J Lipid Atheroscler. 2025 Sep;14(3):372-383
https://doi.org/10.12997/jla.2025.14.3.372
pISSN 2287-2892·eISSN 2288-2561

JLA Journal of Lipid and Atherosclerosis

Original Article

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Factor XI Deficiency in apoE/FXI Double-Knockout Mice Decreases Atherosclerosis by Lowering *MSR1* mRNA Expression Within the Plaque

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Received: Feb 12, 2025
Revised: Mar 18, 2025
Accepted: Mar 30, 2025
Published online: Jun 5, 2025

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ABSTRACT

Objective: Atherosclerosis remains a leading cause of global morbidity and mortality despite cholesterol-lowering drugs and risk factor management. In mice, the absence of coagulation factor XI (FXI) on an apolipoprotein E (apoE)$^{-/-}$ genetic background has been shown to reduce atherosclerosis. This study examined the molecular pathways through which FXI influences the development of atherosclerosis.

Methods: Laser capture microdissection of plaques from the aortic sinus of apoE/factor XI double knockout (DKO) and apoE knockout (KO) mice was performed at 24 weeks. RNA-seq and the NanoString technique were used to analyze the extracted plaques. Immunohistochemical analysis of the atherosclerotic layers was also conducted.

Results: Among 15,353 expressed genes, 64 showed significant differences between the 2 groups. Gene set enrichment analysis, specifically targeting metabolic pathways, identified upregulation of 8 pathways in atherosclerotic plaques of apoE KO mice; seven of these pathways were classified as related to inflammatory processes. Using an immunological panel containing 547 genes linked to inflammatory and immunological processes, a statistically significant difference was observed in the expression of macrophage scavenger receptor 1 (*MSR1*) between DKO mice and apoE KO mice (adjusted *p*-value=0.0015).

Conclusion: Downregulated expression of *MSR1* within the atherosclerotic plaques of apoE/FXI DKO mice compared to apoE KO mice was associated with significantly reduced atherosclerosis. Targeting FXI may therefore represent a promising anti-atherogenic therapeutic strategy in addition to its known antithrombotic effects.

Keywords: Factor XI; Atherosclerosis; Mice; *Msr1* protein

JLA Journal of Lipid and Atherosclerosis

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Funding
None.

Conflict of Interest
The authors have no conflicts of interest to declare.
While preparing this work, the authors used "Grammarly" to improve the manuscript's grammar. After using this tool/service, the author(s) reviewed and edited the content as needed and take full responsibility for the publication's content.

Data Availability Statement
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions
Conceptualization: Harats D, Shaish A, Salomon O; Data curation: Shnerb Ganor R, Paraboschi EM, Asselta R, Salomon O; Formal analysis: Shnerb Ganor R, Paraboschi EM, Stefano D, Asselta R, Schiby G, Steinberg DM, Salomon O; Investigation: Shnerb Ganor R, Paraboschi EM, Stefano D, Asselta R, Schiby G, Leichner GS, Anekstein Spigel M, Tamarin I, Kandel Kfir M, Shaish A, Salomon O; Writing - original draft: Shnerb Ganor R, Shaish A, Salomon O; Writing - review & editing: Paraboschi EM, Harats D, Asselta R, Steinberg DM, Shaish A, Salomon O.

INTRODUCTION

Atherosclerosis is a chronic inflammatory disease and continues to be the leading cause of morbidity and mortality despite considerable progress in early diagnosis and management of major risk factors, including cholesterol-lowering medication, blood pressure control, obesity management, and smoking cessation.[1] The risk of developing atherosclerosis is no longer confined primarily to Western nations but has expanded globally, increasingly affecting younger populations and women.[2]

Atherosclerotic lesions are characterized by the lifelong accumulation and transformation of lipids, foam cells, inflammatory cells, vascular endothelial cells, smooth muscle cells, extracellular matrix, necrotic cellular debris, and surrounding fibrotic tissue beneath a monolayer of endothelial cells lining the vessel lumen.[3,4] Progressively enlarging lesions may critically restrict blood flow to vital organs, potentially causing myocardial infarction or ischemic stroke. Additionally, the chronic condition of atherosclerosis can rapidly transition into acute events when plaque rupture leads to atherothrombosis and thromboembolic incidents.[1]

In contrast to atherothrombosis, where coagulation factors have an established role, the association between coagulation factors and atherosclerosis itself remains unclear, despite coagulation factors being identified within atherosclerotic plaques.[5,6] Previously, we demonstrated that whole-body deletion of factor XI (FXI) protects apolipoprotein E (apoE) knockout (KO) mice—a well-established murine model of atherosclerosis— from developing atherosclerosis in both young and older mice. In these models, FXI deficiency resulted in decreased macrophage infiltration and inflammation within the lesions, although it did not affect fasting plasma cholesterol and triglyceride levels or their lipoprotein distributions.[7,8]

Our results are supported by a recent study showing that pharmacological targeting of FXI using anti-FXI antibody (14E11) or FXI-antisense oligonucleotide (ASO) reduced atherogenesis without altering plasma lipid levels in a low-density lipoprotein receptor knockout ($LDlr^{-/-}$) mouse model of atherosclerosis. However, unlike our model, no change was noted in monocyte infiltration within lesions in that study.[9]

The association of FXI with vascular disease is evident: high levels of FXI correlate with increased risk of cerebrovascular events, whereas reduced FXI levels are associated with decreased incidence of cardiovascular events.[10-12] Nevertheless, the mechanisms by which FXI affects cardiovascular risk remain uncertain.

Given that FXI deficiency, achieved through various methods (e.g., genetic KO, direct antibodies, or FXI antisense oligonucleotides) consistently reduces atherosclerosis in two distinct, established animal models, we sought to investigate the molecular mechanisms underlying this protective effect. To this end, we analyzed mRNA expression within isolated atherosclerotic plaques from the aortic sinus using total RNA sequencing (RNAseq) and NanoString digital counting. We compared mRNA expression between apoE KO and apoE/FXI double knockout (DKO) mice following plaque extraction by laser capture microdissection (LCM).

https://doi.org/10.12997/jla.2025.14.3.372

JLA Journal of Lipid and Atherosclerosis

MATERIALS AND METHODS

1. Mice

ApoE KO mice on a C57BL/6 genetic background (Jackson Laboratory) and apoE/FXI DKO mice—created by crossbreeding apoE KO mice with FXI-deficient mice (FXI KO) on a C57BL/6 background (provided by Prof. David Gailani, Vanderbilt University, Nashville, TN, USA)— were maintained on a chow diet with water provided ad libitum. All animal protocols were approved by the Animal Care and Use Committee of Sheba Medical Center, Tel-Hashomer (approval numbers: 1275/20, 1042/16, 1297/21).

Mice were sacrificed at 24 weeks of age by exposure to isoflurane overdose, administered in a glass jar until complete cessation of respiration was observed. Mice were then monitored for an additional minute to confirm absence of respiration. After perfusion with phosphate-buffered saline containing an RNase inhibitor, hearts were dissected from surrounding tissues. The upper portion of each heart was embedded in OCT compound (Tissue-Tek; Sakura) on dry ice and stored at −80°C until further analysis.

2. LCM

LCM was performed as previously described[13]with some modifications. Sections of 7–10 μm thickness throughout the aortic sinus were transferred to polyethylene naphthalate membrane slides (Zeiss). Slides were air-dried at room temperature for 90 seconds, washed in 75% ethanol for 30 seconds, and then rinsed in diethyl pyrocarbonate (DEPC)-treated water for 30 seconds. Sections were stained with HistoGene Staining Solution (Thermo Fisher Scientific) for 40 seconds, washed again in DEPC-treated water for 30 seconds, and dehydrated sequentially in 75%, 90%, and 100% ethanol for 30 seconds each. Slides were air-dried for 5 minutes before microdissection. Atherosclerotic lesions were isolated using the PALM Microbeam Laser Microdissection system (Zeiss). The LCM software calibrated laser energy automatically and allowed manual fine-tuning of the laser focus to match the tissue sections. The atherosclerotic regions on each section were identified and labeled manually under a 10× objective lens. Each region of interest was cut (energy=53, focus=82) and catapulted (energy=68, focus=82) onto an adhesive cap of a 0.2 mL tube (Zeiss). To minimize RNA degradation, all sections were collected within 50 minutes, after which 20 μL of RNA Lysis Tissue buffer with β-mercaptoethanol was added to the cap and incubated for 30 minutes, followed by centrifugation at 13,400 × *g* for 5 minutes.

3. RNA extraction

RNA was extracted using the RNeasy Micro Kit (Qiagen Inc.). RNA concentration and quality were evaluated using a bioanalyzer equipped with the RNA 6000 Pico Kit (Agilent Technologies). Only samples with an RNA integrity number greater than 8 were selected for library preparation and subsequent sequencing.

4. RNAseq

Library preparation for RNAseq was conducted using the Ovation Solo RNA-Seq system (NuGen). The quality of sequencing libraries was assessed using the Bioanalyzer instrument and a High Sensitivity DNA Chip (Agilent Technologies).

Sequencing was performed at the Department of Genomic Research, University of Illinois, Chicago, using a NovaSeq 6000 instrument (Illumina Inc.) with a paired-end reading strategy of 150 bp and approximately 500 million total reads.

https://doi.org/10.12997/jla.2025.14.3.372

For differential gene expression analysis, STAR software (v.2.7.0a)[14] was used to map sequencing reads to the mouse genome (GRCm38) and quantify transcripts based on mouse transcript annotations from the Ensembl database (GRCm38). Differential expression analysis was carried out using R software[15] and the DESeq2 package.[16] Only genes with at least 5 reads per sample were retained for further analysis. Principal component analysis was conducted following a variance-stabilizing transformation to evaluate sample similarity and potential confounding effects. Data were normalized using DESeq2's median-of-ratios method, accounting for sequencing depth and RNA composition. Genes were considered differentially expressed if their adjusted *p*-value was below 0.05. Upregulated and downregulated genes were classified based on their $\log_2$-fold change ($\log_2$FC) values ($\log_2$FC >0 or $\log_2$FC <0, respectively).

Gene Set Enrichment Analysis (GSEA) (v.4.0.3) was used for enrichment analysis.[17] Gene ontology sets derived from biological processes ontology, comprising 7,481 gene sets, were analyzed for enrichment using RNAseq results. DESeq2-normalized counts served as the input for GSEA, following software guidelines.

Immune infiltration analysis was conducted using ImmQuant software,[18] employing a deconvolution approach on bulk RNAseq data. A sample gene expression matrix and mouse immunological signatures were used to infer immune cell populations based on signature markers.

5. nCounter panel

One nanogram of RNA extracted from LCM-isolated tissue underwent amplification using the nCounter Low RNA Input Amplification Mouse Kit (NanoString; https://nanostring.com/products/ncounter-assays-panels/immunology/immunology-panel). Amplification was performed using primers corresponding to immunological genes included in the panel.

Amplified products were hybridized for 19 hours with probes targeting 561 genes (547 immunological genes and 14 normalization genes). Samples were then immobilized onto the nCounter cartridge, and digital counting of individual target molecules was performed using the nCounter Digital Analyzer.

6. Immunohistochemistry

Immunohistochemical staining of T cells in atherosclerotic plaques was performed using a rabbit anti-CD3 antibody (1:100 dilution; Dako) on 5 μm-thick sections from the aortic sinus. The protocol included blocking with CAS-Block (Thermo Fisher Scientific) for 1 hour, incubation with primary antibody for 1 hour, treatment with secondary horseradish peroxidase-conjugated goat anti-rabbit antibody (1:100 dilution; Abcam) for 1 hour, and color development with 3-amino-9 ethylcarbazole solution for 12 minutes.

7. Statistical methods

Expression levels for each gene were compared using the Wilcoxon test with *p*-values adjusted for multiplicity. A predefined list of 32 genetic pathways was analyzed for consistent upregulation or downregulation by averaging individual Wilcoxon statistics across genes within each pathway; raw p-values were computed through comparison to 10,000 random partitions of mice into two groups of 11 and then adjusted for multiplicity. Single genes surpassing the 0.05 adjusted p-value threshold triggered additional analysis of related gene groups to confirm that the observed associations were not isolated events but part of broader changes. Identified genes also prompted the analysis of specific related genes to evaluate their co-regulation. All

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analyses used a significance threshold of 0.05 for adjusted *p*-values and were conducted using the R Statistical Computing Environment (R Foundation for Statistical Computing).

RESULTS

1. FXI deficiency in mice reduced the expression of genes associated with inflammatory pathways within the atherosclerotic plaque

To investigate how FXI deficiency in mice influences gene expression within atherosclerotic plaques, we utilized LCM to isolate the entire plaque region from the aortic sinus (**Fig. 1**) of 24-week-old apoE/FXI DKO mice, using apoE knockout KO mice as controls. At this age, DKO mice exhibited a reduced lesion area compared to apoE KO mice. Previously, we measured plaque area in 24-week-old mice and observed a 32% reduction in atherosclerotic plaque area in the aortic sinus of DKO mice relative to apoE KO mice (p=0.004).[7] RNA was then extracted from the isolated plaques, and RNAseq was conducted to analyze differential gene expression profiles (**Fig. 1**).

A representative image in **Figure 1** shows a section of the aortic sinus stained with HistoGene Staining Solution at 40× magnification. The lower panel depicts a selected atherosclerotic region at 100× magnification before (left) and after (right) microdissection with LCM. The dashed line marks the boundary of the plaque area isolated from the surrounding tissue.

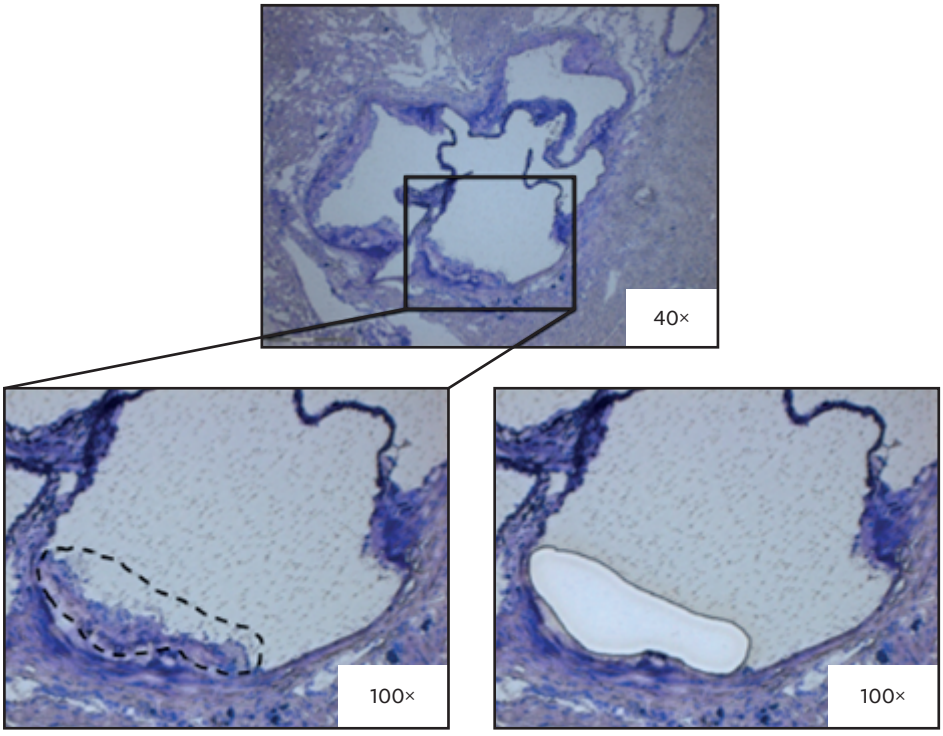

Fig. 1. Isolation of an atherosclerotic plaque from tissue using LCM.
We employed LCM to isolate the entirety of atherosclerotic plaques from the aortic sinus of 24-week-old DKO mice. The accompanying image illustrates a section of the aortic sinus stained with Histogen Staining Solution at 40× magnification. In the lower panel, 100× magnification shows an atherosclerotic region before (left) and after (right) sectioning with LCM. The dashed line delineates the area of the atherosclerotic plaque designated for isolation from the surrounding tissue.
LCM, laser capture microdissection; DKO, double knockout.

https://doi.org/10.12997/jla.2025.14.3.372

JLA Journal of Lipid and Atherosclerosis

The final RNAseq analysis included nine samples: 5 from apoE KO mice and 4 from DKO mice. One DKO mouse sample was excluded due to insufficient alignment with known genome sequences.

Out of 15,353 analyzed genes, 64 showed significant differential expression (adjusted *p*-value <0.05) between the two mouse groups. In DKO mice, 24 genes were significantly upregulated, while 40 genes were significantly downregulated compared to apoE KO mice (**Fig. 2**).

Gene set enrichment analysis (GSEA) identified eight significantly upregulated pathways (false discovery rate [FDR] <0.25) within the atherosclerotic plaques of apoE KO mice. Seven of these pathways are involved in inflammatory processes, specifically: alpha beta T-cell proliferation, natural killer cell-mediated immunity, T-cell differentiation, lymphocyte-mediated immunity, regulation of lymphocyte-mediated immunity, and positive regulation of interferon gamma production (**Table 1**).

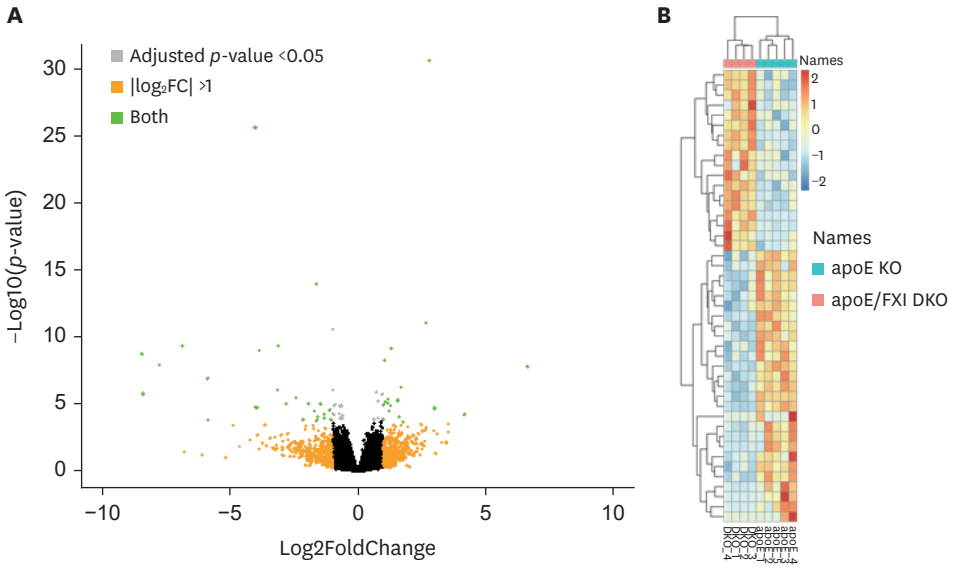

Fig. 2. Differences in gene expression in the atherosclerotic plaque.
(A) Volcano plot illustrating gene expression in the atherosclerotic plaques of DKO mice (n=4) compared to apoE KO mice (n=5). Gray points represent genes with adjusted *p*-value <0.05; orange points represent genes with a fold change greater than 2 or less than 0.5 between the groups; and green points represent genes affected by both conditions. 64 genes were expressed differently in apoE/FXI DKO mice than in apoE KO mice. **Table 1** lists the biological pathways where these genes were found. (B) Heatmap of 45 significantly differentially expressed genes between the 2 genotypes (adjusted *p*-value <0.05, $|\log_2 FC| > 1$).
DKO, double knockout; apoE, apolipoprotein E; KO, knockout; FXI, factor XI; $\log_2 FC$, $\log_2$-fold change.

Table 1. List of enriched pathways (FDR <0.25) as determined by GSEA on the transcriptomic data

Name	Size	NES	Nominal *p*-value	FDR *q*-value
Organic hydroxy compound catabolic process	41	−1.93	0	0.16
Alpha beta T cell proliferation	27	−1.87	0	0.18
Natural killer cells-mediated immunity	39	−1.71	0	0.25
T cell differentiation	203	−1.71	0	0.24
Lymphocyte mediated immunity	185	−1.71	0	0.23
Regulation of lymphocyte-mediated immunity	101	−1.70	0.02	0.23
Positive regulation of interferon gamma production	48	−1.70	0.01	0.22
Regulation of lymphocyte differentiation	138	−1.69	0	0.24

These gene sets originate from biological pathways in Gene Ontology.
FDR, false discovery rate; GSEA, Gene Set Enrichment Analysis; NES, normal enrichment score.

https://doi.org/10.12997/jla.2025.14.3.372

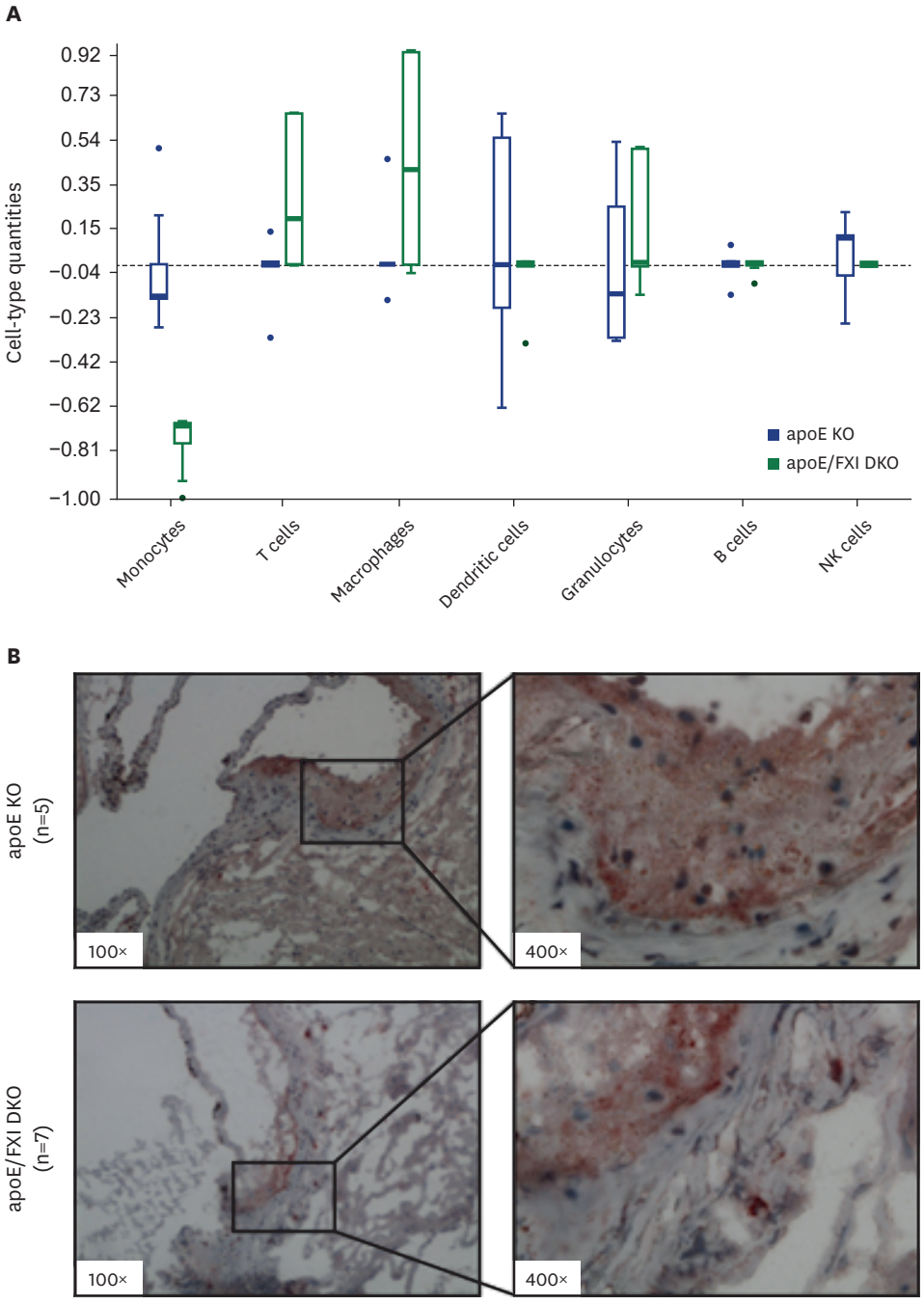

Fig. 3. Decrease in monocyte infiltration in the atherosclerotic plaque of apoE/FXI DKO mice compared to apoE KO mice.
(A) Evaluation of leukocyte infiltration into the atherosclerotic plaque of apoE/FXI DKO and apoE KO mice (n=9) was performed using ImmQuant software. Each box represents the interquartile range, the bold line represents the median, the vertical lines represent the minimum and maximum, and the dots represent outlier values. Significant difference is indicated by an asterisk next to the cell type. (B) Sections representing the atherosclerotic plaque area in the aortic sinus after immunohistochemical staining with CD3 antibody (a marker of T cells). The images on the right are magnified (400×) views of the areas marked by squares in the pictures on the left (100×).
apoE, apolipoprotein E; FXI, factor XI; DKO, double knockout; KO, knockout.

Furthermore, analysis of sequencing data indicated reduced monocyte infiltration within the atherosclerotic plaques of DKO mice compared to apoE KO mice (**Fig. 3**). No significant differences were observed in the infiltration of other cell types (macrophages, T cells,

https://doi.org/10.12997/jla.2025.14.3.372

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dendritic cells, granulocytes, B cells, or natural killer cells). This finding was consistent with immunohistochemical observations showing only sparse T lymphocytes localized outside the atherosclerotic plaques, with no notable difference in their abundance or distribution between DKO and apoE KO control mice (**Fig. 3**).

2. The absence of FXI in mice significantly reduced the expression of macrophage scavenger receptor 1 (*MSR1*) in atherosclerotic plaques

RNAseq results showed decreased expression of genes related to inflammatory processes in DKO mice, prompting further evaluation of inflammatory gene expression using an independent method. For this purpose, samples were analyzed using an immunological panel (NanoString) that assesses 547 genes associated with inflammatory and immunological responses.

In total, 11 mice from each genotype (DKO and apoE KO) were analyzed. Among the 547 examined genes, only *MSR1* showed a statistically significant difference between the groups, with substantially lower expression in DKO mice than in apoE KO control mice (adjusted *p*-value=0.0016).

On average, DKO mice had 98 copies of *MSR1* RNA molecules, whereas apoE KO mice had 3,575 copies of the gene (**Fig. 4**). This result prompted further investigation into whether the MSR1 association reflected broader changes in macrophage-related gene pathways. Therefore, we evaluated expression levels of 20 additional macrophage-specific genes using NanoString: *CD14*, *CD36*, *CD163*, *CD1d1*, *Clec4e*, *Emr1*, *Fcgr1*, *Fcgr2b*, *Fcgr3*, *Fcgr4*, *Fcgrt*, *Icam2*, *Itgam*, *Ly86*, *Ly96*, *Marco*, *S100a8*, *S100a9*, *Trem1*, and *Trem2*. At a group level, no association was found for macrophage genes (p=0.36). Individually, the lowest unadjusted *p*-value observed was 0.023, which did not approach the 0.05 FDR significance threshold after correction.

Moreover, none of the 32 predefined immunological pathways demonstrated convincing or consistent differences between the DKO and apoE KO mouse groups. All unadjusted *p*-values for these pathway analyses exceeded 0.04, clearly surpassing the established FDR threshold (**Fig. 4**).

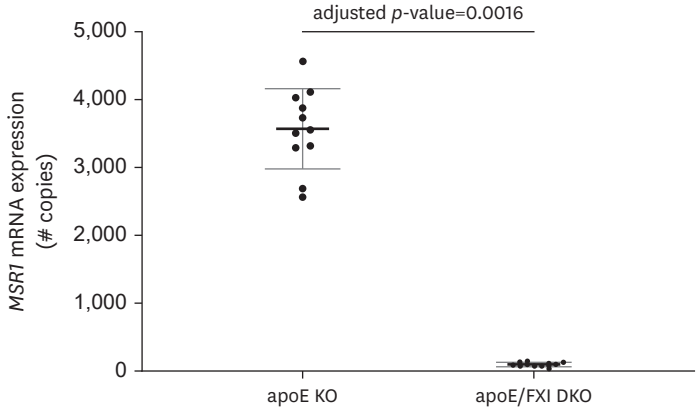

Fig. 4. Low expression of the *MSR1* gene in the atherosclerotic plaque of DKO mice compared to apoE KO mice.
MSR1 gene expression in atherosclerotic plaques of apoE KO and apoE/FXI DKO mice at 24 weeks was measured using NanoString. Each data point on the graph shows *MSR1* mRNA quantity, with error bars indicating mean ± standard error. Two independent experiments were conducted (n=11). The Wilcoxon test adjusted by the false discovery rate addressed multiple comparisons at a 0.05 threshold.
MSR1, macrophage scavenger receptor 1; DKO, double knockout; apoE, apolipoprotein E; KO, knockout; FXI, factor XI.

https://doi.org/10.12997/jla.2025.14.3.372

JLA Journal of Lipid and Atherosclerosis

Fig. 4 illustrates *MSR1* gene expression levels measured by the NanoString immunological panel in atherosclerotic plaques of apoE KO and apoE/FXI DKO mice at 24 weeks of age. Each data point represents the number of *MSR1* mRNA molecules in an individual sample. Error bars denote group means ± standard error. Results represent 2 independent experiments (n=11 per group). Statistical significance was assessed via the Wilcoxon test, adjusted by FDR, with a significance threshold of 0.05.

3. Genes that influence *MSR1* expression

Since *MSR1* expression was significantly lower in DKO mice than in apoE KO mice, we further assessed the expression of genes known to either decrease *MSR1* expression (*TNFα*, *IFNγ*, *TGFβ*, and *PPAR-γ*) or increase its expression (*PDGF*).[19] According to RNAseq data, only *TGFβ* and *PDGF* reached nominal significance based on uncorrected *p*-values in DKO mice but failed to meet the FDR-corrected significance threshold, similar to the other genes examined.

The NanoString panel included only *PPAR-γ* among these genes; however, no significant difference in *PPAR-γ* expression was observed between DKO and apoE KO mice (p=0.52).

DISCUSSION

In a previous study, we demonstrated that FXI deficiency in apoE KO mice reduces atherosclerosis in both young and old mice. To better understand how FXI deficiency confers protection against atherosclerosis, we performed RNA-seq analysis of 15,353 genes derived from atherosclerotic plaques in the aortic sinus of apoE KO and apoE/FXI DKO mice. We identified 8 downregulated pathways in FXI-deficient mice, 7 of which are associated with inflammatory processes according to GSEA. Additionally, sequencing data showed decreased monocyte infiltration within the plaques of DKO mice, consistent with our earlier immunohistochemical observations.[7]

In contrast to the current study, which found reduced monocyte infiltration, Ngo et al.[9] reported no decrease in monocyte infiltration into atherosclerotic lesions within the aorta following treatment with anti-FXI antibody 14E11 or FXI-ASO in a 16-week-old $Ldlr^{-/-}$ mouse model, as indicated by levels of the macrophage marker CD68. However, it is important to note that their study utilized a different mouse model and initiated FXI reduction at eight weeks of age, unlike our approach, where FXI was absent throughout development due to genetic deletion. Nonetheless, another study using an $apoE^{-/-}$ mouse model of plaque rupture (18–20 weeks old, fed a high-fat diet) demonstrated reduced macrophage infiltration into thrombi but not within the underlying plaques.[20]

The observed reduction in expression of immunoglobulin-related and inflammatory pathway genes, coupled with reduced monocyte infiltration in atherosclerotic plaques of DKO mice as identified by RNAseq, prompted us to investigate inflammatory gene expression further using a NanoString immunological panel. Of the 547 inflammatory and immunological genes assessed, only one (*MSR1*) showed almost complete downregulation in DKO compared to apoE KO mice. MSR1 is part of the scavenger receptor class A family, including macrophage type I and II (*MSRA*). During macrophage differentiation, human monocytes predominantly express type I MSR, which is critical for foam cell formation, a key process in atherogenesis. *MSR1* is abundantly expressed in macrophages, expressed to a lesser degree in monocytes, and absent from aortic endothelium in mice and humans.[19] However, *MSR1* is also present in

https://doi.org/10.12997/jla.2025.14.3.372

foam cells and smooth muscle cells within the plaque.[21] It is hypothesized, based on *in vitro* studies, that *MSRA* mediates macrophage interactions with endothelial cells, smooth muscle cells, other macrophages, and extracellular matrix components within plaques, potentially contributing to macrophage recruitment and retention.[19]

The reduced *MSR1* expression in plaques from DKO mice might initially be attributed to fewer macrophages due to FXI deficiency. However, analysis of an additional 20 macrophage-related genes using NanoString technology did not show any significant effect associated with FXI deficiency. Therefore, it is likely that decreased *MSR1* expression results specifically from FXI-mediated regulation rather than simply from fewer macrophages in plaques. Since RNA was extracted from the entire plaque rather than from individual cell types, it is not possible to definitively assign *MSR1* expression changes to a particular cell population. Thus, the decrease in *MSR1* expression might have resulted from reduced expression in other plaque cells, such as smooth muscle cells. Analysis of RNAseq data for 5 genes known to regulate *MSR1* expression did not reveal significant differences between the groups.

The crucial role of *MSR1* in atherogenesis was initially established by Suzuki et al.,[22] who reported a 58% reduction in lesion size in *MSRA*/apoE double knockout mice compared to apoE KO mice.[23] Interestingly, deletion of *MSRA* in Ldlr$^{-/-}$ mice fed a high-fat diet resulted in only a 23%–28% reduction in aortic lesion area,[24] while lethally irradiated C57BL/6 mice transplanted with *MSRA* KO marrow and challenged with a butterfat diet demonstrated a 60% lesion reduction compared to wild-type *MSRA* recipients.[23]

Our research demonstrates for the first time that circulating FXI regulates inflammation-associated genes in atherosclerotic plaques, specifically influencing *MSR1* expression. The substantial decrease in *MSR1* gene expression observed in the plaques of FXI-deficient mice, compared to other macrophage-associated genes, strongly suggests that the difference is unlikely due to a reduced number of macrophages.

Exploring whether the protective effect against atherogenesis observed in apoE/FXI DKO mice surpasses that seen in *MSRA*/apoE DKO mice would be challenging, particularly given the moderate increase in plasma cholesterol associated with *MSRA* deficiency on an apoE-deficient background.[19] In contrast, no difference in plasma cholesterol was observed between apoE and apoE/FXI DKO mice in our previous study.[7]

Unlike *MSRA* KO mice, which exhibit compromised macrophage antibacterial activity and increased susceptibility to sepsis, FXI KO mice have not been challenged with lethal bacterial or viral pathogens. Nevertheless, reduced *MSR1* expression in apoE/FXI DKO mice does not appear to compromise host defense, as these mice have normal lifespans.[22]

A limitation of this study is that residual atherosclerotic lesions in apoE/FXI DKO mice suggest involvement of other scavenger receptors in atherogenesis beyond *MSR1* alone.[22] Additionally, because the plaque core was not analyzed for non-inflammatory genes via NanoString, we may have overlooked genes potentially involved in atherogenesis. Evidence strongly indicates pro-atherogenic roles for T helper 1 cells and anti-atherogenic roles for regulatory T cells.[25] Since we found no difference in total plaque-associated T cells between groups, we did not characterize T cell subsets, representing another limitation and underscoring the need for further investigation. Immunohistochemical analysis revealed few T lymphocytes outside the atherosclerotic core, with no significant difference in number or localization between the 2 groups.

https://doi.org/10.12997/jla.2025.14.3.372

Ultimately, targeting FXI may offer anti-atherogenic properties in addition to established antithrombotic effects. However, further studies in human subjects are necessary to confirm these findings.

ACKNOWLEDGEMENTS

We honor the late Professor Duga for his significant contributions to FXI genetics and vascular biology.

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