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The proteoglycan mimecan is associated with carotid plaque vulnerability and increased risk of future cardiovascular death

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ABSTRACT

Background and aims: A vulnerable plaque is an atherosclerotic plaque that is rupture-prone with a higher risk to cause cardiovascular symptoms such as myocardial infarction or stroke. Mimecan or osteoglycin is a small leucine-rich proteoglycan, important for collagen fibrillogenesis, that has been implicated in atherosclerotic disease, yet the role of mimecan in human atherosclerotic disease remains unknown.

Methods: 196 human atherosclerotic carotid plaques were immunostained for mimecan. Smooth muscle cells, macrophages and intraplaque haemorrhage were also measured with immunohistochemistry. Neutral lipids were stained with Oil Red O and calcium deposits were quantified. Plaque homogenate levels of MCP-1, IL-6 and MMP-1 were measured using a Proximity Extension Assay and MMP-9 levels were measured using Mesoscale. Glycosaminoglycans, collagen and elastin were assessed by colorimetric assays and TGF-β1, β2 and β3 were measured using a multiplex assay. Mimecan gene expression in THP-1 derived macrophages was quantified by qPCR and protein expression in vitro was visualized with immunofluorescence. Cardiovascular events were registered using medical charts and national registers during follow-up.

Results: Mimecan correlated positively with plaque area of lipids, macrophages, intraplaque haemorrhage and inversely with smooth muscle cell staining. Mimecan also correlated positively with plaque levels of MMP-9 and MCP-1. Mimecan was upregulated in THP-1 derived macrophages upon stimulation with MCP-1. Patients with high levels of mimecan (above median) had higher risk for cardiovascular death.

Conclusions: This study indicates that mimecan is associated with a vulnerable plaque phenotype, possibly regulated by plaque inflammation. In line, plaque levels of mimecan independently predict future cardiovascular death.

1. Introduction

The rupture or erosion of an atherosclerotic plaque with subsequent thrombosis leads to myocardial infarction or stroke, which are the most common causes of death globally [1]. Despite recent advances in preventive treatments, there is still a considerable unmet need for new treatment targets to stabilize atherosclerotic plaques, avoiding ruptures or erosions. A plaque that is prone to rupture, often called a vulnerable plaque, is characterized by a large lipid core, covered by a thin fibrous cap, with degraded extracellular matrix (ECM) proteins, poor in smooth muscle cells and rich in inflammatory infiltrates. In contrast, a stable plaque has a smaller lipid core covered by a thick and less inflamed fibrous cap, rich in smooth muscle cells and collagen fibers [2,3]. The balance between the degradation and formation of ECM components is of great importance for plaque stability. Yet, these processes are poorly understood in the context of human atherosclerosis.
Mimecan, also known as osteoglycin, is an ECM component, namely a small leucine-rich proteoglycan (SLRP). Mimecan affects several biological processes including the regulation of collagen fibrillation and angiogenesis [4–6]. Mimecan is expressed in atherosclerotic tissue in rabbits, human coronary arteries and is downregulated in intimal vascular smooth muscle cells (VSMCs) [7,8]. Mimecan is upregulated in rat carotid artery after balloon injury suggesting that it is involved in vascular remodelling [7]. However, in humans the role of mimecan in atherosclerotic plaques is still unknown.

In the present study, we investigated the associations between mimecan and 1) human plaque components in a large number of atherosclerotic lesions obtained from patients undergoing carotid endarterectomy; 2) inflammation in an in vitro model of THP-1 derived macrophages; 3) the risk for future cardiovascular events and death.

2. Materials and methods

2.1. Study design

One hundred and ninety-six human carotid plaques were obtained from 194 patients who underwent carotid endarterectomy between 2005 and 2011 at the Vascular Department at Skåne University Hospital (Malmö, Sweden). Two patients underwent surgery for both right and left carotid plaques and, in those cases, only the first chronological operation was considered for analysis. The indication for carotid endarterectomy was 1) ipsilateral symptoms and stenosis >70% or 2) asymptomatic patient with >80% stenosis.

Table 1

| Clinical characteristics | Low (n = 98) | High (n = 98) | p-value |
|--------------------------|-------------|-------------|---------|
| Mimecan (staining, %)    | 0.17 (0.09-0.27) | 0.91 (0.59-1.33) | 0.017 |
| Age                      | 70 (64-74) | 71 (66-78) | 1.000 |
| Male sex, n (%)          | 63 (64.3) | 63 (64.3) | 0.129 |
| Degree of stenosis, %    | 90 (80-95) | 90 (75-95) | 0.764 |
| BMI                      | 25.8 (23.5-28.7) | 26.8 (24.1-29.0) | 0.080 |
| Current smoker, n (%)    | 38 (38.8) | 27 (27.6) | 0.129 |
| Hypertension, n (%)      | 72 (74.2) | 77 (78.6) | 0.293 |
| Symptoms, n (%)          | 51 (52.0) | 59 (60.2) | 0.314 |
| Fasting plasma levels of lipids | | | |
| Cholesterol, mmol/L      | 4.3 (3.5-5.1) | 4.3 (3.6-5.1) | 0.954 |
| LDL, mmol/L              | 2.4 (2.0-3.2) | 2.5 (1.8-3.1) | 0.430 |
| HDL, mmol/L              | 1.1 (0.9-1.4) | 1.0 (0.8-1.4) | 0.971 |
| TG, mmol/L               | 1.2 (0.9-1.6) | 1.4 (1.0-2.0) | 0.080 |
| Diabetes, n (%)          | 22 (22.4) | 40 (40.8) | 0.009 |
| Hs-CRP, mmol/L           | 4.0 (1.9-6.9) | 3.6 (1.9-6.2) | 0.755 |
| HbA1c, mmol/mol          | 46.9 (43.7-56.3) | 57.3 (45.8-64.6) | 0.038 |
| eGFR mL/min/1.73 m²      | 70.7 (56.9-82.1) | 64.6 (50.5-81.6) | 0.066 |
| Statins                  | 85 (86.7) | 85 (86.7) | 1.000 |
| Anti-hypertensive drugs  | 80 (81.6) | 81 (82.7) | 1.000 |

| BMI | body mass index, LDL = low density lipoprotein, HDL = high density lipoprotein, TG = triglycerides, CRP = c-reactive protein, HbA1c = glycated hemoglobin, eGFR = estimated glomerular filtration rate. |

* Only measured in patients with diabetes. Mann-Whitney U test was used for continuous variables whereas Chi-square test was performed for categorical variables.

All patients were examined with a preoperative ultrasound of the carotid arteries, to evaluate the degree of stenosis, and were clinically assessed by a neurologist before endarterectomy to exclude other causes of thromboembolic events, i.e. atrial fibrillation or flutter. Patients were considered symptomatic if they suffered from amaurosis fugax, transient ischemic attack (TIA) or ischemic stroke. The indication for carotid endarterectomy was 1) ipsilateral symptoms and stenosis >70% or 2) asymptomatic patient with >80% stenosis.

2.2. Sample preparation

Carotid plaques were snap-frozen in liquid nitrogen in the operation theatre directly after surgical removal and stored at −80 °C until sectioning. From the most stenotic part of the plaque, a 1 mm thick section was taken for histology and embedded in optimal cutting medium (OCT, Sakura Finetek Europe BV, Japan). The rest of the plaque was homogenized in a standardised way as described previously [9].

2.3. Histology and immunohistochemistry

The most stenotic part of the plaque (1 mm) was cryosectioned in 8 μm sections for histology as previously described [9]. Sections were then fixed with Histochoice (Amresco, Solon, OH, USA) and stained for smooth muscle cells (smooth muscle α-actin), macrophages (CD68), neutral lipids (Oil Red O) and intraplaque haemorrhage (Glycophorin A) as previously described [9,10]. Calcified areas in plaques were quantified as described previously [11]. Collagen fibers (yellow) were assessed using Russell-Movat Pentachrome staining. Mimecan was analysed in 196 human carotid plaques using immunohistochemistry staining with a rabbit polyclonal antibody (PA5-48255, Invitrogen, Waltham, MA) at 2 μg/mL as a primary antibody and then a MACH3 rabbit probe and horseradish peroxidase-polymer (RP531H, Biocare Medical, Pacheco, CA) was used as a secondary antibody. A rabbit IgG polyclonal isotype control antibody was used as a control for each plaque (ab27478, Abcam, Cambridge, UK).

In general, positive immunoreactivity was visualized using 3,3′-diaminobenzidine (DAB; Vector Laboratories Inc, Burlingame, CA, USA). Sections were counterstained with Mayer’s Hematoxylin (Histo- lab, Gothenburg, Sweden). Stained sections were scanned and digitalized using Aperio ScanScope digital slide scanner (Aperio Technologies, Inc, Vista, CA, version 12. February 3, 8013) and immunoreactivity was quantified using the BioPix iQ version 2.3.1 (Biopix Ab, Gothenburg, Sweden).

2.4. Plaque levels of matrix metalloproteinase 9

Matrix metalloproteinase-9 was analysed with Mesoscale human MMP ultra-sensitive kit (Mesoscale, Gaithersburg, MD, USA) in plaque homogenate supernatants as previously described [2]. All analyses were performed according to the manufacturer’s instructions and the results were normalized to plaque wet weight.

2.5. Biochemical assessments of plaque glycosaminoglycans, collagen and elastin levels

The ECM components glycosaminoglycans, collagen and elastin were analysed in plaque homogenate with colorimetric assays and normalized to plaque wet weight as previously described [9].

2.6. Plaque TGF-β1, -β2 and -β3 analysis

TGF-β1, -β2 and -β3 were assessed in 25 μL of supernatant from plaque homogenate after centrifugation for 5 min at 8000 RPM in 4 °C, using the Milliplex Map TGF-β Magnetic Bead 3 Plex Kit - Immunology Multiplex Assay from MerckMillipore (TGFBMAG-64K-03, Billerica,
MA, USA) according to the manufacturer’s instructions and measured using Luminox 100 IS 2.3 (Austin, TX, USA). Levels of TGF-β1, -β2 and -β3 were normalized to plaque wet weight.

2.7. Cytokine plaque levels and in vitro stimulation of THP-1 cells

MCP-1, IL-6 and MIP-1β were measured in plaque homogenate supernatants using Proximity Extension Assay (PEA) technique using the Proseek Multiplex CVD96x96 reagents kit (Olink Bioscience, Uppsala, Sweden) as previously described [12]. Data are presented as arbitrary units. Human blood monocytes cells (THP-1, 88081201 (U208645, Thermo Fisher Scientific, Waltham, MA) complemented with 10% FBS (10270106, Thermo Fisher Scientific, Waltham, MA) and 50 U/ml penicillin-streptomycin. Prior to the experiment, cells were treated with Phorbol 12-myristate 13-acetate (PMA, cat #78139 Sigma, Saint Louis, USA) for 24 h, to differentiate the cells into macrophages, and then washed 3 times with PBS and medium changed every 48 h for 6 days. Cells were seeded onto 6-well plates and cell culture slides and stimulated with 5 and 15 ng/ml of MCP-1 (RP-8648, Thermo Fisher Scientific, Waltham, USA) and 1 and 2 ng/ml of TGF-β/2 (T2815, Sigma, Saint Louis, USA) for 24 h. Immunofluorescence microscopy was used to detect mimecan staining with a rabbit polyclonal antibody (PA5-48255, Invitrogen, Waltham, MA) as a primary antibody at 1 μg/ml and a secondary polyclonal goat anti-rabbit antibody with Alexa 488 fluorescent dye at 0.5 μg/ml. An isotype rabbit IgG antibody was used as a control antibody (Abcam, 37415) at 1 μg/ml.

For quantification of mimecan in immunofluorescence staining, cells were counted and areas (and cells) exhibiting positive mimecan immunoreactivity were analysed by applying colour threshold measurements (from which positive immunoreactivity corresponding to the isotype control were subtracted) using Adobe Photoshop CS6, Fiji software [13,14] and QuPath v0.1.2 [15]. Cell supernatants were used for mimecan detection, and RNA was extracted from the cells for gene expression analysis.

Total RNA was isolated with the RNeasy Mini Kit (74106, QIAGEN) following manufacturer’s instruction, and 1 μg was retrotranscribed using the High Capacity RNA-to-cDNA Kit (4387406, Applied Biosystems). Quantitative real time PCR (qPCR) was performed using TaqMan Fast Advanced MasterMix (1710122, Applied Biosystems) and the following primers: 18s (Hs00999901_s1), GAPDH (Hs0276624_1), and Osteoglycin (Hs00247901_m1). Each reaction was performed in triplicate (n = 3) and results were normalized by geometric average of two internal controls (18s and GAPDH).

2.8. Follow up

The primary outcome comprised cardiovascular (CV) events with follow-up time of 59 months (interquartile range (IQR): 32–73) and cardiovascular death with a follow-up time of 63 months (IQR: 43–74). Patient were divided in two groups by median. The CV events studied longitudinally included myocardial infarction (MI), transient ischaemic attacks, amaurosis fugax and vascular interventions not planned at the time of the operation such as carotid endarterectomy, carotid artery stenting, coronary artery bypass grafting or percutaneous coronary artery intervention and all deaths with an underlying cardiovascular cause of death. CV events were identified through the Swedish National Patient Register for all hospital discharge codes for all the patients included of death. CV events were identified through the Swedish National Patient Register for all hospital discharge codes for all the patients included. The Swedish National patient register has a high coverage of death. CV events were identified through the Swedish National Patient Register for all hospital discharge codes for all the patients included.

3. Results

3.1. Mimecan was associated to features of plaque vulnerability

Mimecan was detected in human atherosclerotic plaques with immunohistochemistry (0.40% plaque area (IQR 0.17–0.96)). Mimecan was observed in areas of collagen fibers, close to the core and around calcified regions (Fig. 1A and B, Supplementary Fig. 1). Mimecan correlated inversely with the smooth muscle cell marker α-actin (r = −0.192, p = 0.007, Supplementary Table 1, Fig. 1C and D). There were positive correlations between mimecan and neutral lipids (Oil Red O; r = 0.214, p = 0.004), macrophages (CD68; r = 0.179, p = 0.013) and intra-plaque haemorrhage (glycoalbumin A; r = 0.259, p = 0.001, Supplementary Table 1, Fig. 1E–J).

There were no significant correlations between mimecan and total levels of extracellular matrix proteins glycosaminoglycans, elastin or collagen (Supplementary Table 2). However, mimecan correlated positively with plaque levels of MMP-9, one of the extracellular matrix degrading enzymes known to be associated with human plaque vulnerability (r = 0.288, p ≤ 0.0001, Fig. 2A).

3.2. The proinflammatory cytokine MCP-1 upregulates mimecan in THP-1 differentiated macrophages

Since mimecan was positively correlated with macrophage staining, we further examined if mimecan correlated with the pro-inflammatory cytokines MCP-1, IL-6 and MIP-1β, which are well known to affect macrophage polarization. Mimecan correlated with MCP-1 plaque levels (r = 0.157, p = 0.040, Fig. 2B) but not with IL-6 (r = −0.059, p = 0.443) or MIP-1β (r = 0.087, p = 0.379 Supplementary Fig. 2A and B).

Therefore, to examine if MCP-1 could affect mimecan mRNA expression in vitro, THP-1 cells were differentiated into macrophages with PMA and thereafter stimulated with 5 or 15 ng/ml of MCP-1 for 24 h. TGF-β1 is known to be important for maintaining plaque stability and the balance between plaque inflammation and fibrosis [18,19],...
therefore THP-1 cells were stimulated with 1 or 2 ng/ml of TGF-β2 for 24 h to evaluate if this cytokine affects mimecan gene expression. Stimulation with 15 ng/ml of MCP-1 caused a significant increase in mimecan gene expression ($p = 0.0047$; Fig. 2C). However, stimulation with 5 ng/ml of MCP-1 or with TGF-β2 did not significantly affect mimecan gene expression in macrophages. Mimecan upregulation in THP-1 differentiated macrophages was also visualized at protein level with immunofluorescence before and after stimulation with 15 ng/ml of MCP-1 (Fig. 2D and E). The immunofluorescence was quantified and the number of cells with positive immunoreactivity for mimecan was higher in cells stimulated with 15 ng/ml of MCP-1 than in unstimulated cells (10 positive cells IQR (9–14) vs 16 positive cells IQR (14–22), $p = 0.04$, Fig. 2F), and the amount of positive $\mu m^2$ per cell was also significantly higher in cells stimulated with MCP-1 compared to unstimulated cells (3.7 $\mu m^2$ per cell IQR (2.6–6.0) vs 0.7 $\mu m^2$ per cell IQR (0.0–1.3) ($p = 0.001$, Fig. 2G).

### 3.3. Mimecan levels are associated with age and diabetes

Patients with higher plaque levels of mimecan were significantly older compared to patients with lower levels of mimecan (71 years IQR (66–78) vs 70 years IQR (64–74), $p = 0.017$, Table 1). The patient group with mimecan levels above median also had a higher percentage of diabetes compared to the group with lower levels (40.8% vs 22.4%, $p = 0.009$, Table 1) and accordingly, HbA1c levels were significantly higher in patients with mimecan levels above median compared to the group with below median levels of mimecan (57.3 mmol/mol, IQR (45.8–64.6) vs 46.9 mmol/mol IQR (43.7–56.3), (Table 1).

### 3.4. Patients with high mimecan plaque levels have an increased risk of cardiovascular death

A total of 194 patients were followed up for cardiovascular events and cardiovascular death. Two plaques were excluded from the follow-up.
Mimecan is upregulated by the pro-inflammatory cytokine MCP-1 in macrophages.

Scatterplots showing correlations of plaque levels of MMP-9 (A) and MCP-1 (B) with mimecan % plaque area staining. Quantitative real time polymerase chain reaction (qPCR) for mimecan gene expression (C) in THP-1 matured macrophages after stimulation with MCP-1. Values are expressed as fold change relative to unstimulated cells. Immunofluorescence for mimecan (green) and DAPI (blue) in THP-1 cells unstimulated (D) and after (E) stimulation with 15 ng/ml MCP-1 for 24 h. Quantification of immunoreactivity for mimecan in both the number of positive cells (F) and the amount of positive μm² per cell (G) (n = 7 in each group). The boxes in the boxplots represent the median and interquartile range and the whiskers indicate the minimum and maximum values. For the qPCR, each reaction was performed in triplicate (n = 3) and results were normalized by geometric average of two internal controls (18s and GAPDH). Spearman rank correlation test was used in (A and B), one-way ANOVA with Holm Sidak’s adjustment for multiple comparisons was used in (C) and Mann-Whitney test in (D and E) for statistical analysis. Representative immunofluorescence images were taken at 40× magnification. Scale bars (D and E) represent 50 μm.
up analysis since they were from patients who were operated bilaterally. Altogether, 54 patients (27.8%) experienced cardiovascular events and 21 patients (10.8%) suffered from a cardiovascular death. Patients with higher plaque levels of mimecan (above the median) had a higher risk of future cardiovascular events in a log rank test \( (p = 0.005, \text{Fig. 3A}) \) and cardiovascular death \( (p = 0.034, \text{Fig. 3B}) \).

In an unadjusted Cox regression model (Model A), mimecan was associated with both an increased risk for cardiovascular events \((HR (95\% \ CI) 1.812 (1.036–3.170), p = 0.037, \text{Table 2})\) and cardiovascular death \((HR (95\% \ CI) 4.177 (1.404–12.423), p = 0.010, \text{Table 2})\). However, when correcting for age, sex and diabetes (Model B) in a multivariate Cox proportional hazard regression model, the significant association for cardiovascular events was lost. When adjusting for potential confounders (age, sex, diabetes, BMI, HDL, TG and eGFR) in the final model (Model C), above median mimecan levels remained an independent predictor for cardiovascular death \((HR (95\% \ CI) 3.388 (1.055–10.886), p = 0.040, \text{Table 2})\).

![Cardiovascular events and death](https://example.com/cardiovascular_events_death.png)

**Fig. 3.** Patients with high plaque levels of mimecan have an increased risk for cardiovascular events and death. Kaplan-Meier curves for cardiovascular events and cardiovascular death for mimecan (A and B). Groups are divided in above (blue lines) and below median (red lines). Statistical differences between the groups were assessed by Log-rank test. A total number of 194 patients were assessed (above median, \( n = 97 \) and below median, \( n = 97 \)) for both cardiovascular events and death.

| Mimecan (%) staining | CV events \( HR (95\% \ CI) \) | \( p \) | CV death \( HR (95\% \ CI) \) | \( p \) |
|---------------------|-----------------|-----|-----------------|-----|
| Model A             |                 |     |                 |     |
| Above median        | 1.812 (1.036–3.170) | 0.037* | 4.177 (1.404–12.423) | 0.010* |
| Below median        |                 |     |                 |     |
| Model B             |                 |     |                 |     |
| Above median        | 1.566 (0.872–2.810) | 0.133 | 3.210 (1.047–9.842) | 0.041* |
| Below median        |                 |     |                 |     |
| Model C             |                 |     |                 |     |
| Above median        | 1.484 (0.799–2.757) | 0.212 | 3.388 (1.055–10.886) | 0.040* |
| Below median        |                 |     |                 |     |

**Table 2** Uni- and multivariate Cox proportional hazard regression model for cardiovascular events and cardiovascular death for mimecan.

\( CV = \text{cardiovascular}, \text{BMI} = \text{body mass index}, \text{HDL} = \text{high density lipoprotein}, \text{TG} = \text{triglycerides}, \text{eGFR} = \text{estimated glomerular filtration rate}. \)

4. Discussion

In the present study, we showed that mimecan was associated with vulnerable plaque features including macrophages, neutral lipids, intraplaque haemorrhage and MMP-9. Mimecan correlated with proinflammatory cytokine MCP-1 and in vitro mimecan secretion is promoted by various atherogenic conditions. The finding that mimecan was negatively correlated with smooth muscle cell proliferation and enhanced apoptosis in aortic smooth muscle cells \([20]\) could be explained by inhibiting angiogenesis, which may in turn cause dysfunctional neo-vessel formation and, consequently, increased intraplaque haemorrhage. Intra plaque haemorrhage is also suggested to contribute to a vulnerable plaque phenotype \([22]\), again providing a possible link between mimecan and the vulnerable plaque. Intraplaque haemorrhage and smooth muscle cell apoptosis, particularly in the cap, are all deleterious characteristics previously associated with plaque vulnerability \([23]\).

Mimecan could
potentially contribute to plaque vulnerability by affecting collagen fibril size, as seen in mimecan deficient mice [4] where loss of mimecan results in larger collagen fibrils. Mimecan was also shown to affect collagen maturation in cardiac tissue in a mouse model for heart failure after myocardial infarction [24]. However, if mimecan affects the size or maturation of collagen fibrils in atherosclerotic plaques is not known.

Mimecan was also associated with macrophages and correlated with levels of MCP-1, a pro-inflammatory cytokine important for atherosclerotic plaque progression and destabilization [25,26]. In line, mimecan gene expression was upregulated in THP-1 derived macrophages upon MCP-1 stimulation, which supports that a pro-inflammatory environment increases plaque mimecan levels. Moreover, mimecan is expressed in circulating and resident cardiac macrophages and it is also increased in cardiac inflammation during viral myocarditis [27]. Furthermore, mimecan correlated with MMP-9, an extracellular matrix degrading enzyme and a well-known component of vulnerable atherosclerotic plaques, [28,29]. MMP-9 is also produced by macrophages [30,31] and MMP-9 secretion in vitro increased in macrophage-like THP-1 cells and human peripheral blood monocytes after stimulation with MCP-1 [32].

In patients with higher mimecan (above median) levels, there was a higher frequency of diabetes. Interestingly, mimecan has previously been shown to be a coordinator of glucose homeostasis [33], which could potentially explain the associations identified between mimecan, HbA1C and diabetes diagnosis in this study.

The associations found between mimecan and vulnerable plaque phenotype, as well as increased risk for future cardiovascular death, are in line with a study showing that higher serum levels of mimecan were independently predictive for occurrence of major adverse cardiovascular events within one year after coronary angiography [34]. In a prospective study of plaque extracts, mimecan was found to be higher in fibrotic than hemorrhagic plaques, [35]. An explanation for the discrepancy compared to the results of our study could potentially be the smaller sample size of only six plaques in each group, the different method used for measuring mimecan or the criteria for the separation of plaques into hemorrhagic or fibrotic plaques (based upon levels of haemoglobin and fibrous cap thickness).

Since this study is of observational nature, it is not possible to prove causality between mimecan and the increased risk for future cardiovascular death. Moncayo-Arlandi et al. [36] investigated ApoE−/−-mimecan knockout mice and did not see any effect on plaque composition or lesion size, suggesting potential differences in murine and human mimecan function or a compensatory upregulation of other small leucine-rich proteoglycans during development.

In conclusion, mimecan correlated positively with histological features of plaque vulnerability, including macrophages, neutral lipids, and intraplaque haemorrhage, and correlated positively with the extracellular matrix degrading enzyme MMP-9. Mimecan also correlated positively with plaque levels of the pro-inflammatory cytokine MCP-1, and in vitro stimulation by MCP-1 increased mimecan expression in THP-1 derived macrophages. Finally, high plaque levels of mimecan was an independent predictor for future cardiovascular death in patients who had undergone endarterectomy. Taken together, this study suggests an association between mimecan and plaque vulnerability, possibly due to the local inflammatory activity. However, further mechanistic studies are needed to unravel potential treatment targets for patients with atherosclerosis.

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CRediT authorship contribution statement

Christoffer Tengryd: Conceptualization, Investigation, Visualization, Formal analysis, Writing - original draft, Writing - review & editing. Signe Holm Nielsen: Conceptualization, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. Michele Cavalera: Investigation, Writing - review & editing. Eva Bengtsson: Conceptualization, Writing - original draft, Writing - review & editing. Federica Genovese: Writing - review & editing. Morten Karsdal: Funding acquisition, Writing - review & editing. Pontus Duner: Investigation, Writing - review & editing. Marju Orho-Melander: Writing - review & editing. Jan Nilsson: Conceptualization, Writing - review & editing. Andreas Edsfeldt: Conceptualization, Investigation, Formal analysis, Writing - original draft, Supervision, Funding acquisition, Project administration. Isabel Gonçalves: Conceptualization, Investigation, Formal analysis, Writing - original draft, Supervision, Funding acquisition, Project administration.

Declaration of competing interests

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

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Appendix A. Supplementary data

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