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Elevated expression of the metalloproteinase ADAM8 associates with vascular diseases in mice and humans

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HIGHLIGHTS

- ADAM8 associates with cardiovascular diseases in both mice and humans.
- ADAM8 associates with vascular disease markers.
- Soluble ADAM8 serum levels correlate with postoperative organ dysfunction.

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ABSTRACT

Background and aims: Members of the family of a disintegrin and metalloproteinases (ADAMs) and their substrates have been previously shown to modulate the inflammatory response in cardiac diseases, but studies investigating the relevance of ADAM8 are still rare. Our aim is to provide evidence for the inflammatory dysregulation of ADAM8 in vascular diseases and its association with disease severity.

Methods: Western-type diet fed *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice and artery ligation served as murine model for atherosclerosis and myocardial infarction, respectively. Human bypass grafts were used to study the association with coronary artery disease (CAD), with the simplified acute physiology score II (SAPS II) as a measure of postoperative organ dysfunction. Human primary vascular and blood cells were analyzed under basal and inflammatory conditions. mRNA levels were determined by RT-qPCR, ADAM8 protein levels by ELISA, immunohistochemistry or flow cytometry.

Results: *ADAM8*/ADAM8 expression is associated with atherosclerosis and CAD such as myocardial infarction in both mice and humans, especially in endothelial cells and leukocytes. We observed a strong *in vivo* and *in vitro* correlation of *ADAM8* with the vascular disease markers *VCAM-1*, *ICAM-1*, *TNF*, *IL-6*, and *CCL-2*. Serum analysis revealed a significant elevation of soluble ADAM8 serum levels correlating with soluble CXCL16 levels and SAPS II.

Conclusions: We demonstrate a general association of ADAM8 with cardiovascular diseases in mice and humans predominantly acting in endothelial cells and leukocytes. The correlation with postoperative organ dysfunctions in CAD patients highlights the value of further studies investigating the specific function of ADAM8 in cardiovascular diseases.

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1. Introduction

Cardiovascular diseases such as coronary artery disease (CAD) arising from atherosclerosis are a leading cause of death, accounting for more than 30% of deaths worldwide [1,2]. Atherosclerosis is a metabolic and inflammatory disease caused by an initial endothelial dysfunction with subsequent accumulation of low-density lipoprotein and immigration of immune cells. Accumulating apoptotic cells, debris and cholesterol crystals form a necrotic core, which is covered by a fibrous cap consisting of collagen and proliferating smooth muscle cells (SMC). This prothrombogenous environment can result in sudden plaque rupture and cardiac complications [2]. These events are driven by a diverse set of molecular mediators. Dysregulated expression and function of adhesion molecules, cytokines, chemokines, growth factors, scavenger receptors, metabolites and proteolytic enzymes can be observed in the cause of lesion development. Some of these molecular players are thought to represent novel therapeutic targets or factors for risk predictions, which are urgently needed [3].

Many of the mentioned players are subjected to regulated proteolysis close to the cell surface, called shedding, mediated by members of the family of a disintegrin and metalloproteinases (ADAMs) [4,5]. Most ADAMs are expressed as type I transmembrane surface proteins with a typical multidomain structure consisting of an N-terminal metalloproteinase domain, a disintegrin domain, a cysteine-rich domain, an optional epidermal growth factor-like domain followed by a transmembrane domain and a cytoplasmic tail [5]. They may not only act through their shedding activity, but they may interact directly with integrins or components of the extracellular matrix via their disintegrin domain [4,5]. ADAMs are essentially involved in the inflammatory response and have been shown to modulate cardiovascular events on several levels, including leukocyte recruitment, permeability, angiogenesis, neovascularization, and vascular remodeling [4]. ADAM10 and ADAM17, e.g., were found to be elevated in atherosclerotic lesions [6–8], and many of their substrates fulfill essential functions in vascular biology, including intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), tumor necrosis factor (TNF), L-selectin and CD44[4]. In contrast to ADAM10 and ADAM17, ADAM8 is less abundantly expressed and exists as transmembrane and soluble form. Peptide cleavage assays revealed that ADAM8 activity may be associated with the shedding of L-selectin, P-selectin glycoprotein ligand-1, VCAM-1 and TNF [9,10], thereby potentially regulating atherosclerosis development. Further, ADAM8 directly interacts with β_1 integrin and is involved in the inflammatory upregulation of α_L and α_M integrin [11,12]. Increased ADAM8 expression was observed upon atherosclerotic lesion formation, but it remains unclear how and at which stage of disease ADAM8 may fulfill essential functions [10]. Furthermore, gene polymorphism and circulating levels of soluble ADAM8 (sADAM8) were associated with the risk of myocardial infarction in human [13,14].

Here we provide further detailed evidence of an inflammatory dysregulation of ADAM8 expression in different animal models, cultured vascular cells and in tissues from human patients. We could show an association of *ADAM8* expression with atherosclerosis and CAD in both mice and human. Further, we observed a strong correlation of *ADAM8* with vascular disease markers such as *VCAM-1* and *ICAM-1* as well as the previously identified serum marker CXCL16[15]. Thus, ADAM8 functions in endothelial cells and leukocytes may act as proatherosclerotic factor [12,16]. Furthermore, elevated serum levels of sADAM8 correlated with postoperative organ dysfunctions in CAD patients, highlighting the value of further studies investigating the function of sADAM8 as a predictive biomarker in cardiovascular diseases.

2. Materials and methods

2.1. Antibodies and chemokines

Antibodies are listed according to the supplying company. R&D System (Wiesbaden, Germany): mouse monoclonal anti-human ADAM8; Biozol (Eching, Germany): rabbit polyclonal anti-murine ADAM8 (BYT-orb4376); ThermoFisher (Schwerte, Germany): Alexa647-conjugated polyclonal goat anti-mouse. Human TNF-alpha was obtained from Peprotech (Rocky Hill, USA).

2.2. Murine models of vascular diseases

To mimic the development of atherosclerosis, apolipoprotein E-deficient ($Apoe^{-/-}$) and low density lipoprotein receptor-deficient ($Ldlr^{-/-}$) mice on C57BL/6 background were set on a Western-type high-fat diet model as described elsewhere [17]. The chronic model of myocardial infarction was performed as described before [18]. For details of legacy and experimental procedure see supplemental material.

2.3. Human grafts, sample preparation, data collection

23 patients (17 male, 6 female; mean age of 71; Syntax score [19] of 24.5) undergoing bypass surgery and healthy volunteers (8 male, 7 female, mean age of 57; only serum samples) were consecutively enrolled in this observational study after approval of the local institutional review boards (EK 151/09 and 172/18-V2) and informed consent. The study protocol conformed the ethical guidelines of the 1975 Declaration of Helsinki. The left internal thoracic artery (mammary artery) as arterial graft, the remaining parts of the saphenous veins as corresponding control and serum samples were obtained from patients. The simplified acute physiology score (SAPS II), which is associated with a step-wise increase of mortality [20], was calculated as described before [15]. The data were grouped into ≤ 28 and ≥ 29 based on an increase to 10% mortality in patients with a SAPS II score ≥ 29. For further details see supplemental material.

2.4. Cell culture, cell preparation and lentiviral transduction

Isolation and culture of human peripheral blood mononuclear cells (PBMC) and neutrophils as well as human umbilical artery endothelial cells (HUAEC) and arterial SMC (aSMC) are detailed within the supplemental material. Knockdown of ADAM8 in HUAEC was achieved by transduction with shRNA containing lentiviral particles as described before [12].

2.5. Quantitative realtime PCR (RT-qPCR)

The mRNA levels for human and murine ADAM8, ADAM10, ADAM17, TNF, IL-6, CCL-2, VCAM-1 and ICAM-1 were quantified by RT-qPCR analysis. For murine tissue samples, mRNA levels were normalized to the mRNA level of ribosomal protein S29 (RPS29). For human tissue samples normalization was performed against a combination of RNA polymerase II subunit A (POLR2A), cyclin-dependent inhibitor 1B (CDKN1B) and translation initiation factor eIF-2B (EIF2B1) [21], for cell culture samples to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). For sample preparation and details see supplemental material.

2.6. Immunohistochemistry

The expression of Adam8 in murine tissue sections was analyzed by immunohistochemistry. For details see supplemental material.

2.7. ELISA measurements

Levels of sCXCL16 and ADAM8/sADAM8 were determined using commercial ELISA Kits (R&D Systems, DuoSet, Wiesbaden, Germany for human samples; antibodies-online, ABIN415444, Aachen, Germany for murine samples) according to the manufacturer's protocols. ADAM8 in lysates was normalized to the total protein content determined using a commercial BCA protein assay kit (ThermoFisher).

2.8. Flow cytometry

The surface expression of ADAM8 was analyzed by antibody staining and flow cytometry. For details see supplemental material.

2.9. Statistics

Quantitative data are shown as mean ± SD calculated from at least three independent experiments and cell isolates. Animal numbers per group and number of patient samples are indicated within the Fig. legends. The data were analyzed using SAS 9.4 software (SAS Institute, Cary, N·C., USA) and diagrams were created with PRISM 7.0 (GraphPad Software, La Jolla, USA). A p-value < 0.05 was considered significant. All performed correlation analyses are summarized in supplemental table 1. A detailed description of data handling and statistics is included within the supplemental material.

3. Results

3.1. Adam8 gene expression is elevated in murine atherosclerosis and correlates with vessel inflammation

Atherosclerosis is the major cause of CAD development. The early disease stage can be well studied in the murine *Apoe*$^{-/-}$ model, where mice are fed with Western-type high-fat diet (WTD). In the abdominal aorta, WTD in comparison to normal diet led to a slight, but not significant increase of *Adam10* gene expression, whereas *Adam8* and *Adam17* expression was not changed (Fig. 1A). The carotid and aortic arch, belonging to the vessel sites majorly affected by atherosclerosis, showed significantly upregulated *Adam8* expression upon WTD feeding, whereas *Adam10* and *Adam17* were not changed (Fig. 1B and C). Similarly, WTD led to a significant increase of *Adam8* expression in the aortic arch of *Ldlr*$^{-/-}$ mice (Supplemental Fig.1A). Atherosclerosis initiation and progression are accompanied by strong inflammation [2,22]. Prominent markers of vessel inflammation are the proinflammatory cytokine TNF, the chemokine monocyte chemoattractant protein-1 (MCP-1, CCL-2) and the adhesion molecules VCAM-1 and ICAM-1. WTD resulted in significantly increased expression of *Tnf* in all three investigated vessel sites, of *Vcam-1* in both aorta and carotid, and *Ccl-2* in both carotid and aortic arch (Supplemental Fig. 1B-D). The combined correlation analyses for all three vessels revealed a positive correlation of *Adam8* with *Tnf* and *Vcam-1* in murine atherosclerosis (Fig. 1D/E). ADAM8 is not only expressed as transmembrane protein but is released into the extracellular compartment through autocatalytic proteolysis maintaining its proteolytic activity [23]. Indeed, *Apoe*$^{-/-}$ mice displayed enhanced serum levels of sAdam8 (Supplemental Fig.1E). We further questioned which cell type would be responsible for the observed upregulation in *Adam8* gene expression and sAdam8 serum levels. Immunohistochemistry of the aortic arch and the brachiocephalic artery showed an enhanced Adam8 expression in endothelial cells and leukocytes in atherosclerotic regions but not in SMC (Fig. 1 F).

3.2. Adam8 gene expression is elevated in murine myocardial infarction and correlates with vessel inflammation

Myocardial infarction mostly occurs due to atherosclerosis/CAD. Indeed, *Adam8* was significantly increased upon myocardial infarction, with a delayed peak at day 4 and subsequent decline up to day 7, resulting in sigmoidal curve characteristics (Fig. 2A). In contrast, *Adam10* and *Adam17* were only slightly changed (Fig. 2B/C). Next, we analyzed the same subset of inflammatory marker genes in the myocardial infarction model. All four marker genes were significantly enhanced at day 1 post infarction (Supplemental Fig. 2A-D). For *Tnf* and *Vcam-1* (Supplemental Fig.2A/C) we found a similar curve progression as observed for *Adam8*, reflected by the strong and significant correlation of these parameters (Fig. 2D/E). Despite the shift in expression of *Ccl-2* and *Icam-1* (Supplemental Fig.2B/D), *Adam8* correlated with both parameters (Fig. 2F, not significant for *Icam-1* with $r = 0.4882$ and $p = 0.0572$). Immunohistochemistry showed that the enhanced expression of Adam8 mostly occurred in the infarction region, peaking at day 4 post infarction as observed for *Adam8* gene expression (Fig. 2A/G). The upregulation was not restricted to infiltrating leukocytes but was also found in microvascular endothelial cells and cardiomyocytes.

3.3. ADAM8 may act predominantly in endothelial cells and leukocytes

Several cellular changes contribute to atherosclerosis development, including endothelial activation and dysfunction, intimal immune cell infiltration, and SMC migration and proliferation [2,22]. In accordance to the immunohistochemistry data (Fig. 1F/2G), we used different human primary vascular and blood cells to address the question, which cell types in principle express *ADAM8* under basal or inflammatory conditions. The basal *ADAM8* expression level was similar in PBMC, aSMC, and HUAEC, whereas neutrophils showed a much higher expression. Proinflammatory treatment of PBMC and HUAEC with TNF led to significant stimulation of *ADAM8* expression, whereas no effect was observed in neutrophils and aSMC (Fig. 3A). In contrast, *ADAM10* and *ADAM17* expression were not affected (Supplemental Fig.3A/B). Upregulation of adhesion molecules and chemokines by endothelial cells is one prerequisite of immune cell recruitment. In both the atherosclerosis and myocardial infarction model, *ADAM8* was associated with increased expression of vascular disease markers. Indeed, *ADAM8* expression upon proinflammatory stimulation correlated significantly with increased *CCL-2* and *ICAM-1* expression (Fig. 3B/C; *VCAM-1* $r = 0.7143$, $p = 0.0576$) in HUAEC. However, enhanced *ADAM8* expression did not directly cause the upregulation of vessel inflammation markers, as those were not affected by ADAM8 knockdown (Supplemental Fig.3C/D). Additionally, *ADAM8* correlated significantly with *CCL-2* expression in PBMC (Fig. 3D, Supplemental Fig.3E). Elevated sADAM8 serum levels may result from release by leukocytes or endothelial cells. Release of sADAM8 and enhanced ADAM8 surface expression upon proinflammatory stimulation were not observed in HUAEC (data not shown). In both PBMC and neutrophils, the sADAM8 release to the supernatant was significantly increased by proinflammatory stimulation (Fig. 3E). This was accompanied by a reduction of cellular ADAM8 in neutrophils, whereas ADAM8 surface expression was not changed. In contrast, PBMC showed an enhanced ADAM8 surface expression with no change in cellular ADAM8 (Fig. 3F/G). Thus, in vascular inflammation ADAM8 may act predominantly in endothelial cells and leukocytes, including different transcriptional and posttranscriptional mechanisms in distinct cell types.

3.4. Elevated levels of ADAM8 are associated with vascular disease markers and postoperative organ dysfunction in CAD patients

Atherosclerosis consequences in CAD development often require bypass surgery. We investigated human bypass grafts for their mRNA expression of ADAM proteases. The mammary artery was used as pathological vessel and compared to the saphenous vein as internal control. *ADAM8* expression was significantly upregulated in atherosclerotic vessels, whereas *ADAM10* and *ADAM17* remained unchanged (Fig. 4A). Further, *IL-6*, *CCL-2*, *VCAM-1* and *ICAM-1* were similarly elevated in

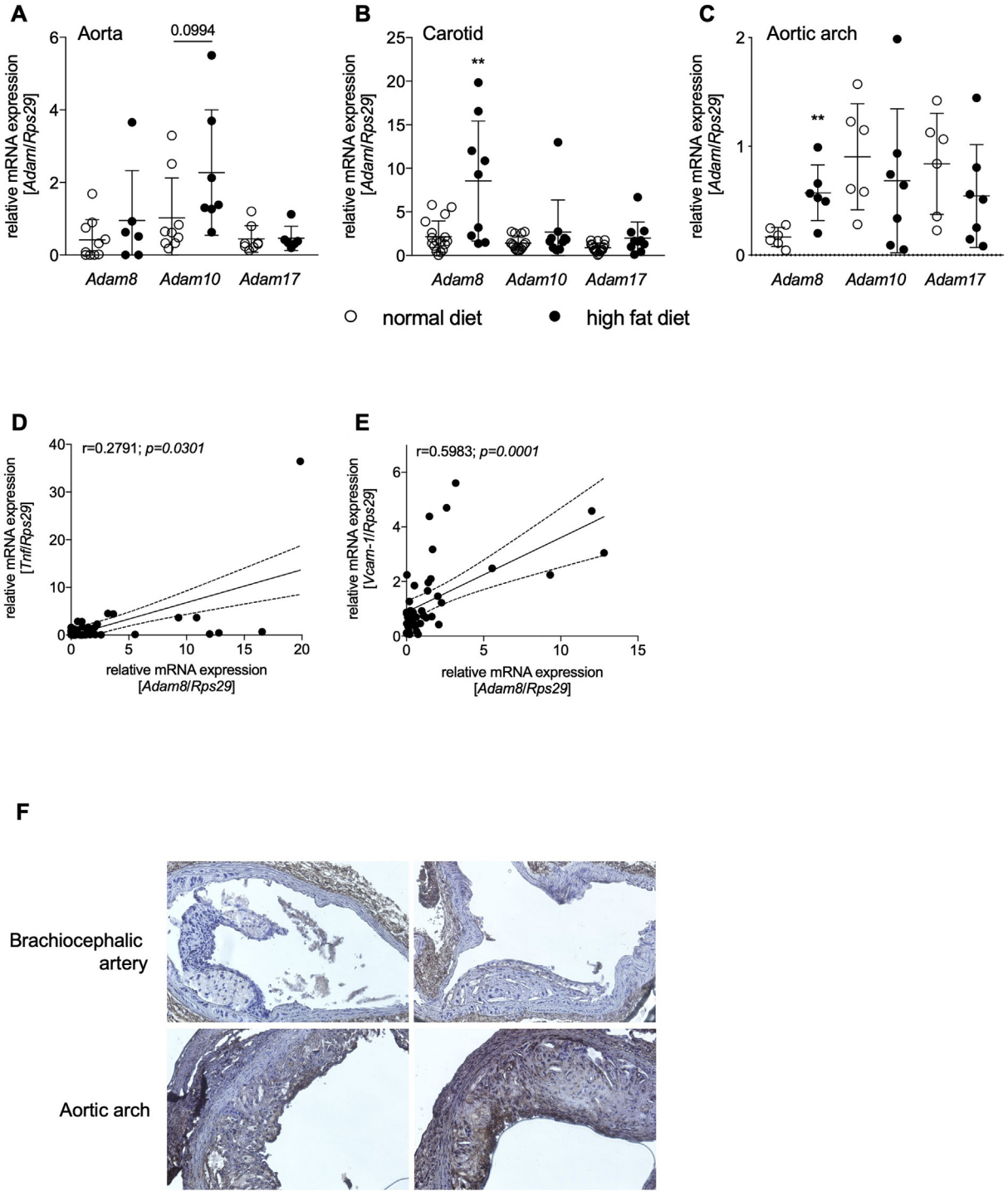

Fig. 1. Adam8 gene and protein expression is elevated in atherosclerosis and correlates with vessel inflammation.
Apoe$^{-/-}$ mice were fed Western-type diet (WTD) or normal diet (ND) for 14 weeks. *Adam8*, *Adam10*, and *Adam17* gene expression in aorta (A, n = 8–10 ND, n = 6–7 WTD), carotid (B, n = 15–17 ND, n = 9–10 WTD) and aortic arch (C, n = 6 ND, n = 6–7 WTD) was measured by RT-qPCR. Data are shown as mean ± SD. The correlation between *Adam8* and the inflammatory markers *Tnf and Vcam-1* (D and E) are depicted as linear regression (black line) with 95% confidence intervals (dashed lines). Representative images of Adam8 immunohistochemistry (20× magnification, n = 6 for each vessel type) are shown in F. Data were analyzed using Mann-Whitney-U test in (A), and Student's t-test in (B and C). One-sided testing was performed in (D and E). Statistical differences in comparison to the appropriate control (normal diet for each gene) are indicated by asterisks (**$p < 0.01$).

mammary artery compared to saphenous vein (Supplemental Fig.5A), resulting in a significant positive correlation of *ADAM8* with all four markers of vascular diseases (Fig. 4B/C, Supplemental Fig.5B/C). Next, we investigated the serum level of sADAM8 in CAD patients in comparison to matched healthy controls. Indeed, sADAM8 was significantly enhanced in CAD patients (Fig. 4D), closely correlating with the measured patients' *ADAM8* expression levels (Supplemental Fig.5D). This suggests that a considerable amount of ADAM8 is released upon its synthesis induction. We could recently show that sCXCL16 is enhanced in patients undergoing cardiac surgery, and is associated with an increased severity of postoperative organ dysfunctions [15]. Indeed, CAD patients revealed significantly increased sCXCL16 serum levels

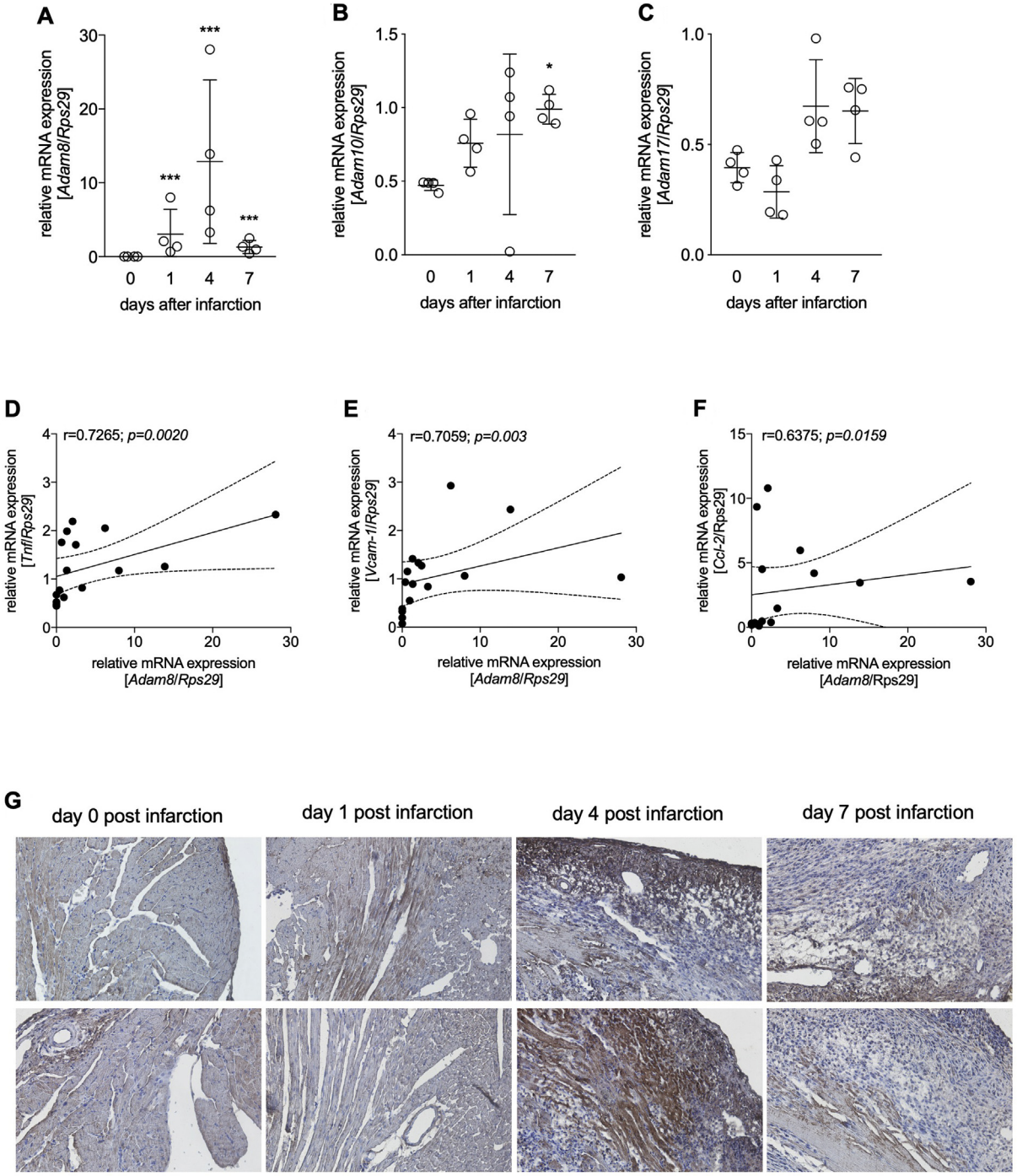

Fig. 2. Adam8 gene and protein expression is elevated in myocardial infarction and correlates with vessel inflammation.
Myocardial infarction was induced in mice, and *Adam8* (A), *Adam10* (B), and *Adam17* (C) gene expression was measured at day 0, 1, 4, and 7 post infarction (n = 4) by RT-qPCR. Data are shown as mean ± SD. The correlations between *Adam8* and the inflammatory markers *Tnf, Vcam-1,* and *Ccl-2* (D–F) are depicted as linear regression (black line) with 95% confidence intervals (dashed lines). Representative images of Adam8 immunohistochemistry (20× magnification, n = 5 for each time-point) are shown in (G). Data were analyzed using one-way ANOVA (A and C) and Kruskal-Wallis test (B) both with Dunnet's correction. Statistical differences in comparison to the appropriate control (time point 0) are indicated by asterisks (*$p < 0.05$, ***$p < 0.001$).

(Supplemental Fig. 5E) and a general significant correlation of serum sCXCL16 and sADAM8 (Fig. 4E) compared to matched healthy controls. Therefore, we next investigated the correlation of sADAM8 and the SAPS II score at day 1 post operation as a marker of organ dysfunction. We found an overall correlation of sADAM8 levels for patients with a SAPS II score ≤28 (Fig. 4F), but not for patients with a SAPS II score ≥29. Thus, ADAM8 associates with vascular diseases markers and in certain cases with postoperative organ dysfunction in CAD patients.

4. Discussion

Previous studies indicated an increase of ADAM8 gene and protein expression upon atherosclerotic lesion formation and an association of ADAM8 variants with the risk of myocardial infarction [10,13,14,24]. However, this was not systemically studied by comparison of *in vitro*, *in vivo* and clinical data. The present study demonstrates that *ADAM8* expression associates with atherosclerosis and CAD such as myocardial

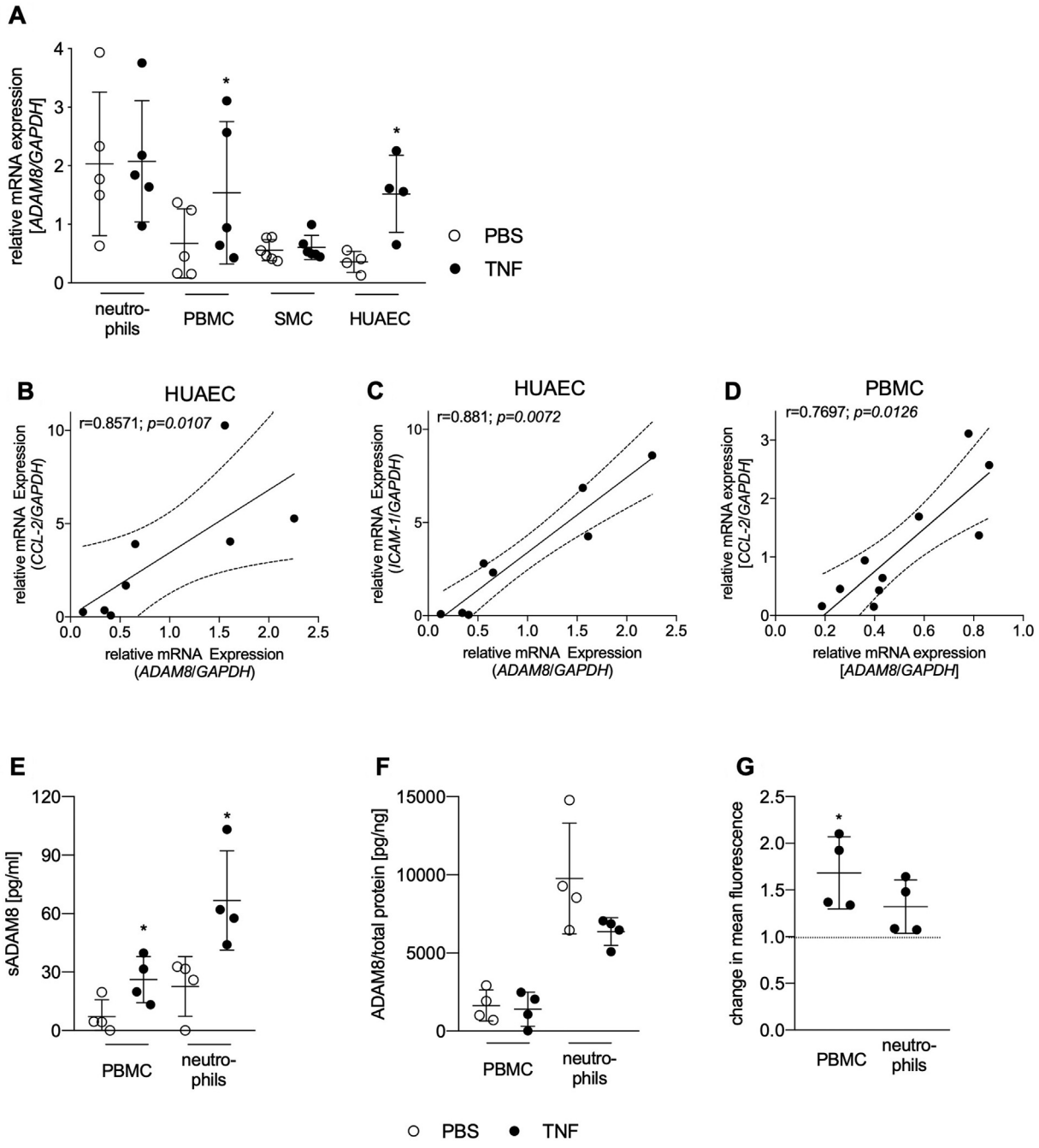

Fig. 3. ADAM8 may act predominantly in endothelial cells and leukocytes.
Primary human cells (neutrophils; PBMC: peripheral blood mononuclear cells; SMC: smooth muscle cells; HUAEC: human umbilical artery endothelial cells) were stimulated with 20 ng/ml TNF or left untreated as control. All cells were investigated for *ADAM8* gene expression by RT-qPCR (A, n = 4–6). The correlations between *ADAM8* and the inflammatory markers *CCL-2 and ICAM-1* in HUAEC (B/C) and *CCL-2* in PBMC (D) are depicted as linear regression (black line) with 95% confidence intervals (dashed lines). Further, PBMC and neutrophils (n = 4) were investigated for the release of sADAM8 to the cell culture supernatant (E) and the amount of total cellular ADAM8 (F) using ELISA as well as ADAM8 surface expression (G, untreated control cells set = 1 for each isolation and indicated as dotted line) using flow cytometry. Data are shown as mean ± SD. Data were analyzed using Student's t-test (A/E-F) and one-sample *t*-test (G, hypothetical value = 1). Statistical differences in comparison to untreated cells are indicated by asterisks (*$p < 0.05$).

infarction in both mice and humans. We observed a strong *in vivo* and *in vitro* correlation with the vascular disease markers *VCAM-1*, *ICAM-1*, *TNF*, *IL-6*, *CCL-2*, and sCXCL16, which drive endothelial inflammation and leukocyte recruitment. The strongly positive correlations *ADAM8*/sADAM8 and sADAM8/SAPS II suggest that the inflammatory dysregulation of ADAM8 may affect the development of atherosclerosis and postoperative organ dysfunctions. Further studies will have to address if and by which mechanisms ADAM8 may affect vascular inflammation, the cell-specific contribution and the value of sADAM8 as initial predictive marker for cardiovascular diseases, especially CAD.

A critical contribution of blood cell or tissue cell expressed ADAM8 to advanced stages of atherosclerosis was recently questioned when studying ADAM8 knockout mice after 10 weeks of high-fat diet. Mouse models used to study atherosclerosis resemble human atherosclerosis. However, they present less advanced lesions, do not allow the investigation of ruptured plaques, which were shown to highly express ADAM8 in humans, and lack the appearance of the medial vasa vasorum in large arteries [25–28]. Thus, it is not excluded that ADAM8

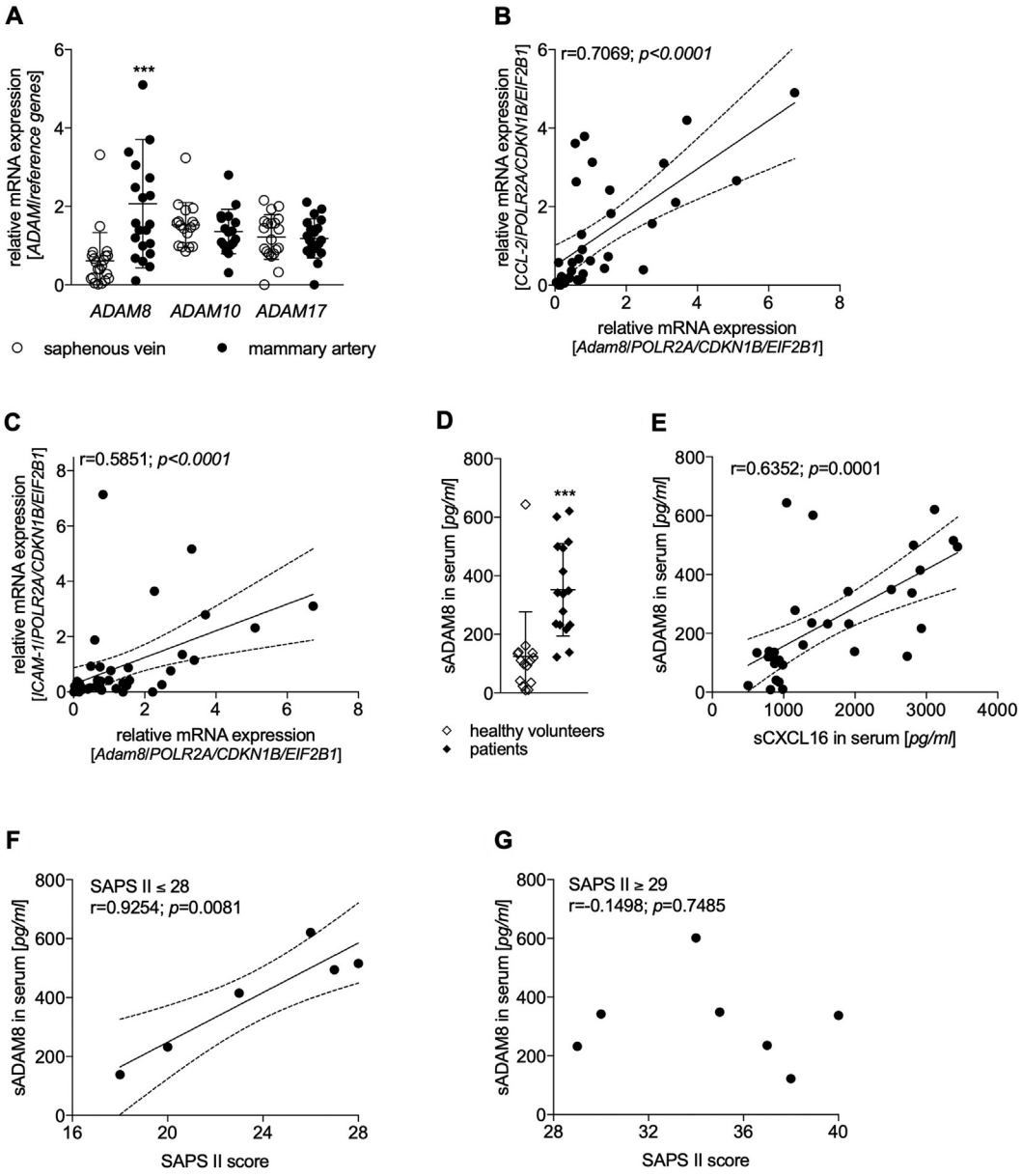

Fig. 4. Elevated levels of ADAM8 are associated with vascular disease markers and postoperative organ dysfunction in CAD patients.
(A–C) Human bypass grafts were analyzed for gene expression of *ADAM8*, *ADAM10*, and *ADAM17* (A, n = 13–21). Mammary artery was used as pathological vessel, saphenous vein served as internal control. The correlations between *ADAM8* and *CCL-2* and *ICAM-1* are shown in B and C. (D and E) Serum levels of sADAM8 (D) and the correlation with sCXCL16 serum levels (E) in patient serum samples (n = 16) in comparison to matched healthy volunteers (n = 15). (F and G) Correlation between circulating sADAM8 levels and SAPS II score on the first postoperative day (POD) (n = 13). Data are shown as mean ± SD, and correlations are depicted as linear regression (black line) with 95% confidence intervals (dashed lines). Data were analyzed using Student's t-test (Mann-Whitney-U test for *ADAM17*). Statistical differences in comparison to saphenous vein (for each gene in A) or healthy volunteers (in D) are indicated by asterisks (***$p < 0.001$).

plays a causal role in more advanced atherosclerotic lesions than those studied earlier [10]. Our clinical analysis revealed a strong correlation of sADAM8 with the postoperative SAPS II score in patients undergoing bypass surgery. The requirement of bypass surgery itself implicates an advanced stage of CAD and a progressive extent of atherosclerosis [29] which is in accordance with our determined SYNTAX score [19]. Thus, the comparison of earlier mouse studies on ADAM8 in atherosclerosis [10] and our data on ADAM8 in CAD leads to the hypothesis of different functions of ADAM8 during different stages of cardiovascular diseases. Of note, a correlation with a SAPS II score ≥29 was lacking. These higher SAPS II scores indicate a more complex disease status, which might not only depend on the extent of atherosclerosis and in which other factors than ADAM8 could contribute to an increased organ dysfunction and increased mortality. Although this aspect is still speculative, the correlation analysis of *ADAM8* and *ICAM-1/VCAM-1* between the murine atherosclerosis model and the human bypass grafts may point towards stage-specific ADAM8 functions. Both ICAM-1 and VCAM-1 are established markers of inflammation and atherosclerosis [30–32]. However, the relevance of the two molecules as markers might differ between different disease stages. A major role for VCAM-1 but not ICAM-1 in early atherosclerosis was shown [32], with endothelial VCAM-1 being more restricted to lesions [33]. The relevance of ICAM-1 seems to be higher upon disease progression also extending to uninvolved vessel areas [33]. Indeed, we observed a correlation of *ADAM8* and *ICAM-1* expression even in the mammary artery as a generally less affected vessel, displaying the advanced disease stage of CAD. In the murine atherosclerotic samples, this correlation was missing, most likely due to the earlier disease stage compared to the human bypass

grafts. In both cases, a correlation with *VCAM-1/Vcam-1* was given, and we observed a correlation within the myocardial infarction model, reflecting the strong inflammatory compound after myocardial infarction [22,34]. The differences in disease progression in the murine atherosclerosis model and CAD may be further reflected by the lack of elevated *Adam*8 expression in the murine aorta in contrast to the human counterpart [13]. It was shown in *Apoe*$^{-/-}$ mice that the extent of lesion formation and thereby the extent of inflammation varies between different vascular beds [35], and the differential upregulation of *Ccl-2* expression indeed points towards a different extent of inflammation in the three investigated vascular beds. Altogether, taking into account earlier insights into the role of ADAM8 in atherosclerosis, our data suggest that ADAM8 may fulfill different functions during the different stages of cardiovascular diseases. The correctness of this assumption, the underlying mechanisms, and whether this is associated with different molecular pathways, remains currently unclear.

Atherosclerosis initiation and progression, as well as myocardial infarction, are accompanied by an intense inflammatory response, involving endothelial activation as well as monocyte and neutrophil recruitment [2,22]. In a previous study, we demonstrated that inhibition of ADAM8 ablation reduces chemokine-induced migration of neutrophils and monocytes *in vivo* and *in vitro* [12]. In the present study, we observed that inflammatory stimulation leads to upregulation of *ADAM8* expression in PBMC but not in neutrophils. We recently showed that ADAM8 in monocytic cells is responsible for the upregulation of integrin α_M and α_L thereby enabling transendothelial transmigration [12]. These integrins are required for the interaction with VCAM-1 and ICAM-1 expressed on the inflamed endothelium. It was previously shown that ADAM8 can be mobilized to the surface and released as soluble protease in activated neutrophils, associated with reduced L-selectin surface expression [36]. Although enhanced neutrophil recruitment upon expression of a non-cleavable L-selectin was observed [37], a more recent study indicated that L-selectin shedding in transmigrating pseudopods is a prerequisite for the polarity in transmigration monocytes [38]. Indeed, we observed an increased release of sADAM8 upon inflammatory stimulation in both PBMC and neutrophils. Thus, such essential contributions of ADAM8 towards cell migration under inflammatory conditions could influence atherosclerosis development. It was recently shown that ADAM8 promotes breast cancer metastasis through upregulation of MMP9[16]. MMP9 is one of the central regulators of the pathological remodeling processes in vascular diseases requiring inflammation and fibrosis, e.g. through direct cleavage of the extracellular matrix and the release of remodeling mediators [39]. An elevation of MMP9 in macrophages through ADAM8 could account for plaque instability and rupture [40], and it was shown that elevated serum levels of MMP9 are related to myocardial infarction and CAD [41]. Thus, there are several feasible scenarios of ADAM8 actions in atherosclerosis or CAD, which have to be investigated in further studies.

It was previously suggested that other proteases such as ADAM17 could compensate for the loss of ADAM8 in knockout animals, covering essential functions of ADAM8 in vascular disease development [10]. Indeed, it is important to note that beside ADAM8 other ADAM proteases could play a crucial role in atherosclerosis development. Myeloid ADAM10 was shown to promote atherosclerosis through an increase of plaque rupture [42]. For ADAM17, counteractive effects in endothelial and myeloid cells were reported [43]. These previous cell-specific investigations and our *in vitro* and knockdown experiments show that further studies are required addressing the function of ADAM proteases in general in a whole-body situation.

In conclusion, we could show a general association of ADAM8 with cardiovascular diseases in mice and humans. However, the relevance and function of ADAM8 at different steps of vascular disease development requires an in-depth investigation from the onset of atherosclerosis development up to plaque rupture and long-term clinical studies. Despite all these uncertainties, our correlation analyses and the general enhancement of sADAM8 serum levels in CAD patients underline the value of future studies investigating the function of sADAM8 as predictive biomarker [10,13].

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Author contributions

DS wrote manuscript and collected data; DD corrected manuscript; AL and HN critically reviewed manuscript; DS, JW, HN, EL, AKV, KG, and TA performed experiments; AG provided human tissue samples; RZ performed the SYNTAX scoring; JWB provided analysis tools; DS, AB and DD analyzed data and performed statistical analysis; AL and DD designed study; all authors approved the final version of the paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.03.008.

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