

# Transcriptional Sex Dimorphism in Human Atherosclerosis Relates to Plaque Type

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RESEARCH LETTER

# Transcriptional Sex Dimorphism in Human Atherosclerosis Relates to Plaque Type

Han Jin , Barend M.E. Mees , Erik A.L. Biessen ,\* Judith C. Sluimer \*

Recently, biological sex-related transcriptomic differences between postmenopausal women and men were derived from human aortic atherosclerotic plaques of the STARNET (Stockholm-Tartu Atherosclerosis Reverse Network Engineering Task) cohort.<sup>1</sup> Unfortunately, sex-specific differences in plaque phenotype were not investigated. Yet, atherosclerotic plaques from males more often exhibit vulnerability traits, that is, necrosis, inflammation, and vascularization, and plaques from females more frequently demonstrate stability traits, that is, fibrosis and smooth muscle cells.<sup>2</sup> Thus, we explored if the identified sex-specific genes possibly also relate to plaque phenotype or sex-hormone response.

We interrogated the identified sex-specific genes in our independent, all-male MaasHPS (Maastricht Human Plaque Study) transcriptomics cohort of carotid endarterectomy plaques, comparing 27 unstable plaque segments with intraplaque hemorrhage to 16 stable segments without intraplaque hemorrhage by quantitative plaque phenotyping and microarray transcriptomics per segment (GEO163154, 23 males, aged  $72.9 \pm 6.3$  years). Clinical and analytical details have been reported.<sup>3</sup> We identified 6,302 differentially expressed genes (MaasHPS plaque phenotype-specific genes, adjusted  $P < 0.01$ ), 3030 upregulated in unstable plaques (unstable-specific genes), and 3272 upregulated in stable plaques (stable-specific genes). Using the STARNET aortic plaque cohort, Hartman et al<sup>1</sup> identified 1837 male-specific and 1891 female-specific genes, 348 male-specific and 464 female-specific key drivers.<sup>1</sup> First, to confirm sufficient comparability between carotid and aortic plaques, we

inspected site-specific differentially expressed genes.<sup>4</sup> We then compared plaque phenotype-specific with sex-specific genes using hypergeometric testing with Benjamini-Hochberg correction and Spearman correlation in R (v4.1.0). PROGENy<sup>5</sup> was used to infer pathway activities of intersecting genes.

## DATA AVAILABILITY

Data and scripts used in this study are available from the corresponding author upon reasonable request.

Previously reported aortic versus carotid site-specific genes<sup>4</sup> ( $n=110$ ) were not overrepresented in either phenotype- or sex-specific gene sets ( $P=0.39$  and  $P=0.38$ , respectively), suggesting these 2 gene sets were not influenced by site-specific differences. Next, we compared plaque phenotype-specific genes and sex-specific genes. In line with the reported sex-dependence of plaque phenotype,<sup>2</sup> we observed a partial dichotomy of sex-specific gene correlation to relevant plaque traits. Male-specific genes were significantly overrepresented among unstable-specific genes (45% overlap, adjusted  $P=7.0 \times 10^{-205}$ ; Figure [A]). Vice versa, 31% of female-specific genes overlapped with stable-specific genes, a similarly significant overrepresentation (adjusted  $P=9.3 \times 10^{-74}$ ). The opposite comparisons (male-specific versus stable-specific; female-specific versus unstable-specific) were not significant. In support of this dichotomy, 40% to 50% of all reported sex-specific genes correlated with MaasHPS phenotype-specific genes. Male-specific genes correlated positively with unstable-specific genes,

**Key Words:** atherosclerosis ■ gene expression ■ phenotype ■ plaque, atherosclerotic ■ sex characteristics ■ transcriptome

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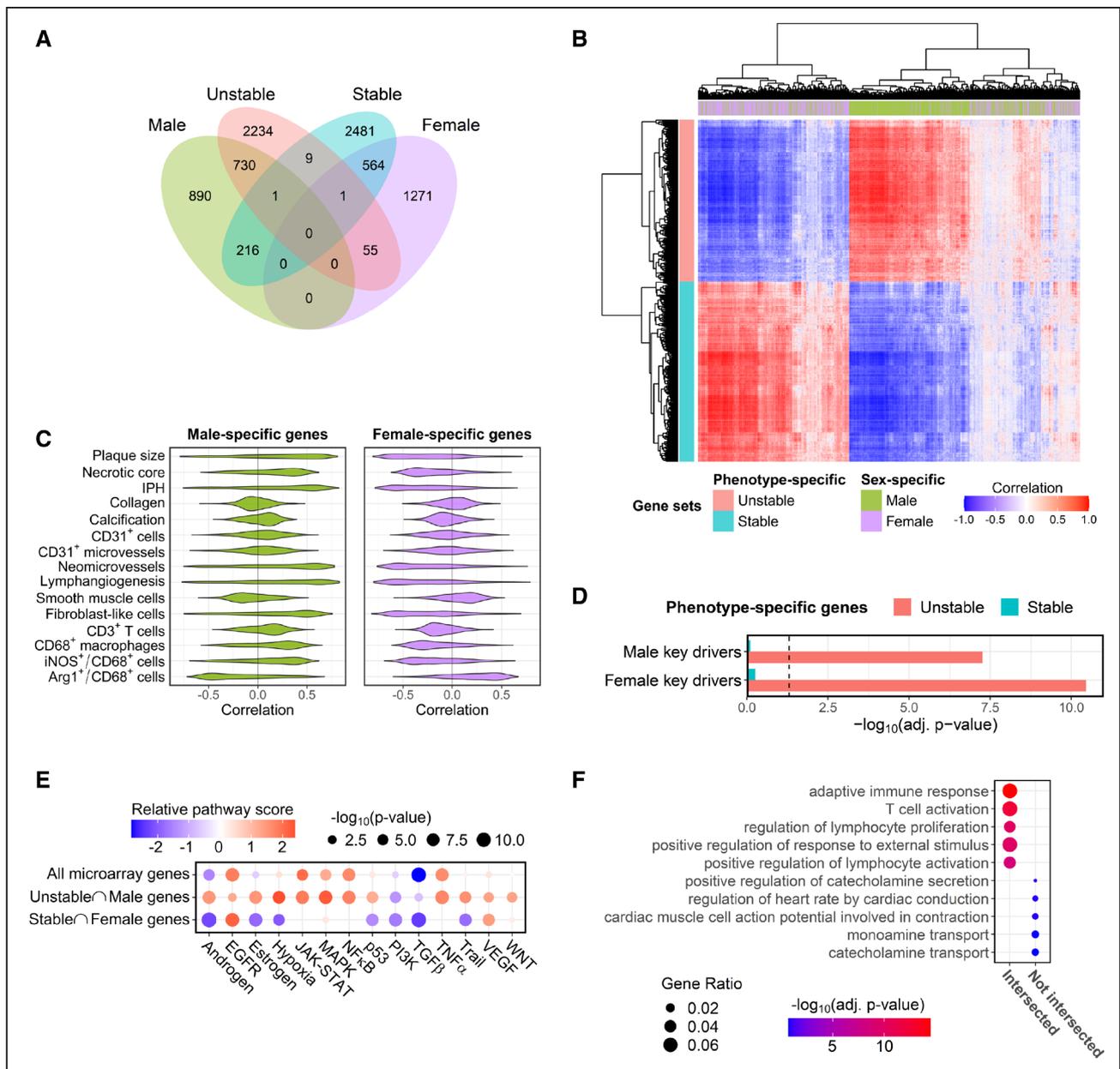
Correspondence to: Judith C. Sluimer, PhD, Department of Pathology, Maastricht UMC+, P. Debyelaan 25, 6229HX, Maastricht, the Netherlands. Email [judith.sluimer@maastrichtuniversity.nl](mailto:judith.sluimer@maastrichtuniversity.nl)

\*E.A.L. Biessen and J.C. Sluimer contributed equally.

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**Figure. Enrichment of sex-specific genes, but not drivers, depends on plaque type, cell death, and inflammation.**

**A**, Venn diagram shows overlap between MaasHPS (Maastricht Human Plaque Study) differentially expressed transcripts (unstable/stable, 6768 transcripts mapped to 6302 genes for intersection) and STARNET (Stockholm-Tartu Atherosclerosis Reverse Network Engineering Task) sex-specific genes (male/female). **B**, Heatmap shows correlations between sex-specific and phenotype-specific genes based on MaasHPS microarray. **C**, Violin plots show distribution of correlations between sex-specific genes and plaque traits in MaasHPS. Parameters reflect n/mm<sup>2</sup>, unless indicated. Necrotic core: area with no or low nuclei/plaque area (%); intraplaque hemorrhage (IPH): bright-pink area/plaque area (%); collagen: Sirius Red/plaque area (%); calcification: Alizarin Red/plaque area (%); CD31<sup>+</sup> cells: CD31<sup>+</sup> lumen-lining cells/plaque area; CD31<sup>+</sup> microvessels: CD31<sup>+</sup>-lined structures with lumen/plaque area; neomicrovessels: CD105<sup>+</sup>CD31<sup>+</sup> microvessels/total CD31<sup>+</sup> microvessels (%); lymphangiogenesis: D2-40<sup>+</sup> microvessels/plaque area; smooth muscle cells: αSMA<sup>+</sup> cells/plaque area; fibroblast-like cells: αSMA<sup>+</sup>PDGFRα<sup>+</sup> cells/plaque area; CD3<sup>+</sup> T cells: CD3<sup>+</sup> cells/plaque area; CD68<sup>+</sup> macrophages: CD68<sup>+</sup> cells/plaque area; iNOS<sup>+</sup>/CD68<sup>+</sup> cells: iNOS<sup>+</sup> area/CD68<sup>+</sup> area (%); Arg1<sup>+</sup>/CD68<sup>+</sup> cells: Arg1<sup>+</sup> area/CD68<sup>+</sup> area (%). **D**, Overrepresentation by hypergeometric testing of sex-specific key drivers in phenotype-specific genes (dashed line=0.05). **E**, Differential pathways in unstable relative to stable plaques driven by (1) all MaasHPS microarray genes; (2) intersection of unstable- and male-specific genes (unstable∩male); and (3) intersection of stable- and female-specific genes (stable∩female), analyzed by PROGENy. Significance level was assessed by a linear model fitting the pathway scores to plaque type. **F**, Ten most representative gene ontology terms of sex-specific genes that do/do not intersect with phenotype-specific genes. All correlations were calculated by Spearman  $\rho$ . Arg1 indicates arginase 1; αSMA, alpha-smooth muscle actin; EGFR, epidermal growth factor receptor; iNOS, inducible nitric oxide synthase; JAK-STAT, Janus kinase and signal transducer and activator of transcription protein; MAPK, mitogen-activated protein kinase; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; PDGFRα, platelet derived growth factor receptor alpha; PI3K, phosphoinositide 3-kinase; TGFβ, transforming growth factor beta; TNFα, tumour necrosis factor alpha; VEGF, vascular endothelial growth factor; WNT, wntless and int-1.

but negatively with stable-specific genes, and vice versa for female-specific genes (Figure [B]). Distribution-based visualization of the correlations between sex-specific genes and individual plaque traits confirmed the dichotomy of male-specific genes related to an unstable plaque type and vice versa for female-specific genes (Figure [C]).

Unlike the dual dependence of the proposed sex-specific gene set on both plaque phenotype and biological sex, sex-specific key drivers did not depend on plaque phenotype. Both male- and female-specific key drivers were significantly overrepresented in unstable-specific genes (adjusted  $P < 1.0 \times 10^{-7}$ ; Figure [D]), but not in stable-specific genes. This suggests key drivers are related to biological sex, possibly owing to the 175 common genes shared between the 348 male- and 464 female-specific key drivers. Thus, our analysis suggests that 30–50% of the previously identified sex-specific genes are related to plaque phenotype, while the remainder and the key drivers are likely explained by biological sex.

Based on PROGENY<sup>5</sup> functional pathways analysis, the MaasHPS microarray showed JAK-STAT (Janus kinase and signal transducer and activator of transcription protein) as the most prevalent pathway in unstable plaques, versus profibrotic epidermal growth factor receptor (EGFR) and transforming growth factor beta (TGF $\beta$ ) pathways in stable plaques (Figure [E]). When confining to the intersects ( $n$ ) of unstable $\cap$ male and stable $\cap$ female genes, largely inverse patterns in underlying pathways appear: inflammation pathways (eg, TNF $\alpha$  [tumour necrosis factor alpha], NF $\kappa$ B [nuclear factor kappa-light-chain-enhancer of activated B cells], JAK-STAT, mitogen-activated protein kinase [MAPK], and WNT [wingless and int-1] signaling) dominate in unstable $\cap$ male intersect, and profibrotic pathways in the stable $\cap$ female intersect. Unexpectedly, both androgen and estrogen pathways were driven positively by the unstable $\cap$ male intersect, and negatively by stable $\cap$ female intersect, making these pathways an unlikely explanation for plaque-type associations (Figure [E]). Gene ontology analysis of genes that were driven by biological sex only, ie, sex-specific genes that do not intersect with phenotype-specific genes, showed enrichment of catecholamine secretion and muscle cell contraction terms, suggesting these processes to be sex-dependent (Figure [F]).

To summarize, the proposed sex-specific genes relate not only to biological sex but also to plaque phenotype.

The latter is partly accountable to genes implicated in fibrosis, and inflammation, rather than sex-hormone response genes. Regarding study limitations, STAR-NET and MaasHPS differed in sample size (320 versus 43) and site (aortic versus carotid), although both were sufficiently powered for the approach, and site-specific genes were not influential.<sup>4</sup> Besides, both studies describe cross-sectional associations. Hence, the causality dilemma of whether sex-specific genes drive plaque phenotype, or vice versa, remains to be answered by longitudinal studies.

## ARTICLE INFORMATION

### Affiliations

Cardiovascular Research Institute Maastricht (CARIM), Maastricht University Medical Center + (MUMC +), the Netherlands (H.J., B.M.E.M., E.A.L.B., J.C.S.). Institute for Molecular Cardiovascular Research, RWTH Aachen University, Germany (E.A.L.B.). Centre for Cardiovascular Science, University of Edinburgh, United Kingdom (J.C.S.).

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### Disclosures

None.

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