expressed genes across quiescent and proliferative VSMCs. Adjacency matrices were derived from Pearson correlations and soft-thresholded to approximate scale-free topology. Network preservation analysis was performed across sexes within matched conditions using the medianRank statistic implemented in Weighted Gene Coexpression Network Analysis,[26] with modules in the bottom 20th percentile of preservation considered sex-biased.

Atherosclerosis-Relevant VSMC Phenotypes

VSMC phenotypes relevant to atherosclerosis, including calcification, migration, and proliferation, were quantified as previously described.[14] Associations between gene expression and phenotypes were evaluated using linear models incorporating gene-by-sex interaction terms, with log-transformed expression values used to account for skewness.

Single-Cell RNA-Sequencing Analysis

Single-cell RNA-sequencing of 20 female and 26 male human atherosclerotic plaques from Athero-Express Biobank was analyzed using Seurat[27] with standard quality control, normalization, and clustering workflows. Cell populations were annotated using canonical markers and reference-based methods (SingleR), with SMCs identified by expression of ACTA2 (α-actin 2) and TAGLN (smooth muscle 22α). Subpopulations were defined through secondary clustering and differential expression analysis.

BN Generation and KD Analysis

Directed gene regulatory relationships were inferred using BN modeling (bnlearn)[28] with a hill-climbing algorithm and bootstrap aggregation. Networks were pruned based on edge strength thresholds to approximate scale-free topology. KD analysis was performed to identify highly connected regulatory

genes, with the top 10% of nodes ranked by neighborhood connectivity defined as KDs.[29]

Xenium Prime In Situ Gene Expression Analysis

Spatial transcriptomic profiling of 4 symptomatic and 4 asymptomatic atherosclerotic plaques from patients undergoing carotid endarterectomy was performed using the 10x Genomics Xenium platform. Data were processed using Seurat with standard filtering, normalization, integration, and clustering approaches. Cell segmentation and gene expression quantification were performed using the Xenium Analyzer, and differential expression analysis was conducted using Wilcoxon rank-sum testing.

Clustered Regularly Interspaced Short Palindromic Repeats Interference and RNA-Sequencing

Clustered regularly interspaced short palindromic repeats interference[30] targeting MYH9 was performed in primary VSMCs from 3 female and 3 male donors using a guide RNA directed at the transcriptional start site, with a nontargeting guide as a control. After perturbation, RNA was extracted and sequenced, and gene expression was quantified using pseudoalignment (Kallisto)[31] and analyzed with DESeq2.[32] Gene set enrichment analysis[33] was performed to identify pathways associated with MYH9 perturbation and sex-specific regulatory effects.

Statistical Analysis

Statistical analyses were performed in R. Paired or unpaired 2-tailed tests were applied as appropriate, using Student *t* tests for normally distributed data and Mann-Whitney *U* tests otherwise. Gene expression values were log-transformed when necessary to improve normality and variance stability. Differential and pathway-level analyses were performed using false discovery rate (FDR) correction or permutation-based methods as specified. Unless otherwise noted, statistical significance was defined as $P \leq 0.05$.

RESULTS

Generation of Sex-Specific Gene Expression Modules in Human VSMCs

We constructed sex-specific gene coexpression networks from RNA-sequencing (RNA-seq) data of aortic SMCs isolated from 119 male and 32 female heart transplant donors from distinct genetic ancestries (Figure 1). SMCs were cultured in quiescent or proliferative conditions to mimic different vascular phenotypes represented in atherosclerosis.[14] For each sex and culture condition, we input 11 300 genes for Weighted Gene Coexpression Network Analysis[24,25] to identify groups of genes with high topological overlap (see Methods). The female quiescent and proliferative SMC conditions resulted in 10 885 and 10 829 coexpressed genes segmented into 55 and 68 modules, respectively. The male quiescent and proliferative conditions resulted in 10 633 and 9942 coexpressed genes segmented into 43 and 53 modules, respectively (Table S1). The female data sets resulted in more modules, potentially due to smaller sample sizes.

To test the potential effects of using different sample sizes for males and females, we performed a sensitivity analysis on the quiescent and proliferative male data sets. We randomly subsampled 32 male samples 5× and reconstructed coexpression networks. We observed an increase in the number of modules compared with the coexpression networks constructed from all 119 male samples, suggesting that sample size affects module membership (Table S2). Therefore, we studied whether these module differences resulted in limitations in capturing biologically meaningful information. We performed pathway enrichment analyses across the modules using Gene Ontology Terminology,[34] Kyoto Encyclopedia of Genes and Genomes,[35] and Hallmark[36] pathways. Across all 3 pathway databases, modules generated from a smaller sample size (n=32) led to fewer enriched pathways detected (FDR≤0.05). However, of these enriched pathways, 70% were reproduced within the pathway enrichment results from modules generated with 119 male samples (Table S2). Although some information may be missed when using 32 randomly selected male samples, this analysis suggests that there is enough power to detect groups of coexpressed genes enriched for biologically relevant information.

Prioritization of Female-Specific Modules in Human VSMCs

We assessed whether the 55 GRNs identified in quiescent female VSMCs were preserved in the 43 GRNs identified in quiescent male VSMCs. We performed a similar analysis for the proliferative female (68) and male (53) GRNs. We used medianRank,[26] a composite statistic of preservation, to rank the preservation of each GRN across sexes in each phenotypic state. A low medianRank score represents a highly preserved GRN expected to capture similar biological regulation and processes shared across sexes, whereas a high medianRank score represents a less preserved GRN expected to identify genes and biological pathways most likely to be specific to female VSMCs. We identified 10 female-biased GRNs in the quiescent condition and 12 female-biased GRNs in the proliferative condition, scoring in the bottom 20th percentile of preservation (Figure 2A and 2B; Table S3).

We next sought to determine which of these networks was most likely to be functional in atherosclerosis. First, to reproduce our GRNs in an independent data set, we compared the gene membership of our 22 unpreserved GRNs with 7 previously described female-biased modules identified in a bulk RNA-seq study of carotid atherosclerotic plaques from 158 age-matched female and male donors in the Athero-Express cohort.[8] Fisher

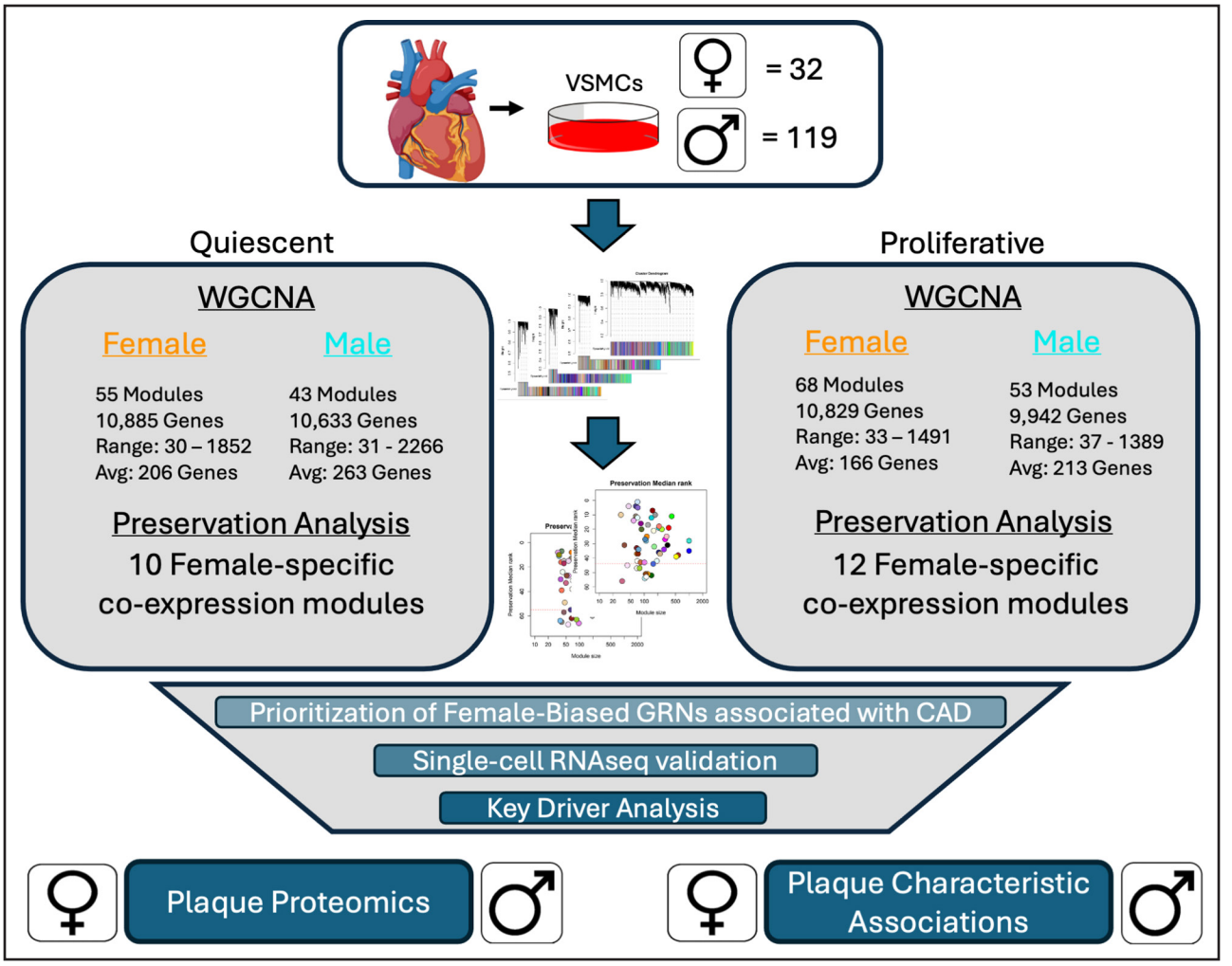

Figure 1. Overview of study design.
Vascular smooth muscle cells (VSMCs) from 32 female and 119 male ascending aorta donors were cultured under quiescent and proliferative conditions. RNA-sequencing (RNA-seq) data were used to construct sex-specific coexpression modules using Weighted Gene Coexpression Network Analysis (WGCNA). Network preservation analysis ranked modules by sex-specificity, and modules were prioritized based on coronary artery disease (CAD) association and reproducibility across data sets. Selected modules were reconstructed into Bayesian networks for key driver (KD) analysis. Proteomics and carotid plaque histopathology were integrated to link candidate KDs to plaque characteristics and disease risk. GRN, gene regulatory network. Created in BioRender. Watts, K. (2026) https://BioRender.com/k4c6y00

exact tests revealed that 10 of the 22 VSMC GRNs were reproduced in the disease-associated female modules from Athero-Express (Table S4).

We then ranked the 10 GRNs according to their relevance to CAD genetic susceptibility and known atherosclerosis biology. Using a manually curated list of genes located in CAD Genome-wide Association Study (GWAS) loci[37–41] (Table S5), we found that the violet and brown4 modules were enriched for CAD GWAS genes (Table S6). Because hub genes of GRNs act as central regulators within a network,[25] CAD GWAS genes that serve as hub genes are strong candidates for genetically driven disease mechanisms. Four GRNs contained a CAD GWAS gene as a hub gene (Table S7). We then evaluated the relevance of the GRNs for atherosclerosis by testing for enrichment of curated atherosclerosis gene sets (DisGeNET[42] and Ingenuity Pathway Analysis,[7] Table S5) and for biological pathways relevant to vascular remodeling and SMC phenotypic switching (Gene Ontology). $GRN_{floralwhite}$ was enriched for known atherosclerosis genes (Table S6). Three GRNs were enriched for biological pathways relevant to SMC-mediated plaque biology: $GRN_{floralwhite}$ for inflammatory and immune response pathways,[16] $GRN_{yellowgreen}$ for actin filament–based and vascular remodeling processes,[43] and $GRN_{thistle1}$ for mitogen-activated protein kinase (MAPK) signaling pathways.[44] We used FDR≤0.25 to capture all potential biologically relevant modules (Tables S8 and S9). Figure 2C summarizes the cumulative results of this scoring, identifying $GRN_{floralwhite}$, $GRN_{yellowgreen}$, and GRN_{violet} as the networks with the strongest disease-associated evidence. However, only $GRN_{floralwhite}$ and $GRN_{yellowgreen}$ linked CAD-associated genes with biological processes directly related to SMC-driven plaque progression in the

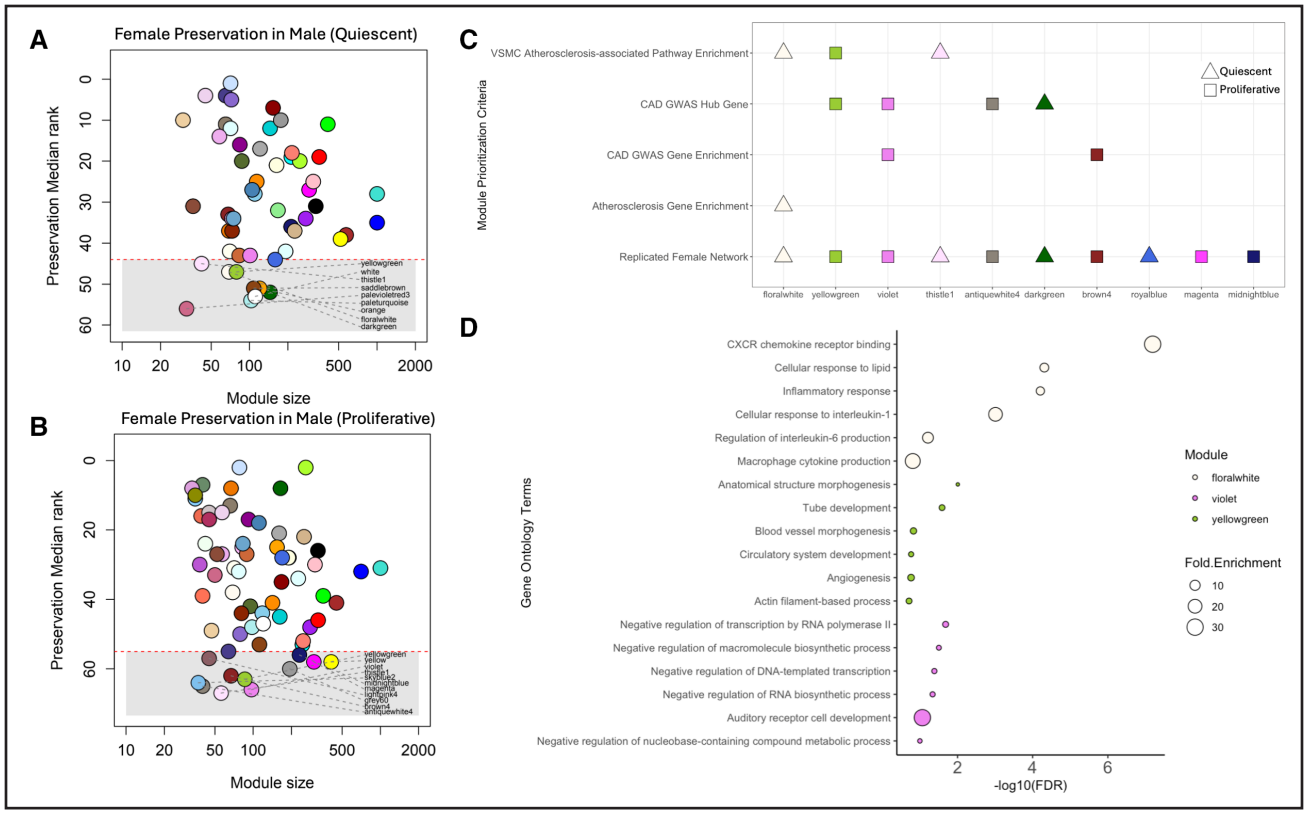

Figure 2. Identification and prioritization of female-specific vascular smooth muscle cell (VSMC) coexpression modules. **A** and **B**, MedianRank preservation scores comparing female quiescent (**A**) and proliferative (**B**) VSMC modules with corresponding male modules. The red line indicates the bottom 20th percentile; modules below this threshold are least preserved. **C**, Prioritization of unpreserved female modules based on replication in female-biased plaque modules, enrichment for atherosclerosis-associated and coronary artery disease (CAD) Genome-wide association study (GWAS) genes, presence of CAD GWAS hub genes, and enrichment of VSMC-related Gene Ontology (GO) pathways (Biological Process and Molecular Function). **D**, GO pathway enrichment (false discovery rate [FDR] ≤0.25) of the top 3 prioritized modules (floralwhite, yellowgreen, violet). Point size reflects fold enrichment.

vascular wall (Figure 2D). Because of this, we selected these 2 GRNs for downstream analyses and did not pursue GRN_{violet} further.

VSMC Phenotypic Characterization of $GRN_{floralwhite}$ and $GRN_{yellowgreen}$

We next examined how gene expression within these 2 GRNs related to atherosclerosis-associated cellular phenotypes. Using the same cohort of 151 SMCs, we quantified 12 phenotypes relevant to VSMC behavior, including proliferation, migration, and calcification.[14] For each gene in the 2 GRNs, we first fit linear models testing the interaction between gene expression and sex for each phenotype to assess sex-dependent associations between gene expression and cellular behavior. In $GRN_{floralwhite}$, linear modeling revealed that osteogenic calcification exhibited the highest number of significant gene-by-sex interaction effects (17 genes, $P \leq 0.05$; Table S10). In $GRN_{yellowgreen}$, the phenotype with the greatest number of significant gene-by-sex interactions was proliferation in response to TGF-β1 (transforming growth factor beta 1) treatment (16 genes; Table S10). For each respective GRN and phenotype, we investigated sex-specific correlations of each gene. Twelve genes in $GRN_{floralwhite}$ showed significant female-specific correlations with calcification (Figure 3A). Twenty-four genes in $GRN_{yellowgreen}$ were significantly correlated with female gene expression under TGF-β1-stimulated proliferation, whereas only 2 genes showed comparable associations in males (Figure 3B). $GRN_{yellowgreen}$, enriched for actin filament organization and vascular remodeling pathways, showed the strongest association with TGF-β-induced proliferation—consistent with the well-established role of TGF-β signaling in driving cytoskeletal reorganization, matrix remodeling, and phenotypic modulation of VSMCs in the vessel wall.[45,46] In contrast, $GRN_{floralwhite}$, enriched for inflammatory and immune response pathways, showed the strongest association with osteogenic calcification. Although the inflammation driven calcification relationship is less direct than the TGF-β–cytoskeletal connection observed in $GRN_{yellowgreen}$, chronic inflammatory signaling is known to promote osteogenic reprogramming of SMCs and to contribute to late-stage plaque mineralization.[47]

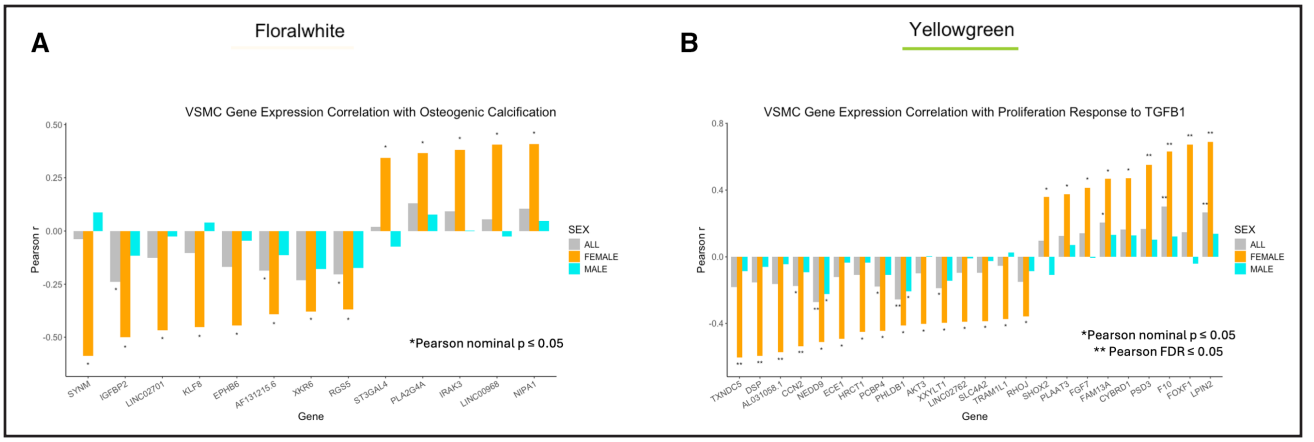

Figure 3. Female-specific correlations between gene expression and vascular smooth muscle cell (VSMC) phenotypes. **A**, Pearson correlation coefficients (*r*) between gene regulatory network $(GRN)_{floralwhite}$ genes and osteogenic calcification in female VSMCs (orange). Correlations for male (cyan) and combined samples (gray) are shown for comparison. **B**, Pearson correlation coefficients (*r*) between $GRN_{yellowgreen}$ genes and proliferation response to TGF-β1 (transforming growth factor beta 1) in females (orange), with male (cyan) and combined (gray correlations displayed. *Pearson nominal $P \leq 0.05$. **False discovery rate (FDR) ≤0.05.

Independent Sex-Specific Evidence for Prioritized GRNs

To study these 2 prioritized female-biased GRNs in more detail, we used single-cell RNA-sequencing data from carotid plaques of 20 women and 26 men (Figure 4A). We first calculated the module score of both GRNs within ACTA2+ SMCs to validate our sex-biased results. The module score is a proxy for the average expression of the genes within each GRN for each cell.[48] We performed this analysis in a sex-stratified manner to identify if there were differences between cell type clusters and gene expression signatures within each GRN due to sex. For ACTA2+ SMCs, the genes in $GRN_{yellowgreen}$ had a higher module score in females compared with males, whereas the genes in the $GRN_{floralwhite}$ had a lower module score in females compared with males (Figure 4B and 4C). The percentage of SMCs compared with all cell types within the carotid plaques was nearly equal across male samples (5.69%) and female samples (5.55%). These results support the fibrous plaque phenotypic contributions hypothesized by the $GRN_{yellowgreen}$ and $GRN_{floralwhite}$ pathway enrichments.

Single-cell RNA-sequencing identifies genes representative of SMC dedifferentiation and unique phenotypic states.[49] Therefore, we reexamined the female and male module scores from each GRN within the 4 distinct SMC cell states (Figure 4D). We observed a difference between female and male module scores in the SMC4 cluster for genes within the $GRN_{yellowgreen}$ but not for $GRN_{floralwhite}$ (Figure 4E and 4F). The module score was higher for females compared with males. The percentage of SMC4 cells compared with all SMC cells was nearly equal across male samples (21.30%) and female samples (20.18%). SMC4 is enriched for both contractile biological processes and cytoskeleton and vasculature development pathways (FDR≤0.05; Figure S2), suggestive of a less synthetic and more plaque-stabilizing phenotypic state.

In Vivo Cues Influencing Regulation of $GRN_{yellowgreen}$

The cultured VSMCs used to define the female-biased GRN cannot fully replicate the complexity of the atherosclerotic plaque environment, where mechanical, cytokine, and hormonal cues collectively shape SMC phenotypes.[50] Therefore, we investigated the role of in vivo stimuli in regulating the yellowgreen network. To assess how $GRN_{yellowgreen}$ responds to ECM differences, we analyzed publicly available gene expression data obtained from human coronary artery VSMCs from 4 donors cultured within either a soft hydrogel matrix or a stiffer hydrogel matrix representative of early atherosclerotic lesions (GSE100081).[51] $GRN_{yellowgreen}$ was significantly enriched for genes upregulated in stiff hydrogels compared with soft hydrogels (Fisher exact $P = 2.8 \times 10^{-2}$), with 20 of 86 network genes showing stiffness-dependent activation (adjusted *P*-value [P_{adj}]≤0.05, log2(Fold Change [FC])≥0.5; Table S12). We next examined the influence of TGF-β, a potent cytokine known to regulate SMC differentiation and ECM production, previously linked to $GRN_{yellowgreen}$. Using publicly available gene expression data from four human aortic SMC cultures treated with or without TGF-β (GSE246269),[52] we found that $GRN_{yellowgreen}$ was enriched for TGF-β-responsive genes (Fisher exact $P = 3.0 \times 10^{-3}$, with 12 of 86 network genes significantly upregulated in treatment versus control conditions ($P_{adj} \leq 0.05$, log2FC≥0.5; Table S13). Finally, we analyzed single-nucleus data from the aortic arches of 6-month-old Four-Core Genotypes[53] mice on an ApoE-deficient background (Four-Core Genotypes $ApoE^{-/-}$)

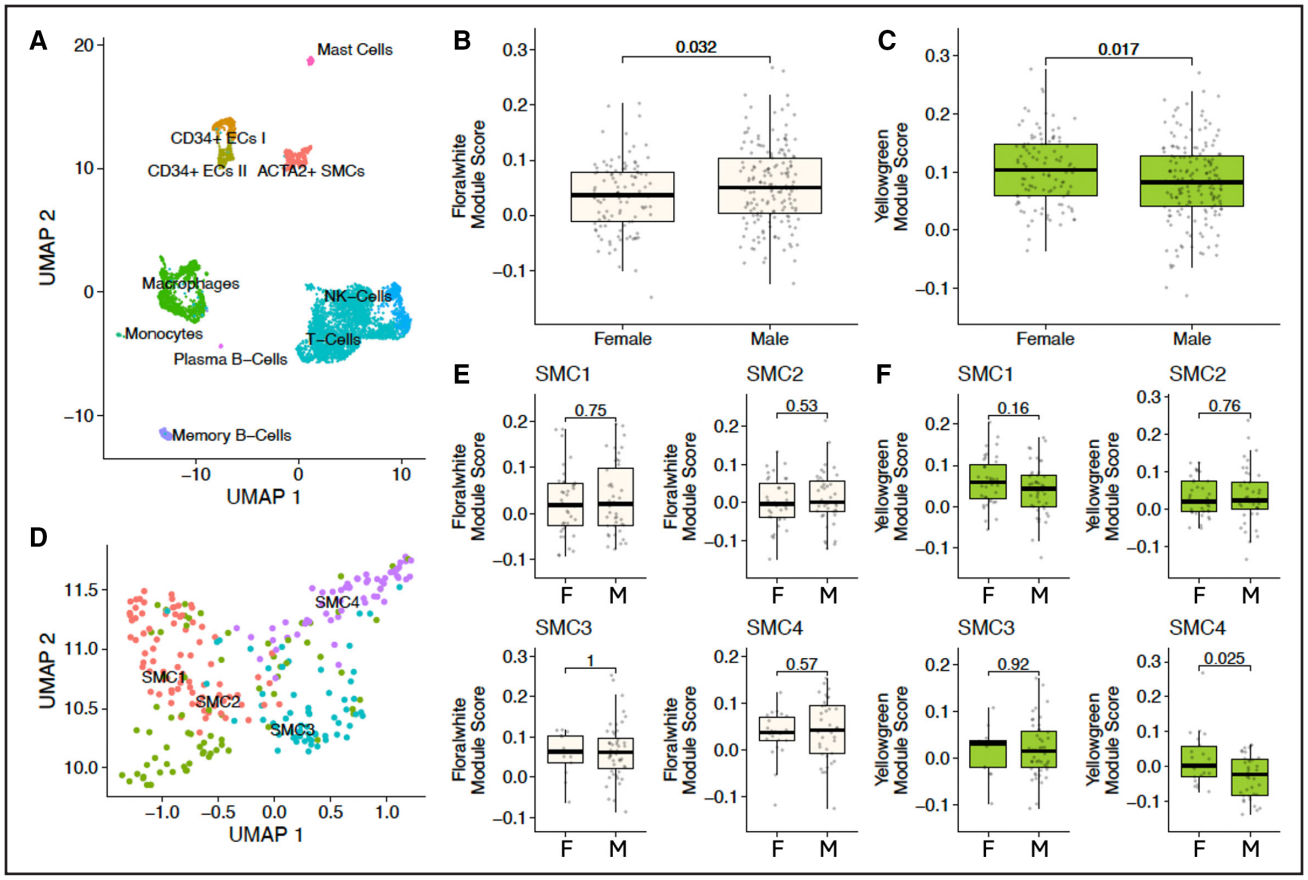

Figure 4. Sex-specific validation of gene regulatory network (GRN)$_{floralwhite}$ and GRN$_{yellowgreen}$ in single-cell RNA-sequencing of atherosclerotic plaques.

A, Uniform Manifold Approximation and Projection (UMAP) of 4948 carotid plaque cells (20 female [F] and 26 male [M]). **B** and **C**, Module score expression for GRN$_{floralwhite}$ (**B**) and GRN$_{yellowgreen}$ (**C**) genes in ACTA2 (α-actin 2)+ smooth muscle cells (SMCs) comparing female (F) and male (M) single-cell expression data. **D**, UMAP of 672 smooth muscle cells showing 4 SMC subclusters. **E** and **F**, Module score expression for GRN$_{floralwhite}$ (**E**) and GRN$_{yellowgreen}$ (**F**) genes across SMC subtypes, comparing F and M single-cell expression data. Statistical differences were assessed using 2-tailed, unpaired *t* tests. EC indicates endothelial cell.

fed a standard chow diet. This model enables us to analyze the gonadal effects on atherosclerosis development, independent of chromosomal complement, by generating XX mice with ovaries or testes and XY mice with ovaries or testes (XX-Ov, XX-Ts, XY-Ov, XY-Ts). Four Core Genotypes *ApoE*$^{-/-}$ mice (n=9 per group) were profiled using single-nuclei RNA-sequencing of aortic arch tissue. Among the three major SMC states identified—SMC, Modulated SMC, and Transitioning—we focused on the transitioning SMC population (Figure S3A through S3E; Tables S14 and S15), which most closely resembles the proliferative VSMC phenotype observed in vitro. The GRN$_{yellowgreen}$ module score was significantly higher in mice with ovaries compared with those with testes, indicating that female sex hormones may enhance activation of the GRN$_{yellowgreen}$ network (Figure S3F). Together, this data supports a fibrous- and sex-associated regulation on GRN$_{yellowgreen}$ that may regulate SMC plasticity and remodeling to contribute to the more fibrous plaque phenotype observed in females.

Topology Analysis of Prioritized Female-Specific Yellowgreen GRN

Coexpression networks indicate which genes change together, but not which ones might be influencing the others. To address this, we used BN algorithms,[28,54] which can suggest likely directions of influence, to assign directionality and refine the regulatory interactions within the GRN$_{yellowgreen}$. We used a hill-climbing score–based algorithm with 10 000 iterations to infer edge weights and directionality for each of the 86 genes within GRN$_{yellowgreen}$. We then trimmed low-weight edges to yield a scale-free topological distribution representative of biological networks where most nodes have few connections, while a small number of nodes have many connections[55] (Figure 5A; Table S16). Next, we performed KD analysis on BN$_{yellowgreen}$ to identify the genes with the highest regulatory potential that may govern fibrous plaque–associated pathways[29] (Supplemental Methods). We defined KD as genes within the top 10% of KD scores, resulting in 6 KD genes for BN$_{yellowgreen}$

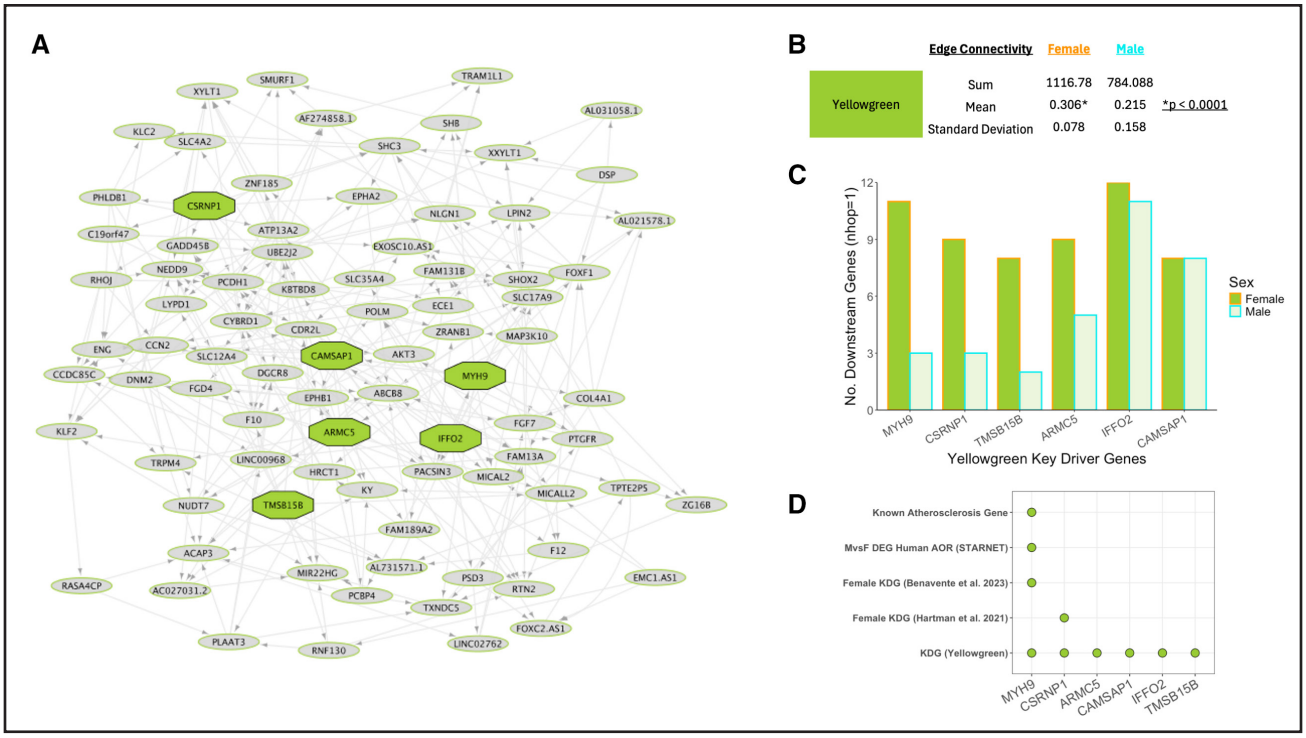

Figure 5. Bayesian network and key driver analysis identify MYH9 (myosin heavy chain 9) as a female-specific regulator of gene regulatory network (GRN)$_{yellowgreen}$.
A, Bayesian network (BN) constructed from GRN$_{yellowgreen}$ genes using female gene expression data. Octagonal nodes represent key driver genes (KDG). **B**, Network topology comparison between female and male BN$_{yellowgreen}$. Mean edge connectivity is higher in the female network (2-tailed unpaired Mann-Whitney *U* test, P=2.2×10^{-16}). **C**, Number of downstream genes one node away from each key driver gene identified in **A** in both female- and male-generated BNs. **D**, Prioritization of female-specific key drivers based on replication in female-biased modules (STARNET [Stockholm-Tartu Atherosclerosis Reverse Network Engineering Task], Athero-Express), DEG (differential expression) between female and male aortas with coronary artery disease (CAD; STARNET), and prior evidence linking the gene to atherosclerosis. AOR indicates aortas.

(*TMSB15B*, *MYH9*, *CAMSAP1*, *IFFO2*, *CSRNP1*, and *ARMC5*; Table S17). These 6 genes are expected to have a more significant effect in regulating downstream gene expression and the function of biological pathways within the yellowgreen network. To ensure that these KD genes are specific to female networks, we used the expression values for the same genes from the male donors and recreated the BN$_{yellowgreen}$ (Table S11). We then compared the topology of the male and female networks and identified KDs in each network. The female-specific network had significantly higher mean edge connectivity compared with the male network when using untrimmed edge weights (P=2.2×10^{-16}; Figure 5B). There were no overlapping KD genes between female and male BN$_{yellowgreen}$ (Table S17). We separately compared the number of genes directly downstream of the female KDs in the male and female networks. The 6 female BN$_{yellowgreen}$ KD genes had 57 downstream edges, 1 node away in the female network, versus only 32 downstream edges, 1 node away in the male network (Figure 5C). *MYH9* exhibited the largest difference in predicted downstream gene connectivity between the female and male networks. Each female KD was then evaluated and prioritized according to 3 independent criteria: (1) replication as a female-specific KD in an external data set, (2) differential expression between males and females in human atherosclerotic tissue, and (3) previous implication in atherosclerosis-related pathways or plaque biology. Among the 6 candidate KDs, *MYH9* showed the strongest cumulative evidence. It was replicated as a female-specific KD in carotid plaques (Athero-Express),[7,23] and the only KD differentially expressed between males and females in atherosclerotic aorta samples from 160 age-matched patients with CAD in the STARNET cohort (P_{adj}=3.0×10^{-2}). *MYH9* is also the only known atherosclerosis-associated gene of the 6 KDs, previously implicated in cytoskeletal remodeling and mechanotransduction[56] (Figure 5D). Together, these lines of evidence led us to prioritize *MYH9* as the most likely sex-specific regulatory driver within the GRN$_{yellowgreen}$.

MYH9 Protein Level in Atherosclerotic Plaques Is Correlated With Fibrous-Associated Biological Processes

To better understand how biological processes and potential disease mechanisms are regulated by MYH9, we incorporated untargeted LC-MS (liquid chromatography–mass spectrometry) proteomics data from 200

patients (149 male, 51 female) undergoing carotid artery endarterectomy[22] (Supplemental Methods). We first assessed whether MYH9 protein was differentially expressed between male and female plaques and found that MYH9 was significantly higher in females (logFC=0.4, P_{adj}=1.5×10^{-2}; Figure S4A). We next performed a sex-stratified, MYH9-centered correlation analysis to explore potential functional associations within the broader proteomic data set. Using 1499 quantified proteins, we calculated pairwise correlations with MYH9 separately in female and male samples. Proteins significantly correlated with MYH9 in females were enriched for actin filament organization and cytoskeletal remodeling processes, whereas no comparable enrichments were detected in males (FDR≤0.05; Figure S4B and S4C). When combining female and male data, we identified 642 positively and 133 negatively correlated proteins (P_{adj}≤0.05; Table S18). Gene Ontology Term analysis of positively correlated proteins showed enrichments for actin filament organization, cell-matrix adhesion, and extracellular matrix organization, capturing similar biological processes as GRN$_{yellowgreen}$ and SMC4 subcluster (FDR≤0.05; Figure S5A). Proteins negatively correlated were enriched primarily for a variety of immune response pathways (FDR≤0.05; Figure S5B). Although these proteomic findings do not establish causality, they provide orthogonal support for MYH9 contributions in fibrous-associated and sex-associated regulation in atherosclerosis.

MYH9 Protein Level in Atherosclerotic Plaques Is Associated With Fibrous Plaque Characteristics

We investigated the relationship between MYH9 and carotid plaque characteristics assessed in 200 patients from the Athero-Express biobank. The carotid plaques in this biobank have been histologically characterized for SMC content using ACTA2 staining and for lipid content using H&E and picrosirius red staining.[18] We first calculated the association between MYH9 protein levels and plaque composition in male and female samples. MYH9 abundance was positively correlated with ACTA2+ SMC content in males and showed a near-significant correlation in females, likely reflecting differences in sample size (149 male versus 51 female samples; Figure 6A). When all samples were combined, the association between MYH9 and ACTA2+ SMC content was significant (P=7.6×10^{-3}; Figure S6A). For associations with lipid content in plaque, we found a sex-specific association. Increased levels of MYH9 were associated with lower levels of lipid content only in females (P=1.5×10^{-2}; Figure 6B; Figure S6B). The carotid plaques in this biobank were further classified based on plaque vulnerability and overall cellular composition to define fibrous versus atheromatous plaques and asymptomatic versus symptomatic plaques. We found a female-specific association between elevated MYH9 levels and fibrous plaques versus atheromatous plaques (P=1.0×10^{-2}; Figure 6C and Figure S6C). We then investigated the difference in MYH9 abundance in symptomatic and asymptomatic plaques and observed that MYH9 expression was higher in asymptomatic plaques (P=2.0×10^{-2}; Figure 6D and Figure S6D). Together, these data provide evidence that MYH9 plays a distinct and critical role in the development of fibrous plaques and more stable, asymptomatic plaque characteristics.

MYH9 Expression in SMCs and the Fibrous Cap Suggests a Role in Regulating Plaque Erosion–Related Phenotypes

We analyzed spatial transcriptomics data from asymptomatic (n=4) and symptomatic (n=4) carotid plaques obtained from endarterectomy samples (Supplemental Methods). *MYH9* expression was significantly upregulated in asymptomatic compared with symptomatic plaques (P<2.2×10^{-30}, fold change=1.62; Figure 7A; Figure S7A). Clustering of spatial transcriptomic profiles using the Leiden algorithm in Seurat[27] revealed that *MYH9* expression was highest within *MYH11*$^{+}$ SMCs (Figure 7B; Figure S7B). Figure 7C further demonstrates *MYH9* colocalization within the fibrous cap region, consistent with its enrichment in GRN$_{yellowgreen}$ and its potential involvement in maintaining fibrous plaque structure.

To functionally evaluate *MYH9*'s contribution to SMC behavior relevant to plaque erosion, we performed a ≈20% knockdown of MYH9 expression in VSMCs from 3 female and 3 male donors (Figure 7D). The level of *MYH9* reduction was chosen to approximate the sex difference in expression observed in human aortic tissue (STARNET cohort).[7] We performed Gene Set Enrichment Analysis of the RNA-seq data comparing male and female scramble controls (Figure 7E) and *MYH9* knockdown samples (Figure 7F). Gene Set Enrichment Analysis identified 6 pathways differentially regulated between male and female MYH9 knockdown cells at FDR≤0.1. There were no significant results at FDR≤0.05. However, 2 of these pathways—the Gleevec response pathway and the TPO (thrombopoietin) signaling pathway—were not differentially enriched in the scramble male versus female comparison, even under a relaxed threshold (FDR≤0.25). The Gleevec pathway, which is associated with inhibition of proliferation and modulation of cytoskeletal signaling,[57] and the TPO pathway, which regulates thrombosis and platelet activation,[58] both connect mechanistically to vascular remodeling and fibrous cap integrity. These results suggest that *MYH9* modulates sex-dependent SMC phenotypes in plaque stability and erosion through regulation of proliferative and thrombotic signaling pathways. Spatial localization and functional perturbation data further support a role for MYH9 in fibrous cap-associated

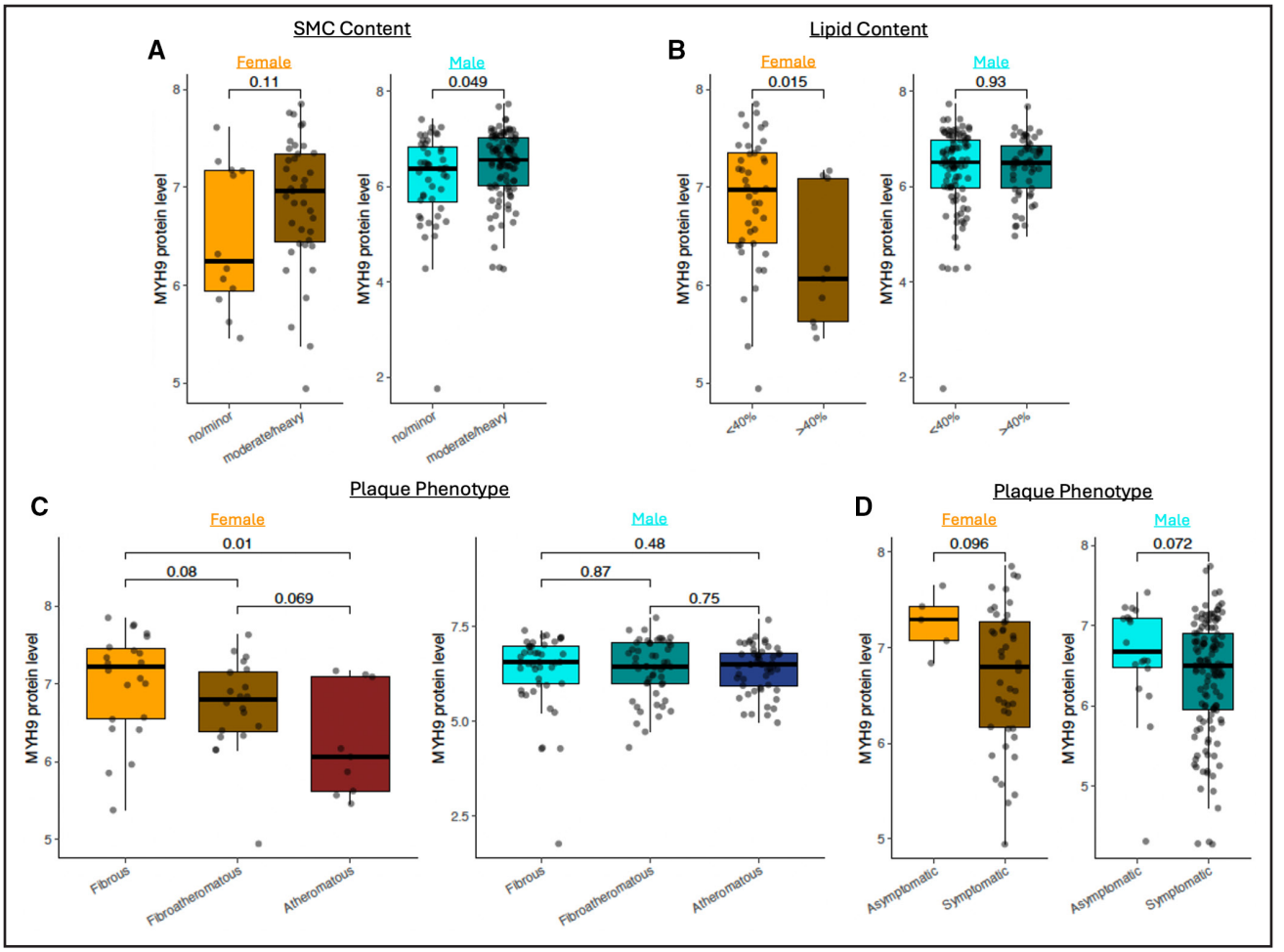

Figure 6. Associations between MYH9 (myosin heavy chain 9) protein abundance and carotid plaque characteristics. **A**, MYH9 protein levels in plaques with no/minor (female n=12, male n=48) and moderate/heavy ACTA2 (α-actin 2)+ cell content (female n=39, male n=101) in females (orange) and males (cyan). **B**, MYH9 protein levels in plaques with lipid core < 40% (female n=42, male n=93) or > 40% (female n=9, male n=56) of total plaque area. **C**, MYH9 levels across fibrous (female n=22, male n=44), fibroatheromatous (female n=20, male n=52), and atheromatous (female n=9, male n=53) plaques. **D**, MYH9 levels in asymptomatic (female n=5, male n=18) and symptomatic (female n=44, male n=127) plaques. *P* values were calculated using 2-tailed unpaired Mann-Whitney *U* tests (95% confidence). SMC indicates smooth muscle cell.

SMC behavior and female-biased mechanisms underlying stable, erosion-prone plaques.

DISCUSSION

While it is clear that males and females exhibit differences in plaque, the underlying molecular mechanisms remain unclear.[2] Recent studies investigated how GRNs generated from plaque tissue RNA-sequencing data differ between males and females in the development and presentation of atherosclerosis. Sex differences in tissue composition of atherosclerotic plaques have been described before.[59] Therefore, comparisons of RNA-sequencing data obtained from plaques may be confounded by cellular composition differences rather than gene expression levels in each cell type found in plaques. SMC content is a key deterministic feature distinguishing thin versus thick fibrous caps that influence plaque erosion and plaque rupture phenotypes.[17] Sex-stratified SMC GRNs can better detect the sex differences that exist on a cellular level to unravel molecular driving forces describing the higher prevalence of fibrous plaques in females.

In this study, we utilized RNA-sequencing data from human VSMCs isolated from male and female heart transplant donors, allowing us to construct sex-specific GRNs. Preservation statistics between GRNs can identify unique aspects of differential conditions that methods such as differential expression analysis and Gene Set Enrichment Analysis are unable to capture. Within this cohort of VSMCs, only 8 genes were shown to be differentially expressed between males and females.[60] On the other hand, our method of characterizing the differences in the topology of GRNs identified female-biased gene connectivity enriched for inflammatory pathways and vascular remodeling processes and associated

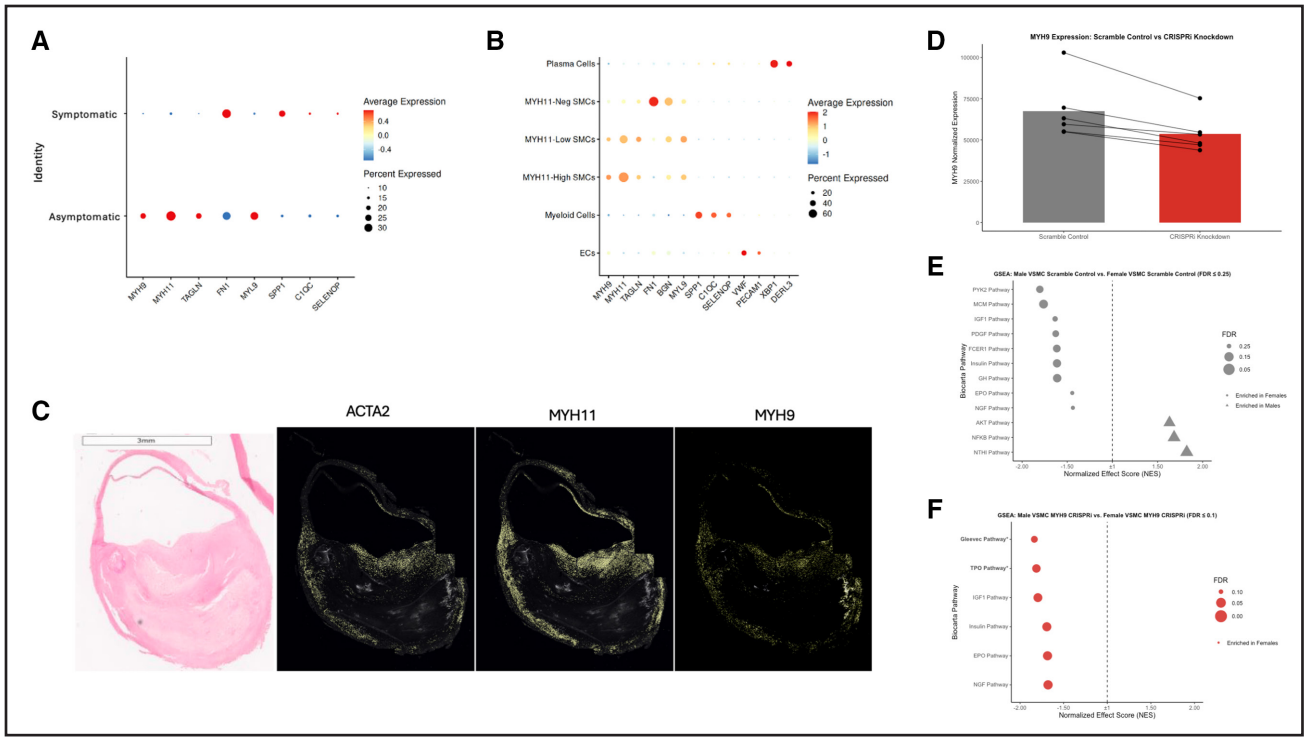

Figure 7. Spatial transcriptomics and clustered regularly interspaced short palindromic repeats interference (CRISPRi) perturbation implicate MYH9 (myosin heavy chain 9) plaque erosion–related phenotypes.
A, Xenium spatial transcriptomic analysis comparing *MYH9* and smooth muscle cell (SMC) marker expression between asymptomatic (n=4) and symptomatic (n=4) carotid plaques. *MYH9* was significantly upregulated in asymptomatic plaques (Wilcoxon rank-sum test, $P<2.2\times10^{-30}$, fold change=1.62). **B**, Average expression of *MYH9* and cell type marker genes across spatially resolved cell populations. **C**, Sirius Red staining and spatial transcriptomic expression of *ACTA2*, *MYH11*, and *MYH9* in a carotid plaque with a fibrous cap. Representative images were selected for clarity and signal quality and are intended to illustrate observed trends; all quantitative analyses were performed across the full data set. **D**, Normalized *MYH9* expression in vascular smooth muscle cells (VSMCs) after CRISPRi with scramble (n=6) or *MYH9*-targeting gRNAs (n=6). Black lines denote paired samples. Statistical significance was assessed using a paired Wilcoxon signed-rank test (18.1% reduction, $P=3.6\times10^{-2}$). **E**, Gene Set Enrichment Analysis (GSEA) of Biocarta pathways comparing male and female scramble controls (false discovery rate [FDR] ≤0.25). **F**, GSEA of Biocarta pathways comparing male and female *MYH9* CRISPRi knockdown samples (FDR≤0.10). Pathways uniquely enriched in the knockdown comparison are shown in bold. ACTA2 indicates α-actin 2; EC, endothelial cell; NFκb, nuclear factor kappa B; and TPO, thrombopoietin.

with key atherosclerosis-relevant phenotypes of calcification and TGF-β-induced proliferation. Each of these phenotypes is associated with sex-specific mechanisms of plaque development. Females have fewer coronary calcifications, leading to more stable lesions,[61] and TGF-β signaling responses are linked to sex hormone-specific regulation.[13] We were able to reproduce the female-biased GRN results within single-cell RNA-seq data. This analysis added a critical layer of validation to our approach and represents the power of utilizing network preservation statistics to identify sex differences. The single-cell RNA-seq data also supported the fibrous plaque-association hypothesis of $GRN_{yellowgreen}$, potentially pointing toward a role in regulating VSMC phenotypic switching. During the early stages of atherosclerosis, VSMCs undergo a transient dedifferentiation into a synthetic, proliferative, and migratory state to facilitate repair and matrix deposition.[10] Our findings support a hypothesis that after the initial response to injury and VSMC migration to contribute to fibrous cap formation, local in vivo cues seem to drive redifferentiation toward a more contractile, *ACTA2* and *MYH11* expressing phenotype. Several observations support this model. First, genes within $GRN_{yellowgreen}$ are highly responsive to extracellular matrix stiffness, consistent with the known mechanosensitive control of SMC differentiation. Second, TGF-β signaling strongly regulates this network, linking it to canonical pathways that promote SMC contractile gene expression and extracellular matrix remodeling.[62] Third, ovarian hormonal signaling exhibits a stronger association with $GRN_{yellowgreen}$ than testicular signaling, suggesting that female hormonal environments may enhance this differentiation program. Estrogen, for instance, has been linked to a protective effect on the vascular wall by inhibiting the proliferation and migration of VSMCs through MAPK/ERK (extracellular signal–regulated kinase) and PI3K/AKT (phosphoinositide 3-kinase/protein kinase B) pathways.[63] Collectively, these findings suggest that the $GRN_{yellowgreen}$ integrates ECM, TGF-β, and hormonal cues to stabilize SMCs in a fibrous, less migratory state,

providing a potential molecular basis for the observed sex differences in fibrous plaque phenotypes.

Further analysis of $GRN_{yellowgreen}$ using BN methods and proteomics identified MYH9 as a KD predicted to regulate actin, ECM, and vascular remodeling processes relevant to fibrous plaque progression in females. In carotid plaques, MYH9 was associated with a higher prevalence of fibrous plaques compared with atheromatous plaques in females. MYH9 may act as a central effector in this regulatory axis by controlling actin cytoskeletal dynamics and mechanotransduction. MYH9 could help translate mechanical and hormonal signals into transcriptional programs that promote SMC redifferentiation and fibrous cap formation. Consistent with this model, MYH9 knockdown in VSMCs perturbed the Gleevec-responsive pathway, which suppresses proliferation, and modulated the TPO pathway linking cytoskeletal remodeling to endothelial integrity and plaque erosion. Together, these findings suggest that the MYH9-TGFβ axis governs the transition of VSMCs from a proliferative state to a more fibrous, contractile phenotype, influencing plaque stability in a sex-specific manner. Future studies will be needed to determine how ECM stiffness, hormonal cues, and MYH9-dependent cytoskeletal remodeling interact to regulate fibrous cap formation and plaque erosion risk.

Deciphering the exact mechanisms of sex-specific contributions to atherosclerosis phenotypes associated with MYH9 requires further investigation. Sex chromosomes contribute to sex-specific gene expression through mechanisms such as X-inactivation, where certain genes escape inactivation on the X chromosome, leading to differential expression in females compared with males.[64] Protein correlations for MYH9 revealed RPS4X (ribosomal protein S4 X-linked) as the highest correlated protein in both sexes. *RPS4X*, an X-linked gene known to regulate translation, has been shown to escape X-inactivation in females and may help explain the differential protein regulation of MYH9 between females and males.[64] On the other hand, sex hormones could also play key mechanistic roles. Estrogen has been linked to delaying the formation and development of plaques[63] by inhibiting VSMC proliferation and migration. It has also been shown to enhance cholesterol efflux and reduce foam cell formation derived from VSMCs.[65] This could potentially describe the contractile and more fibrous phenotype associated with $GRN_{yellowgreen,}$ as well as the female-specific association between MYH9 and fat content within plaques. The interplay between sex chromosomes and sex hormones likely creates a multifaceted regulatory environment that shapes atherosclerotic disease progression in a sex-specific manner.

Quantifying VSMC phenotypes in cell culture relevant to atherosclerosis has inevitable limitations. Culture conditions lack the key interactions with other cell types and environmental conditions in the vessel wall and atherosclerotic plaque. Previous scRNA-seq of human atherosclerotic plaques has implicated SMCs, particularly SMC phenotypic switching, as a major contributor to the sex differences of CAD, motivating our continued investigation.[7,8] However, endothelial cells and immune cells also play a key role in plaque formation and stability.[3] It is unknown if these sex-specific regulators of CAD involve crosstalk between cell types. As MYH9 is involved in mechanotransduction and cell-cell adhesion, it is possible that the sex-specific regulation could propagate to other cell types.[56] In addition, atherosclerosis takes decades to develop; therefore, it cannot be adequately replicated in vitro. Animal models, particularly $ApoE^{-/-}$ and $Ldlr^{-/-}$, have been instrumental in studying genetic and molecular drivers of atherosclerosis, as they reliably develop lipid-rich lesions. However, these models do not fully recapitulate the advanced human plaque phenotypes of fibrous cap thickening, erosion, or rupture. Mouse plaques rarely undergo spontaneous thrombosis, and the mechanical and cellular hallmarks of plaque erosion are absent in these systems. As a result, there remains a gap in our ability to model sex-biased fibrous plaque and erosion phenotypes in vivo, reinforcing the value of our human VSMC-based approach.

Cultured human artery SMCs have been successfully used in previous studies to investigate genetic determinants of CAD.[66] Due to the difficult nature of capturing vascular wall phenotypes in cellular detail in the arteries of humans and the current limitations in murine models, our approach, studying cultured VSMCs isolated from an ancestrally diverse population of 151 heart transplant donors, provides a reasonable proxy for in vivo characteristics of VSMCs. However, detailed donor-level metadata such as age, cardiovascular risk factors, and medication were not available within this cohort, restricting our ability to assess confounding factors. In addition, the sample size for female donors was relatively small (n=32), which may limit the generalizability of our findings. However, this limitation guided our efforts to validate our findings in independent data sets, increasing confidence in our major findings. To our knowledge, this work is the first to propose a comprehensive approach exploring GRNs observed in female VSMCs that are not preserved in male VSMCs. There may be male-specific contributions to plaque rupture phenotypes that we did not investigate. Our findings should be considered under the current limitations of employing systems genetics and network analyses. There are many decisions made during the model-building phase and prioritization of GRNs that can affect the results and conclusions. Further, several analytical limitations should be considered when interpreting these results. FDR thresholds were selected based on analytic intent, with more liberal cutoffs (eg, FDR≤0.25) used for initial discovery analyses to avoid excluding potentially relevant pathways, and more stringent thresholds (FDR≤0.05) applied in downstream, hypothesis-driven validation steps. In addition, some RNA-seq analyses incorporated small sample

sizes, limiting statistical power and precision; these analyses were therefore used to support trends and inform hypothesis-driven experiments rather than serve as standalone evidence.

Our study highlights the role of sex-specific GRNs in VSMC biology and their contribution to fibrous plaque formation and plaque erosion. Using a multidimensional approach, we identify MYH9 as a KD of actin and vascular remodeling processes that regulate SMC-rich fibrous plaques. Further investigation is needed to define the mechanistic relationship between MYH9 and TGFβ signaling in promoting stable fibrous caps associated with plaque erosion. Additional studies addressing the limitations of in vitro models, small sample sizes, and sex-specific contributions to other plaque phenotypes, including plaque rupture, will be important. Greater insight into how sex chromosomes, sex hormones, and GRNs interact will improve our understanding of sex differences in atherosclerosis and may inform more targeted strategies for the prevention of cardiovascular disease.

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Author Contributions

R.N. Perry and M. Civelek conceived and designed the study. R.N. Perry and G. Lenert performed computational/statistical analyses (gene regulatory networks, preservation, Bayesian networks). R.N. Perry and N. Barbera conducted experimental analyses and interpreted results. E.D. Benavente, M. Mokry, D.P.V. de Kleijn, M.P.J. de Winther, and H.M. den Ruijter oversaw and interpreted AtheroExpress data. A. Bölük, T. Örd, M. Taipale, N. Sachs, J. Pauli, L. Maegdefessel, and M.U. Kaikkonen oversaw and interpreted spatial transcriptomics data. L. Ma. and J.L.M. Björkegren oversaw and interpreted STARNET (Stockholm-Tartu Atherosclerosis Reverse Network Engineering Task) data. M. Mokry led proteomics data collection/oversight. R. Hernandez, V. Mendoza, and K. Reue oversaw the Four-Core Genotype mouse data. H.M. den Ruijter and M. Civelek supervised the study. R.N. Perry drafted the manuscript; all authors reviewed, edited, and approved the final version.

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Disclosures

None.

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