and carotid artery stenosis. However, it is unknown whether cFAS is similarly elevated in patients with DM and advanced peripheral artery disease. This study aims to evaluate whether cFAS content and enzyme activity are biomarkers for clinical severity in patients with DM and chronic limb-threatening ischemia (CLTI).

Methods: Serum samples were prospectively collected from patients undergoing arterial revascularization procedures and maintained in an institutional review board-approved institutional biobank. The cFAS content and enzyme activity were evaluated using colorimetric ELISA assays. Multivariable logistic regression was used to evaluate DM and CLTI outcomes while adjusting for patient clinical characteristics. Hosmer-Lemeshow tests and C-index assessed goodness of fit and classification accuracy. All tests were two sided and a *P* value of less than .05 was considered significant.

Results: A total of 86 patients underwent cFAS analysis (67 content; 63 activity). Mean age was 65.0 ± 8.5 years with 67.4% male, 46.5% had DM, and 47.7% had CLTI. Bivariable analyses demonstrated association of CLTI with cFAS content ($P < .01$), DM ($P = .01$), and insulin use ($P = .01$); DM was associated with body mass index ($P = .001$), CKD ($P = .001$), and current smokers ($P < .05$). On multivariable analysis, CLTI was associated with cFAS content (odds ratio [OR] 1.16; 95% confidence interval [CI], 1.02-1.32; $P = .02$) and closely with DM (OR, 2.72; 95% CI, 0.94-7.89; $P = .066$), whereas DM was associated with body mass index (OR, 1.13; 95% CI, 1.05-1.23; $P < .01$) and CKD (OR, 4.86; 95% CI, 1.72-13.73; $P < .01$). No interactions were observed to be significant. Both models showed good fit ($P > .15$) and classification (Fig).

Conclusions: Serum cFAS content is associated with an increased risk of CLTI but not DM. Each unit increase in cFAS content increases the odds of CLTI by 16%. Future analysis with a larger sample and statistical bootstrapping will determine whether cFAS content is a clinically relevant biomarker of CLTI severity in patients with advanced atherosclerosis.

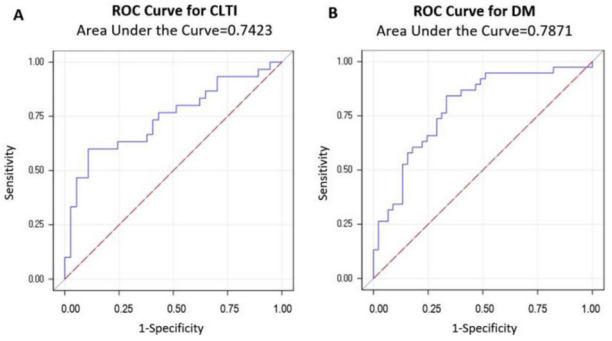

Fig. C-index and area under the receiver operating characteristic curve (AUROC) for crotocal limb-threatinging ischemia (*CLTI*) and diabetes mellitus (DM) multivariable logistic regression models. **A**, CLTI model with cFAS content and DM, AUROC = 0.7423 (95% conifdence interval, 0.6184-0.8663). **B**, DM model with BMI and chronic kidney disease (CKD); AUROC, 0.7871 (95% confidence limit, 0.8869).

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ZEB2 Regulates Activation and Exhaustion Programming of CD8 T Cells in Atherosclerosis

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Background: T cells are among the most prevalent immune cells found in human atherosclerotic lesions, yet their role remains obscure. In previous single-cell immunophenotyping studies we found that CD8$^+$ T cells of carotid atherosclerotic plaques display a spectrum of functionally heterogeneous states that vary based on differentiation, activation, and exhaustion. Furthermore, CD8$^+$ T-cell profiles varied between patients without (asymptomatic) and with (symptomatic) recent cardiovascular events (ie, transient ischemic attack and stroke), suggesting that T cells might contribute to adverse outcomes. The transcriptional regulator *ZEB2* has dual roles in T-cell differentiation and cardiovascular disease, as genome-wide association studies have reported *ZEB2* polymorphisms as independent risk alleles for coronary artery disease and myocardial infarction.

Methods: Our independent analysis identified *ZEB2* as a key driver of CD8$^+$ T-cell alterations in atherosclerotic lesions.

Results: We found that *ZEB2* was differentially regulated between patient types, with asymptomatic patients expressing higher *ZEB2* and *GZMB* levels compared with symptomatic (Fig, *A*). *ZEB2^{high}* CD8$^+$ T cells upregulated genes involved in cytotoxic functions, and conversely *ZEB2^{low}* CD8$^+$ T cells upregulated *PD-1* signaling in T-cell exhaustion (Fig, *B*). To probe the mechanistic implications of *ZEB2*, we performed in vitro chronic stimulation assays of human primary CD8$^+$ T cells using depleted of *ZEB2* using CRISPR/CAS9. *ZEB2* knockout cells had reduced cytotoxic function and had a dampened activation state upon acute stimulation.

Conclusions: Persistent stimulation-induced exhaustion in these cells showed that *ZEB2* knockout increased PD-1 expression levels, a protein marker that is critical for T-cell exhaustion (Fig, *C*). Collectively, these experiments suggest that *ZEB2* may contribute to the regulation of T-cell activation and exhaustion states, which is different between clinical phenotypes.

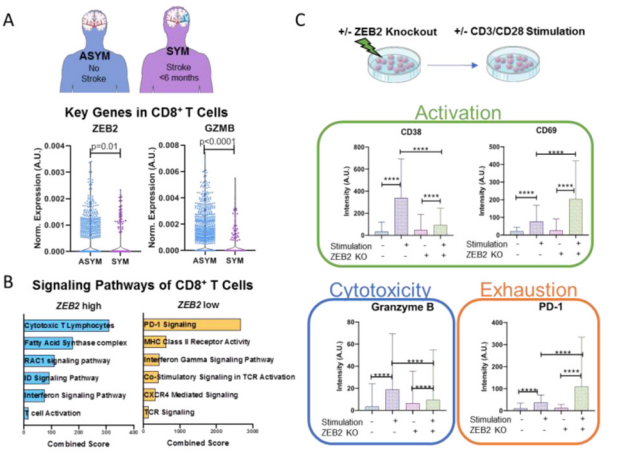

Fig. ZEB2 is a key driver of CD8* T cells in atherosclerosis. **A**, Expression of key genes from CD8* T cells stratified by patient type. Statistics by Welch's *t*-test. **B**, Signaling pathways upregulated in ZEB2^{high} and ZEB2^{low}CD8 T cells from scRnaseq data. **C**, ZEB2 knockout by CRISPR/CAS9 in human PBMCs and evaluated for expression of expression of activation (CD38, CD69), cytotoxicity (granzyme B), and exhaustion (PD-1) markers.

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The Role of Mast Cells in Atherosclerotic Plaque Calcification

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Background: Vascular calcification is a key feature of atherosclerosis and has been associated with major adverse cardiovascular events.

Unstable carotid atherosclerotic plaques cause stroke and lesions from those patients are abundant with activated mast cells at the sites of rupture. Recent data from our group showed a statistically significant upregulation of activated mast cells in low calcified whereas resting mast cells were upregulated in high calcified plaques, indicating that mast cells fractions may associate with various aspects of plaque pathology. Our hypothesis is that mast cell fractions associate with key features of plaque vulnerability such as calcification, intraplaque hemorrhage and other immune cell fractions.

Methods: The Biobank of Karolinska Endarterectomies prospectively enrolls patients (n = 1300) treated for carotid atherosclerosis in Stockholm, comprising BioBank with paraffin-embedded plaque tissues for histology, ImageBank with quantified diagnostic computed tomography images using VascuCap software and DataBank of 100 clinical variables as well as transcriptomics and proteomics large-scale datasets.

Results: Histologic stainings of plaque tissue microarrays confirmed the presence of mast cells in atheromatous lesions and revealed that mast cells were systematically found in Perls$^+$ regions. The average total number of mast cells per square millimeter per patient correlated negatively with the calcification content. In addition, immunohistochemical analysis demonstrated that mast cells correlate positively with CD3$^+$ cells while they did not correlate with markers of other immune cells. By stratifying the results according to patient symptoms, we found that activated mast cells were elevated in both symptomatic and asymptomatic patients and increased with severe symptoms of plaque instability. However, patients' medication does not impact mast cell regulation.

Conclusions: Systematic enumeration of mast cell fractions in human plaques indicates that activated mast cells associate with increased vulnerability, both when it comes to clinical patient symptoms and morphological plaque features.

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Inflammatory Activity of Human Perivascular Adipose Tissue in Abdominal Aortic Aneurysms

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Background: Perivascular adipose tissue (PVAT) contributes to vascular homeostasis and is increasingly linked to vascular pathology. PVAT density and volume were associated with abdominal aortic aneurysm (AAA) presence and dimensions in imaging techniques. However, mechanisms underlying the role of PVAT in AAA have not been clarified. Our study aimed to explore differences in PVAT from AAA using gene expression and functional tests.

Methods: Human aortic PVAT and control subcutaneous adipose tissue were collected during open AAA surgery. Gene analyses and functional tests were performed. The control group consisted of healthy aorta from nonliving renal transplant donors. Gene expression tests were performed to study genes potentially involved various inflammatory processes and AAA related genes. Live PVAT and subcutaneous adipose tissue from AAA were used for ex vivo co-culture with smooth muscle cells (SMC) retrieved from nonpathologic aortas.

Results: Adipose tissue was harvested from 27 AAA patients (n[gene expression] = 22, n[functional tests] = 5) and 5 control patients. An increased inflammatory gene expression of *PTPRC* ($P = .008$), *CXCL8* ($P = .033$), *LCK* ($P = .003$), and *CCL5* ($P = .004$) and an increase in extracellular matrix breakdown marker *MMP9* ($P = .016$) were found in AAA compared with controls. Also, there was a decreased anti-inflammatory gene expression of *PPARC* in AAA compared with controls ($P = .040$). SMC co-cultures from nonpathologic aortas with PVAT from AAA showed increased *MMP9* ($P = .033$) and *SMTN* ($P = .008$) expression and subcutaneous adipose tissue increased *SMTN* expression in these SMC.

Conclusions: Our data revealed that PVAT from AAA shows an increased proinflammatory and matrix metallopeptidase gene expression and decreased anti-inflammatory gene expression. Furthermore, increased expression of genes involved in aneurysm formation was found in healthy SMC co-culture with PVAT of AAA patients. Therefore, PVAT from AAA might contribute to inflammation of the adjacent aortic wall and thereby plays a possible role in AAA pathophysiology. These proposed pathways of inflammatory induction could reveal new therapeutic targets in AAA treatment.

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Polygenic Risk Score Identifies Patients at Increased Risk for Abdominal Aortic Aneurysm and May Benefit from Ultrasound Screening

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Background: Abdominal aortic aneurysm (AAA) is a significant heritable cause of cardiovascular related mortality, yet published genome-wide association studies have only identified 10 genome-wide significant ($P < 5 \times 10^{-8}$) risk loci to date. In addition, current AAA screening recommendations remain limited to men age 65 to 75 with a history of smoking. Genetic variants affecting multiple biological pathways are associated with AAA risk and may help to identify asymptomatic individuals at higher risk for disease.

Methods: Using electronic health record data, we identified individuals with and without clinical AAA in Million Veteran Program (MVP) participants. Individuals were genotyped on a customized Affymetrix array, and we tested 18 million genotyped and imputed DNA variants for association with AAA using logistic regression models adjusting for age, sex and population structure. We then performed replication in external datasets and set a $P < 5 \times 10^{-8}$ for statistical significance. In downstream analyses, we tested and validated a series of AAA polygenic risk scores (PRS) and assessed the associated AAA risk per standard deviation increase in PRS using prevalent data from an independent set of MVP participants (1656 AAA cases; 44,908 controls). We set a *P* value of less than .05 for statistical significance.

Results: We identified 7642 AAA cases and 172,172 controls. Following replication, we identified 14 novel AAA loci implicating known risk factors including lipids (*LPA, PCSK9*) and smoking (*CHRNA3*). We generated a 29 variant PRS and observed that a 1 standard deviation increase in the AAA PRS was associated with a 32% increased risk of AAA (odds ratio, 1.32; P_{PRS}