Title

Common variants in *OSMR* contribute to carotid plaque vulnerability

Short Title

Common variants in *OSMR* and plaque vulnerability

AUTHORS

D. van Keulen, MSc[1,2,3,4], I. D. van Koeverden, MD PhD[1], A. Boltjes PhD[2], H. M. G. Princen, PhD[4], A. J. van Gool, PhD[5], G.J. de Borst, MD PhD[6], F.W. Asselbergs, MD PhD[7,8,9], D. Tempel, PhD[2,3,10], G. Pasterkamp, MD PhD[2]*, S.W. van der Laan, PhD[2]*.

* these authors contributed equally

AFFILIATIONS

[1]Laboratory of Experimental Cardiology, University Medical Center Utrecht, University of Utrecht, Utrecht, the Netherlands;

[2]Laboratory of Clinical Chemistry and Hematology, University Medical Center Utrecht, University of Utrecht, Utrecht, the Netherlands;

[3]Quorics B.V., Rotterdam, The Netherlands;

[4]TNO-Metabolic Health Research, Gaubius Laboratory, Leiden, The Netherlands;

[5]Translational Metabolic Laboratory, Radboudumc, Nijmegen, The Netherlands;

[6]Department of Vascular Surgery, University Medical Center Utrecht, University of Utrecht, Utrecht, the Netherlands;

[7]Department of Cardiology, Division Heart & Lungs, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands;

[8]Institute of Cardiovascular Science, Faculty of Population Health Sciences, University College London, London, United Kingdom;

[9]Health Data Research UK and Institute of Health Informatics, University College London, London, United Kingdom

[10]SkylineDx B.V., Rotterdam, The Netherlands.

CORRESPONDING AUTHORS

Prof. dr. G. Pasterkamp

Laboratory of Clinical Chemistry, Division Laboratories, Pharmacy, and Biomedical Genetics, University Medical Center Utrecht, Utrecht University

Heidelberglaan 100, 3508 GA, Utrecht, the Netherlands

Telephone: +31 (0) 88 75 571 55

Fax: +31 (0)30 252 26 93

E-mail: g.pasterkamp@umcutrecht.nl

dr. S.W. van der Laan

Laboratory of Clinical Chemistry, Division Laboratories, Pharmacy, and Biomedical Genetics, University Medical Center Utrecht, Utrecht University

Heidelberglaan 100, 3508 GA, Utrecht, the Netherlands

Telephone: +31 (0) 88 75 676 96

Fax: +31 (0)30 252 26 93

E-mail: s.w.vanderlaan-2@umcutrecht.nl

MANUSCRIPT CONTENTS

Manuscript of 5,854 words, 3 tables, 2 figures and Supplemental Material.

JOURNAL SUBJECT TERMS

Epigenetics, Genetics, Atherosclerosis, Cardiovascular disease

Abstract

Background

Oncostatin M (OSM) signaling is implicated in atherosclerosis, however the mechanism remains unclear. We investigated the impact of common genetic variants in *OSM* and its receptors, *OSMR* and *LIFR,* on overall plaque vulnerability (based on macrophage, collagen, smooth muscle cell and fat content) and on seven individual atherosclerotic plaque phenotypes (calcification, collagen, atheroma size, macrophages, smooth muscle cells, vessel density and intraplaque hemorrhage).

Methods and results

We queried Genotype-Tissue Expression (GTEx) data and selected one variant, rs13168867 (C allele), that associated with decreased *OSMR* expression and one variant, rs10491509 (A allele), that associated with increased *LIFR* expression in arterial tissue. No variant was associated to significantly altered *OSM* expression.

We associated these two variants with plaque characteristics from 1,443 genotyped carotid endarterectomy patients in the Athero-Express Biobank Study. The rs13168867 variant in *OSMR* was significantly associated with an increased overall plaque vulnerability ($\beta = 0.118 \pm$ s.e. $= 0.040$, $p = 3.00 \times 10^{-3}$, C allele). With respect to different plaque phenotypes, this variant showed strongest associations with intraplaque fat ($\beta = 0.248 \pm$ s.e. $= 0.088$, $p = 4.66 \times 10^{-3}$, C allele) and collagen content ($\beta = -0.259 \pm$ s.e. $= 0.095$, $p = 6.22 \times 10^{-3}$, C allele). No associations were found for rs10491509 in the *LIFR* locus.

Conclusion

Our study suggests that genetically decreased arterial *OSMR* expression, possibly resulting in decreased OSM signaling, contributes to increased carotid plaque vulnerability.

Keywords: Atherosclerosis, plaque, OSM, OSMR

1. Introduction

Oncostatin M (OSM) is an inflammatory cytokine[1] that is released by activated monocytes[2], macrophages[2], T-lymphocytes[3] and neutrophils[4], and mediates its effects through binding to either the glycoprotein (gp) 130/ oncostatin M receptor (OSMR) heterodimer or the gp130/ leukemia inhibitory factor receptor (LIFR) heterodimer[5–7]. Binding of OSM to either of the receptor heterodimers can activate multiple pathways, including the janus kinase (JAK)/ signal transduction and activator of transcription (STAT), the mitogen-activated protein kinase (MAPK), and the Phosphoinositide 3-kinase (PI3K)/AKT pathway[6]. It is suggested that the ratio of the two receptor types expressed on the cell membrane is a potential regulatory mechanism for the multiple and sometimes opposing effects that are exerted by OSM[8]. The cytokine is associated to multiple inflammatory diseases, including chronic periodontitis[5,9], rheumatoid arthritis[10] and inflammatory bowel disease[11].

There are multiple indications, that OSM is involved in atherosclerosis. OSM is present in both murine and human atherosclerotic plaques[12] and OSMR$^{-/-}$ApoE$^{-/-}$ mice show reduced plaque size and improved plaque stability compared to their *OSMR* expressing littermates[13], indicating that OSM drives atherosclerosis development. To our knowledge, no studies have been performed to investigate the involvement of LIFR in OSM driven atherosclerosis development. However, we previously showed that OSM signals through both receptors simultaneously to induce activation in human endothelial cells, suggesting that also LIFR is involved in atherosclerosis development[14].

Little is known about the effect of OSM on plaque composition. Since OSM affects multiple cell types and processes, it is difficult to predict how OSM contributes to atherosclerotic plaque formation. As OSM promotes angiogenesis[15], endothelial activation[14], vessel permeability[16] and osteoblastic differentiation[17], it hypothetically results in a higher intraplaque microvessel density and intraplaque hemorrhages and plaque calcification, thereby contributing to the formation of a vulnerable plaque[18,19]. On the other hand, OSM also promotes fibroblast proliferation[20], collagen formation[20], smooth muscle cell

proliferation[12] and M2 macrophage polarization[21], hypothetically resulting in enhanced fibrosis and attenuates inflammation, thereby contributing to plaque stabilization[22–24].

We aimed to investigate these theorized opposing effects of OSM signaling on the atherosclerotic plaque using data from the Athero-Express Biobank Study, which comprises a large collection of human plaque specimens obtained through carotid endarterectomy[25]. Common genetic variation in gene expression is key to disease susceptibility, and *cis*-acting genetic variants, single-nucleotide polymorphisms (SNPs), have been mapped to expression quantitative trait loci (eQTLs)[26]. Likewise, eQTLs modulate transcriptional regulation of *OSM*, *OSMR*, and *LIFR* in arterial tissues. We hypothesized that eQTLs for these genes, can be used as proxies of gene expression to examine the effect on overall plaque vulnerability[27] and individual plaque characteristics, including collagen, lipid, macrophage and smooth muscle cell content, calcification, and intraplaque microvessel density and hemorrhage.

2. Materials and methods

2.1 Sample collection

The Athero-Express Biobank Study (https://www.atheroexpress.nl) contains plaque material of patients that underwent carotid endarterectomy (CEA) or femoral endarterectomy at two Dutch tertiary referral centers[25]. Details of the study design were described before. Briefly, blood and plaque material were obtained during endarterectomy and stored at -80℃. Only CEA patients were included in the present study. All patients provided informed consent and the study was approved by the medical ethics committee.

2.2 Athero-Express genotyping, quality control, and imputation

Details of genotyping have been previously described[28]. In short, DNA was extracted from EDTA blood or (when no blood was available) plaque samples of 1,858 consecutive patients from the Athero-Express Biobank Study and genotyped in 2 batches. For the Athero-Express Genomics Study 1 (AEGS1) 836 patients, included between 2002 and 2007, were

genotyped using the Affymetrix Genome-Wide Human SNP Array 5.0 (SNP5) chip (Affymetrix Inc., Santa Clara, CA, USA). For the Athero-Express Genomics Study 2 (AEGS2) 1,022 patients, included between 2002 and 2013, were genotyped using the Affymetrix Axiom□ GW CEU 1 Array (AxM). Both studies were carried out according to OECD standards. After genotype calling, we adhered to community standard quality control and assurance (QCA) procedures of the genotype data from AEGS1 and AEGS2. Samples with low average genotype calling and sex discrepancies (compared to the clinical data available) were excluded. The data was further filtered on 1) individual (sample) call rate > 97%, 2) SNP call rate > 97%, 3) minor allele frequencies (MAF) > 3%, 4) average heterozygosity rate ± 3.0 s.d., 5) relatedness (pi-hat > 0.20), 6) Hardy–Weinberg Equilibrium (HWE $p < 1.0\times10^{-6}$), and 7) population stratification (based on HapMap 2, release 22, b36) by excluding samples deviating more than 6 standard deviations from the average in 5 iterations during principal component analysis and by visual inspection as previously described[28]. After QCA 657 samples and 403,789 SNPs in AEGS1, and 869 samples and 535,983 SNPs in AEGS2 remained. Before phasing using SHAPEIT2[29], data was lifted to genome build b37 using the liftOver tool from UCSC (https://genome.ucsc.edu/cgi-bin/hgLiftOver). Finally, data was imputed with 1000G phase 3[30] and GoNL 5[31] as a reference.

2.3 Variant selection

We queried data from the Genotype-Tissue Expression (GTEx) Portal (https://gtexportal.org)[26] for variants that alter *OSM* expression in the blood, and *OSMR* or *LIFR* expression in arterial tissue. We selected common variants with a MAF >3%, which yielded 2 variants in total. We harmonized the effect alleles and effect sizes from these eQTLs to the Athero-Express Biobank Study data.

2.4 Plaque phenotyping

The (immuno)histochemical analyses of plaque phenotypes have been described previously[25,28,32]. Briefly, the culprit lesion was identified directly after dissection, fixed in

4% formaldehyde and embedded in paraffin. The tissue was cut in 5µm sections on a cryotome for (immuno)histochemical analysis by pathology experts. Calcification (hematoxylin & eosin, H&E) and collagen content (picrosirius red) were semi-quantitatively scored and defined as no/minor or moderate/heavy. Atheroma size (H&E and picrosirius red) was defined as <10% or ≥10% fat content. The amount of macrophages (CD68) and smooth muscle cells (ACTA2) were quantitatively scored and classified as percentage of plaque area. The presence of intraplaque hemorrhage (H&E and fibrin) was defined as absent or present, and vessel density was classified as the number of intraplaque vessels (CD34)/ hotspot.

2.5 Plaque vulnerability

Assessment of overall plaque vulnerability was performed as previously described[27]. In short, the amount of macrophages and smooth muscle cells were also semi-quantitatively defined as no/minor or moderate/heavy. Each plaque characteristic that defines a stable plaque (i.e. no/minor macrophages, moderate/heavy collagen, moderate/heavy smooth muscle cells and <10% fat) was given a score of 0, while each plaque characteristic that defines a vulnerable plaque (i.e. moderate/heavy macrophages, no/minor collagen, no/minor smooth muscle cells and ≥10% fat) was given a score of 1. The score of each plaque characteristic was summed resulting in a final plaque score ranging from 0 (most stable plaque) to 4 (most vulnerable plaque). Intraobserver and interobserver variability were examined previously and showed good concordance (κ 0.6-0.9)[33].

2.6 Statistical analyses

Quantitatively scored characteristics (macrophages, smooth muscle cells, and the vessel density) were Box-Cox transformed[34] to obtain a normal distribution. Association of the common variants with continuous parameters were statistically tested with linear regression and the categorical parameters with logistic regression. Data was corrected for age, sex, genotyping chip, and genetic ancestry using principal components 1 through 4. Correction for multiple testing resulted in a corrected p-value of p = 0.05/((7 plaque

phenotypes + plaque vulnerability) x 2 common variants) = 3.13 x 10^{-3}. The power of the study was estimated at ±75% based on a sample size of 1,443, a minor allele frequency (MAF) of 0.409 and a relative risk of 1.28 (http://csg.sph.umich.edu/abecasis/cats/gas_power_calculator/, Supplemental Figure 1).

3. Results

3.1 Baseline characteristics

A total of 1,443 patients that underwent carotid endarterectomy were genotyped and included in this study. The genotyped groups (AEGS1 and AEGS2) are not overlapping. As we previously showed that the baseline characteristics of both groups are comparable[28], the groups were combined for overall plaque vulnerability and phenotype analyses. Baseline characteristics of the combined groups are shown in Table 1.

3.2 Common variants altering *OSM*, *OSMR* and *LIFR* expression

OSM is secreted by neutrophils[4], monocytes[2], macrophages[2] and T-cells[3], and acts through binding to OSMR and LIFR[5–7] in the arterial wall[13,35]. Thus we queried data from the Genotype-Tissue Expression project (GTEx)[26] for SNPs that alter *OSM* expression in whole blood and *LIFR* and *OSMR* expression in arterial tissue. There were no significant eQTLs for *OSM*, but there were two eQTLs that associated with either altered *OSMR* (rs13168867) or *LIFR* (rs10491509) expression in arterial tissue. The C allele of rs13168867 showed the strongest association with decreased *OSMR* expression in the tibial artery (Figure 1A), and the A allele of rs10491509 showed the strongest association with increased *LIFR* expression in the aortic artery (Figure 1B). Cross-tissue meta-analysis showed that these variants have > 0.9 m-values in both tibial and aortic artery tissue, indicating a high probability that they are single *cis*-eQTLs in both tissues (Supplemental Figure 2 and 3).

3.3 Genetic association with plaque vulnerability

To determine the effect of OSM signaling on the overall plaque vulnerability, we correlated the rs13168867 and rs10491509 genotypes to the overall plaque vulnerability, which was

given a score ranging from 0 (least vulnerable plaque) to 4 (most vulnerable plaque). The effect allele of variant rs13168867 in the *OSMR* locus was significantly correlated with an increased overall plaque vulnerability ($\beta = 0.118 \pm$ s.e. $= 0.040$ (C allele), $p = 3.00 \times 10^{-3}$, Table 2), which is visualized in Figure 2. No association was observed with rs10491509 and overall plaque vulnerability.

3.4 Genetic association with plaque phenotypes

To determine the effect of OSM signaling on the plaque phenotype, we assessed the association between rs13168867 and rs10491509 and seven plaque phenotypes in the Athero-Express Biobank Study. The strongest associations were observed between the effect allele of variant rs13168867 in the *OSMR* locus and intraplaque fat ($\beta = 0.248 \pm$ s.e. $= 0.088$ (C allele), $p = 4.66 \times 10^{-3}$), and collagen content ($\beta = -0.259 \pm$ s.e. $= 0.095$ (C allele), $p = 6.22 \times 10^{-3}$, Table 3). No associations were observed between rs10491509 and any of the plaque phenotypes.

4. Discussion

We investigated whether common variants associated to arterial gene expression, eQTLs, near *OSM, OSMR* and *LIFR* affect overall plaque vulnerability and phenotype. We showed that one *cis*-acting eQTL (rs13168867), associated with reduced *OSMR* expression arterial tissue, is associated with increased plaque vulnerability. This suggests that a decrease in *OSMR* expression and therefore possibly a decrease in OSM signaling, increases the chance on a vulnerable plaque. To gain further insight into the role of genetically decreased *OSMR* expression on plaque vulnerability, we examined the effect of rs13168867 on individual plaque characteristics in more detail. The strongest associations were found for rs13168867 with increased intraplaque fat and decreased collagen content, suggesting that reduced OSM signaling results in a larger lipid core and less fibrosis - in line with a more vulnerable plaque phenotype. None of the other plaque characteristics were associated with rs13168867.

The increase in intraplaque fat content that is associated with genetically decreased arterial *OSMR* expression can be related to the effect of OSM signaling on endothelial cells. OSM enhances ICAM-1 expression, but not VCAM-1 expression on endothelial cells[36,37], suggesting that OSM enhances recruitment of the non-classical monocyte subset[38,39]has a protective effect on the endothelium[38,39] Furthermore, this monocyte subset is biased to turn into the M2 macrophage subset[40], which is associated with plaque regression[41–43]. OSM could even accelerate this process as OSM induces M2 macrophage polarization [21]. Yet, this hypothesis remains to be investigated and future studies should explore whether genetically reduced OSM signaling indeed increases intraplaque lipid content by reduced recruitment of non-classical monocytes and impaired M2 macrophage polarization. The decrease in collagen content associated to genetically decreased OSM signaling could be attributed to the increase in fibroblast proliferation and enhancement of collagen formation that is induced by OSM[20]. Moreover, OSM enhances liver fibrosis in mice[44] and is upregulated in patients with pulmonary fibrosis[45]. Future studies are required to investigate if decreased OSM signaling indeed contributes to a decreased collagen content in plaques by reduced fibroblast proliferation and collagen formation.

No significant associations were found for rs10491509 in the *LIFR* locus and overall plaque vulnerability or any of the investigated plaque phenotypes. This suggests that an increase in *LIFR* expression does not affect OSM signaling or that OSMR is the dominant receptor in OSM signaling regarding the OSM related effects on atherosclerotic plaque vulnerability and phenotypes.

Recent developments in single-cell expression analyses might extend on the present study by investigating which cell types, that are present in the plaque, most abundantly express *OSM, OSMR* and *LIFR.* Furthermore, it would be interesting to investigate if the *OSMR/LIFR* expression ratio correlates with plaque vulnerability and if this ratio might be a predictor of plaque vulnerability.

Interestingly, a previous study showed that OSMR deficient mice have more stable plaques[13], which runs counter to our findings. This controversy could be explained by the

differences in human and murine OSM signaling. In humans OSM binds to OSMR and LIFR with the same affinity, while murine OSM has a much stronger affinity for OSMR[46] In point of fact, while homologous, the sequence similarity of human OSM, OSMR, LIFR with murine is moderate at best (64.75%, 70.79%, 78.51%, respectively based on data from GeneCards[47]) and might directly impact receptor affinity[48].

Based on our data we can conclude that the variant rs13168867 in the *OSMR* locus is associated with increased plaque vulnerability. Given the multiple testing burden for individual plaque characteristics, it remains unclear through which precise biological mechanisms OSM signaling exerts its effects on plaque morphology, although our data point towards lipid metabolism and extracellular matrix remodeling. Compared to genome-wide association studies that include thousands of individuals, the Athero-Express Biobank Study is relatively small (n = 1,443), and given its design finite in size. However it is well suited to examine the effect of common disease associated genetic variation on plaque morphology and characteristics. Indeed, we estimated the power at ±75% given a MAF = 0.40 (approximately the frequency of rs13168867) and relative risk = 1.28[49].

5. Conclusion

We associated one eQTL in the *OSMR* locus, which associates with decreased arterial *OSMR* gene expression, with increased human plaque vulnerability in the Athero-Express Biobank Study. Further analyses of plaque phenotypes showed the strongest associations of this eQTL with increased intraplaque fat and decreased collagen content. No associations were found between an eQTL in the *LIFR* locus and either plaque vulnerability or any of the investigated plaque characteristics. In contrast to earlier mouse studies, our observations in human derived samples suggest that genetically decreased *OSMR* arterial expression contributes to increased carotid plaque vulnerability. Further, our study underscores the need to study prospective therapeutic targets derived in experimental models through human genetics.

Funding

SWvdL is funded through grants from the Netherlands CardioVascular Research Initiative of the Netherlands Heart Foundation (CVON 2011/B019 and CVON 2017-20: Generating the best evidence-based pharmaceutical targets for atherosclerosis [GENIUS I&II]). This work was supported by ERA-CVD, grant number: 01KL1802. FWA is supported by UCL Hospitals NIHR Biomedical Research Centre. DvK, HP and DT are funded through the FP7 EU project CarTarDis (FP7/2007-2013) under grant agreement 602936. HP received funding from the TNO research program "Preventive Health Technologies".

Disclosures

DvK is employee of Quorics B.V. and DT is employee of SkylineDx B.V and Quorics B.V. Quorics B.V. and SkylineDx B.V. had no part whatsoever in the conception, design, or execution of this study nor the preparation and contents of this manuscript.

Acknowledgments

We would like to thank dr. Jessica van Setten and acknowledge her graciously for imputing our datasets using an in-house developed imputation pipeline. Evelyn Velema and Petra Homoet-Van der Kraak are graciously acknowledged for the immunohistochemical stainings. We also acknowledge the support from the Netherlands CardioVascular Research Initiative from the Dutch Heart Foundation, Dutch Federation of University Medical Centres, the Netherlands Organisation for Health Research and Development and the Royal Netherlands Academy of Sciences ("GENIUS I & II", CVON2011-19) and the TNO research program "Preventive Health Technologies".

References

1. Guihard P, Boutet M-A, Brounais-Le Royer B, Gamblin A-L, Amiaud J, Renaud A, et al. Oncostatin m, an inflammatory cytokine produced by macrophages, supports intramembranous bone healing in a mouse model of tibia injury. Am J Pathol. 2015;185(3):765–75.

2. Kastl SP, Speidl WS, Kaun C, Katsaros KM, Rega G, Afonyushkin T, et al. In human macrophages the complement component C5a induces the expression of oncostatin M via AP-1 activation. Arterioscler Thromb Vasc Biol. 2008;28(3):498–503.

3. Brown TJ, Lioubin MN, Marquardt H. Purification and characterization of cytostatic lymphokines produced by activated human T lymphocytes. Synergistic antiproliferative activity of transforming growth factor beta 1, interferon-gamma, and oncostatin M for human melanoma cells. J Immunol. 1987;139(9):2977–83.

4. Grenier A, Dehoux M, Boutten A, Arce-Vicioso M, Durand G, Gougerot-Pocidalo MA, et al. Oncostatin M production and regulation by human polymorphonuclear neutrophils. Blood. 1999;93(4):1413–21.

5. Thoma B, Bird TA, Friend DJ, Gearing DP, Dower SK. Oncostatin M and leukemia inhibitory factor trigger overlapping and different signals through partially shared receptor complexes. J Biol Chem. 1994;269(8):6215–22.

6. Dey G, Radhakrishnan A, Syed N, Thomas JK, Nadig A, Srikumar K, et al. Signaling network of Oncostatin M pathway. J Cell Commun Signal. 2013;7(2):103–8.

7. Hermanns HM, Radtke S, Haan C, Schmitz-Van de Leur H, Tavernier J, Heinrich PC, et al. Contributions of leukemia inhibitory factor receptor and oncostatin M receptor to signal transduction in heterodimeric complexes with glycoprotein 130. J Immunol. 1999;163(12):6651–8.

8. Mosley B, De Imus C, Friend D, Boiani N, Thoma B, Park LS, et al. Dual oncostatin M (OSM) receptors. Cloning and characterization of an alternative signaling subunit conferring OSM-specific receptor activation. J Biol Chem. 1996;271(51):32635–43.

9. Pradeep AR, Thorat Manojkumar S, Garima G, Raju A. Serum levels of oncostatin M (a gp 130 cytokine): an inflammatory biomarker in periodontal disease. Biomarkers. 2010;15(3):277–82.

10. Hui W, Bell M, Carroll G. Detection of oncostatin M in synovial fluid from patients with rheumatoid arthritis. Ann Rheum Dis. 1997;56(3):184–7.

11. West NR, Hegazy AN, Owens BMJ, Bullers SJ, Linggi B, Buonocore S, et al. Oncostatin M drives intestinal inflammation and predicts response to tumor necrosis factor-neutralizing therapy in patients with inflammatory bowel disease. Nat Med. 2017;23(5):579–89.

12. Albasanz-Puig A, Murray J, Preusch M, Coan D, Namekata M, Patel Y, et al. Oncostatin M is expressed in atherosclerotic lesions: a role for Oncostatin M in the pathogenesis of atherosclerosis. Atherosclerosis. 2011;216(2):292–8.

13. Zhang X, Li J, Qin J-J, Cheng W-L, Zhu X, Gong F-H, et al. Oncostatin M receptor β deficiency attenuates atherogenesis by inhibiting JAK2/STAT3 signaling in macrophages. J Lipid Res. 2017;58(5):895–906.

14. van Keulen D, Pouwer MG, Pasterkamp G, van Gool AJ, Sollewijn Gelpke MD, Princen HMG, et al. Inflammatory cytokine oncostatin M induces endothelial activation in macro- and microvascular endothelial cells and in APOE*3Leiden.CETP mice. PLoS One. 2018;13(10):e0204911.

15. Vasse M, Pourtau J, Trochon V, Muraine M, Vannier JP, Lu H, et al. Oncostatin M induces angiogenesis in vitro and in vivo. Arterioscler Thromb Vasc Biol. 1999;19(8):1835–42.

16. Takata F, Sumi N, Nishioku T, Harada E, Wakigawa T, Shuto H, et al. Oncostatin M induces functional and structural impairment of blood-brain barriers comprised of rat brain capillary endothelial cells. Neurosci Lett. 2008;441(2):163–6.

17. Guihard P, Danger Y, Brounais B, David E, Brion R, Delecrin J, et al. Induction of osteogenesis in mesenchymal stem cells by activated monocytes/macrophages depends on oncostatin M signaling. Stem Cells. 2012;30(4):762–72.

18. Hutcheson JD, Maldonado N, Aikawa E. Small entities with large impact: microcalcifications and atherosclerotic plaque vulnerability. Curr Opin Lipidol. 2014;25(5):327–32.

19. Virmani R, Kolodgie FD, Burke AP, Finn AV, Gold HK, Tulenko TN, et al. Atherosclerotic Plaque Progression and Vulnerability to Rupture [Internet]. Vol. 25, Arteriosclerosis, Thrombosis, and Vascular Biology. 2005. p. 2054–61. Available from: http://dx.doi.org/10.1161/01.atv.0000178991.71605.18

20. Scaffidi AK, Mutsaers SE, Moodley YP, McAnulty RJ, Laurent GJ, Thompson PJ, et al. Oncostatin M stimulates proliferation, induces collagen production and inhibits apoptosis of human lung fibroblasts. Br J Pharmacol. 2002;136(5):793–801.

21. Shrivastava R, Asif M, Singh V, Dubey P, Ahmad Malik S, Lone M-U-D, et al. M2 polarization of macrophages by Oncostatin M in hypoxic tumor microenvironment is mediated by mTORC2 and promotes tumor growth and metastasis. Cytokine [Internet]. 2018. Available from: http://dx.doi.org/10.1016/j.cyto.2018.03.032

22. Wal A van der, van der Wal A. Atherosclerotic plaque rupture – pathologic basis of plaque stability and instability [Internet]. Vol. 41, Cardiovascular Research. 1999. p. 334–44. Available from: http://dx.doi.org/10.1016/s0008-6363(98)00276-4

23. Medbury HJ, James V, Ngo J, Hitos K, Wang Y, Harris DC, et al. Differing association of macrophage subsets with atherosclerotic plaque stability. Int Angiol. 2013;32(1):74–84.

24. Lafont A. Basic aspects of plaque vulnerability [Internet]. Vol. 89, Heart. 2003. p. 1262–7. Available from: http://dx.doi.org/10.1136/heart.89.10.1262

25. Verhoeven BAN, Velema E, Schoneveld AH, de. JPP, de Bruin P, Seldenrijk CA, et al. Athero-express: Differential atherosclerotic plaque expression of mRNA and protein in relation to cardiovascular events and patient characteristics. Rationale and design. Eur J Epidemiol. 2004;19(12):1127–33.

26. GTEx Consortium. The Genotype-Tissue Expression (GTEx) project. Nat Genet. 2013;45(6):580–5.

27. Verhoeven B, Hellings WE, Moll FL, de Vries JP, de Kleijn DPV, de Bruin P, et al. Carotid atherosclerotic plaques in patients with transient ischemic attacks and stroke have

unstable characteristics compared with plaques in asymptomatic and amaurosis fugax patients. J Vasc Surg. 2005;42(6):1075–81.

28. van der Laan SW, Foroughi Asl H, van den Borne P, van Setten J, van der Perk MEM, van de Weg SM, et al. Variants in ALOX5, ALOX5AP and LTA4H are not associated with atherosclerotic plaque phenotypes: the Athero-Express Genomics Study. Atherosclerosis. 2015;239(2):528–38.

29. O'Connell J, Gurdasani D, Delaneau O, Pirastu N, Ulivi S, Cocca M, et al. A general approach for haplotype phasing across the full spectrum of relatedness. PLoS Genet. 2014;10(4):e1004234.

30. 1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, et al. A global reference for human genetic variation. Nature. 2015;526(7571):68–74.

31. Genome of the Netherlands Consortium. Whole-genome sequence variation, population structure and demographic history of the Dutch population. Nat Genet. 2014;46(8):818–25.

32. van Lammeren GW, den Ruijter HM, Vrijenhoek JEP, van der Laan SW, Velema E, de Vries J-PPM, et al. Time-dependent changes in atherosclerotic plaque composition in patients undergoing carotid surgery. Circulation. 2014;129(22):2269–76.

33. Hellings WE, Pasterkamp G, Vollebregt A, Seldenrijk CA, De Vries J-PPM, Velema E, et al. Intraobserver and interobserver variability and spatial differences in histologic examination of carotid endarterectomy specimens. J Vasc Surg. 2007;46(6):1147–54.

34. Box GEP, Cox DR. An Analysis of Transformations. J R Stat Soc Series B Stat Methodol. 1964;26(2):211–52.

35. Rolfe BE, Stamatiou S, World CJ, Brown L, Thomas AC, Bingley JA, et al. Leukaemia inhibitory factor retards the progression of atherosclerosis. Cardiovasc Res. 2003;58(1):222–30.

36. Ruprecht K, Kuhlmann T, Seif F, Hummel V, Kruse N, Brück W, et al. Effects of oncostatin M on human cerebral endothelial cells and expression in inflammatory brain lesions. J Neuropathol Exp Neurol. 2001;60(11):1087–98.

37. Modur V, Feldhaus MJ, Weyrich AS, Jicha DL, Prescott SM, Zimmerman GA, et al. Oncostatin M is a proinflammatory mediator. In vivo effects correlate with endothelial cell expression of inflammatory cytokines and adhesion molecules. J Clin Invest. 1997;100(1):158–68.

38. Yang J, Zhang L, Yu C, Yang X-F, Wang H. Monocyte and macrophage differentiation: circulation inflammatory monocyte as biomarker for inflammatory diseases. Biomarker Research. 2014;2(1):1.

39. Quintar A, McArdle S, Wolf D, Marki A, Ehinger E, Vassallo M, et al. Endothelial Protective Monocyte Patrolling in Large Arteries Intensified by Western Diet and Atherosclerosis. Circ Res. 2017;120(11):1789–99.

40. Italiani P, Boraschi D. From Monocytes to M1/M2 Macrophages: Phenotypical vs. Functional Differentiation. Front Immunol. 2014;5:514.

41. Chinetti-Gbaguidi G, Baron M, Bouhlel MA, Vanhoutte J, Copin C, Sebti Y, et al. Human Atherosclerotic Plaque Alternative Macrophages Display Low Cholesterol Handling but High Phagocytosis Because of Distinct Activities of the PPARγ and LXRα Pathways. Circ Res. 2011;108(8):985–95.

42. Bobryshev YV, Ivanova EA, Chistiakov DA, Nikiforov NG, Orekhov AN. Macrophages and Their Role in Atherosclerosis: Pathophysiology and Transcriptome Analysis. Biomed Res Int. 2016;2016:9582430.

43. Rahman K, Vengrenyuk Y, Ramsey SA, Vila NR, Girgis NM, Liu J, et al. Inflammatory Ly6Chi monocytes and their conversion to M2 macrophages drive atherosclerosis regression. J Clin Invest. 2017;127(8):2904–15.

44. Matsuda M, Tsurusaki S, Miyata N, Saijou E, Okochi H, Miyajima A, et al. Oncostatin M causes liver fibrosis by regulating cooperation between hepatic stellate cells and macrophages in mice. Hepatology. 2018;67(1):296–312.

45. Mozaffarian A, Brewer AW, Trueblood ES, Luzina IG, Todd NW, Atamas SP, et al. Mechanisms of oncostatin M-induced pulmonary inflammation and fibrosis. J Immunol. 2008;181(10):7243–53.

46. Walker EC, Johnson RW, Hu Y, Brennan HJ, Poulton IJ, Zhang J-G, et al. Murine Oncostatin M Acts via Leukemia Inhibitory Factor Receptor to Phosphorylate Signal Transducer and Activator of Transcription 3 (STAT3) but Not STAT1, an Effect That Protects Bone Mass. J Biol Chem. 2016;291(41):21703–16.

47. Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, et al. The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. Curr Protoc Bioinformatics. 2016;54:1.30.1–1.30.33.

48. Drechsler J, Grötzinger J, Hermanns HM. Characterization of the rat oncostatin M receptor complex which resembles the human, but differs from the murine cytokine receptor. PLoS One. 2012;7(8):e43155.

49. Johnson JL, Abecasis GR. GAS Power Calculator: web-based power calculator for genetic association studies [Internet]. 2017. Available from: http://dx.doi.org/10.1101/164343

50. Carithers LJ, Moore HM. The Genotype-Tissue Expression (GTEx) Project. Biopreserv Biobank. 2015;13(5):307–8.

Table 1. Baseline characteristics of genotyped CEA patients from the Athero-Express Biobank Study. Cerebrovascular disease history is defined by ischemic stroke prior to surgery. Coronary artery disease history includes coronary artery disease, myocardial infarction, percutaneous coronary intervention, and coronary artery bypass graft. Peripheral disease history includes diagnosed peripheral arterial occlusive disease, femoral artery interventions, and ankle-brachial index <70. Type 2 diabetes mellitus includes all individuals with diagnosed type 2 diabetes mellitus and those on appropriate medication. Hypertension includes all individuals with self-reported hypertension. Current smokers include all individuals smoking up to 6 months until the surgery date. BMI, kg/m^2. eGFR rate was based on the Modification of Diet in Renal Disease formula, mL/min/1.73m^2. Anti-hypertensives include all anti-hypertension medication. Anti-thrombotics include clopidogrel, dipyridamole, acenocoumarin, ascal, and anti-platelet drugs. Missingness shows the percentage of the patients of which we lack information on the specific patient characteristic.

Patient characteristics	**AEGS 1 and 2** **N = 1,443**	**Missingness (%)**
Sex, male, n (%)	976 (64.0)	5.7
Age in years, mean (SD)	68.84 (9.33)	5.7
History		
Cerebrovascular disease, n (%)	478 (33.2)	5.7
Coronary artery disease, n (%)	430 (29.9)	5.8
Peripheral artery disease, n (%)	297 (20.7)	5.8
Risk factors		
Type 2 diabetes mellitus, n (%)	332 (23.1)	5.7
Hypertension, n (%)	1017 (73.0)	8.7

Current smoker, n (%)	492 (34.9)	7.5
BMI, median [IQR]	26.0 [24.0-28.4]	11.5
eGFR, median [IQR]	72.3 [58.7-85.4]	8.1
Total cholesterol in mmol/L, median [IQR]	4.38 [3.60-5.25]	22.8
LDL in mmol/L, median [IQR]	2.40 [1.81-3.13]	27.8
HDL in mmol/L, median [IQR]	1.06 [0.87-1.30]	25.0
Triglycerides in mmol/L, median [IQR]	1.50 [1.08-2.04]	24.6
Medication		
Antihypertensives, n (%)	1110 (77.2)	5.8
Lipid lowering drugs, n (%)	1112 (77.4)	5.8
Antithrombotics, n (%)	1272 (88.6)	6.0
Symptoms		
Asymptomatics, n (%)	195 (13.6)	6.0
Ocular, n (%)	221 (15.4)	6.0
TIA, n (%)	634 (44.2)	6.0
Stroke, n (%)	384 (26.8)	6.0

Table 2. *OSMR* and *LIFR* variants and their association with overall plaque vulnerability

For each variant, the association with overall plaque vulnerability is given. NEF: the normalized effect size on expression (from GTEx Portal, www.gtexportal.org)[26]); Alleles: the effect allele and the other allele, respectively; EAF: effect allele frequency; Info: estimated imputation score; β: effect size; s.e.: standard error; *P*: p-value of association.

Gene	Variant	NEF	Alleles	EAF	Info	β (s.e.)	*P*
OSMR	rs13168867	-0.13	C/T	0.393	0.999	0.118 (0.040)	**3.00×10^{-3}**
LIFR	rs10491509	0.29	A/G	0.351	0.989	0.001 (0.043)	0.981

Table 3. *OSMR* and *LIFR* variants and their association with plaque phenotypes

For each variant, the association with plaque phenotypes is given. NEF: the normalized effect size on expression; Alleles: the effect allele and the other allele, respectively; EAF: effect allele frequency; Info: estimated imputation score; β: effect size; s.e.: standard error; *P*: p-value of association. Calcification and collagen were classified as no/minor vs moderate/heavy, fat content as 10% vs >10% fat of plaque area, intraplaque hemorrhage was classified as absent vs present. Smooth muscle cells and macrophages were classified as Box-Cox transformed percentage of plaque area and vessel density as Box-Cox transformed number of vessels/ hotspot.

Gene	Variant	NEF	Alleles	EAF	Info	Phenotype	β (s.e.)	*P*
OSMR	rs13168867	-0.13	C/T	0.393	0.999	Calcification	0.036 (0.077)	0.637
						Collagen	-0.259 (0.095)	6.22×10^{-3}
						Fat content	0.248 (0.088)	4.66×10^{-3}
						Intraplaque hemorrhage	-0.014 (0.080)	0.862
						Smooth muscle cells	0.001 (0.011)	0.913
						Vessel density	-0.000 (0.004)	0.976
						Macrophages	0.004 (0.015)	0.809
LIFR	rs10491509	0.29	A/G	0.351	0.989	Calcification	0.046 (0.082)	0.577

						Collagen	0.134 (0.104)	0.194
						Fat content	0.086 (0.094)	0.363
						Intraplaque hemorrhage	0.071 (0.086)	0.414
						Smooth muscle cells	-0.003 (0.012)	0.840
						Vessel density	0.002 (0.004)	0.577
						Macrophages	0.015 (0.016)	0.354

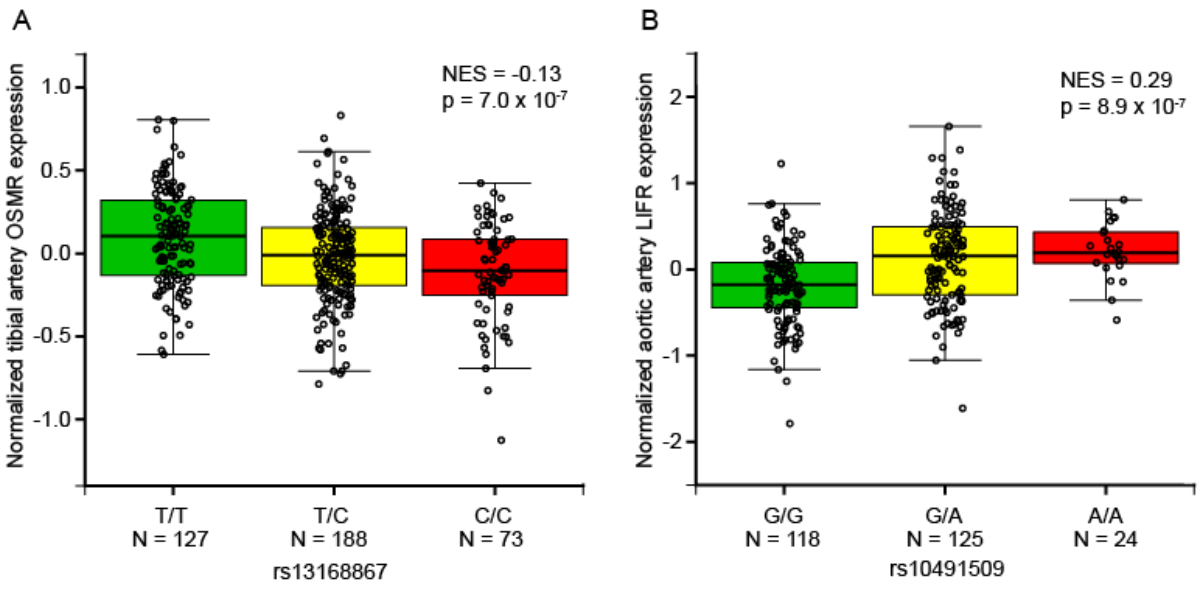

Figure 1: Association of *OSMR* and *LIFR* variants in arterial tissues. Per variant, the normalized expression of *OSMR* (A) and *LIFR* (B) is given in arterial tissue. Data from GTEx Portal (www.gtexportal.org). NES = Normalized effect size. In aortic arterial tissue, rs13168867 had a NES of -0.123 and in tibial arterial tissue, rs10491509 had a NES of 0.0881 (Supplementary Figure 2 and 3).

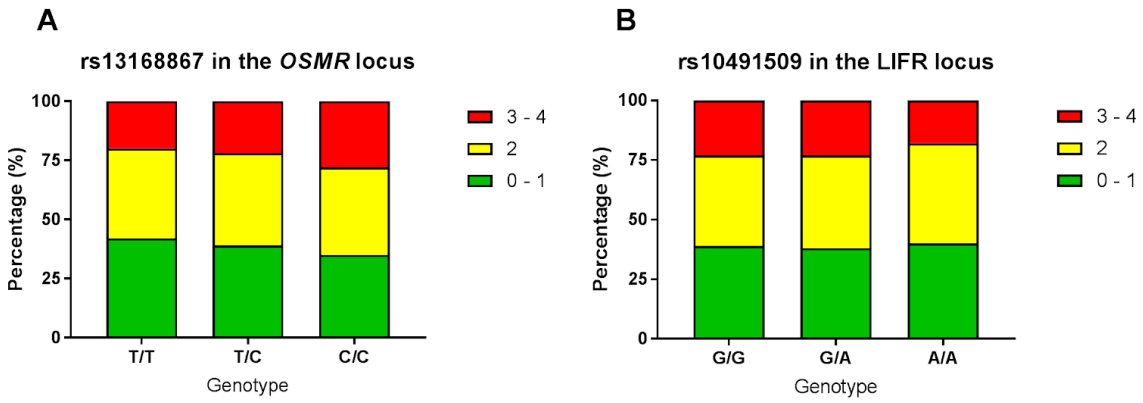

Figure 2: Association of *OSMR* and *LIFR* variants with overall plaque vulnerability. The plaques were given a score ranging from 0 (least vulnerable plaque) to 4 (most vulnerable plaque) and divided in three groups (Group 1 contains plaque vulnerability scores 0 and 1, group 2 contains the plaque vulnerability score 2 and group 3 contains the plaque vulnerability scores 3 and 4). The bars represent the proportion of each group per genotype for rs13168867 in the *OSMR* locus (A) and rs10491509 in the *LIFR* locus (B). A bar chart showing the proportion of each individual score is shown in Supplemental Figure 4.