Biomarkers of High-Risk Carotid Atherosclerosis

By

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Abstract

Carotid atherosclerosis is responsible for 15-25% of the nearly 8 million first-ever ischemic strokes that occur each year worldwide. This proportion has remained constant over the past three decades, thus suggesting that some patients with carotid atherosclerosis currently receive suboptimal treatment to prevent stroke. Therefore, new strategies are needed not only to improve stroke risk stratification but also to better understand the pathobiology of carotid atherosclerosis. The overall objective of this three-part doctoral thesis was to provide new robust evidence to support the use of both imaging and blood biomarkers for clinical decision-making in the routine management of patients with carotid atherosclerosis.

In the first part of our work, we used a meta-analysis approach to demonstrate that ipsilateral non-stenotic carotid plaques with high-risk imaging features are common in patients with embolic stroke of unknown source and might be the actual cause of the stroke. We relied on the same approach to show that high-risk plaques are common in asymptomatic carotid stenosis and the associated risk of ipsilateral ischemic cerebrovascular events is higher than commonly perceived. In the second part, we conducted systematic literature reviews and identified several biomarkers that have been associated with carotid plaque vulnerability or progression and with cerebrovascular events in patients with carotid atherosclerosis. Such biomarkers included interleukin-1β, interleukin-6, C-reactive protein, uric acid, lipoprotein associated phospholipase A2, and lectin-like oxidized LDL receptor among others. However, none of the biomarkers had a validated threshold for use in the clinical management of patients with carotid atherosclerosis and only a few were targetable with existing drugs, notably interleukin-6. In the third part, we used a prediction modelling approach to demonstrate that interleukin-6 predicts carotid plaque severity, vulnerability, and progression, independent of dyslipidemia and other cardiovascular risk factors. We also showed that 2.0 pg/mL represents a promising candidate clinical cut-off that could help select patients with carotid atherosclerosis who would benefit from anti-IL6

drugs as an adjuvant stroke prevention strategy. Furthermore, we provided preliminary evidence that monocyte transcriptomics analysis could help define a distinctive molecular profile for high-risk carotid atherosclerosis to inform in-depth pathobiological investigations and potentially support the identification of new biomarkers and therapeutic targets.

Altogether, our research has four core implications for clinical practice and research. First, routine assessment of carotid atherosclerosis beyond the grade of stenosis using multimodal neurovascular imaging should be implemented in clinical practice to support the etiological classification of embolic strokes of unknown source, improve stroke risk stratification in asymptomatic carotid stenosis, and optimize stroke prevention strategies. Second, revascularization trials using multimodal neurovascular imaging for risk stratification before randomization in patients with asymptomatic carotid stenosis are warranted. Third, biomarkers have a role to play in the management of carotid atherosclerosis, but more research is needed to accelerate their integration into routine clinical practice, notably the definition and validation of thresholds, the combination into panels, and the integration into diagnostic and prognostic tools. Finally, trials of anti-interleukin-6 drugs as an adjuvant stroke prevention strategy in people with carotid atherosclerosis are warranted.

Preface

This thesis is original research work by Joseph Kamtchum Tatuene.

Chapter 1 has been published as "Kamtchum-Tatuene J, Wilman A, Saqqur M, Shuaib A, Jickling GC. Carotid Plaque with High-Risk Features in Embolic Stroke of Undetermined Source: Systematic Review and Meta-Analysis. *Stroke* 2020; 51(1): 311-314." Kamtchum Tatuene J and Jickling GC conceived the study. Kamtchum Tatuene J performed the literature search, selected the articles, extracted the data, performed the analyses, and drafted the manuscript. Wilman A, Saqqur M, Shuaib A, and Jickling GC contributed to data interpretation and critically revised the manuscript. Kamtchum Tatuene J is the guarantor of the review.

Chapter 2 has been published as "Kamtchum-Tatuene J, Noubiap JJ, Wilman AH, Saqqur M, Shuaib A, Jickling GC. Prevalence of High-Risk Plaques and Risk of Stroke in Patients with Asymptomatic Carotid Stenosis: A Meta-analysis. *JAMA Neurol* 2020; 77(12):1524-1535." Kamtchum Tatuene J and Jickling GC conceived the study. Kamtchum Tatuene J prepared the protocol and performed the literature search. Kamtchum Tatuene J and Noubiap JJ selected the studies and assessed the risk of bias. Kamtchum Tatuene J extracted the data, performed the analyses, and drafted the manuscript. Noubiap JJ, Wilman AH, Saqqur M, Shuaib A, Jickling GC contributed to data interpretation, critically revised successive versions of the manuscript, and approved the final version. Kamtchum Tatuene J is the guarantor of the review.

Chapter 3 has been published as "Kamtchum-Tatuene J, Jickling GC. Blood Biomarkers for Stroke Diagnosis and Management. *Neuromolecular Med* 2019; 21(4): 344-368." Kamtchum Tatuene J performed the literature search, selected relevant sources, and wrote the manuscript. Jickling GC revised the manuscript.

Chapter 4 has been published as "Kamtchum-Tatuene J, Nomani AZ, Falcione S, Munsterman D, Sykes G, Joy T, Spronk E, Vargas MI, Jickling GC. Non-stenotic Carotid Plaques in Embolic Stroke of Unknown Source. *Front Neurol* 2021; 12: 719329." Kamtchum Tatuene J did the literature search and wrote the manuscript. Vargas MI and Kamtchum-Tatuene J prepared the figure. Nomani AZ, Falcione S, Munsterman D, Sykes G, Joy T, Spronk E, Vargas MI, and Jickling GC critically revised the manuscript.

Chapter 5 has been submitted as "Kamtchum-Tatuene J, Saba L, Heldner MR, Poorthuis MHF, de Borst GJ, Rundek T, Kakkos SK, Chaturvedi S, Topakian R, Polak JF, Jickling GC. Circulating interleukin-6 predicts carotid plaque severity, vulnerability, and progression in the Cardiovascular Health Study." The related research project referred to as the Carotid Atherosclerosis and Stroke Collaboration (CASCO) received research ethics approval from the University of Alberta Human Research Ethics Board (Pro00106520). Kamtchum Tatuene J contributed to project conception and design, data curation and analysis, project administration, data visualisation, and manuscript writing. Saba L, Heldner MR, Poorthuis MHF, de Borst GJ, Rundek T, Kakkos SK, Chaturvedi S, and Topakian R critically revised the manuscript. Polak JF contributed to data curation, project administration, supervision, and manuscript review & editing. Jickling GC contributed to project administration, supervision, and manuscript review & editing.

Chapter 6 has been submitted as "Kamtchum-Tatuene J, Falcione S, Munsterman D, Sykes G, Joy T, Jickling GC. Monocyte transcriptomic analysis of high-risk carotid atherosclerosis." The related project referred to as the Molecular Signatures of high-risk Carotid Atherosclerosis (MOSCATO) study was nested in the University of Alberta Stroke Genomics (UASG) study which was approved by the University of Alberta Human Research Ethics Board (Pro00066577). Kamtchum Tatuene J and Jickling GC conceived the study. Kamtchum Tatuene J and Munsterman D consented and enrolled the participants. Kamtchum Tatuene J performed the laboratory experiments and the data analyses and drafted the manuscript. Falcione S, Munsterman D, Sykes G, Joy T, and Jickling GC contributed to data interpretation and critically revised the manuscript. Jickling GC is the principal investigator of the UASG and supervised all aspects of the MOSCATO study.

Dedication

To the Lord

&

To my late mother

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Thank you also to those whose names might have been mistakenly omitted on this non-exhaustive list.

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LIST OF ABBREVIATIONS

ADAMTS = A Disintegrin And Metalloproteinase with Thrombospondin type-1 motif

AHA = American Heart Association

aHR = adjusted hazard ratio

ANP = atrial natriuretic peptide

aOR = adjusted odds ratio

APOA1-UP = apolipoprotein A1 unique peptide

AUC = area under the receiver operating curve

BNP = B-type natriuretic peptide

CANTOS = Canakinumab Anti-inflammatory Thrombosis Outcome Study

CASCO = carotid atherosclerosis and stroke collaboration

Cdc42Hs = cell division control 42 *Homo sapiens*

CDM = clinical diffusion mismatch

c-Fn = cellular fibronectin

CHS = cardiovascular health study

CITL = calibration in the large

CRP = C-reactive protein

CT = computerized tomography

CVE = cerebrovascular events

DHC = decompressive hemicraniectomy

DNA = desoxyribonucleic acid

ELISA = enzyme-linked immunosorbent assay

END = early neurological deterioration

ESUS = embolic stroke of unknown source

EVT = endovascular thrombectomy

FC = fold change

GFAP = glial fibrillary acid protein

GFR = glomerular filtration rate

HbA1c = glycated hemoglobin

HDAC9 = histone deacetylase 9

HDL-C = high-density lipoprotein cholesterol

HFABP = heart-type fatty acid binding protein

HR = hazard ratio

HT = hemorrhagic transformation

ICAM-1 = intercellular adhesion molecule

ICH = intracerebral hemorrhage

IL-10 = interleukin-10

IL-6 = interleukin-6

IS = ischemic stroke

K2 EDTA = dipotassium ethylenediaminetetraacetate

LAA = large artery atherosclerosis

LDL-C = low-density lipoprotein cholesterol

lncRNA = long non-coding RNA

Lp-PLA2 = lipoprotein-associated phospholipase A2

MBL = mannose-binding lectin

MCA = middle cerebral artery

MiRNA = microRNA

MMP9 = matrix metalloproteinase 9

MRI = magnetic resonance imaging

mRNA = messenger RNA

MR-proANP = mid-regional pro-atrial natriuretic peptide

mRS = modified Rankin scale

MS = mass spectrometry

N/A = not applicable or not available

NASCET = North American Symptomatic Carotid Endarterectomy Trial

NDKA = nucleoside diphosphate kinase A

NfL = neurofilament light

NIHSS = national institutes of health stroke scale

NIPBL = nipped B-like factor

NLRP3 = nucleotide-binding domain leucine-rich repeat and pyrin domain containing receptor 3

NMDA = N-methyl-D-aspartate

NRDC = nardilysin convertase

NSE = neuron-specific enolase

NT-proBNP = N-terminal B-type natriuretic peptide

OR = odds ratio

PAI-1 = plasminogen activator inhibitor 1

PARK7 = Parkinson disease protein 7

PBMC = peripheral blood mononuclear cells

PBP = platelet basic protein

POLM = DNA polymerase Mu

RBP4 = retinol-binding protein 4

RNA = ribonucleic acid

S100B = serum calcium binding protein

Se = sensitivity

SLC44A1 = solute career family 44 member 1

Sp = specificity

TAFI = thrombin-activatable fibrinolysis inhibitor

TIA = transient ischemic attack

TNF-α = tumor necrosis factor α

TNK2 = non-receptor tyrosine kinase 2

tPA = tissue plasminogen activator

UASG = University of Alberta stroke genomics study

VCAM = vascular cell adhesion molecule

VCAM = vascular cell adhesion molecule

VEGF = vascular endothelial growth factor

vWF = von Willebrand factor

ZFAS1 = zinc finger antisense 1

General introduction

Carotid atherosclerosis is responsible for 15-25% of the nearly 8 million first-ever ischemic strokes that occur each year worldwide.[1-3] The current management of carotid atherosclerosis is guided by only two parameters: the grade of stenosis and the occurrence of ipsilateral cerebrovascular events (stroke, transient ischemic attack, amaurosis fugax). Patients with moderate or severe (> 50% stenosis) symptomatic carotid atherosclerosis are offered surgical revascularization with either endarterectomy or stenting while those with mild (<50% stenosis) or asymptomatic carotid atherosclerosis receive best medical therapy consisting mainly of cardiovascular risk factor control and lifestyle modifications.[4,5] Such strategies likely help to prevent some first-ever or recurrent strokes but are obviously insufficient to suppress the overall risk of stroke attributable to carotid atherosclerosis. Indeed, the proportion of ischemic strokes caused by carotid stenosis has remained constant over the past three decades.[6-9] This observation suggests that a significant proportion of patients with carotid atherosclerosis currently receive suboptimal treatment to prevent stroke and this is potentially explained by an underestimation of their true risk. Therefore, new strategies are needed not only to improve stroke risk stratification and intervene earlier but also to better understand the pathobiology of carotid atherosclerosis and design new interventions. Biomarkers seem to be an interesting approach to address both needs.

On one hand, various studies have reported a wide range of imaging biomarkers that could improve stroke risk stratification in patients with carotid atherosclerosis.[10,11] However, the relevance and feasibility of routine vascular imaging to detect those biomarkers remained unclear, thus explaining why physicians have been reluctant to use imaging biomarkers for clinical decision-making. On the other hand, several blood biomarkers of high-risk carotid atherosclerosis have been described in studies of various sizes and quality[12-23] but none has been adopted to support clinical decision-making, thus suggesting the need for more robust and convincing evidence of utility and relevance. Therefore, the overall objective of this doctoral thesis was to provide new robust evidence to support the use of both imaging and blood biomarkers in the routine clinical management of patients with carotid atherosclerosis. The work was organized into 3 parts.

Part A focused on imaging biomarkers and aimed to answer two questions. First, how many patients with a post-stroke work-up reported as unremarkable could potentially be at high risk of stroke recurrence due to the presence of an undertreated ipsilateral mild carotid stenosis with high-risk features? In other words, how often do we find an ipsilateral carotid plaque with high-risk features in patients with an embolic stroke of unknown source (ESUS)? Second, is it relevant and feasible to use multimodal neurovascular imaging to perform a risk-oriented selection for revascularization in patients with asymptomatic carotid atherosclerosis? In other words, are high-risk features frequent enough in asymptomatic carotid stenosis and is the associated risk of stroke high enough to make multimodal neurovascular imaging a reasonable strategy to identify patients who would benefit from surgical revascularization or more aggressive pharmacological interventions?

In part B, we performed two comprehensive reviews of biomarker research in the field of stroke and carotid atherosclerosis. Those reviews aimed to identify the most promising biomarkers that would deserve further investigation in clinical practice and trials. The ideal biomarker would (i) predict first-ever and recurrent ischemic events, (ii) predict carotid plaque severity, vulnerability, and progression, (iii) distinguish strokes due to large artery atherosclerosis from strokes of other causes, (iv) help establish a causal link between a carotid plaque and an ischemic stroke, which would be most relevant in patients with ESUS and an ipsilateral mild carotid stenosis, and of course (v) be a potential therapeutic target for stroke prevention trials. Interleukin-6 (IL-6) was one biomarker fulfilling several of these criteria.[24-30]

In part C, we aimed to provide additional evidence to support the relevance of IL-6 as a biomarker for clinical decision-making in patients with carotid atherosclerosis and define a candidate cut-off that would facilitate its adoption in routine clinical practice. Additionally, we explored the potential of monocyte transcriptomics to improve our understanding of the pathobiology of carotid atherosclerosis and to unravel new candidate biomarkers and therapeutic targets for stroke risk stratification and stroke prevention. Specifically, two questions were addressed in this part of the thesis. First, is IL-6 an independent predictor of carotid plaque severity, vulnerability, and progression and if so, what would be a reasonable threshold for its use as a biomarker in clinical practice? Second, could monocyte transcriptomics help define a distinctive molecular profile of high-risk carotid atherosclerosis that would facilitate the etiological diagnosis of stroke?

Considering the above, our thesis is organized in six chapters corresponding to 6 manuscripts that are either published or under review.

PART A: Imaging biomarkers of high-risk carotid atherosclerosis

Chapter 1: Prevalence of carotid plaques with high-risk features in embolic stroke of undetermined source[1]

1.1. Introduction

Embolic stroke of undetermined source (ESUS) represents 17% (9-25%) of all ischemic strokes [31]. An ipsilateral mild carotid stenosis (plaque with <50% luminal narrowing) is identified in nearly 40% of patients with ESUS and may represent a source of athero-embolism [32,33]. Vascular imaging is used to assess carotid plaque features other than degree of stenosis that may be important to estimate the stroke risk, notably intraplaque hemorrhage, large lipid-rich necrotic core, thin or ruptured fibrous cap, silent embolic infarcts, progression, irregularity or ulceration, echolucency, neovascularization, inflammation, large juxta-liminal hypoechoic area, large plaque volume, microembolic signals, and impaired cerebrovascular reserve [11]. Patients with ESUS that have a high-risk plaque may benefit from specific interventions to prevent stroke. We aimed to summarize data on the frequency of mild carotid stenosis with high-risk features in ESUS.

1.2. Methods

This report is compliant with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The data supporting the findings of this study are available from the corresponding author upon reasonable request**.**

We searched Medline and Ovid-Embase for observational studies reporting carotid plaque imaging results in ESUS, from inception to July 15, 2019 (Appendix 1, Table I). The titles and abstracts were screened, and full texts of potentially eligible records were retrieved for further assessment. Disagreements regarding study inclusion were resolved through consensus among authors. The risk of bias was assessed using the Risk of Bias Tool for Prevalence Studies (Appendix 1, Table II) with the aim of excluding all studies with high-risk of bias from the quantitative synthesis.

We extracted first author's name, year of publication, study design, sample size, mean age, proportion of women, frequency of cardiovascular risk factors, type of index event (stroke or TIA), imaging modality, onset-to-imaging time, side, and frequency of mild carotid stenosis with high-risk features.

[1] This chapter has been published as "Kamtchum-Tatuene J, Wilman A, Saqqur M, Shuaib A, Jickling GC. Carotid Plaque with High-Risk Features in Embolic Stroke of Undetermined Source: Systematic Review and Meta-Analysis. *Stroke* 2020; 51(1): 311-314."

Analyses were performed with STATA (version 13, StataCorp, College Station, TX, USA). Heterogeneity between studies was assessed using the χ^2 test on the Cochran's Q statistic and quantified by the I^2 index. The prevalence of ipsilateral and contralateral mild carotid stenosis with high-risk features was pooled using random-effect meta-analysis after stabilizing the variance of each study with the Freeman-Tukey double arc-sine transformation. Small-study effect was assessed by visual inspection of funnel plots and formally tested using Egger's test. Statistical tests were two-sided and statistical significance was defined as $p \leq 0.05$.

1.3. Results

The initial search identified 181 records. Eight articles met the inclusion criteria [34-41] (Appendix 1, Figure I).

All studies were prospective and enrolled 323 participants with unilateral anterior circulation ischemic stroke (Table 1.1.). Plaque imaging was performed within 14 days of stroke onset using MRI [34,37-39], CTA [36] or ultrasound [35]. Ulceration, intraplaque hemorrhage, thrombus, fibrous cap rupture, echolucency, or plaque thickness ≥ 3 mm were the high-risk features considered.

Table 1.1. Characteristics of the included studies

PMID	Author	Year	Sample size	Age (mean)	Age (median)	Women %	HTN %	DM %	Smoking %	DLP %	CAD %	Plaque imaging	Imaging delay (days)	High-risk features	ROB
24330333	Bayer-Karpinska [34]	2013	32	NA	74	32	72	22	49	28	22	MRI (HRBB)	< 7	ulceration, intraplaque hemorrhage, thrombus	9
29307510	Buon [35]	2018	44	NA	46.5	43	14	2	59	16	NA	Carotid US	NA	ulceration, echolucency, thrombus	9
27412144	Coutinho [36]	2016	85	NA	70	52	60	28	NA	34	20	CTA	< 10	plaque thickness ≥ 3 mm	10
22498329	Freilinger [37]	2012	32	71.7	NA	31	59	22	63	47	22	MRI (HRBB)	5.8	ulceration, intraplaque hemorrhage, thrombus	10
26077590	Gupta [38]	2015	27	71	NA	48	78	22	4	56	11	MRI (3D-TOF)	2.6	intraplaque hemorrhage	8
29571754	Singh [39]	2018	35	74.3	NA	54	74	29	6	80	49	MRI (HRBB)	NA	intraplaque hemorrhage	9
26897689	Gupta [40]	2016	50	69.5	NA	50	NA	NA	NA	NA	NA	MRI (3D-TOF)	1	intraplaque hemorrhage	9
26433367	Hyafil [41]	2016	18	70	NA	63	72	22	17	28	22	MRI (HRBB)	< 14	fibrous cap rupture, intraplaque hemorrhage, thrombus	9

3D-TOF = 3-dimensional time of flight, CAD = Coronary artery disease, DLP = Dyslipidemia, DM = Diabetes mellitus, HRBB = High-resolution black blood, HTN = Hypertension, MRI = Magnetic Resonance Imaging, NA = Not available, PMID = PubMed accession number, ROB = Risk of bias score (maximum 10, 8-10 = low risk of bias/high-quality, 5-7 = moderate risk of bias/moderate quality, ≤4 = high risk of bias/low quality), US = Ultrasound

The pooled prevalence of mild carotid stenosis with high-risk features was 32.5% (95% CI: 25.3 – 40.2) in the ipsilateral carotid (Figure 1.1.) and 4.6 % (95% CI: 0.1 – 13.1) in the contralateral carotid (Appendix1, Figure II). There was no small-study effect (Appendix 1, Figure III). The odds ratio of finding a mild carotid stenosis with high-risk features in the ipsilateral versus the contralateral carotid was 5.5 (95% CI: 2.5 – 12.0) (Figure 1.2.). The odds ratio of finding a ruptured fibrous cap in the ipsilateral versus the contralateral carotid was 17.5 (95% CI: 2.2 – 140.1) (Appendix 1, Table III). In the sensitivity analysis, similar results were obtained after excluding studies with sample size < 20 or with potential population overlap [40,41] (Appendix 1, Figures IV and V).

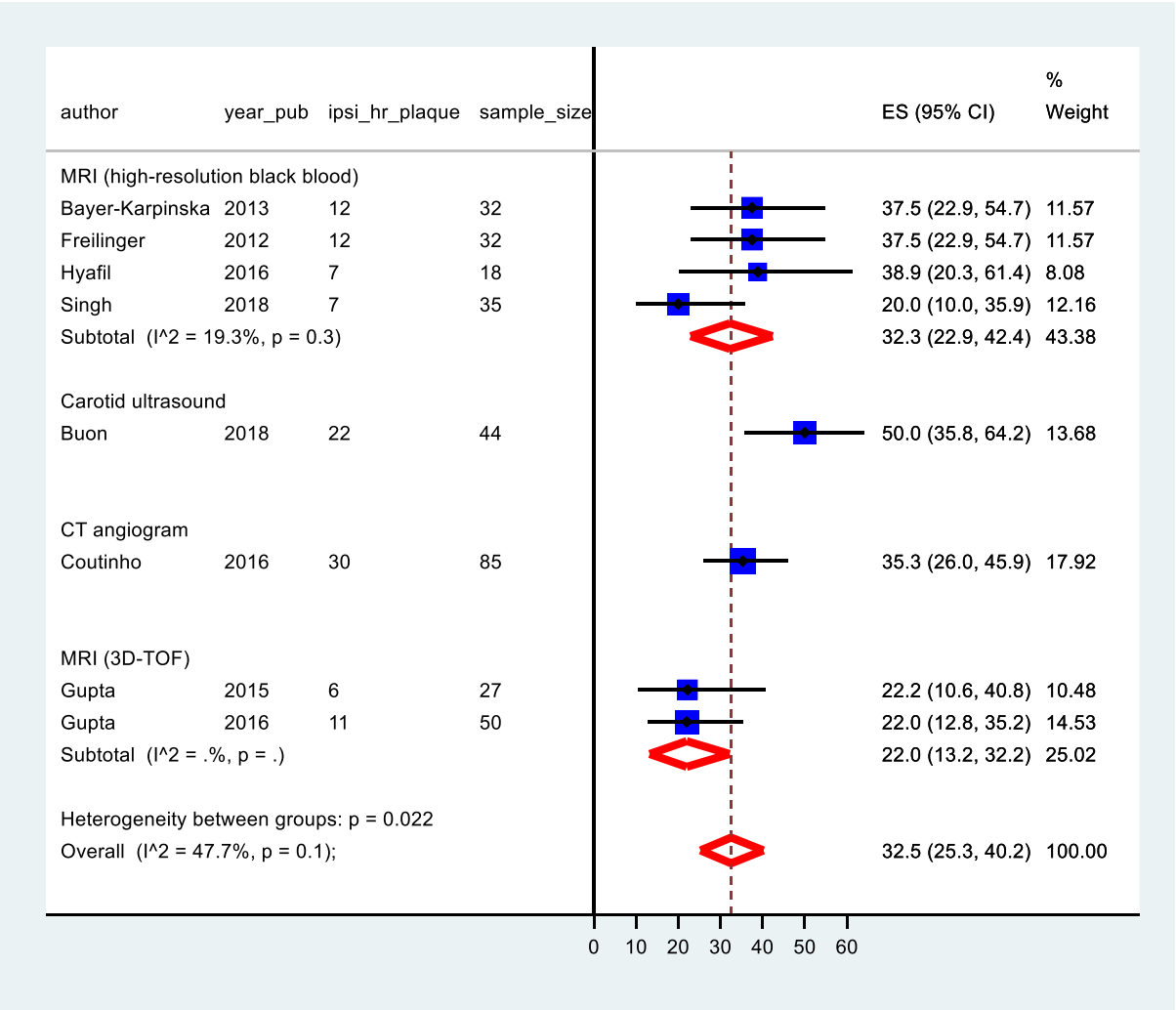

Figure 1.1. Prevalence of ipsilateral carotid plaque with high-risk features in ESUS

3D-TOF = 3-dimensional time of flight, CI = Confidence interval, CT = Computed tomography, ES = Effect size, ipsi_hr_plaque = ipsilateral carotid plaque with high-risk features, MRI = Magnetic resonance imaging, sample_size = number of participants in the study, year_pub = year of publication

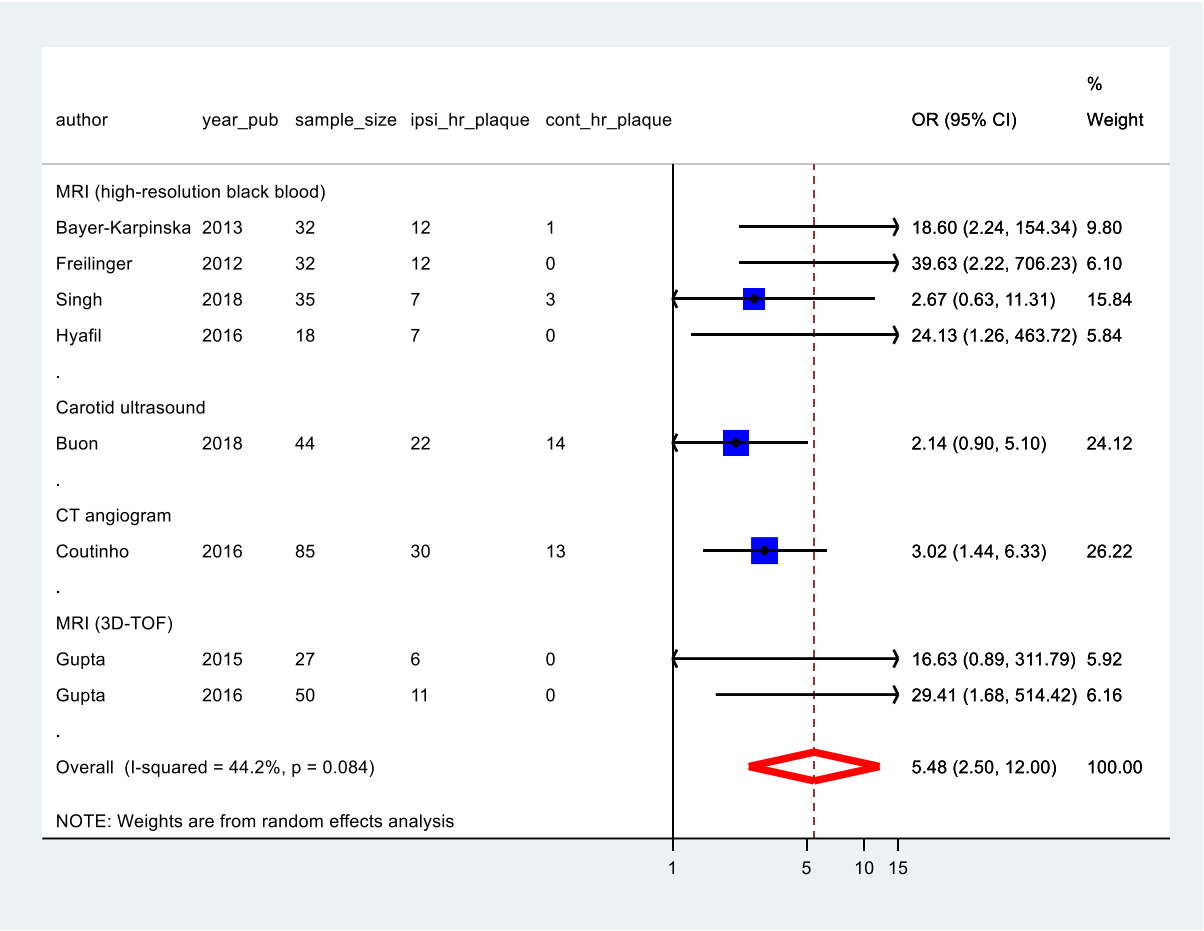

Figure 1.2. Odds-ratio of finding plaque with high-risk features in the ipsilateral versus the contralateral carotid in ESUS

3D-TOF = 3-dimensional time of flight, CI = Confidence interval, CT = Computed tomography, cont_hr_plaque = contralateral carotid plaque with high-risk features, ipsi_hr_plaque = ipsilateral carotid plaque with high-risk features, MRI = Magnetic resonance imaging, OR = Odds ratio, sample_size = number of participants in the study, year_pub = year of publication

1.4. Discussion

Mild stenosis with high-risk features were five times more prevalent in the ipsilateral compared to the contralateral carotid in ESUS, suggesting a relationship to stroke risk. Our findings align with the results of the Prediction of Atrial Fibrillation in patients with Embolic Stroke of Undetermined Source (AF-ESUS) study showing that patients with ESUS and ipsilateral mild carotid stenosis had a lower 10-year probability of atrial fibrillation detection, thus making a cardioembolic source less probable [32]. Moreover, in the New Approach Rivaroxaban Inhibition of Factor Xa in a Global Trial Versus Aspirin to Prevent Embolism in Embolic Stroke of Undetermined Source (NAVIGATE-ESUS) trial, patients with ESUS and ipsilateral mild carotid stenosis did not benefit from anticoagulation [33]. In the Cardiovascular Outcomes for People Using Anticoagulation Strategies (COMPASS) trial [42], Rivaroxaban-Aspirin combination was more effective than Aspirin or Rivaroxaban for prevention of non-cardioembolic strokes and represents a potential therapeutic option in patients with ESUS and an ipsilateral mild carotid stenosis. However, recent strokes were excluded, and some participants had asymptomatic ≥ 50% carotid stenosis [43]. Therefore, further trials are needed to investigate the benefit of Rivaroxaban-Aspirin combination in patients with recent ESUS and an ipsilateral mild carotid stenosis. Dual antiplatelet therapy with high-dose statins, endarterectomy or stenting also represent potential treatment options.

1.5. Limitations and future directions

All studies used a single plaque imaging modality which may have led to an underestimation of the prevalence of high-risk plaques in ESUS since various imaging modalities have different sensitivity and specificity for the detection of high-risk features [11]. Besides features visible on plaque MRI, high-risk features identified by other imaging modalities may be useful: microembolic signals (transcranial Doppler), large plaque volume (3D ultrasound), plaque neovascularization (contrast-enhanced ultrasound), and plaque inflammation (PET-CT) [11]. A combination of vascular imaging and blood biomarkers may also be useful to refine stroke risk stratification in patients with ESUS and ipsilateral mild carotid stenosis. RNA biomarker panels that predict stroke etiology with >90% sensitivity and specificity [44] can be integrated into multiparameter scores to predict causality of an ipsilateral mild carotid stenosis in ESUS and better stratify the risk of recurrence prior to inclusion in trials.

Chapter 2: Prevalence of high-risk plaques and risk of stroke in asymptomatic carotid stenosis[2]

2.1. Introduction

Over the past three decades, numerous randomized controlled trials have evaluated the benefit of carotid endarterectomy and stenting for stroke prevention in patients with asymptomatic carotid stenosis.[45,46] Following the results of those trials, current international guidelines recommend considering revascularization in patients with severe asymptomatic carotid stenosis provided the periprocedural risk of cerebrovascular events (CVE) is less than 3% (Class IIa).[4,5] However, critics assert that owing to improvement in best medical therapy, the risk of CVE in patients with asymptomatic carotid stenosis could now be lower than 1%, meaning revascularization may be harmful in some patients.[6,47] As a consequence, the management of asymptomatic carotid stenosis has become a matter of debate and controversy with some clinicians advocating for no revascularization outside of ongoing clinical trials.[48-51] Several studies, including meta-analyses, have shown that features of carotid plaque beyond the degree of stenosis can help select patients with asymptomatic carotid stenosis that are at increased risk of stroke and could, therefore, benefit from revascularization.[10,11,52-63] However, due to small sample sizes and low number of events, such studies have not provided definitive evidence that the annual incidence of ipsilateral ischemic CVE in patients with high-risk plaques exceeds the periprocedural risk of CVE. Moreover, they have not provided a robust estimate of the prevalence of high-risk plaque features to inform the design of clinical trials using a risk-oriented selection of patients prior to randomization. In this study, we provide estimates of the prevalence of plaques with high-risk features and the annual incidence of CVE in asymptomatic carotid stenosis.

[2] This chapter has been published as "Kamtchum-Tatuene J, Noubiap JJ, Wilman AH, Saqqur M, Shuaib A, Jickling GC. Prevalence of High-Risk Plaques and Risk of Stroke in Patients with Asymptomatic Carotid Stenosis: A Meta-analysis. *JAMA Neurol* 2020; 77(12):1524-1535."

2.2. Methods

2.2.1. Search strategy and selection criteria

This report is compliant with the guidelines for reporting systematic review and Meta-analysis Of Observational Studies in Epidemiology (MOOSE).

We searched PUBMED and Ovid-EMBASE to identify all prospective studies reporting the prevalence of plaques with high-risk features and the associated risk of CVE in subjects with asymptomatic carotid stenosis, from inception to July 31, 2019 (search strategy available in Appendix 2, Table I). For each study, we relied on the definitions used by the authors for quantifying the grade of stenosis or identifying the specific high-risk features, provided they were scientifically valid. These definitions are reported in Appendix 2, Table II. Details of the study selection criteria are provided in Appendix 2, Figure I.

Two investigators (JK-T and JJN) independently screened the titles and abstracts of the records retrieved by database searches. Then, the full texts of potentially eligible articles were obtained and further assessed for final inclusion. Methodological quality and risk of bias were independently assessed by two investigators (JK-T and JJN) using an adapted version of the Risk of Bias Tool for Prevalence Studies (Appendix 2, Table III).[64] We aimed to include only studies with low risk of bias in the meta-analysis. The inter-rater agreement for study selection was assessed using a non-weighted Cohen's kappa.[65-67] Disagreements regarding study inclusion were resolved through consensus.

2.2.2. Data extraction and analysis

Aggregated data were extracted using a pre-designed standard form (list of variables provided with Appendix 2, Table II, see footnote). The definition of ipsilateral ischemic CVE was consistent across studies. In our analysis, retinal infarcts and amaurosis fugax were considered as equivalent of stroke or transient ischemic attack, respectively. For studies reported in two or more articles, only the most comprehensive with the largest sample size was considered.

The number of person-years in each cohort was obtained by multiplying the sample size or the number of patients with high-risk features by the mean duration of follow-up. The prevalence of high-risk features and the incidence of ipsilateral ischemic CVE (in the overall sample and in patients with high-risk plaques) were determined by pooling study-specific estimates using random-effects meta-analysis after stabilizing the variance of each study with the Freeman-Tukey

double arc-sine transformation.[68-71] Publication bias was assessed by inspecting funnel plots and performing the Egger's test.[72] In our analysis, the odds ratio was preferred as the measure of association between plaque features and stroke risk because the adjusted hazard ratio was reported only in 7 cohorts out of 22 eligible for meta-analysis. Nevertheless, we also performed a meta-analysis of the reported adjusted hazard ratios to verify that the magnitude of association remains the same (Appendix 2, Figure II).

In studies that included a subset of more than 30 patients with symptomatic carotid atherosclerosis, we also computed the prevalence of each high-risk plaque feature. Although it was not the primary objective of this study, this ancillary analysis was used to verify the hypothesis that the prevalence of high-risk plaque features is higher in patients with symptomatic carotid stenosis compared to asymptomatic patients examined under the same conditions (period of recruitment, medical management, imaging technique used, and expertise of the investigators).

Subgroup analyses were performed to identify parameters influencing the prevalence of high-risk plaques and the associated risk of CVE. Of special interest was the quantification of the stroke risk associated with the presence of high-risk plaque in studies enrolling only patients with severe stenosis. To define the subgroups, the levels of the factor variable were used for categorical parameters (e.g., decade of publication, type of high-risk feature, or grade of stenosis) while the median across relevant studies was used as the cut-off for continuous parameters (e.g., mean age of participants or proportion on statins). This is a standard methodological approach to increase transparency and avoid arbitrary or biased cut-off selection. Heterogeneity between studies and subgroups was assessed using the χ^2 test on the Cochran's Q statistic and quantified by the I^2 index.[73] Values of I^2 <25%, 25-75%, > 75% were interpreted as low, medium, and high heterogeneity, respectively.

Univariable random-effects meta-regression models were performed to test the difference of pooled prevalence, incidence, or odds ratio between subgroups. This was achieved by first recoding the subgroups as numerical ordinal variables guided by the observed trend in prevalence, incidence, or odds ratio. Then a linear meta-regression of pooled prevalence, incidence, or odds ratio (dependent variable) over the ordinal variable (independent variable) was performed and the hypothesis that the slope of the fitted regression line differs from zero was tested. The meta-regression accounts for the weight of each study in the initial meta-analysis.

All statistical tests were two-sided and statistical significance was defined as $p \leq 0.05$. Analyses were performed with the software STATA (version 13, StataCorp, College Station, TX, USA).

2.3. Results

2.3.1. Characteristics of the included studies

Overall, 68 studies enrolling 21210 participants (age 29-95 years) were included in the qualitative synthesis (Table 2.1.). The individual characteristics of the included studies are presented in the appendix (Appendix 2, Table II). There was 99.2% agreement between investigators for study inclusion ($\varkappa = 0.86$). A subset of 64 studies was included in the meta-analysis (Appendix 2, Figure I).

Table 2.1. Characteristics of included studies

Characteristics	Cross-sectional studies (n = 42)	Cohort studies (n = 26)
Year of publication	1992 – 2019	1995 – 2017
Country	Australia (n=1), Austria (n=1), Canada (n=1), China (n=6), France (n=4), Germany (n=6), Italy (n=6), Japan (n=2), Netherlands (n=4), Switzerland (n=2), United Kingdom (n=4), United States of America (n=4), Multiple countries (n=1)	Australia (n=1), Canada (n=2), China (n=2), Denmark (n=1), Germany (n=2), Italy (n=2), Japan (n=1), Norway (n=1), Switzerland (n=1), United Kingdom (n=7), United States of America (n=4), Multiple countries (n=2)
Period of enrolment	1986 – 2018 (from 27 studies)	1989 – 2010
Enrolment before endarterectomy	No (n=33), Yes (n=9)	No (n = 26)
Settings	Hospital (n=38), population (n=4)	Hospital (n=23), population (n=3)
Grade of stenosis eligible [a]	Any grade (n=21), mild only (n=1), mild and moderate (n=3), moderate only (n=1), moderate and severe (n=10), severe only (n=6)	Any grade (n=4), moderate only (n=2), moderate and severe (n=11), severe only (n=9)
High-risk features considered [b]	AHA type IV, V, VI (n=2), echolucency (n=9), IPH (n=13), ipsilateral SBI (n=6), irregularity (n=1), LRNC (n=7), MES (n=8), mural thrombus (n=1), neovascularization (n=10), Thin or rupture fibrous cap (n=6), ulceration (n=6)	AHA type IV, V, VI (n=1), echolucency (n=8), IPH (n=3), impaired CVR (n=5), LRNC (n=4), MES (n=7), ipsilateral silent brain infarcts (n=1), thin or ruptured fibrous cap (n=2), ulceration (n=3)
Vascular imaging modality [c]	CT(n=4), MRI 1.5T (n=6), MRI 3.0T (n=8), US (n=24)	CT (n=2), MRI 1.5T (n=4), MRI 3.0T (n=1), US (n=24)
Method to ascertain the occurrence of cerebrovascular events	Not applicable	CT or MRI (n=13), phone interview (n=3), review of medical records (n=5), not indicated (n=5)
Number of patients with asymptomatic carotid stenosis	9756	11454
Age range (years)	29 – 95 (from 11 studies)	30 – 88 (from 5 studies)
Mean age (years) [d]	55.0 – 76.5 (from 34 studies)	60.7 – 74.0 (from 21 studies)
Male participants	45 – 87% (from 35 studies)	18 – 85% (from 21 studies)
Hypertension	16 – 93% (from 29 studies)	33 – 90% (from 20 studies)
Diabetes mellitus [e]	3 – 100% (from 31 studies)	7 – 100% (from 20 studies)

Smoking	6 – 83% (from 28 studies)	12 – 62% (from 19 studies)
Dyslipidemia [f]	38 – 100% (from 20 studies)	23 – 87% (from 11 studies)
Coronary artery disease [g]	0 – 100% (from 23 studies)	0 – 61% (from 15 studies)
Peripheral artery disease	8 – 71% (from 10 studies)	0 – 40% (from 8 studies)
Atrial fibrillation	0 – 13% (from 10 studies)	0 – 7% (from 8 studies)
Patients on statins	29 – 100% (from 15 studies)	28 – 100% (from 12 studies)
Patients on antiplatelets	11 – 38% (from 17 studies)	21 – 100% (from 11 studies)
Mean duration of follow-up (years)	Not applicable	0.7 – 6.5
Patients lost to follow-up	Not applicable	0-12% (from 25 studies)

[a] Any grade (0-99%), mild only (< 50%), mild and moderate (0-69%), moderate only (50 – 69%), moderate and severe (≥ 50%), severe only (≥ 70%).
[b] Some studies explored more than one high-risk feature. AHA means American Heart Association; CVR, cerebrovascular reserve; IPH, intraplaque hemorrhage; SBI, silent brain infarct; LRNC, lipid-rich necrotic core; and MES, microembolic signals. The diagnostic criteria for the high-risk plaque features are presented for each study in the Appendix, Supplementary Table 3.
[c] CT indicates computerized tomography; MRI, magnetic resonance imaging; and US, ultrasound.
[d] From this row, data are presented for asymptomatic patients only and displayed as range (number of studies).
[e] One cohort and one cross-sectional studies included only patients with diabetes mellitus
[f] One cross-sectional study included only patients with familial hypercholesterolemia.
[g] One cross-sectional study included only patients with coronary artery disease.

2.3.2. Prevalence of plaque with high-risk features

The pooled prevalence of high-risk plaques was 26.5% (95% CI: 22.9-30.3) in 20751 participants with asymptomatic carotid stenosis (Table 2.2). The most prevalent high-risk plaque features were neovascularization (43.4%, 31.4-55.8, n = 785), echolucency (42.3%, 32.2-52.8, n = 12364), and lipid-rich necrotic core (36.3%, 27.7-45.2, n = 3728) (Table 2.2. and Appendix 2, Figures II to XI). The visual inspection of the funnel plot suggested that there is no publication bias as confirmed by the Egger's test (p = 0.58) (Table 2.2 and Appendix 2, Figure XII).

The prevalence of high-risk plaques was not affected by the demographic characteristics of the study population (mean age and proportion of men), the grade of stenosis, and the circumstances of enrolment (setting and planned endarterectomy) as shown in Appendix 2, Table IV. It was significantly higher in studies enrolling more than 78% participants on antiplatelet therapy (34.6% versus 17.8%, p = 0.002) (Appendix 2, Table IV). There was a non-significant trend towards a higher prevalence of high-risk plaques in studies published after the year 2000 and in studies with a higher proportion of patients with hypertension and diabetes mellitus (Appendix 2, Table IV).

There were 18 cross-sectional and 2 cohort studies that also provided relevant data on 1652 patients with symptomatic carotid stenosis. In these 20 studies, the pooled prevalence of high-risk plaques was 43.3% (95% CI: 33.6-53.2) in symptomatic patients versus 19.9% (95% CI: 14.5-25.8) in asymptomatic patients (Appendix 2, Table V). The odds of finding a high-risk plaque in a symptomatic versus an asymptomatic carotid stenosis was 3.4 (95% CI: 2.5-4.6).

Table 2.2. Prevalence of high-risk features in asymptomatic carotid stenosis

	Number of studies	Number of participants	Number of cases	Prevalence (95% CI)	I^2	p-values	
						Heterogeneity	Egger's test
Overall analysis							
Any high-risk feature	64	20751	NA [a]	26.5 (22.9-30.3)	97.8	<0.001	0.58
Specific high-risk features							
AHA type IV, V, VI	3	168	57	30.8 (15.6-48.4)	81.3	<0.001	0.07
Echolucency	16	12364	4223	42.3 (32.2-52.8)	99.1	<0.001	0.24
Impaired cerebrovascular reserve [b]	5	348	109	29.2 (15.1-45.7)	89.9	<0.001	0.86
Intraplaque haemorrhage	16	3245	934	19.1 (13.8-25.0)	91.2	<0.001	<0.001
Ipsilateral silent brain infarcts	7	2226	428	21.9 (15.6-28.8)	90.2	<0.001	0.35
Lipid-rich necrotic core	11	3728	1514	36.3 (27.7-45.2)	95.7	<0.001	0.54
Microembolic signals	14	1648	245	14.3 (10.0-19.2)	81.1	<0.001	0.82
Mural thrombus	1	41	3	7.3 (2.5-19.4)	NE	NE	NE
Neovascularization	8	785	360	43.4 (31.4-55.8)	90.9	<0.001	0.53
Plaque irregularity	1	44	15	34.1 (21.9-48.9)	NE	NE	NE
Thin/ruptured fibrous cap	8	670	177	24.1 (12.0-38.7)	93.8	<0.001	0.96
Ulceration	8	2086	197	13.1 (3.5-27.1)	98	<0.001	0.34

[a] The number of patients with at least one high-risk feature was not provided in each study and cannot be computed because some participants had more than one high-risk feature. The overall prevalence is obtained by pooling the prevalence of specific high-risk features across studies.
[b] Studies reporting impaired cerebrovascular reserve only included patients with severe carotid stenosis.
AHA means American Heart Association; CI, confidence interval; NA, not applicable; and NE, not estimable because of the small number of studies ($n \leq 3$).

2.3.3. Presence of high-risk features and risk of ipsilateral ischemic events

A total of 22 cohorts enrolling 10381 participants with asymptomatic carotid stenosis provided relevant data for the meta-analysis of risk of CVE associated with high-risk plaque features. The average duration of follow-up was 2.8 years. The incidence of ipsilateral ischemic CVE was 3.2 per 100 person-years (95% CI: 2.2-4.3) in the overall population of patients with asymptomatic carotid stenosis (Appendix 2, Figure XIII and XIV). There was evidence of publication bias (p-value for Egger's test < 0.001). Studies with a higher number of person-years reported lower incidence rates (Appendix 2, Figure XV).

The incidence of ipsilateral ischemic CVE was higher in patients with high-risk features (4.3 per 100 person-years, 95% CI: 2.2-4.3, Figures 2.1 and Appendix 2, Figure XVI) than in those without (1.2 per 100 person-years, 95% CI: 0.6-1.8, Appendix 2, Figure XVII) with a corresponding odds ratio of 3.0 (95% CI = 2.1-4.3, I^2 = 48.8%; Figure 2.2). This magnitude of association between presence of high-risk plaque and risk of CVE was confirmed by pooling data from the 7 studies reporting the adjusted hazard ratio (pooled HR = 3.0, 95% CI: 1.8-4.2, I^2 = 0%, Appendix 2, Figure XVIII). This increased risk was also observed specifically for ischemic stroke (OR = 2.0, 95% CI: 1.5-2.7, I^2 = 0%) and transient ischemic attack (OR = 2.4; 95% CI: 1.2-4.9, I^2 = 13.0%) as shown in Table 2.3. There was a non-significant trend towards a higher risk of ipsilateral ischemic CVE in studies with a greater proportion of participants with hypertension, diabetes, smoking or on statins or antiplatelet therapy (Table 2.3). The presence of AHA plaque type IV, V, or VI was the strongest predictor of ipsilateral ischemic CVE (OR = 28.7, 95% CI: 1.6-513.3), followed by microembolic signals (OR = 5.6, 95% CI: 2.0-15.3, I^2 = 68.0%) as shown in Table 2.3. The incidence of ipsilateral ischemic CVE in asymptomatic carotid stenosis with high-risk features is presented for each type of high-risk feature in Appendix 2, Figure XIX and for each decade since 1990 in Appendix 2, Figure XVI. In the subgroup of studies focusing on severe stenosis only, the incidence of ipsilateral ischemic CVE was also higher in patients with high-risk features (7.3 per 100 person-years, 95% CI: 2.0-15.0, Figure 1) than in those without (1.7 per 100 person-years, 95% CI: 0.6-3.3, Appendix 2, Figure XVII), with an odds ratio of 3.2 (95% CI: 1.7-5.9, I^2 = 39.6%, Table 2.3).

The incidence of ipsilateral ischemic CVE in patients with high-risk plaques was not modified by study characteristics including the mean age and the proportion of males, the frequency of various cardiovascular risk factors, and the use of statin or antiplatelet therapies (Appendix 2, Table VI). The incidence of ipsilateral ischemic stroke and transient ischemic attack in the overall

population of patients with asymptomatic carotid stenosis and in those with high-risk plaques is provided in the appendix (Appendix 2, Figures XX to XXIII).

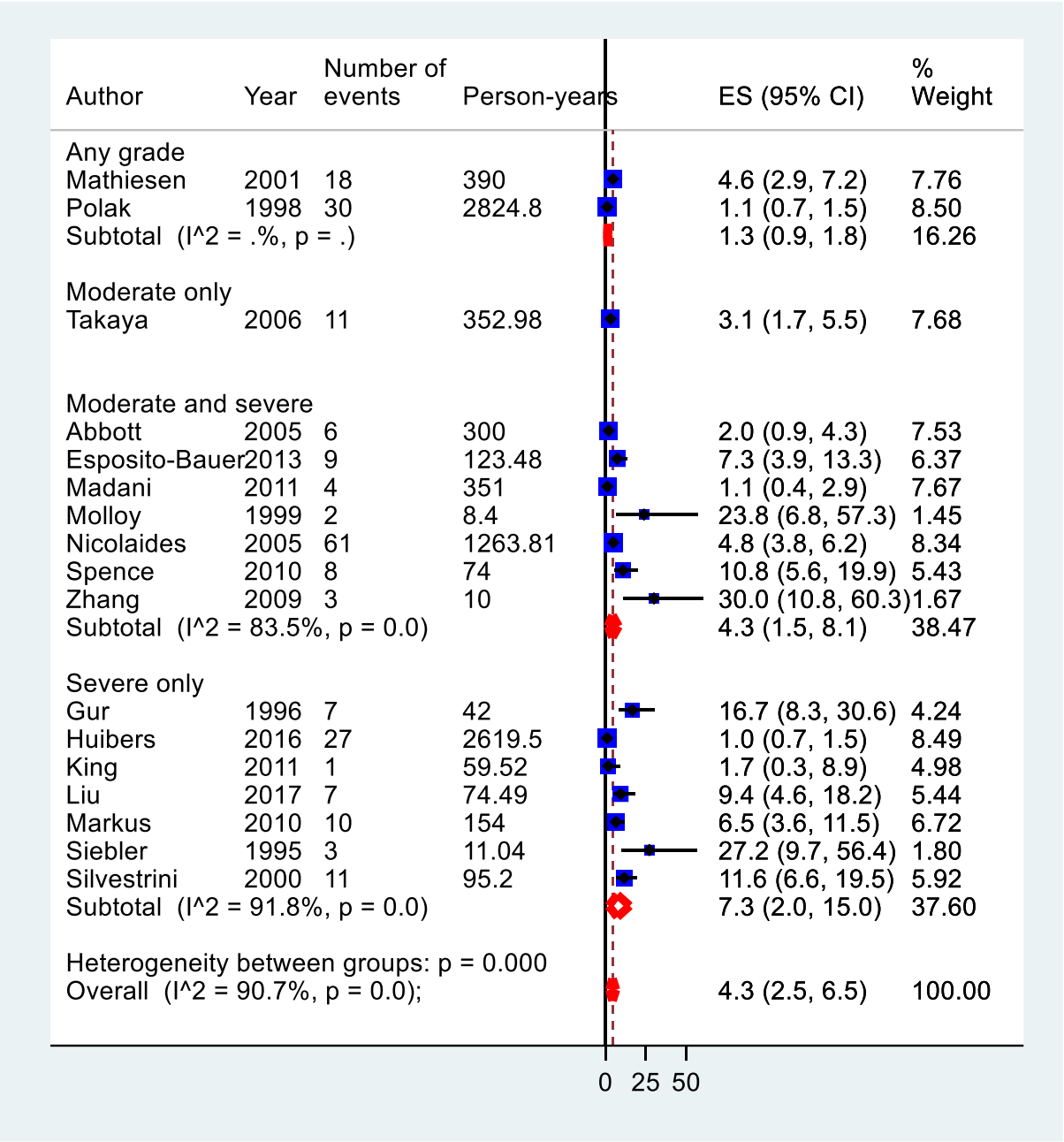

Figure 2.1. Incidence of ipsilateral ischemic cerebrovascular events in asymptomatic carotid stenosis with high-risk features by grade

CI means confidence interval and ES means effect-size (representing the incidence in this case).

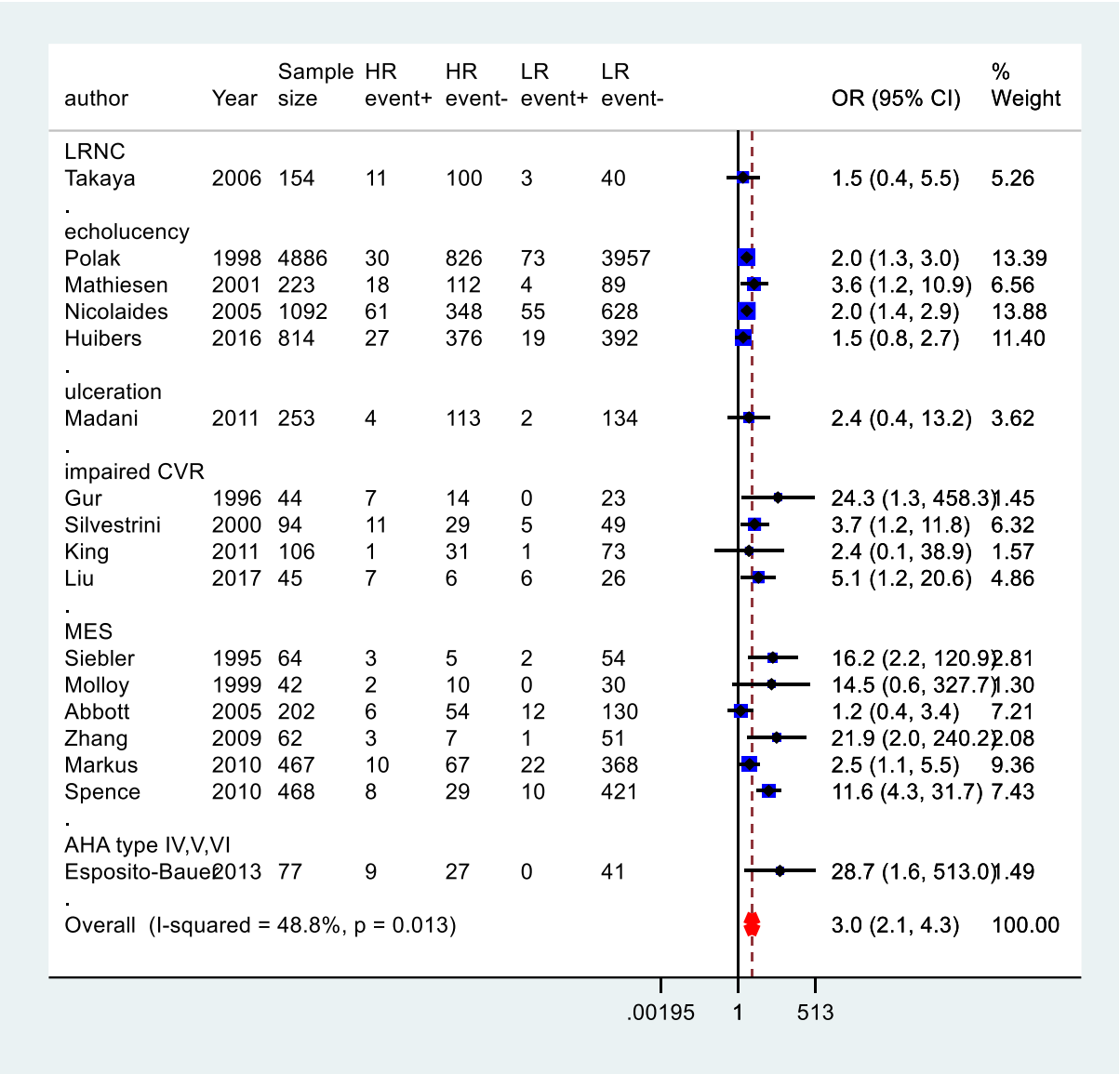

Figure 2.2. Risk of ipsilateral ischemic cerebrovascular events in asymptomatic carotid stenosis with high-risk features

The specific odds ratio for each high-risk feature is presented in Table 2.3.

CI = confidence interval, HR event- = high-risk without event, HR event+ = high-risk with event, LR event- = low-risk without event, LR event+ = low-risk with event, OR = odds ratio

Table 2.3. Subgroup analysis of the risk of ipsilateral ischemic CVE associated with high-risk plaques in asymptomatic carotid stenosis

	Number of studies	Number of participants	High-risk		Low-risk		OR (95%CI)	I^2	p-values			
			Event	No event	Event	No event			Heterogeneity		Egger's test	Meta-regression
									Within subgroup	Between subgroups		
Decade of publication												
1990-1999	4	5036	42	855	75	4064	7.1 (1.5-34.2)	62.8	0.05	0.01	0.04	0.36
2000-2009	6	1827	110	650	80	987	2.3 (1.4-3.7)	27.8	0.23		0.20	
2010-2019	7	2230	66	649	60	1455	3.7 (1.7-7.8)	60.9	0.02		0.21	
Grade of stenosis												
Moderate only	1	154	11	100	3	40	1.5 (0.4-5.5)	NE	NE	0.01	NE	0.49
Moderate and severe	7	2196	93	588	80	1435	4.5 (1.8-10.9)	68.9	0.004		0.05	
Severe only	7	1634	66	528	55	985	3.2 (1.7-5.9)	39.6	0.13		0.04	
Any grade	2	5109	48	938	77	4046	2.1 (1.4-3.2)	0	0.32		NE	
Type of event												
Ischemic stroke	10	7423	104	1803	120	5396	2.0 (1.5-2.7)	0	0.53	NA	0.04	NA
Transient ischemic attack	9	7170	46	1744	39	5341	2.4 (1.2-4.9)	13.0	0.33		0.17	
Any ischemic event	17	9093	218	2154	215	6506	3.0 (2.1-4.3)	48.8	0.01		0.002	
Type of high-risk marker												
LRNC	1	154	11	100	3	40	1.5 (0.4-5.5)	NE	NE	0.01	NE	0.01
Ulceration	1	253	4	113	2	134	2.4 (0.4-13.2)	NE	NE		NE	
Echolucency	4	7015	136	1662	151	5066	1.9 (1.5-2.5)	0	0.58		0.42	
Impaired CVR	4	289	26	80	12	171	4.6 (2.0-10.4)	0	0.64		0.38	

MES	6	1305	32	172	47	1054	5.6 (2.0-15.3)	68.0	0.008		0.11	
AHA type IV, V, VI	1	77	9	27	0	41	28.7 (1.6-513.0)	NE	NE		NE	
Mean age of participants (years)												
Below 70	7	1159	50	286	25	798	7.0 (3.8-12.7)	7.6	0.37	0.01	0.19	0.25
At least 70	7	5986	78	1134	116	4658	2.1 (1.5-2.9)	0	0.43		0.20	
Not indicated	3	1948	90	734	74	1050	1.9 (1.3-2.8)	15.0	0.31		0.33	
Proportion of male participants												
Below 65%	8	6150	94	1227	98	4731	4.1 (2.0-8.1)	58.2	0.02	0.01	0.06	0.30
At least 65%	6	1747	58	562	61	1066	2.2 (1.3-3.8)	33.2	0.19		0.19	
Not indicated	3	1196	66	365	56	709	5.7 (1.0-34.1)	61.3	0.08		0.23	
Proportion of participants with hypertension												
Below 70%	7	6200	89	1369	107	4635	2.7 (1.6-4.7)	42.6	0.11	0.01	0.03	0.99
At least 70%	6	1474	45	308	48	1073	3.2 (1.3-7.9)	63.6	0.02		0.41	
Not indicated	4	1419	84	477	60	798	3.6 (1.5-9.0)	48.6	0.12		0.12	
Proportion of participants with diabetes mellitus												
Below 21%	8	7260	89	1501	141	5529	2.7 (1.5-4.6)	60.8	0.01	0.01	0.23	0.17
At least 21%	5	414	45	176	14	179	4.3 (1.8-10.6)	31.0	0.22		0.01	
Not indicated	4	1419	84	477	60	798	3.6 (1.5-9.0)	48.6	0.12		0.12	
Proportion of smokers												
Below 27%	6	1540	36	308	47	1149	3.5 (1.4-8.4)	59.1	0.03	0.01	0.50	0.69
At least 27%	6	657	59	279	20	299	4.1 (2.2-7.9)	16.9	0.31		0.004	
Not indicated	5	6896	123	1567	148	5058	2.0 (1.4-3.0)	36.6	0.18		0.05	
Proportion of participants with dyslipidemia												
Below 64%	4	260	34	76	11	139	5.6 (2.5-12.7)	0	0.40	0.01	0.01	0.25
At least 64%	3	824	25	183	25	591	2.8 (0.6-13.3)	82.9	0.003		0.67	
Not indicated	10	8009	159	1895	179	5776	2.3 (1.7-3.1)	22.5	0.24		0.02	
Proportion of participants with coronary artery disease												
Below 37%	5	1510	52	469	36	953	4.9 (1.5-15.9)	73.7	0.004	0.01	0.27	0.92

At least 37%	5	931	37	240	39	615	2.8 (1.2-6.6)	50.8	0.09		0.05	
Not indicated	7	6652	129	1445	140	4938	2.3 (1.7-3.3)	13.8	0.32		0.04	
Proportion of participants with peripheral artery disease												
Below 34%	3	747	23	110	22	592	5.9 (0.9-39.2)	82.4	0.003	0.01	0.51	0.49
At least 34%	3	984	31	412	22	519	3.3 (0.7-15.6)	60.3	0.08		0.54	
Not indicated	11	7362	164	1632	171	5395	2.4 (1.8-3.2)	11.0	0.34		0.008	
Proportion of participants treated with statins												
Below 64%	5	1734	57	624	40	1013	3.1 (1.2-7.9)	71.1	0.008	0.01	0.66	0.21
At least 64%	2	183	10	58	1	114	8.0 (0.6-107.1)	39.7	0.20		NE	
Not indicated	10	7176	151	1472	174	5379	2.7 (1.8-4.0)	38.8	0.10		0.002	
Proportion of participants treated with antiplatelets												
Below 88%	5	1593	51	470	49	1023	3.8 (1.4-10.9)	78.4	0.001	0.01	0.22	0.16
At least 88%	3	227	17	72	1	137	11.3 (2.2-59.6)	0	0.37		0.35	
Not indicated	9	7273	150	1612	165	5346	2.2 (1.7-2.8)	0	0.49		0.01	

AHA means American Heart Association; CI, confidence interval; CVR, cerebrovascular reserve; IPH, intraplaque hemorrhage; LRNC, lipid-rich necrotic core; MES, microembolic signals; NA, not applicable; NE, not estimable because of the small number of studies ($n \leq 3$); and OR, odds ratio.

2.4. Discussion

To our knowledge, this is the first comprehensive summary of data on the prevalence of high-risk plaque and the associated risk of stroke in the specific population of patients with asymptomatic carotid stenosis. The prevalence of high-risk features was not affected by the grade of stenosis, suggesting that they reflect the underlying pathomechanism of plaque formation, remodelling, and destabilization which is the same across all grades of stenosis.[74,75] This also indicates that, although revascularization is not beneficial in all patients with mild stenosis,[76] there may be a subset of patients in this group that could benefit from specific interventions in addition to best medical therapy.

The prevalence data reported in this study are important for sample size calculation of future interventional trials where asymptomatic patients at higher risk of stroke are randomized to therapy. The low prevalence of well-validated high-risk features like microembolic signals[77] and intraplaque hemorrhage[62] suggests that a combination of high-risk features in a multimodal imaging approach might be necessary to optimize a screening strategy relying on imaging biomarkers for risk stratification. This assumption is supported by the higher prevalence of high-risk plaques in studies using the American Heart Association classification. Indeed, by pooling together plaques type IV, V, and VI, those studies combined features equivalent to lipid-rich necrotic core, thin or ruptured fibrous cap, ulceration, intraplaque haemorrhage, and mural thrombus.[74,75] The combination of imaging modalities is already part of routine clinical practice in many centres, but it adds cost and time. Therefore, a simple alternative may be desirable, such as a blood-based biomarker that correlates with vascular imaging findings and is associated with the risk of stroke.[78] Such a biomarker still needs to be developed and may include a panel of markers reflecting risk of plaque rupture and thromboembolism.

A key finding of this meta-analysis is that the risk of ipsilateral ischemic CVE in the overall population of patients with asymptomatic carotid stenosis (3.2%) and in the subsets of patients with (4.3%) and without (1.2%) high-risk plaque features is greater than the commonly accepted rate of 1%.[6,47] The latter was computed using the 10-year follow-up data from the Asymptomatic Carotid Surgery Trial (ACST-1).[79] In this trial, the decrease in stroke incidence in patients randomized to deferral of carotid intervention has been attributed solely to improvement in best medical therapy. However, it is also probable that the composition of the trial population became progressively skewed towards a predominance of patients without high-risk plaque features since those with high-risk plaques had a stroke earlier or underwent surgery. The bias

underlying the report of low incidence rates in long-lasting closed cohorts was confirmed in our meta-analysis of incidence data. Current evidence indicate that patients with asymptomatic carotid stenosis have a lower periprocedural risk of stroke and better outcomes after revascularization when compared to symptomatic patients.[80,81] Thus, in future carotid revascularization trials, it may be relevant to perform additional subgroup analyses based on the presence of high-risk features at baseline (CREST-2, NCT02089217; ECST-2, ISRCTN97744893; ACST-2, NCT00883402; and ACTRIS, NCT02841098).[63,82]

In the subgroup analyses, we observed a non-significant trend towards a higher prevalence of high-risk features and a higher incidence of CVE in studies enrolling a greater proportion of patients with hypertension or diabetes mellitus. The risk of CVE was also higher in studies with a greater proportion of smokers. These findings highlight the role of cardiovascular risk factors in atherosclerotic plaque progression and destabilization. It also emphasizes the importance of vascular risk factor control in the management of patients with carotid stenosis.[4,5,45] The drop in the incidence of ipsilateral ischemic CVE after the year 2000, despite a rise in the prevalence of high-risk features may also be attributable to an improvement of medical therapy over time. An alternative explanation could be the increase of revascularization procedures after the release of results from the Asymptomatic Carotid Atherosclerosis Study,[83] meaning that fewer asymptomatic patients were left untreated and available for enrolment into observational studies. With the available data, it was not possible to explore if the change in prevalence of high-risk plaque features before and after 2000 was a true increase due to various changes in lifestyle and environmental factors, or if the rise should be attributed to better availability of vascular imaging and better reporting of high-risk plaque features.

An unexpected finding was the higher risk of stroke in studies that included a greater proportion of patients on statins and antiplatelet drugs. This might be attributable to the fact that patients with high-risk plaques were more likely to be offered these treatments and the authors recorded the prescription of antiplatelet drugs and statins before and after the identification of the high-risk features without distinction. This hypothesis is in line with our findings that the prevalence of high-risk plaques was significantly higher in studies including a greater proportion of patients on antiplatelet drugs. A complementary hypothesis could be that, although useful, statins and antiplatelet therapy remain insufficient to curb the higher risk of stroke associated with high-risk plaques.[84] Testing such a hypothesis would require the collection of individual patient data on the

nature and doses of statins and antiplatelet drugs, the control status of cardiovascular risk factors, the adherence to treatment, and the resistance to antiplatelet drugs.[45,46,85-88]

2.5. Limitations

Despite the large sample size and the rigorous methodology, the findings of this review should be interpreted in the context of its limitations. Firstly, there was substantial heterogeneity for the meta-analysis of prevalence of high-risk features which is explained by differences across studies regarding the definition criteria and the imaging modalities used. Moreover, the prevalence of high-risk plaques was obtained by pooling the specific prevalence of each high-risk feature under the pragmatic assumption that high-risk plaques are equal, irrespective of how they were identified. There likely is heterogeneity in stroke risk between high-risk features. However, high-risk features do have some relationship to each other as they represent aspects of the same atherosclerotic disease. Combining different views provides a better assessment of the underlying biology. Consensus imaging recommendations are needed to help decrease the heterogeneity of carotid imaging data across studies and to facilitate international clinical research collaborations. Secondly, it is possible that some of the CVE reported were not due to athero-embolism from the index asymptomatic carotid stenosis. But the proportion of CVE due to other causes is likely small since most studies excluded patients with atrial fibrillation, and CVE deemed attributable to other causes were also excluded from the analyses.[89-93]

2.6. Conclusion and future directions

This study provides evidence that, in patients with asymptomatic carotid stenosis, high-risk plaques are common, and the associated risk of ipsilateral ischemic CVE is higher than the currently accepted estimates. Therefore, a routine assessment of asymptomatic carotid stenosis beyond the grade of stenosis could help identify patients at a higher risk of stroke who require an intensification of medical therapy for the control of cardiovascular risk factors. Further trials using multimodal neurovascular imaging for risk stratification before randomization are warranted to determine the optimal strategy for stroke prevention in asymptomatic carotid stenosis.

PART B: Comprehensive literature reviews

Chapter 3: Blood biomarkers for stroke diagnosis and management[3]

3.1. Introduction

Biomarkers are objective indicators used to assess normal or pathological processes, evaluate responses to medical interventions, and predict outcomes [94]. They can refer to molecules present in body fluids (blood, cerebrospinal fluids, urine) but also to physical measurements on tissues (e.g., imaging, electrophysiology). Molecular biomarkers include proteins, metabolites, lipids, and ribonucleic acids (RNA) (Table 3.1) [95-97]. They can be used alone or in combination (panels, scores or indices) to improve their diagnostic accuracy or their capacity to estimate disease risk or clinical outcome [98]. Several blood biomarkers are used to aid clinical decisions. For example, high-sensitive cardiac troponin T guides the diagnosis of myocardial infarction [99], D-dimers are informative for the diagnosis of pulmonary embolism [100], plasma creatinine is used to assess and monitor kidney function, antibodies targeting acetylcholine receptors help to diagnose myasthenia gravis [101], B-type natriuretic peptide (BNP) is used to assess heart failure, and C-reactive protein levels reflect the response to antibiotic therapy in bacterial infection [102].

There is currently no blood biomarker used for the diagnosis of stroke. This is in part because the characteristics required are challenging including high sensitivity and specificity in a heterogenous disorder and the need for a very rapid turnaround. [96,103,104]. Several reviews have summarized biomarkers studied to date in stroke [96-98,103-115]. The current review does not intend to be an exhaustive description of stroke biomarkers. It is focused on blood biomarkers that show promise for translation into clinical practice and describe newly reported markers that could add to routine stroke care. Avenues for the discovery of new biomarkers and future research are discussed. The description of the biomarkers is organized according to their applications in clinical practice: diagnosis, treatment decisions, and outcome prediction.

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Table 3.1. Classification of the blood biomarkers used in stroke management

Nature	Origin	Subgroup	Classical illustrative examples	References
Proteins	Brain-specific biomarkers	Related to glial activation	Serum calcium-binding protein (S100B)	[116,117]
			Glial fibrillary acid protein (GFAP)	[118,119]
			Myelin basic protein (MBP)	[120]
		Related to neuronal injury	Neuron-specific enolase (NSE)	[121,122]
			Heart fatty acid-binding protein (HFABP)	[123,124]
			Anti-N-methyl-D-Aspartate (anti-NMDA) receptors antibodies	[125]
	Biomarkers not specific for the central nervous system	Related to hemostasis and endothelial dysfunction	Von Willebrand Factor (vWF) and its cleaving protein ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13)	[126,127]
			D-Dimer	[128,129]
			Fibrinogen	[130,131]
			Plasminogen activator inhibitor (PAI)	[132]
			Thrombomodulin	[133,134]
			Fibronectin	[135,136]
			Thrombin activatable fibrinolysis inhibitor	[137]
			Adhesion molecules: soluble intercellular adhesion molecule (ICAM), Vascular cell adhesion molecule (VCAM)	[138]
		Related to inflammation	C-reactive protein (CRP)	[139,140]
			Matrix metalloproteinase 9 (MMP-9)	[141]
			Lipoprotein-associated phospholipase A2	[142]
			Cytokines: Interleukins (IL-6, IL-10) and tumor necrosis factor alpha (TNF-α)	[143,144]
			Plasma ferritin	[145]

		Related to apoptosis	Caspase-3	[146]
		Neuroendocrine markers	Copeptin	[147,148]
			Natriuretic peptides and their precursors: atrial natriuretic peptide (ANP), midregional pro-atrial natriuretic peptide (MR-proANP), B-type natriuretic peptide (BNP), N-terminal pro-B-type natriuretic peptide (NT-proBNP).	[149,150]
			Adiponectin	[151]
RNAs	Differential expression of messenger RNA (mRNA)	40 gene panel	ADAMTSL4, AP3S2, ARHGEF12, ARHGEF5, BANK1, C16orf68, C19orf28, CD46, CHURC1, CLEC18A, COL13A1, EBF1, ENPP2, EXT2, FCRL1, FLJ40125, GRM5, GSTK1, HLA-DOA, IRF6, LHFP, LHFP, LOC284751, LRRC37A3, OOEP, P2RX5, PIK3C2B, PTPN20A, TFDP1, TMEM19, TSKS, ZNF185, ZNF254	[152]
		41 gene panel	ALS2CR11, C18orf49, CALM1, CCDC114, CCDC78, CCL2, CCL3, CHML, FAM179A, FAM70B, FLJ13773, GBP4, GTF2H2, HLA-DQA1, HLADRB4, IL8, LAG3, LAIR2, LGR6, LRRC8B, MPZL3, OASL, PDXDC1, PROCR, PRSS23, QKI, RASEF, RUNX3, SCAND2, STK4, STX7, TGFBR3, TSEN54, TTC12, UBA7, UGCG. UTS2, VAPA	[153]
	Differential expression of non-coding RNA	MicroRNAs (miRNA)	miR-200c	[23]
			Let-7i	[154]
		Long non-coding RNAs (lncRNA)	Zinc Finger Antisense 1 (ZFAS1)	[155]
Lipids			Oxidized low-density lipoprotein	[156]
			Free fatty acids	[157]
			Lysophosphatidylcholine	[158]
Metabolites			Plasma glutamate	[159]
			Nitric oxide	[160]

3.2. Biomarkers for stroke diagnosis

Clinicians are often faced with challenges in the diagnosis and management of stroke. A diagnostic test for stroke is needed not only to confidently identify stroke mimics that explain more than 40% of cases presenting with an acute neurological deficit [161], but also to aid in the distinction between hemorrhagic and ischemic stroke in circumstances where access to brain imaging is limited. Early identification of patients with acute ischemic stroke is important because revascularization therapies are time-sensitive, currently limited to 4.5 hours for intravenous thrombolysis, [162,163] and up to 24 hours for endovascular thrombectomy. [164] Another important challenge in stroke diagnosis is determining stroke etiology which remains cryptogenic in as many as one third of patients even after a comprehensive workup [165,166]. Moreover, the pathophysiological processes involved in brain damage and repair in the context of human stroke remain poorly understood, limiting the design of adjunctive drug therapies to improve the recovery process. Several molecules are being evaluated as blood biomarkers for stroke diagnosis (Table 3.2).

Table 3.2. Biomarkers used for the differential diagnosis of stroke

Clinical purpose	Type	Biomarker	Sample size	Cut-off	First sample collection (after onset)	Se (%)	Sp (%)	Ref.
Stroke versus no stroke (Mimics, Controls)	Protein	Anti-NMDA	360 samples from 105 strokes and 255 controls	≥ 2 μg/L	< 3h	97	98	[125]
		NSE	66 strokes	N/A	< 3h	N/A	N/A	[167]
		HFABP	22 strokes, 22 controls, 22 acute myocardial infarctions	OD > 0.531	< 24h	68.2	100	[124]
		NDKA	622 strokes, 165 controls	≥ 2.55 μg/L	< 24h	73	97	[168]
		PARK7	622 strokes, 165 controls	≥ 1.55 μg/L	< 24h	85	97	[168]
		Glycogen phosphorylase isoenzyme BB	172 strokes, 133 controls	7.0 ng/mL	< 12h	93	93	[169]
		Serum APOA1-UP	94 strokes, 37 controls	Ratio APOA1-UP to reference protein > 1.80	N/A	91	97	[170]
		PBP	35 TIA/minor IS, 12 controls	1500 ng/mL	< 48h	91	57	[171]
	4-protein panel	S100B, vWF, MM9, VCAM	65 strokes, 157 controls	N/A	< 6h	90	90	[172]
	18-gene panel (mRNA)	ARG1, BCL6, CA4, CKAP4, ETS-2, HIST2H2AA, HOX1.11, F5, FPR1, LY96, MMP-9, NPL, PYGL, RNASE2, S100A9, S100A12, S100P, SLC16A6	70 strokes, 107 controls	>|1.5| FC	< 3h	93.5	89.5	[173]
	MicroRNAs	Downregulated: miR-122, miR-148a, let-7i, miR-19a, miR-320d, and miR-4429 / Upregulated: miR-363 and miR-487b are increased	24 strokes, 24 controls	>|1.2| FC	< 72h	N/A	N/A	[174]

	Long non-coding RNA	ZFAS1	176 strokes, 111 controls	N/A	< 48h	89.4	48	[155]
Ischemic versus Hemorrhagic stroke	Protein	GFAP	205 patients (39 ICH, 163 IS, 3 mimics)	≥ 0.29 μg/L	< 24h	84.2	96.3	[175]
		S100B	46 ICH, 71 IS	≥ 67 pg/mL	< 6h	95.7	70.4	[176]

3.2.1. Distinction between acute stroke, healthy controls, and stroke mimics

Many blood proteins have the potential to distinguish stroke from disorders mimicking stroke or healthy controls, notably antibodies against the NR2A/NR2B subunits of the N-Methyl-D-Aspartate (NMDA) receptor [125], neuron-specific enolase – NSE [167], heart-type fatty acid binding protein – HFABP [124], Parkinson disease protein 7 – PARK7, and nucleoside diphosphate kinase A – NDKA [168]. However, none of these protein biomarkers has made it to the clinical setting because they either showed suboptimal sensitivity and specificity in studies with small sample size and were not independently validated or because the interpretation of their performance was limited by selection or classification biases [106]. As an example, PARK 7 (also called DJ-1 or Parkinsonism-associated deglycase-1), a redox-sensitive molecular chaperone measured by enzyme-linked immunosorbent assay, was shown to discriminate stroke from controls with 85% sensitivity and 97% specificity in a multi-center retrospective observational sudy that included 622 patients with stroke or transient ischemic attack and 165 controls. The diagnostic cut-off used was 1.55 μg/L [168]. These promising results have not been robustly replicated to establish the benefit of measuring PARK7 in patients with suspected acute stroke in the emergency setting.

In a prospective study of 172 strokes and 133 controls, glycogen phosphorylase isoenzyme BB was found to discriminate stroke from controls with 93% sensitivity and specificity when measured within 12 hours of onset (cut-off of 7.0 ng/mL) [169]. Glycogen phosphorylase breaks down glycogen into glucose-1-phosphate to provide the needed metabolic energy. It is not specific for brain injuries as its plasma concentration also increases in acute coronary syndromes [177] which were excluded using troponin T screening. Serum apolipoprotein A1 unique peptide (APOA1-UP) was also shown to discriminate acute ischemic stroke patients from controls with a sensitivity of 91% and a specificity of 97% in a sample of 94 ischemic strokes and 37 controls [170]. Platelet basic protein identified by mass-spectrometry seems to adequately discriminate patients with transient ischemic attacks from healthy controls. The results obtained on a sample of 20 TIAs, 15 minor strokes and 12 controls (migraine, seizures) need to be confirmed on larger cohorts [171]. Another study using mass spectrometry showed that a set of 30 proteins related to inflammation, coagulation, atrial fibrillation and neurovascular unit injury improved discrimination between strokes ($n = 20$) and controls ($n = 20$) compared to a model based on age alone ($p < 0.001$, cross-validated area under the ROC curve = 0.93 vs. 0.78) [178].

Researchers have also attempted to combine protein biomarkers into panels to improve their diagnostic properties. A panel of four biomarkers including serum calcium-binding protein B –

S100B (glial activation), von Willebrand Factor – vWF (thrombosis), Matrix Metalloproteinase 9 – MMP9, and vascular cell adhesion molecule – VCAM (inflammation) was shown to discriminate stroke from controls with 90% sensitivity and specificity [172]. In the STROKE-CHIP study (n = 1308), none of the 21 biomarkers tested showed sufficient accuracy to differentiate between real strokes and stroke mimics and between ischemic and hemorrhagic strokes in the hyperacute phase [179]. A logistic regression model including the patients' demographics and cardiovascular risk factors outperformed the model including biomarkers only, for the differentiation between ischemic stroke and ICH. The 21-biomarker panel did not include glial-specific markers such as the glial fibrillary acid protein (GFAP) which is currently the most robust biomarker of ICH (discussed below).

Transcriptional changes induced by the interaction between white blood cells and various cellular (damaged brain cells, platelets, blood clot) and humoral factors (cytokines, hormones) before or immediately after a stroke could also provide a molecular signature of stroke [111,180]. These transcriptional changes could be observed either at the level of messenger RNAs (mRNAs or coding RNAs) or at the level of non-coding RNAs. To date, only mRNAs, microRNAs (miRNAs), and long non-coding RNAs (lncRNAs) have been studied as potential diagnostic biomarkers for stroke. The first study of mRNA expression in acute stroke was conducted in rat models of ischemic stroke, intracerebral hemorrhage, status epilepticus, hypoxia, and hypoglycemia. Whole-genome microarray was used to assess mRNA expression in leukocytes isolated within 24 hours after the index event. The study demonstrated that many mRNAs are differentially expressed in the various conditions explored but an accurate distinction of each specific condition from the others could not be done using a single mRNA. The study of a gene expression profile (a group or panel of genes) was indispensable to fully characterize each type of brain injury [181].

Using an 18-gene panel, a subsequent human study confirmed that the assessment of mRNA expression profile in peripheral blood mononuclear cells (PBMC) isolated at various time points after ischemic stroke (3, 5 and 24 hours) could discriminate acute strokes (45 samples) from controls (15 samples) with a sensitivity and a specificity greater than 85% [182]. However, the genes differentially expressed in humans after an ischemic stroke were different from those reported in rats, meaning that only human studies are appropriate for subsequent transcriptomics studies of human stroke [111]. Therefore, a larger validation study was performed including 70 stroke patients (199 samples) and 107 controls (17 with acute myocardial infarction, 52 with various

cardiovascular risk factors, 38 healthy individuals). The same 18-gene panel was used to explore mRNA expression in whole blood and had a sensitivity of 93.5% and a specificity of 89.5% for stroke diagnosis [173]. In further clinical studies, differential mRNA expression also displayed 100% sensitivity and specificity for the discrimination of patients with transient ischemic attacks from controls with a similar profile of cardiovascular risk factors. The genes differentially expressed were associated with inflammation and platelet or prothrombin activation [183,184].

Considering that miRNAs have a direct influence on mRNA translation [185], it is expected that the modifications of mRNA expression observed in stroke patients would also be reflected at the level of miRNA expression. Indeed, it has been shown that miR-122, miR-148a, let-7i, miR-19a, miR-320d, and miR-4429 are decreased while miR-363 and miR-487b are increased in patients with acute stroke when compared to controls with a similar profile of cardiovascular risk factors [174]. These miRNAs were predicted to regulate various aspects of the inflammatory and coagulation responses in stroke. Changes in the miRNA machinery might even precede the modifications of mRNA expression. Further research is needed to refine our understanding of the role of miRNAs (both intracellular and extracellular) in stroke.

Long non-coding RNAs have also been explored as potential diagnostic biomarkers for stroke. Wang and collaborators have reported that the expression levels of the lncRNA Zinc Finger Antisense 1 (ZFAS1) had a sensitivity of 89.4% for discriminating patients with stroke due to large artery atherosclerosis from healthy subjects but with only 48% specificity [155]. In an analysis of whole-blood RNA samples from 133 patients with ischemic stroke and 133 controls matched for vascular risk factors, 299 lncRNAs and 97 lncRNAs were differentially expressed between stroke patients and controls in males and females, respectively. There was proximity between the differentially expressed lncRNAs and some putative stroke-risk loci, including lipoprotein, lipoprotein(a)-like 2, ABO (transferase A, α1-3-N-acetylgalactosaminyltransferase; transferase B, α1-3-galactosyltransferase) blood group, prostaglandin 12 synthase, and α-adducins [186].

3.2.2. Distinction between ischemic stroke and intracerebral hemorrhage

Distinguishing ischemic from hemorrhagic stroke is important as it guides therapeutic decisions. Patients with ischemic stroke benefit from intravenous thrombolysis, which is contraindicated in hemorrhagic stroke. Currently, a plain CT scan of the head is used to identify hemorrhagic stroke. This requires patients to be transported to a CT-equipped hospital which can delay the treatment. Studies have explored the use of biomarkers to quickly rule out an intracerebral

hemorrhage (ICH). Such biomarkers could be useful in remote regions where transport to the nearest CT scanner could take hours.

Glial fibrillary acid protein (GFAP) is a leading candidate to identify hemorrhagic stroke. GFAP is a brain-specific intermediate filament protein maintaining astroglial cell structure [187]. It is only found at very low concentrations in the plasma of healthy individuals because it is not actively secreted from cells [188]. However, an immediate destruction of glial cells, as is the case in ICH, causes a release of great amounts of GFAP and other glial proteins in the bloodstream within minutes. Considering that necrotic cell death and cell lysis can be delayed in ischemic stroke, the difference in GFAP release kinetics between hemorrhagic and ischemic stroke creates a diagnostic window [189]. In the BE FAST 1 and 2 trials, the sensitivity-specificity of GFAP to distinguish hemorrhagic and ischemic stroke was 84.2%-96.3% and 77.8%-94.2% at a threshold of 0.29 μg/mL and 0.03 μg/mL, respectively [175,190]. The ability of GFAP to discriminate hemorrhagic from ischemic stroke has been confirmed by subsequent studies using different cut-points [191,192] and in a meta-analysis [193]. Unfortunately, its diagnostic performance varies from one cohort to the other and is influenced by the delay between symptom onset and sample collection, the nature of the specimen used (serum or plasma), the volume of the hematoma, the severity of the stroke, the measurement method, and eventually the ethnicity [194]. Also, when compared to CT scan, the sensitivity of GFAP does not seem to be high enough for it to serve as a stand-alone test to decide whether initiating intravenous thrombolysis is safe or not.

Further studies have not clearly improved the diagnostic performance of GFAP by combining it with various other biomarkers, notably retinol-binding protein 4 (RBP4) [118], anti-NMDA [195], and ubiquitin carboxyl-terminal hydroxylase-L1 [196]. However, no study has investigated the combination with S100B, another glial-specific protein expressed by mammalian astrocytes that discriminates ischemic stroke from intracerebral hemorrhage with a sensitivity and specificity of 95.7% and 70.4%, respectively, at a cut-point of 67 pg/mL [176]. A panel combining glial-specific and neuron-specific biomarkers might be useful to investigate in the acute stroke setting.

In a study of mRNA expression in 99 whole-blood samples from patients with ischemic strokes (n = 33), ICH (n = 33) and vascular risk factors-matched controls (n = 33), a panel of 107 differentially expressed transcripts related to T-cell receptors function could differentiate ICH from ischemic strokes and controls [197]. Further transcriptomic work is needed to better understand its potential as a biomarker to rapidly distinguish ischemic from hemorrhagic stroke.

3.2.3. Identification of stroke etiology

Stroke is a heterogeneous disorder with multiple underlying etiologies. In hemorrhagic stroke, hypertension accounts for 50-70% of cases. Other etiologies include cerebral amyloid angiopathy, vascular malformations, brain neoplasm, and disorders of coagulation [198,199]. In ischemic stroke, etiologies include cardioembolism, large vessel atherosclerosis (LAA), small vessel disease, or other determined cause (e.g. dissection, mitochondrial disorder, genetic mutation) [200]. Often, no clear cause of stroke can be identified despite extensive investigation, resulting in over 30% of patients having unclear or cryptogenic causes of stroke. Furthermore, multiple potential etiologies can exist in the same patient leaving uncertainty as to the exact cause. This is highlighted by the causative stroke classification system. In lacunar stroke, clinicians rely on indirect features to ascribe etiology (e.g., infarct size and location) without clear methods to image the underlying small vessel pathology. Biomarkers could potentially improve stroke etiology assignment (Table 3.3).

Table 3.3. Biomarkers used for the etiologic diagnosis of stroke

Clinical purpose or question	Type	Biomarker	Sample size	Cut-off	First sample collection (after onset)	Se (%)	Sp (%)	Ref.
Cardioembolic stroke	Protein	MR-proANP	362 IS	≥ 180 pg/mL	< 72h	71	60.3	[149]
		NT-proBNP	1840 IS (meta-analysis)	N/A	N/A	55	93	[201]
Cardioembolic versus LAA stroke	40-gene panel	ADAMTSL4, AP3S2, ARHGEF12, ARHGEF5, BANK1, C16orf68, C19orf28, CD46, CHURC1, CLEC18A, COL13A1, EBF1, ENPP2, EXT2, FCRL1, FLJ40125, GRM5, GSTK1, HLA-DOA, IRF6, LHFP, LHFP, LOC284751, LRRC37A3, OOEP, P2RX5, PIK3C2B, PTPN20A, TFDP1, TMEM19, TSKS, ZNF185, ZNF254	76 cryptogenic strokes (194 samples)	>|1.2| FC	< 3h	95	95	[152]
Lacunar versus non-lacunar stroke	41-gene panel	ALS2CR11, C18orf49, CALM1, CCDC114, CCDC78, CCL2, CCL3, CHML, FAM179A, FAM70B, FLJ13773, GBP4, GTF2H2, HLA-DQA1, HLADRB4, IL8, LAG3, LAIR2, LGR6, LRRC8B, MPZL3, OASL, PDXDC1, PROCR, PRSS23, QKI, RASEF, RUNX3, SCAND2, STK4, STX7, TGFBR3, TSEN54, TTC12, UBA7, UGCG. UTS2, VAPA	184 cryptogenic strokes	>|1.5| FC	< 72h	> 90	> 90	[153]
LAA stroke	Proteins (tested in independent studies)	CRP, fibrinogen, P-selectin (CD62P), adiponectin, ICAM-1, Lp-PLA2	Variable sample sizes	N/A	N/A	N/A	N/A	[14,15,202-206]
Lacunar stroke	Proteins (tested in independent studies)	Homocysteine, vWF, D-dimer, PAI-1, CRP, IL-6, TNF-α	Variable sample sizes	N/A	N/A	N/A	N/A	[128]
Cervical artery dissection	Protein	Fibrillin-1	214 IS (99 with cervical artery dissection), 20 controls	≥ 88.5 ng/mL	N/A	78	80	[207]

For cardioembolic stroke, natriuretic peptides have been studied. There are three types of natriuretic peptides: atrial natriuretic peptide (ANP) synthesized mainly in the heart atria, B-type natriuretic peptide (BNP) synthesized mainly by the heart ventricles, and C-type natriuretic peptide (CNP) synthesized by the central nervous system and vascular tissues. ANP and BNP exist as pro-hormones that are cleaved into N-terminal inactive fragments (NT-proANP, NT-proBNP) and biologically active hormones (ANP, BNP) before their release into the bloodstream [208]. The plasma concentration of the inactive fragments can be measured by immunoassays using antibodies targeting epitopes on their N-terminal end or their mid-region. The mid-regional epitopes are more stable to degradation by exoproteases than the N-terminal ones and may therefore allow a more precise estimation of the serum concentration of proANP or proBNP [209]. In a prospective cohort including 362 consecutively enrolled patients with ischemic stroke (36% cardioembolic), midregional-proANP (MR-proANP) had a sensitivity of 71% and a specificity of 60.3% for identifying cardioembolic stroke at a cut-point of 180 pg/mL [149]. NT-proBNP and D-dimers have also shown good performance for the identification of cardioembolic strokes [150,210] and the discrimination of patients that benefit the most from anticoagulation with warfarin as compared to aspirin [211]. A systematic review found that NT-proBNP has a summary sensitivity of 55% and a summary specificity of 93% for distinguishing cardioembolic from non-cardioembolic strokes [201]. These discriminative properties are currently used in the ARCADIA trial (Atrial cardiopathy and antithrombotic drugs in prevention after cryptogenic stroke – NCT03192215), a multicenter, biomarker-driven, randomized, double-blinded, phase III trial comparing apixaban and aspirin in participants who have evidence of atrial cardiopathy and a recent stroke of unknown cause [212]. Other potential protein biomarkers of cardioembolic stroke include von Willebrand factor, tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6) and interleukin 1 beta (IL1β). However, their diagnostic properties have not been described in detail [144,213].

Transcriptomics studies have also described biomarkers for the etiologic classification of stroke. By analyzing changes in mRNA expression in 76 patients with ischemic stroke (194 samples), a 40-gene panel could discriminate cardioembolic from large vessel atherosclerotic stroke with more than 95% sensitivity and specificity within the first 24 hours of stroke onset [152]. A separate 37-gene panel was able to distinguish atrial fibrillation from non-atrial fibrillation cardioembolic strokes with a sensitivity and a specificity both greater than 90%. Functional analysis of the genes highlighted differences in the inflammatory profile observed in the various stroke subtypes [152]. After defining the gene expression profile of lacunar strokes [153], the profiles of mRNA expressed

were applied to 131 cryptogenic strokes patients classified as having a small deep infarct/possibly lacunar (n = 32) or a non-small deep infarct/likely embolic (n = 99). A 41-gene panel predicted lacunar stroke in 15 of the 32 small deep infarcts. The 40-gene panel was then applied to the remaining 116 embolic strokes of undetermined significance/ESUS and predicted 76 to be cardioembolic, 24 to be LAA, and 16 to remain of unclear etiology. These results suggest that up to 50% of patients diagnosed with cryptogenic stroke may have a cardioembolic source and a subset of patients in this group might benefit from anticoagulation. The NAVIGATE-ESUS trial showed no difference between aspirin and anticoagulation with rivaroxaban for the prevention of stroke recurrence in patients with an ESUS [214]. Whether patients could be pre-selected by cardioembolic stroke biomarkers before randomization remains unclear. Non-coding RNAs have also been associated with cardioembolic stroke. A set of 15 miRNAs were differentially expressed in 16 patients with cardioembolic stroke compared to controls [215].

Biomarkers of lacunar and LAA strokes have also been described. When compared to controls, stroke patients with LAA stroke have higher levels of C-reactive protein (CRP) [205], fibrinogen [202], P-selectin or CD62P [206], adiponectin [203], intercellular adhesion molecule 1 (ICAM-1) [204], and lipoprotein-associated phospholipase A2 [14,15]. ICAM-1 is also increased in symptomatic versus asymptomatic carotid plaques collected post-endarterectomy [13]. ICAM-1 is not specific to large vessel atherosclerosis as it is increased in other stroke subtypes and other diseases [216]. Various other markers of endothelial dysfunction (homocysteine, vWF), coagulation/fibrinolysis (D-dimer, plasminogen activator inhibitor – PAI), and inflammation (CRP, IL-6, TNF-α) have also been associated with lacunar stroke (higher levels compared to non-stroke) [128].

Plasma levels of fibrillin-1 discriminate strokes due to carotid dissection (n = 99) from stroke of other causes (n = 115) and healthy controls (n = 20) with a 78% sensitivity and an 80% specificity [207]. Thus, plasma fibrillin-1 could aid in the diagnosis of stroke due to dissection in situations where there is a high level of clinical suspicion, but conventional neurovascular imaging is inconclusive or not affordable.

3.3. Biomarkers for acute stroke treatment

Once the diagnosis of stroke is made, the appropriate treatment must be administered promptly to ensure the greatest benefits for patients and to avoid complications. For ICH, treatment options include reversal of anticoagulation if required, control of blood pressure, treatment of increased intracranial pressure, respiratory support if required, and supportive care and

monitoring to prevent complications such as infection, seizure, hyperglycemia, and metabolic derangements [217-219]. In ischemic stroke, acute treatments include intravenous administration of tissue plasminogen activator (tPA or alteplase) and endovascular thrombectomy (EVT) [220]. Treatment algorithms are becoming complex with the need to consider various clinical, imaging and biological parameters, notably the time of symptom onset [163], the infarct size/volume in relation to that of the penumbra [164,221,222], and the risk of hemorrhagic transformation [223]. Various biomarkers have been explored to refine the estimation of these parameters and deliver patient-specific treatment recommendations (Table 3.4).

Table 3.4. Biomarkers used to guide stroke treatment

Clinical purpose	Type	Biomarker	Sample size	Cut-off	First sample collection (after onset)	Se (%)	Sp (%)	Ref.
Estimation of the penumbral volume	Panel (Protein/cytokine, metabolite/neurotransmitter)	IL-10, Glutamate	226 IS (61 with clinical-diffusion mismatch)	IL-10 ≥ 23 pg/mL, glutamate ≥ 130 μmol/L	< 12h	96	98	[224]
Prediction of recanalization after thrombolysis	Protein	PAI-1	44 IS (proximal MCA occlusion)	≥ 34 ng/mL	< 3h	84.6	70	[137]
		ADAMTS13	108 IS	Activity ≥ 75%	< 4.5h	69	55	[126]
Prediction of spontaneous HT (no tPA)	Protein	MMP9	250 IS	≥ 140 ng/mL	< 15h	87	90	[225]
		S100 B	458 IS	≥ 11.89 pg/mL	< 48h	92	48	[226]
		NSE		≥ 24.05 μg/mL	< 48h	24	95	
		VEGF		< 177.43 pg/mL	< 48h	53	82	
Prediction of HT after thrombolysis	Protein	c-Fn	87 strokes	≥ 3.6 μg/L	< 4.5h	100	96	[227]
	2-protein panel	PAI-1 and TAFI	77 strokes	PAI-1 < 21.4 ng/mL, TAFI activity > 180%	< 3h	75	98	[132]
	6-gene panel (mRNA)	SMAD4, INPP5D, VEGI, AREG, MARCH7, MCFD2	44 strokes	>|1.2| FC	< 3h	80	70.2	[228]

3.3.1. Estimation of the time of stroke onset and the volume of the ischemic penumbra

In acute ischemic stroke, patient selection for endovascular therapy (EVT) utilizes advanced imaging to identify regions of salvageable brain (ischemic penumbra) in comparison to the size of permanently infarcted tissue [164,221,222]. Although perfusion and vascular imaging are important for the triage of acute stroke patients, they are not always readily available in all care centers. A biomarker could complement acute stroke imaging in the selection of patients for reperfusion therapy.

To date, there is no validated blood biomarker to estimate the time of stroke onset and find if a penumbra is still present in human acute ischemic stroke. Most attempts to define the molecular characteristics of the ischemic penumbra have been performed on animal brains (rodents and monkeys) and have reported increased levels of various proteins, cytokines and metabolites (lactate, glutamate, heat shock proteins such as HSP70, neuregulin, IL-1 and IL-6, TNF-α, hypoxia-inducible factor-1/HIF-1, chemokine stromal-derived factor-1/SDF-1/CXCL12, prostacyclin synthase/PGIS) or upregulation of early inducible genes (e.g. c-fos and c-jun) and anti-apoptotic genes (e.g. Bcl-2 and Bcl-xl) [229,230]. Only one study attempted to validate some of the reported protein biomarkers of ischemic penumbra in human stroke. In 226 adults with acute hemispheric ischemic stroke (median onset to enrolment time: 3.6 hours), including 61 with clinical-diffusion mismatch (CDM), serum interleukin-10 ≥ 23pg/mL and glutamate ≥ 130 μmol/L predicted CDM with a sensitivity of 96% and a specificity of 98%. Patients with CDM also had higher levels of IL-10, TNF-α and lower levels of NSE, IL-6, and active matrix metalloproteinase-9 (MMP-9) [224]. However, the authors did not comment on the performance of the biomarker for discriminating between different estimated sizes of penumbra (small, medium, or large CDM defined by a combination of admission NIHSS and lesion volume on diffusion-weighted imaging). Such distinction is important because the cost-benefit and/or the risk-benefit ratios might sometimes be against the administration of recanalization therapy in patients with small CDMs. In the DEFUSE-3 trial evaluating the benefit of EVT performed 6 to 16 hours after stroke onset, patients were only enrolled if they had a penumbra to infarct volume ratio of 1.8 or greater, with a penumbra volume > 15 mL and a core volume < 70 mL [222]. Further studies are needed to refine the molecular characterization of the ischemic penumbra in human acute stroke as this could pave the way for the optimization of patients triage in the acute setting or the design of therapeutic intervention aimed at extending the therapeutic window by improving neuronal tolerance to ischemia.

3.3.2. Prediction of recanalization following intravenous thrombolysis

The rates of arterial recanalization within the first 2 hours following tPA administration are generally < 35% and depend on the location (proximal versus distal), length and composition of the thrombus [231]. In patients with proximal internal carotid artery, basilar artery or carotid T occlusions, the rate of recanalization could be as low as 4% [232]. Patients with a thrombus longer than 8 mm or with a higher proportion of platelets also have lower rates of recanalization [233,234]. Biomarkers to predict recanalization could inform the design of adjuvant therapies to improve the efficacy of tPA in areas where EVT is not readily available or when EVT is not indicated (distal clots with low NIHSS at presentation and high pretreatment modified Rankin scale - mRS) [235].

As an example, lower levels of α_2-antiplasmin, and thrombin-activatable fibrinolysis inhibitor (TAFI) have been associated with successful recanalization [236]. A study of acute stroke in mice has demonstrated that the administration of a diabody targeting PAI-1 and TAFI improves the efficacy of tPA without increasing the risk of hemorrhagic transformation [237]. Another TAFI inhibitor is currently being evaluated in a multicenter randomized double-blind placebo-controlled phase 1b/2 trial (NCT02586233) aiming to recruit 130 patients with acute stroke presenting beyond 4.5 hours of onset and therefore not eligible for tPA [231]. Plasma levels of plasminogen activator inhibitor 1 (PAI-1) > 34 ng/mL have also been shown to predict proximal middle cerebral artery (MCA) recanalization resistance with a sensitivity of 84.6% and a specificity of 70% [137]. These results could be explained by the inhibitory effect of PAI-1 on tPA that guided the design of tenecteplase (TNK). The latter is a genetically modified tPA with increased fibrin specificity and resistance to PAI-1. TNK has a longer half-life allowing a single bolus administration at a lower dose (0.25 mg/kg, maximum 25 mg) [238]. In the EXTEND-IA TNK trial (NCT02388061), recanalization rates were twice as high in the group receiving TNK (22%, n = 101) than in the group receiving tPA (10%, n = 101). The patients receiving tenecteplase also had better 90-days functional outcomes with similar rates (1%) of hemorrhagic transformation [239].

More recently, a study recruiting 108 tPA-treated acute ischemic stroke patients demonstrated that higher plasma levels of ADAMTS13 (A Disintegrin And Metalloproteinase with ThromboSpondin type-1 motif, member 13) were associated with successful recanalization assessed by the Thrombolysis In Brain Ischemia (TIBI) flow grading system using transcranial Doppler. A cut-off of 75% predicted recanalization 2 hours after tPA treatment with 69%

sensitivity and 55% specificity [126]. The administration of ADAMTS13 has shown promise as a standalone therapy in mouse models of stroke due to arterial platelet-rich thrombi that are tPA-resistant [234]. Further studies should inform on the possibility to use this molecule alone or in combination with alteplase in human acute stroke.

3.3.3. Prediction of hemorrhagic transformation in ischemic stroke

Hemorrhagic transformation (HT) is a feared complication of reperfusion therapy. It occurs when blood extravasates into the brain parenchyma across a disrupted cerebral vessel. Depending on the severity and the type, HT is observed in 3-45% of patients with acute ischemic stroke [240,241]. Cases of HT can be divided into asymptomatic versus symptomatic according to a set of clinical and imaging criteria. In the European Cooperative Acute Stroke Study, a symptomatic HT was defined by a neurological deterioration within the first 36 hours of stroke onset associated with a greater than 4 points increase of the NIHSS score [242]. The administration of tPA leads to a 10-fold increase in the rate of symptomatic hemorrhagic transformation [243]. Many factors and clinical scores to predict the risk of HT have been reported, including stroke severity, administration of tPA or antithrombotics, hyperglycemia, hypertension, and cerebral white matter disease [223].

Several protein and transcriptomics biomarkers to predict the occurrence of HT in ischemic stroke have been described. Plasma levels of MMP-9 ≥ 140 ng/mL, cellular fibronectin (c-Fn) ≥ 3.6 μg/mL and serum levels of S100B ≥ 11.89 pg/mL, neuron specific enolase (NSE) ≥ 24.05 μg /mL, and vascular endothelial growth factor < 177.43 pg/mL predict HT with a sensitivity-specificity of 87%-90% [225], 100%-96% [227], 92%-48%, 24%-95%, 53%-82% [226], respectively. When combining levels of PAI-1 < 21.4 ng/mL and TAFI > 180%, symptomatic HT was predicted with 75% sensitivity and 98% specificity [132]. An mRNA expression panel comprising 6 genes (SMAD4, INPP5D, VEGI, AREG, MCFD2, and MARCH7) measured within 1.5 hour of stroke onset could identify patients that developed tPA-related HT at 24 hours with 80% sensitivity and 70.2% specificity [228].

The biomarkers associated with risk of HT could inform the development of therapies to prevent HT despite the complexity of the underlying pathophysiology. For example, lower levels of PAI-1 are associated with higher rates of recanalization [137] and higher rates of HT [132]. This means that enhancing the activity of PAI-1 may decrease the risk of HT while reducing the effect of tPA if administered concurrently. Minocycline, an inhibitor of MMP-9, reduces rates of HT in

animal stroke models and has shown similar effects in human stroke (MINOS trial) [223,244]. Confirmatory data on the neuroprotective effects of minocycline are awaited from ongoing trials (e.g. NCT03320018). Finally, there are currently four major clinical trials aiming to determine the optimal time to start anticoagulation in patients with acute ischemic stroke: ELAN (NCT03148457; Switzerland), OPTIMAS (EudraCT, 2018-003859-38; UK), TIMING (NCT02961348; Sweden), and START (NCT03021928; USA) [245]. Whether a blood biomarker could help stratify the risk of HT and guide the timing of anticoagulation warrants study.

3.4. Biomarkers for stroke prognosis

Predicting the outcome is important to guide treatment and communicate with patients and their families regarding the expected effects of a stroke. Biomarkers offer the potential to predict prognosis in stroke, including patient response to treatment, development of complications, and long-term functional outcomes.

3.4.1. Prediction of early complications

Patients with an acute stroke can suffer a wide range of complications in the hours following the onset of symptoms, including hemorrhagic transformation (discussed above), malignant cerebral edema, infarct growth with early neurological deterioration (END), and infection (e.g. pneumonia, urinary tract infection).

Approximately 10-20% of patients with complete large MCA infarcts develop a malignant cerebral edema [240]. Decompressive hemicraniectomy (DHC), when performed early (< 48 hours), can reduce mortality by 50% [235,246]. Early treatment is associated with improved outcomes [247]. Studies of biomarkers to aid in the selection of candidates for DHC are scarce and generally included a small number of participants. For instance, plasma levels of S100B > 1.03 μg/L predicted a malignant course of infarction in acute MCA occlusion with 94% sensitivity and 83% specificity when measured 24 hours after stroke onset in a sample of 51 stroke [248]. Plasma levels of c-Fn > 16.6 μg/mL on admission also predicted the development of fatal malignant MCA infarction with 90% sensitivity and 100% specificity in a sample of 40 patients [136]. These studies require replication.

An average of one-third of acute stroke patients experience an early neurological deterioration (END) which means a worsening of their neurological status within the first 72 hours following symptom onset [249]. The causes of early neurological deterioration are variable, including infarct

growth, recurrent stroke, and infection. Identifying biomarkers to predict END could help clinicians to refine patients' selection for specific management. In a study of 197 patients with acute hemispheric infarction (<12 hours), plasma glutamate > 200 μmol/L on admission was the most powerful and independent predictor of infarct growth on DWI [159]. Glutamate release in the extracellular space in the context of ischemic stroke may cause infarct growth by activating the neuronal nitric oxide synthase pathway leading to the generation of toxic free radicals and by inducing a spreading depolarization in the peri-infarct tissue thus increasing the metabolic demand in the context of reduced oxygen supply. This leads to the accumulation of lactate and free radicals causing protein denaturation, inflammation and ultimately cell death if the recovery machinery (heat shock proteins and neuregulin) fails to restore cell function [229]. Inflammatory markers have also been associated with END, notably plasma ferritin > 275 ng/mL (sensitivity of 93% and specificity of 80%) in a study with 100 participants [145], TNF-α > 14 pg/mL, ICAM-1 > 208 pg/mL [250].

Chest and urinary infections are the most common medical complications in stroke, occurring in 13 – 45% of patients [251]. In a recent systematic review, standardized CRP at 24–48 hours was independently associated with infection (OR 1.93-30.41 depending on the model) [139].

3.4.2. Prediction of short and long-term outcome

Several biomarkers have been associated with short- and long-term clinical outcome after stroke (Table 3.5) but most of them have not improved the prediction capacities of clinical variables. Some of these biomarkers include neuroglial proteins such as S100B and HFABP [123,252]; inflammatory markers such as IL-6, CRP, and TNF-α [107,252,253]; cardiac markers such as NT-proBNP and MR-proANP [107,149,253]; and hemostatic markers such as fibrinogen and D-dimer [249,252]. Copeptin, a neuroendocrine marker released by the hypothalamus in equimolar concentration with vasopressin, represents an exception since it could improve the prediction capacity of the NIHSS score for the 90-day functional outcome and the mortality [254,255].

Leptin/adiponectin ratio > 1.16 on day 1 has been associated with good 90-day functional outcome (mRS: 0-2) in 35 patients with atherothrombotic acute ischemic stroke [256]. High serum levels of mannose-binding lectin (MBL), a component of the complement activation cascade, were associated with mortality and poor 90-day functional outcome in 220 patients with acute ischemic stroke [257]. In another cohort of 220 patients with acute ischemic stroke, low levels of 25-hydroxyvitamin D (25-OHD) were associated with mortality and poor 90-day functional

outcome [258]. Other biomarkers of mortality and/or poor 90-day functional outcome in patients with acute ischemic stroke include high serum levels of progranulin, a multipotent growth factor (n = 216) [259]; YKL-40, a glycoprotein associated with acute and chronic inflammation (n = 141, large artery atherosclerotic stroke) [260]; RBP4 (n = 299, cut-point of 37.4 μg/mL, 50% sensitivity, 90% specificity) [261]; and neurofilament light, a neuronal scaffolding protein (n = 110) [262]. High serum levels of neurofilament light also correlated with infarct volume and recurrent ischemic lesions on MRI. High levels of glycated hemoglobin or HbA1c (n = 308) [263] and low activity of ADAMTS13 have also been associated with mortality or poor functional outcome [264]. All these biomarkers improved the performance of the NIHSS and other traditional risk factor models for the prediction of poor functional outcome and mortality. Further studies are needed to validate these results and clarify their clinical implications.

Many protein biomarkers have been reported for outcome prediction in patients with ICH. For example, serum fibulin-5, an extracellular matrix protein, predicted mortality (cut-off 80.7 μg/mL, sensitivity 78%, specificity 93%) and poor 90-day functional outcome (cut-off 48.5 μg/mL, sensitivity 86%, specificity 54%) in a cohort of 68 patients with acute ICH. Serum levels of fibulin-5 were also associated with disease severity (positive correlation with the NIHSS and the hematoma volume, negative correlation with the Glasgow Coma Scale) [265]. Another study of 1262 patients with ICH demonstrated that admission serum levels of calcium ≤ 2.41 mmol/L could predict a poor composite 90-day prognosis (death or major disability) with 89% sensitivity and 78% specificity [266].

Table 3.5. Biomarkers to predict early complications and the 90-day functional outcome [a]

Outcome	Type	Biomarker	Sample size	Cut-off	First sample collection (after onset)	Se (%)	Sp (%)	Ref.
Malignant cerebral edema in MCA occlusion	Protein	S100 B	51 strokes	≥ 1.03 μg/L	< 24h	94	83	[248]
		c-Fn	75 IS	≥ 16.6 μg/mL	< 9h	90	100	[136]
Early neurological deterioration or infarct growth	Neurotransmitter	Glutamate	197 IS	> 200 μmol/L	< 12h	aOR = 89.7	95% CI: 19.8 – 406.6	[159]
	Protein	Ferritin	100 IS	> 275 ng/mL	< 16h	93	80	[145]
		ICAM-1	113 lacunar strokes	> 208 pg/mL	< 24h	aOR = 315	95%CI = 17 - 5748	[250]
	Protein (cytokine)	TNF-α	113 lacunar strokes	> 14 pg/mL	< 24h	aOR = 511	95% CI: 17 – 4937	[250]
Infection	Protein	CRP	697 IS (meta-analysis)	N/A (> fourth quartile)	24-48h	aOR = 3.21	95%CI: 1.93–5.32	[102]
Poor functional outcome at 90 days	Proteins (individually associated with poor 90-day functional outcome but did not improve the prediction capacity of clinical parameters or NIHSS)	S100B, IL-6, CRP, TNF-α, NT-proBNP, MR-proANP, fibrinogen, D-dimers	Variable sample sizes	N/A	N/A	N/A	N/A	[107,123,149,249,252,253]
	Proteins (individually associated with poor 90-day functional outcome with improvement of the prediction capacity of the NIHSS)	MBL, progranulin, YKL-40, NfL, HbA1c, ADAMTS13	Variable sample sizes	Variable	N/A	N/A	N/A	[257,259,260,262-264]
	Protein	Copeptin	359 strokes	N/A (per log unit	< 72h	aOR = 4.23	95% CI: 1.61 –	[255]

				increase)			11.15	
		RBP4	299 IS	≥ 37.4 μg/mL	< 48h	50	90	[261]
		Serum fibulin-5	73 ICH	≥ 80.7 μg/mL	< 72h	78	96	[265]
	Vitamin	25-hydroxyvitamin D	220 IS	N/A (per unit increase)	< 12h	OR = 0.79	95% CI: 0.73–0.85	[258]
	Electrolyte	Calcium	1262 ICH	≤ 241 mmol/L	< 12h	89	78	[266]

[a] Wherever appropriate, the sensitivity (Se) and specificity (Sp) have been replaced by the adjusted odds ratio (aOR) and the corresponding 95% confidence interval (CI), respectively.

3.4.3. Risk stratification for secondary prevention

Stroke survivors are at increased risk for recurrent cerebrovascular events [240]. Biomarkers may help to stratify the risk of recurrent stroke, myocardial infarction, and death in patients with TIA, ischemic stroke, and intracerebral hemorrhage (Table 3.6).

a) Transient ischemic attacks

In patients with TIA, the risk of recurrence ranges from 2-15% within the first 90 days [267]. Clinical scores such as the ABCD2 and ABCD3-I are used to predict the risk of stroke after TIA and identify high-risk groups in need of urgent evaluation and therapy [268,269]. Biomarkers may offer the possibility to improve the accuracy of ABCD2 or ABCD3-I. For example, the neuroendocrine hormone copeptin improved the ABCD3-I capacity to predict stroke recurrence after TIA [147,148,270]. Lower plasma levels of lysophosphatidylcholine predict recurrent stroke in TIA and add to the predictive ability of the ABCD2 score [158]. In the CHANCE trial, high levels of high-sensitive CRP (marker of inflammation) and soluble CD40L (marker of atherosclerotic plaque instability) were also identified as independent predictors of stroke recurrence [271,272]. In the same trial, patients with increased levels of glycated albumin (GA > 15.5%, n = 1907) had similar rates of stroke recurrence whether they were in the aspirin group or the aspirin plus clopidogrel group [85].

b) Large artery atherosclerotic stroke

Large artery atherosclerosis is responsible for approximately 15-25% of all ischemic strokes and encompasses cervical artery atherosclerosis affecting the anterior (carotid arteries) or the posterior circulation (vertebral arteries) and stroke due to intracranial atherosclerosis [2,3]. The risk is not the same in all these subcategories and depends on the topography of the stenosis, its grade, and the characteristics of the atherosclerotic plaque [10,273].

Several protein and RNA markers of carotid plaque instability or progression have been reported. In 173 adult patients with ischemic stroke, low serum levels of omentin-1, a protein regulating vascular inflammation, were associated with the presence of instability features on carotid plaques assessed by ultrasound (ulceration and/or hypoechogenicity) [274]. In 70 acute ischemic stroke patients, serum levels of complement complex C5b-9 were associated with plaque instability, plaque burden, and degree of carotid stenosis [275]. High levels of ICAM-1, high-sensitivity CRP, and lipoprotein-associated phospholipase A2 (Lp-PLA2) have also been associated with progressive or symptomatic LAA [12-15]. However, the specificity of these markers

for carotid atherosclerosis is uncertain as they may also reflect the inflammatory response to brain ischemia. In the STABILITY trial, an inhibitor of Lp-PLA2 (darapladib) did not reduce the risk of ischemic stroke [276], raising questions regarding the relationship of Lp-PLA2 to the risk of ischemic stroke. The trial was designed to demonstrate the incremental effect of darapladib for the prevention of cardiovascular events in patients already receiving optimal secondary prevention therapy, including statins in 96% and coronary revascularization in 75% prior to randomization. Statins have been shown to reduce the levels of Lp-PLA2 by up to 35% [277,278]. Therefore, the events rate might have been lower than expected in both arms of the trial, thus limiting the probability to observe a significant effect of the adjunctive therapy.

MicroRNAs may also inform the risk of stroke in patients with carotid atherosclerosis. In 60 patients with >50% asymptomatic carotid artery stenosis, increased plasma levels of miR-199b-3p, miR-27b-3p, miR-130a-3p, miR-221-3p, and miR-24-3p were associated with progression of carotid stenosis [279]. These miRNAs play roles in inflammation, angiogenesis, endothelial and smooth muscle cell proliferation, migration, and differentiation [280-282]. In another study of 170 healthy participants, increased plasma levels of miR-29c was independently associated with subclinical atherosclerosis defined as carotid intima-media thickness ≥ 0.9 mm after adjusting for age, body mass index, systolic blood pressure, total cholesterol, fasting blood-glucose, and CRP. Expression levels of miR-29c and CRP levels were positively correlated [283]. Carotid intima-media thickness is a well-described and validated surrogate marker of atherosclerosis and a predictor of future cardiovascular events [284,285]. In a study of miRNA expression in 22 carotid plaques from patients undergoing carotid endarterectomy, higher levels of miR-200c were found in unstable carotid plaques (n = 12) defined according to findings on preoperative contrast-enhanced ultrasound and medical history (symptomatic or not, risk factors, treatment) [23]. Moreover, when analysing mRNA expression levels of selected biomarkers, miR-200c was positively correlated with biomarkers of plaque instability (MMP1, MMP9, IL-6, and monocyte chemoattractant 1 or MCP-1) and negatively correlated with biomarkers of plaque stability (zinc finger E-box binding homeobox 1 or ZEB1, endothelial nitric oxide NO synthase or eNOS, forkhead boxO1 or FOXO1, and Sirtuin1 or SIRT1). Plasma levels of miR-200c decreased after 24 hours post-endarterectomy but returned to preoperative levels at 1 month.

c) Other stroke subtypes

A plasma level of high-sensitive CRP > 4.86 mg/L was associated with stroke recurrence in the Levels of Inflammatory Markers in the Treatment of Stroke (LIMITS) trial (n = 1244) [286]. In the Northern Manhattan Study (NOMAS), high plasma procalcitonin was associated with an increased risk of lacunar stroke and high plasma MR-proANP was related to an increased risk of cardioembolic stroke [287]. An increase in plasma levels of free fatty acids was associated with a higher risk of stroke recurrence in patients with cardioembolic stroke (n = 105) [157]. Further studies are required to confirm these findings and clarify the mechanism by which free fatty acids increase the risk of stroke in patients with cardioembolism.

An analysis of data from 2176 participants of the Stroke Prevention by Aggressive Reduction of Cholesterol Levels (SPARCL) trial demonstrated that osteopontin, neopterin, and myeloperoxidase are were independently associated with the risk of recurrent stroke and improved the prediction capacity of the Stroke Prognostic Instrument II (area under the receiver operating characteristic curve increased by 0.023, P=0.015 and continuous net reclassification improvement of 29.1%, P<0.0001) [288,289]. Finally, low ADAMTS13 activity was associated with a higher risk of first-ever ischemic stroke of any type in 5941 individuals aged 55 years or older from the Rotterdam study [127].

Table 3.6. Biomarkers used to predict stroke recurrence, plaque instability and response to antithrombotic treatments

Outcome to predict	Type	Biomarker	Sample size	Cut-off	First sample collection (after onset)	aHR	95% CI	Ref.
Stroke recurrence	Protein	Copeptin [a]	107 TIAs	≥ 9 pmol/L	< 72h	Se = 80%	Sp = 76%	[147]
		High-sensitive CRP	3044 TIA/minor IS	> 3 mg/L	< 36h	1.5	95%CI: 1.1 – 2.0	[271]
		Soluble CD40L	3044 TIA/minor IS	> third tertile	< 36h	1.5	95%CI: 1.1 – 2.0	[272]
	Proteins (tested in independent studies)	procalcitonin, osteopontin, neopterin, myeloperoxidase, ADAMTS13	Variable sample sizes	N/A	N/A	N/A	N/A	[127,287,288]
	Lipids	Free fatty acids	105 cardioembolic IS	N/A (per unit increase)	< 7 days	2.7	95%CI: 1.1 – 7.0	[157]
	Metabolite	Lysophosphatidylcholine [b]	293 TIA	< 1,14,000 MS counts	< 24h	OR = 3.5	95% CI: 1.5 – 8.9	[158]
Plaque progression or instability	Proteins (tested in independent studies)	Omentin-1, complement complex C5b-9, ICAM-1, CRP, Lp-PLA2	Variable sample sizes	N/A	N/A	N/A	N/A	[12-15,274,275]
	MicroRNAs	MiR-199b-3p, miR-27b-3p, miR-130a-3p, miR-221-3p, miR-24-3p, miR-29c, miR-200c	Variable sample sizes	Variable thresholds of FC	N/A	N/A	N/A	[23,279,283]
Response to dual antiplatelets antiplatelet therapy	Protein	Glycated albumin	1907 TIA/minor IS	> 15.5%	< 36h	0.8 (vs 0.4)	95%CI: 0.6 – 1.1 (vs 0.3 – 0.6)	[85]
Benefit from anticoagulation	Protein	NT-proBNP	1028 IS	> 750 pg/mL	< 30 days	0.3 (vs 1.2)	95%CI: 0.1 – 0.8 (0.9 – 1.7)	[211]

[a] The adjusted hazard ratio (aHR) and the corresponding 95% confidence interval (CI) have been replaced by the sensitivity (Se) and specificity (Sp), respectively.

[b] The adjusted hazard ratio (aHR) has been replaced by the odds ratio (OR).

3.5. Conclusion and future directions

Efforts to overcome the limitations of expert clinical judgement and multimodal neuroimaging in stroke medicine have resulted in the identification of several blood biomarkers that could improve the diagnosis and the management of stroke patients. These biomarkers are mainly proteins, RNA, lipids, and metabolites involved in various aspects of stroke, including brain injury and repair. For the diagnosis of stroke, the best discrimination between stroke and mimics have been observed when markers are combined in panels. GFAP is a leading candidate for the distinction between ischemic and hemorrhagic strokes and might perform better if combined with selected brain-specific markers. Likewise, to determine stroke etiology, panels of markers may also achieve sufficient sensitivity and specificity to address the heterogeneity in human stroke. For stroke treatment, serum IL-10 and glutamate may identify patients with a clinical-diffusion mismatch, but further studies are needed to better define the blood biomarkers of ischemic penumbra. Several blood markers to predict HT have been described and future studies will clarify if they could inform the development of therapies to prevent HT or assist decision-making regarding the timing of anticoagulation after stroke. For stroke prognosis, plasma copeptin can add to age and NIHSS to predict functional outcome and to the ABCD2/ABCD3-I scores to predict stroke recurrence after TIA. Other markers of functional outcome include YKL-40, RBP4, and neurofilament light which require validation. Several RNA markers have been associated with atheroma plaque instability and further work is needed to determine if they could refine patient selection for carotid endarterectomy or stenting. The development of blood biomarkers to improve stroke diagnosis and management is ongoing. Additional results regarding the role of biomarkers to aid in the diagnosis, risk stratification, and treatment decisions are expected from several larger trials mentioned in this review.

Chapter 4: Non-stenotic Carotid Plaques in Embolic Stroke of Unknown Source[4]

4.1. Introduction

Ischemic stroke is considered cryptogenic when no definite cause is identified during the baseline etiological workup.[200] According to the Cryptogenic Stroke/Embolic Stroke of Undetermined Source International Working Group, the baseline etiological workup should include brain imaging with computed tomography (CT) or magnetic resonance imaging (MRI), assessment of the heart rhythm with 12-lead ECG and continuous cardiac monitoring for at least 24 hours with automated rhythm detection, transthoracic cardiac ultrasound, and imaging of cervical and intracranial vessels supplying the infarcted brain region (using CT, MRI, conventional angiography, or ultrasonography).[2]

Cryptogenic strokes represent approximately 30% of all ischemic strokes. They could be further classified into three subgroups: stroke with no cause despite complete baseline workup, stroke with multiple possible underlying causes, and stroke with incomplete baseline workup.[290] In the subgroup of cryptogenic strokes with complete workup, embolic stroke of unknown source (ESUS) is a clinical construct referring to non-lacunar ischemic strokes (size >1.5 cm on CT or > 2.0 cm on diffusion MRI) of presumable embolic origin (superficial/cortical brain lesion) despite the absence of any obvious sources of cardiac or arterial embolism (e.g., atrial fibrillation, carotid or intracranial stenosis > 50%) (Figure 4.1).[2] ESUS represent approximately 17% of all ischemic strokes with a recurrent stroke rate of 4.5% per year despite antithrombotic therapy.[31,214,291]

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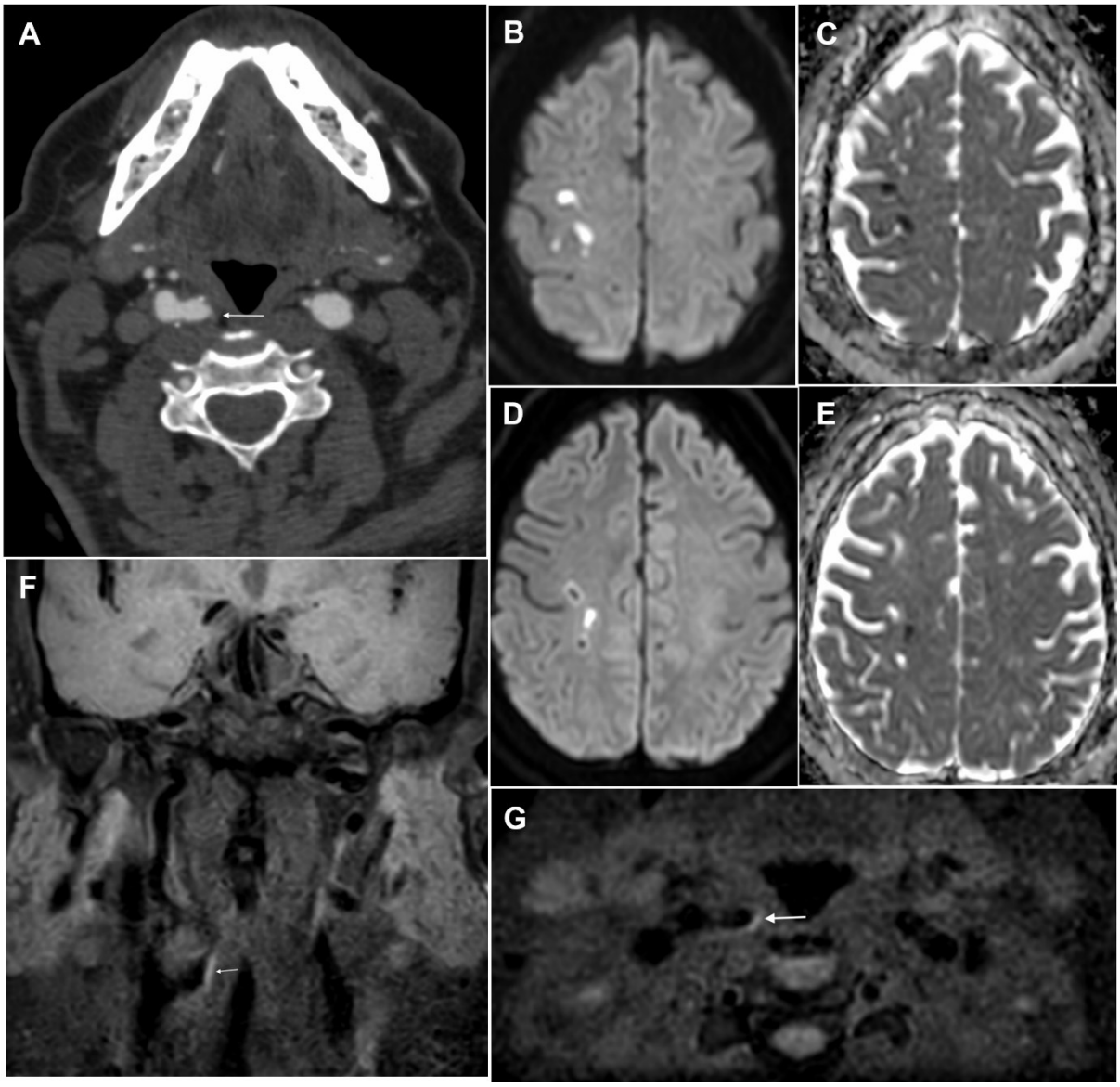

Figure 4.1. Brain and plaque imaging findings in 64-year-old man with ESUS

A: Axial angio-CT scan slice showing a hypodense nonstenotic carotid plaque in the right internal carotid artery (white arrow).
B, C, D, and E: Axial diffusion-weighted imaging slices (with corresponding ADC maps) showing multiple embolic strokes in the right pre-and post-central area.
F and G: Coronal and axial T1-weighted black blood sequence showing hyperintensity of the nonstenotic plaque in the right internal carotid artery (white arrow), thus confirming the presence of intraplaque hemorrhage.

The definition of ESUS was based on the assumptions that cryptogenic strokes may be related to covert atrial fibrillation and that a relationship between nonstenotic atherosclerotic plaques (causing less than 50% stenosis) and stroke was unlikely. However, new evidence suggest that ESUS represents a heterogeneous group including patients with various other potential causes of stroke besides atrial fibrillation.[292-294] Such causes include atrial cardiopathy,[295] patent foramen ovale (PFO),[296] cancer,[297] and nonstenotic plaques affecting the aortic arch or carotid, vertebral, or intracranial arteries.[292,298,299] Atrial cardiopathy is a concept referring to a dysfunction of the left atrium that is thought to precede and favor the onset of atrial fibrillation and its eventual detection by electrocardiographic devices. The diagnosis is based on the identification of imaging markers (e.g., left atrial enlargement, spontaneous echocontrast in the left atrium or the left atrial appendage, atrial fibrosis with delayed gadolinium enhancement on MRI), electrocardiographic markers (e.g., paroxysmal supraventricular tachycardia, increased P-wave terminal force in V1, interatrial block, prolonged PR), and blood biomarkers (e.g., N-terminal pro-brain natriuretic peptide, highly sensitive cardiac troponin T).[295]

Nonstenotic carotid plaques are found in 40% of patients with ESUS and 10-15% of patients with ESUS have mild stenosis (20-49%).[2,32,33,300] Here we review the evidence supporting the relationship between nonstenotic carotid plaque with high-risk features and stroke in patients with ESUS. We present the remaining challenges in the process of formally establishing the causal link between nonstenotic plaques and ESUS, notably those related to the identification of blood biomarkers of vulnerable plaque. Finally, we discuss the management of nonstenotic carotid plaques in patients with ESUS and highlight areas for future research.

4.2. Nonstenotic carotid plaques as a potential cause of ESUS

The relationship between nonstenotic carotid plaques and ESUS is supported by a set of three clinical observations.

First, in patients with ESUS, carotid plaques are more prevalent on the side of the stroke than on the contralateral side. In a cross-sectional study of 85 patients with ESUS, nonstenotic carotid plaques thicker than 3 mm were present in 35% of ipsilateral carotid arteries versus 15% of the contralateral carotid arteries.[36] A similar finding was observed in a review of 138 ESUS cases from the prospective multicenter INTERRSeCT study (The Predicting Early Recanalization and Reperfusion With IV Alteplase and Other Treatments Using Serial CT Angiography). The

investigators found a nonstenotic carotid plaque ipsilateral to the stroke in 29.2% of patients and contralateral to the stroke in 18.7%.[300]

Second, in patients with ESUS, there is a lower incidence of atrial fibrillation detected during follow-up in patients with ipsilateral nonstenotic carotid plaques than in those without, thus suggesting that nonstenotic carotid plaques may be contributory. In 777 participants of the New Approach Rivaroxaban Inhibition of Factor Xa in a Global Trial Versus ASA to Prevent Embolism in Embolic Stroke of Undetermined Source (NAVIGATE-ESUS) trial who were followed up for a median of 2 years, the incidence of atrial fibrillation was 2.9 per 100 person-years in patients with ipsilateral nonstenotic carotid plaque versus 5.0 per 100 person-years in those without (overall rate: 8.5% versus 19.0%; adjusted hazard ratio: 0.57, 95% CI 0.37 – 0.84).[32]

Third, plaques with high-risk features are more prevalent on the side of the stroke in patients with ESUS. In a meta-analysis of 8 studies enrolling 323 patients with ESUS, plaques with high-risk features were present in 32.5% of the ipsilateral carotid arteries versus 4.6% of the contralateral carotid arteries. More specifically, the odds of finding a nonstenotic carotid plaque with a ruptured fibrous cap in the ipsilateral versus the contralateral carotid artery was 17.5, reinforcing the idea that nonstenotic carotid plaques should not be considered as benign coincidental findings in patients with ESUS.[298]

High-risk plaques have features on brain or vascular imaging that are associated with a higher risk of stroke in patients with either symptomatic or asymptomatic carotid atherosclerosis, independent of the grade of stenosis.[11,62,301-304] The most common high-risk plaque features are echolucency, impaired cerebrovascular reserve, intraplaque hemorrhage, silent brain infarcts, lipid-rich necrotic core, large juxtaluminal black hypoechoic area, large plaque volume, plaque thickness, microembolic signals, mural thrombus, neovascularization, plaque irregularity, plaque inflammation or hypermetabolism, thin or ruptured fibrous cap, and ulceration.[10,11,301,305-310] The American Heart Association combines some of these features to derive a classification of atherosclerotic plaques into 6 types reflecting increasing instability and risk of cardiovascular events (Table 4.1).[34,37,41,74,75,311] On average, high-risk plaque features are three times more prevalent in patients with symptomatic versus asymptomatic carotid stenosis (OR = 3.4, 95% CI: 2.5-4.6).[301] They are detected using various vascular imaging modalities (Table 4.2). To date, there are no data on the risk of recurrent stroke associated with each of the high-risk features in

patients with ESUS. Analysis of secondary outcome data from the Carotid Plaque Imaging in Acute Stroke study (CAPIAS; NCT01284933) might help to address this knowledge gap.[34,312]

Table 4.1. American Heart Association comprehensive morphological classification scheme for atherosclerotic lesions[74,75,311]

Plaque type	Description							
		Lipid rich necrotic core	**Fibrous cap**	**Calcification**	**Erosion/rupture**	**Intraplaque hemorrhage**	**Thrombus**	**Regression to normal**
Type I (Initial lesion)	Initial lesion, accumulation of smooth muscle cells and isolated foam cells, absence of a necrotic core.	Absent	Absent	Absent	Absent	Absent	Absent	Possible
Type II (Intimal xanthoma)	Multiple layers of foam cells, previously referred to as "fatty streak"	Absent	Absent	Absent	Absent	Absent	Absent	Possible
Type III (pre-atheroma)	Smooth muscle cells in a proteoglycan-rich extracellular matrix, multiple layers of foam cells, non-confluent extracellular lipid pools)	Absent	Present (ill-defined)	Absent	Absent	Absent	Absent	Possible
Type IV (atheroma)	Confluent extracellular lipids	Present (well-formed)	Present (well-defined)	Absent	Absent	Absent	Absent	Not possible
Type Va (Fibroatheroma)	Confluent extracellular lipids with prominent proliferative fibromuscular layer	Present (well-formed)	Present (thick)	Possible [a]	Absent	Absent	Absent	Not possible
Type VI (Complicated atheroma)	Inflammatory lesion with at least one high-risk feature	Present (large)	Present (thin or eroded)	Possible (partial calcification)	Possible (VIa if present alone)	Possible (VIb if present alone)	Possible (VIc if present alone)	Not possible

[a] The plaque is assigned category Vb if predominantly calcified (fibro-calcific) or category Vc if predominantly fibrous (collagen-rich atheroma with smaller lipid core).
[b] The plaque is assigned category VIabc if erosion/ulceration, intraplaque hemorrhage and luminal thrombus are present concurrently.

Table 4.2. High-risk plaque features commonly used in clinical practice[10,11,298,305-310]

High-risk plaque features [a]	Imaging modality of choice	Description [b]	Alternative imaging modalities	Prevalence (%)in patients with ESUS
AHA type IV,V,VI[34,37,41]	MRI	Plaque with large lipid-rich necrotic core (>40% of the vessel circumference), rupture fibrous cap, mural thrombus, or intraplaque hemorrhage (see below).	CT, US	In 3 studies including 82 patients with ESUS, an AHA plaque type IV-VI was found in the ipsilateral carotid in 38% of cases on average.[34,37,41]
Echolucency	US	Hypoechoic area within the plaque on B-mode (reference = sternocleidomastoid muscle)	Not applicable	In a study of 44 patients with ESUS, an ipsilateral echolucent nonstenotic carotid plaque was found in 50.0% [35]
Impaired cerebrovascular reserve	TCD	<10% increase of blood flow in the ipsilateral MCA while breathing 5% CO_2 for 2 minutes.	BOLD-MRI	Not applicable for nonstenotic plaques
Intraplaque haemorrhage	MRI	Intraplaque hyperintensity on T1WFAT SAT (black blood) and 3D-TOF	MRI	In 5 studies including 162 patients, intraplaque hemorrhage was found in the ipsilateral carotid in 24.4% of cases.[298]
Ipsilateral silent brain infarcts	MRI	Non-lacunar hyperintensity of the brain parenchyma, in the territory of the internal carotid artery, visible on T2W and FLAIR, or DWI (if acute)	CT (would appear as a hypodensity)	No data available for patients with ESUS
Lipid-rich necrotic core	MRI	Collection of foam cells, cholesterol crystals and apoptotic cells that appears iso/hyper-intense on T1W and iso/hypo-intense on T2W.	CT, US (although it is difficult to make the difference with intraplaque hemorrhage on these	No data available for patients with ESUS

			modalities)	
Microembolic signals	TCD	Random audible transient increase (variable threshold) of the Doppler signal within the monitored arterial blood flow, generating a high-intensity signal on the doppler imaging (PWV and M-Mode), visible and moving in the direction of the flow. Duration of recording ≥ 1 hour. [c]	Not applicable	No data available for patients with ESUS
Mural thrombus	MRI	Filling defect on contrast MRI, hyperintense signal adjacent to the lumen on T1W	CT, US	In 3 studies enrolling 94 patients with ESUS, plaque thrombus was identified in the ipsilateral carotid in 6.9% of cases.[298]
Neovascularization	CEUS	Enhancement of the plaque on pulse inversion harmonic imaging (microbubbles carried into the plaque by the blood entering the neovessels)	DCE-MRI	No data available for patients with ESUS
Plaque irregularity	MRI	0.3-0.9 mm fluctuations of the surface of the plaque	CT, CEUS	No data available for patients with ESUS
Thin/ruptured fibrous cap	MRI	Disrupted or invisible dark band adjacent to the lumen on 3D-TOF	CEUS	In 2 studies enrolling 50 patients with ESUS, a thin or ruptured fibrous cap was found in the ipsilateral carotid in 23.6% of cases.[298]
Ulceration	MRI	Depression > 1 mm on the surface of the plaque	CTA, CEUS (the threshold is 2 mm in ultrasound studies)	No data available for patients with ESUS

[a] The following high-risk features are used less often: juxta-luminal black hypoechoic area and plaque volume assessed by ultrasound, plaque inflammation measured by standardized ^{18}F-FDG uptake on positron emission tomography-computed tomography, carotid temperature assessed by microwave radiometry.
[b] For simplicity, the description of each high-risk feature is based on its appearance on the imaging modality of choice.

[c] The sound threshold and the number of MES for a positive examination is variable across studies.
AHA means American Heart Association; BOLD, blood oxygen level-dependent; CEUS, contrast-enhanced ultrasound; CI, confidence interval; CT, computed tomography; DCE, dynamic contrast-enhanced; ESUS, embolic stroke of undetermined source; FLAIR, fluid-attenuated inversion recovery; MCA, middle cerebral artery; MRI, Magnetic Resonance Imaging; T1W, T1-weighted imaging; T2W, T2-weighted imaging; TCD, transcranial Doppler ultrasound; and 3D-TOF, 3-dimensional time of flight.

4.3. Challenges of establishing causal link with stroke

4.3.1. Puzzling clinical associations

Although studies of high-risk features have provided evidence of an association between nonstenotic carotid plaques and brain infarction in patients with ESUS, establishing causality remains challenging in most cases. The dilemma rests on four clinical observations. First, high-risk features are often found in plaques in the absence of related clinical symptoms.[301,313] In a meta-analysis of 8 studies enrolling 323 patients with ESUS, a nonstenotic carotid plaque with high-risk features was identified in the contralateral carotid artery in 4.6% of cases (95% CI: 0.1-13.1).[298] Likewise, in a meta-analysis of 64 studies enrolling 20,571 patients with asymptomatic carotid stenosis of various grades, 26.5% of patients were found to have at least one high-risk plaque feature (95% CI: 22.9-30.3). The highest prevalence was observed for neovascularization (43.4%, 95% CI: 31.4-55.8) and the lowest for mural thrombus (7.3%, 95% CI: 2.5-19.4). On average, intraplaque hemorrhage was found in 1 out of 5 patients.[301] Second, high-risk plaque features are not specific for symptomatic carotid plaques. In a meta-analysis of data from 20 prospective studies enrolling 1652 patients with symptomatic carotid stenosis, high-risk plaque features were identified in less than 1 in 2 patients (43.3%, 95% CI: 33.6-53.2).[301] Third, in patients with stroke, there is an association between the presence of high-risk plaque features and atrial fibrillation. In a study of 68 patients with embolic stroke, including 45 ESUS, the presence of high-risk plaque features on carotid ultrasound (ulceration, thickness $\geq$ 3 mm, and echolucency) was independently associated with detection of atrial fibrillation on admission or during follow-up (OR = 4.5, 95% CI: 1.0-19.6).[314] Fourth, in some patients with ESUS diagnosed using the current clinical definition, nonstenotic carotid plaque often coexist with other potential causes of stroke, including atrial fibrillation (8.5%),[32] intracranial atherosclerosis (8.4%),[315] PFO (5-9%),[316,317] and atrial cardiopathy (2.4%).[318]

4.3.2. Lack of reliable biomarkers

The identification of an ipsilateral nonstenotic carotid plaque with or without high-risk features is not sufficient to reclassify ESUS as stroke due to large vessel disease. Further research is, therefore, needed to determine whether the combination of vascular imaging findings, clinical data, and candidate biomarkers of plaque progression/instability or atheroembolism [15-18,27-30,78,86,152,274,275,279,319-341] into multiparameter scores could improve the ability to (1) establish a causal link between ESUS and a nonstenotic carotid plaque, (2) predict plaque progression or stroke recurrence, and (3) select patients who might benefit from adjuvant anti-inflammatory and lipid-lowering therapies as briefly discussed in section 4.3. Some biomarkers of plaque progression

and instability that warrant further investigation specifically in patients with ESUS are presented in Table 4.3. There are several ongoing projects exploring biomarkers in patients with ESUS or cryptogenic stroke, notably the Searching for Explanations for Cryptogenic Stroke in the Young: Revealing the Etiology, Triggers, and Outcome study (SECRETO, NCT01934725),[342] the Carotid Plaque Imaging in Acute Stroke study (CAPIAS, NCT01284933),[34] and the Biomarkers of Acute Stroke Etiology study (BASE, NCT02014896).[343] Efforts to establish a causal relationship between nonstenotic carotid stenosis and ESUS using biomarkers and multimodal vascular imaging in well-phenotyped prospective cohorts will also benefit from research aiming to identify alternative causes of stroke in patients with ESUS.[212,299,333,344-350]

Table 4.3. Biomarkers of potential interest for the study of nonstenotic carotid plaques in ESUS

Biomarker	Type	Main source	Key evidence	Specific target of a drug previously tested in human trials	References
Lectin-like oxidized LDL receptor (LOX-1)	Protein	Endothelial cells, smooth muscle cells, fibroblasts	In 4703 participants from the Malmo Diet and Cancer Cohort, higher plasma levels of soluble LOX-1 were associated with higher risk of stroke during a mean follow-up of 16.5 years (HR = 1.5, 95% CI: 1.3-2.4). In 202 patients undergoing carotid endarterectomy, plasma levels of soluble LOX-1 were correlated with the plaque content of oxidized LDL, proinflammatory cytokines, and matrix metalloproteinases.	No	17,18,319,320,327,335
Omentin-1	Protein	Visceral adipose stromal vascular cells, lung, heart, placenta, ovaries	In 173 patients with acute ischemic stroke, serum levels of omentin-1 were lower in subjects with unstable plaque (n= 38, echolucent, thin fibrous cap, ulcerated) than in those with stable plaques (median of 53 vs 62 ng/mL).	No	274
Lipoprotein-associated phospholipase A2 (Lp-PLA2)	Protein	Monocytes, macrophages, T lymphocytes, and mast cells	In 1946 participants of the Northern Manhattan study, there was a dose-response relationship Lp-PLA2 mass and the risk of first-ever stroke due to large vessel atherosclerosis (HR = 1.4, 4.5, and 5.1 for quartiles 2, 3, and 4 compared with quartile 1 in multivariable survival analysis).	Yes (Darapladib)	15,86,276
Chitinase-3-like-1 (YKL-40)	Protein	Inflammatory cells	In 1132 patients with carotid atherosclerotic plaques of various grades, higher levels of YKL-40 were associated with plaque instability (n= 855, echolucency) after adjusting for various demographic and cardiovascular risk factors (OR=2.1 and 1.7 for quartiles 3 and 4, respectively).	No	324,327
Granzyme B	Protein	T lymphocytes	In 67 patients with severe carotid stenosis undergoing revascularization, higher plasma levels of granzyme B were found in patients with unstable plaques (n=16, echolucent) than in those with stable plaques (median of	No	

			492.0 vs 143.8 pg/mL)		
Vimentin	Protein	Endothelial cells, macrophages, and astrocytes	In 4514 patients with carotid plaques in the Malmo Diet and Cancer Cohort, higher plasma levels of vimentin at baseline were associated with the incidence of ischemic stroke after a mean follow-up of 22 years (HR = 1.66, 95% CI: 1.23-2.25).	Yes (Withaferin-A)	[331,351]
Macrophage chemoattractant protein (MCP-1/CCL2)	Protein	Monocytes	In the Athero-EXPRESS biobank, higher plaque levels of MCP-1 levels were found in symptomatic (versus asymptomatic) plaques and in vulnerable (versus stable) plaques.	No	[328]
Matrix metalloproteinase 9 (MMP9)	Protein	Macrophages, foam cells	Serum levels of MMP9 were higher in large artery atherosclerosis strokes (n=26, 1137 ng/mL) versus cardioembolic strokes (n=86, 517 ng/mL). MMP9 >1110 ng/mL had 85% sensitivity and 52% specificity for differentiating large vessel from cardioembolic strokes.	No	[327,332]
Complement 5b-9	Protein	Liver	In 70 patients with acute ischemic stroke, serum C5b-9 levels were higher in patients with unstable plaques (n=37) than in those with stable plaques (median of 875 vs 786 ng/mL). There was also a positive correlation with plaque burden and grade of stenosis.	Yes (Eculizumab)	[275,352]
Interleukin 1β (IL-1β)	Protein	Monocytes, macrophages	A higher expression of IL-1β and other components of the NLRP3 inflammasome was observed in 30 plaques when compared with 10 healthy mesenteric arteries, both at the protein and the mRNA level.	Yes (Anakinra, Rilonacept, Canakinumab)	[336,353-355]
Interleukin 6 (IL-6)	Protein	Monocytes, macrophages	In a sub-analysis of data from 703 participants of the population-based Tromsø study, higher plasma levels of IL-6 were independently associated with plaque progression after a 6-year follow-up (OR 1.4, 95% CI 1.1-1.8 per SD increase in IL-6 level).	Yes (Ziltivekimab, Tocilizumab)	[27-30]
C-Reactive Protein (CRP)	Protein	Hepatocytes, white blood cells, adipocytes, smooth muscle cells	In a prospective observational study enrolling 271 participants, higher levels of CRP (quartile 4 versus 1) were associated with plaque progression after a follow-up of 37 months (OR = 1.8, 95% CI: 1.03-2.99).	No	[337,356]

CD36	Protein	Various cells including monocytes, endothelial cells, adipocytes, platelets.	In 62 patients with severe carotid stenosis undergoing revascularization, plasma levels of soluble CD36 were higher in those with symptomatic (n=31) and unstable (echolucent, n=20) plaques.	No	[16]
Lipoprotein (a)	Lipoprotein	Food/Liver	In 876 consecutive patients with carotid atherosclerosis (2.5% occlusions), plasma lipoprotein (a) was an independent predictor of carotid occlusion (OR=1.7, 95% CI: 1.2-2.3 per 1 SD increase), suggesting that it plays a role in plaque destabilization/rupture, thrombosis, and impaired fibrinolysis. In 225 patients with coronary artery disease who underwent intra-coronary optical coherence tomography imaging of culprit plaque, the prevalence of thin fibrous cap atheroma was significantly higher in the group with higher serum lipoprotein (a) levels (> 25 mg/dL, n=87): 23% versus 11%.	Yes (AKCEA-Apo(a)-LRx)	[338-340,357,358]
Non-HDL cholesterol (Non-HDL-C)	Lipoproteins	Food/Liver	In 2888 patients with carotid plaque, including 1505 with vulnerable plaques (echolucent, irregular, or ulcerated), higher serum levels of non-HDL cholesterol were independently associated with plaque vulnerability (OR=1.5 for tertile 3 versus 1, 95% CI: 1.2-1.8).	Yes (various classes of lipid-lowering drugs)	[322,359,360]
Uric acid	Xanthine (purine derivatives)	Various cells	In a study including 88 patients with carotid plaques (44 symptomatic), serum uric acid levels were significantly higher in patients with symptomatic plaques (7.4 versus 5.4 mg/dL) who also had higher plaque expression of xanthine oxidase as assessed by immunohistochemistry.	Yes (allopurinol)	[341]
Neutrophil count	Cells	NA	In 60 patients with recently symptomatic carotid artery disease, higher neutrophil count (>5900/μL) was associated with detection of microembolic signals on transcranial Doppler monitoring.	No	[326]
miR-199b-3p, miR-27b-3p, miR-130a-3p, miR-	RNA	Various cells	In 60 patients with moderate or severe asymptomatic carotid stenosis, higher plasma levels of the micro-RNAs were associated with plaque progression (n=19) after 2	No	[279]

221-3p, and miR-24-3p			years of follow-up.		
miR-200c	RNA	Various cells	In 22 patients undergoing carotid endarterectomy, higher levels of miR-200c were found in patients with unstable plaques (echolucent symptomatic) and were positively correlated with biomarkers of plaque instability (matrix metalloproteinase – MMP1, MMP9; interleukin 6, macrophage chemoattractant protein 1 – MCP-1)	No	[23,327]
Resistin and chimerin mRNA	RNA	Various cells	An analysis of 165 carotid plaque (67% unstable based on histological criteria), Resistin and chemerin mRNA expression was 80% and 32% lower, respectively, in unstable versus stable plaques.	No	[334]

4.4. Challenges of secondary stroke prevention

As a result of the challenges to determine the root cause of an ESUS, the optimal treatment strategy for patients with ESUS remains unclear, and a tailored approach would likely be the most appropriate.[294] In this section, we briefly describe the strategies that have been explored so far and discuss possible future directions.

4.4.1. Dual antiplatelet therapy and antiplatelet switch

Following the results of the Platelet-Oriented Inhibition in New TIA and Minor Ischemic Stroke (POINT)[361] and the Clopidogrel in High-Risk Patients with Acute Nondisabling Cerebrovascular Events (CHANCE)[362] trials, patients with ESUS are treated with Aspirin-based dual antiplatelet therapy for 21 days provided that their baseline NIHSS is low. After 3 weeks, patients ideally return to single antiplatelet therapy and switching from Aspirin to Clopidogrel is considered in patients who had an ESUS while on Aspirin.[363] A meta-analysis of data from CHANCE and POINT showed that extending the treatment beyond 3 weeks might increase the bleeding risk without additional benefit for secondary stroke prevention.[364] Whether the presence of ipsilateral nonstenotic carotid plaque with or without high-risk features would modify the magnitude (absolute risk reduction) and duration (beyond 21 days) of the benefits derived from dual antiplatelet therapy in patients with ESUS remains unknown. In patients allergic to Clopidogrel and in carriers of a CYP2C19 loss of function allele, Ticagrelor might be an alternative according to findings of the Acute Stroke or Transient Ischaemic Attack Treated with Ticagrelor and ASA [acetylsalicylic acid] for Prevention of Stroke and Death (THALES) trial.[365-368] The ongoing Clopidogrel with Aspirin in High-risk patients with Acute Non-disabling Cerebrovascular Events II (CHANCE-2, NCT04078737) trial is evaluating the superiority of the Ticagrelor-Aspirin combination over Clopidogrel-Aspirin therapy in CYP2C19 loss of function carriers with minor stroke or transient ischemic attack (TIA).[369] There is currently no evidence supporting the use of dual antiplatelet therapies not containing Aspirin or triple antiplatelet therapies (with or without Aspirin) for secondary stroke prevention in patients with acute stroke or TIA.[370]

4.4.2. Anticoagulation

The New Approach Rivaroxaban Inhibition of Factor Xa in a Global Trial Versus ASA [Acetylsalicylic Acid] to Prevent Embolism in Embolic Stroke of Undetermined Source (NAVIGATE-ESUS) and the Randomized Double-Blind Evaluation in Secondary Stroke Prevention Comparing The Efficacy Of Oral Thrombin Inhibitor Dabigatran Etexilate for Secondary Stroke Prevention in Patients With Embolic Stroke of Undetermined Source (RE-

SPECT-ESUS) trials have shown that universal full-dose oral anticoagulation is not an effective strategy to reduce the risk of stroke recurrence in patients with ESUS.[214,291] These results are likely explained by the heterogeneity of stroke mechanisms in patients with ESUS as discussed earlier, with atrial fibrillation being diagnosed in only 24.8% of cases at 24 months using insertable cardiac monitors.[371] Moreover, there is no evidence that patients with ESUS and ipsilateral nonstenotic carotid stenosis should be treated differently than those without plaque. In a subgroup analysis of data from 2,905 patients with nonstenotic carotid plaque enrolled in the NAVIGATE-ESUS trial, there was no difference between Rivaroxaban and Aspirin with respect to the prevention of ipsilateral ischemic stroke (Hazard ratio [HR] = 0.6, 95% CI: 0.2-1.9). Major bleeding complications were significantly more frequent in patients taking anticoagulation (HR = 3.7, 95% CI: 1.6-8.7).[33]

In the Cardiovascular Outcomes for People Using Anticoagulation Strategies (COMPASS) trial, the combination Rivaroxaban-Aspirin (2.5 mg twice daily plus Aspirin 100 mg once per day) was superior to Aspirin alone (100 mg once daily) for the prevention of cardioembolic strokes (HR = 0.4, 95% CI: 0.2-0.8) and ESUS (HR = 0.3, 95% CI: 0.1-0.7) but there was no effect on the incidence of stroke due to moderate-to-severe carotid stenosis (HR = 0.9, 95% CI: 0.5-1.6).[372] Although these results suggest that the combination of Aspirin and low-dose Rivaroxaban could be an effective secondary stroke prevention strategy, they are not directly applicable to patients with ESUS since all patients with acute stroke (< 1 month) were excluded from the trial due to the perceived higher risk of major intracranial bleeding.[43] Furthermore, the baseline proportion of patients with nonstenotic carotid plaque, with or without high-risk features, was not reported. The prevalence of ipsilateral nonstenotic carotid plaque in participants diagnosed with ESUS during follow-up was also not reported.

According to currently available data, patients with ESUS and features of atrial cardiopathy, notably atrial enlargement, constitute the only subgroup that may benefit from anticoagulation.[373] However, since these results are derived from a post-hoc analysis of the NAVIGATE-ESUS trial, they might not be used to justify universal prescription of anticoagulation until confirmation is obtained in dedicated trials. The ongoing Atrial Cardiopathy and Antithrombotic Drugs in Prevention After Cryptogenic Stroke (ARCADIA, NCT03192215),[212] Apixaban for Treatment of Embolic Stroke of Undetermined Source (ATTICUS, NCT02427126), and A Study on BMS-986177 (oral factor XIa inhibitor) for the Prevention of a Stroke in Patients Receiving Aspirin and Clopidogrel (AXIOMATIC-SSP, NCT03766581) trials will, hopefully, provide conclusive

results to guide patient care. Likewise, in the Oxford Vascular Study, a large patent foramen ovale is present in 36% of patients with a cryptogenic stroke aged > 60 years[374] and associated with a 2.5 times higher risk of recurrent ischemic stroke,[375] thus suggesting it might be worth trialing PFO closure or anticoagulation in elderly patients with a large PFO. However, the causal relationship between the PFO and the recurrent stroke was not formally established and the prevalence of ipsilateral nonstenotic carotid plaque was not reported. Because PFO closure or anticoagulation are not expected to prevent strokes due to large vessel atherosclerosis, trials of PFO closure or anticoagulation in elderly patients with a large PFO should carefully plan subgroup analyses according to the presence of alternative candidate causes of the recurrent stroke, notably an atrial cardiopathy or an ipsilateral nonstenotic carotid plaque that may coexist with PFO.[316,317,376]

4.4.3. Other therapies and interventions

Currently, patients with ESUS receive intensive lipid-lowering therapy (e.g., statins, ezetimibe) to achieve a level of LDL cholesterol < 70 mg/dL (1.8 mmol/L) as early as possible after stroke.[235,377,378] The treatment is maintained long-term if well tolerated, even in older adults.[379-382] Specific targets of LDL cholesterol have not been assessed in patients with ESUS and it is unknown if the presence of an ipsilateral nonstenotic carotid plaque would modify the effect of lipid-lowering drugs as suggested by findings of the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL).[383] Furthermore, the potential role of newer classes of lipid-lowering drugs for plaque stabilization and secondary stroke prevention is yet to be defined. Such drugs include proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors (small interfering RNA - inclisiran or monoclonal antibodies - evolocumab or alirocumab) and Apo(a) antisense oligonucleotides that reduce plasma levels of both LDL cholesterol and lipoprotein(a) [Lp(a)]; as well as anti- angiopoietin-like 3 monoclonal antibodies that do not affect Lp(a) levels and bempedoic acid. [360,384-389] Like ezetimibe, [359,390] the new lipid-lowering drugs may be useful as add-on or statin-sparing agents in cases of allergy or intolerance to statins, familial hypercholesterolemia, refractory hypercholesterolemia, or in patients with high Lp(a) levels at the time of stroke since statins increase plasma levels of Lp(a).[358,391] There are reports of an association between high Lp(a) levels and cryptogenic stroke[392,393] suggesting that Lp(a) could represent a biomarker to guide optimization of lipid-lowering therapy in patients with ESUS as is the case in other cardiovascular diseases.

Systemic inflammation, a hallmark of atherosclerosis, modulates the risk of stroke and the effect of lipid-lowering agents.[394-396] This explains the benefit of various anti-inflammatory drugs (e.g., canakinumab, colchicine) for the prevention of atherosclerotic cardiovascular diseases.[353,354,397] In patients with ESUS and ipsilateral nonstenotic carotid plaque, the effect of anti-inflammatory agents is worth exploring, especially in those with high-risk plaque features since they would not be offered revascularization procedures as first-line treatment according to current guidelines.[4,5,398] Data from the ongoing Colchicine for Prevention of Vascular Inflammation in Non-Cardioembolic Stroke (CONVINCE, NCT02898610) might answer the question of whether patients with ESUS with or without ipsilateral nonstenotic carotid plaques would benefit from the addition of low-dose colchicine to best medical therapy for secondary stroke prevention.[399] The relevance of serial vascular imaging to monitor carotid plaque progression and stability is another aspect of the management that remains unexplored.

Besides pharmacological treatments, there is a variety of lifestyle interventions that are beneficial for cardiovascular risk reduction and are recommended by the American Heart Association for secondary stroke prevention no matter the suspected underlying etiology. Such interventions include smoking cessation, regular physical activity, weight loss, improved sleep hygiene, avoidance of noise and air pollution, reduction of salt and sugar intake, higher consumption of fish, fruits, and vegetables.[400-407]

4.5. Conclusion and future directions

ESUS is a common subtype of stroke that is frequently associated with an ipsilateral nonstenotic carotid plaque. Evidence suggests that advanced multimodal vascular imaging and biomarkers might help reclassify some ESUS as large vessel strokes. However, the precise algorithm for this reclassification remains to be designed. Despite significant research efforts since the term ESUS was coined in 2014, the optimal management strategy for patients with ESUS remains unclear. There are several ongoing trials investigating various interventions. While waiting for more evidence to support the design of tailored therapeutic guidelines for the various well-phenotyped subgroups of patients with ESUS, clinicians should continue to fully implement all previously validated stroke prevention strategies, whether an ipsilateral nonstenotic carotid plaque is present or not. Such strategies include short-term dual antiplatelet therapy if appropriate, long-term intensive lipid-lowering therapy, control of modifiable cardiovascular risk factors (e.g., hypertension, diabetes, smoking, obesity), and lifestyle changes.

PART C: Blood biomarkers of high-risk carotid atherosclerosis

Chapter 5: Circulating interleukin-6 predicts carotid plaque severity, vulnerability, and progression in the Cardiovascular Health Study[5]

5.1. Introduction

Abnormal lipid profile and chronic systemic inflammation are key features of atherosclerosis, a major contributor to the burden of cardiovascular disease.[395] Historically, interventions to slow the progression of atherosclerosis have focused on controlling vascular risk factors and reducing circulating levels of cholesterol.[235] Recent clinical and experimental studies have demonstrated that anti-inflammatory drugs targeting the nucleotide-binding domain leucine-rich repeat and pyrin domain-containing receptor 3 (NLRP3) inflammasome can decrease cardiovascular events independent of lipid lowering and blood pressure control.[355] Additional analyses have revealed that this atheroprotective effect is mediated by the reduction in circulating levels of interleukin-6 (IL-6).[408,409] Furthermore, genetic studies substantiate the causal relationship between IL-6 signaling and atherosclerotic cardiovascular disease.[410-414] These observations position human monoclonal antibodies targeting IL-6 as promising adjuvant agents to prevent ischemic stroke in patients with carotid atherosclerosis.[28-30]

The potential of anti-inflammatory drugs to prevent carotid atherosclerosis-related stroke warrants further evaluation. To date, trials assessing anti-inflammatory drugs to reduce cardiovascular risk have used composite endpoints and not specifically stroke caused by carotid atherosclerosis. In a subgroup analysis of the CANTOS trial, canakinumab did not show benefit for stroke prevention potentially because of non-atherothrombotic causes of stroke. Second, there is no population-based study demonstrating the specific association of IL-6 levels with carotid atherosclerosis-related ischemic stroke. Third, a recent population-based study with a modest sample size demonstrated that high levels of IL-6 are associated with carotid plaque progression but did not demonstrate the relationship of IL-6 with plaque severity and vulnerability.[27]

To know if therapies targeting IL-6 are worth evaluating as an adjuvant primary or secondary stroke prevention strategy in patients with carotid atherosclerosis, it is important to demonstrate the relationship between levels of IL-6 and high-risk plaque features associated with stroke

[5] This chapter has been submitted as "Kamtchum-Tatuene J, Saba L, Heldner MR, Poorthuis MHF, de Borst GJ, Rundek T, Kakkos SK, Chaturvedi S, Topakian R, Polak JF, Jickling GC. Circulating interleukin-6 predicts carotid plaque severity, vulnerability, and progression in the Cardiovascular Health Study."

risk.[301] Our primary objective was to investigate if circulating levels of IL-6 are independently associated with plaque severity, vulnerability, and progression at 5 years in the Cardiovascular Health Study (CHS). As a secondary objective, we aimed to identify a candidate threshold for the use of circulating IL-6 as a biomarker for clinical decision-making in the management of carotid atherosclerosis.

5.2. Methods

5.2.1. Study design and participants

The Cardiovascular Health Study (CHS) is a prospective population-based cohort study aiming to identify risk factors of cardiovascular disease in people aged 65 years or older, recruited and followed up between 1989 and 1999.[415] The 5888 participants were randomly sampled from Medicare eligibility lists in four communities: Forsyth (North Carolina), Sacramento (California), Washington (Maryland), and Pittsburgh (Pennsylvania). Eligible participants were non-institutionalized, able to give informed consent, not requiring a proxy respondent at baseline, and expected to remain in their area of residence for at least 3 years following enrolment. Individuals who were wheelchair-dependent, institutionalized or receiving anticancer treatment at baseline were excluded. For the analyses reported in this article, we excluded participants with a missing value of baseline circulating IL-6 levels or incomplete carotid ultrasound data (baseline and follow up at 5 years).

5.2.2. Clinical and laboratory assessment

All participants underwent clinical and laboratory evaluations at baseline to identify the presence and severity of cardiovascular risk factors as well as subclinical and clinical cardiovascular disease. The diagnosis of prevalent cardiovascular diseases at baseline and during follow up was centrally adjudicated.[415] Information on prescription medication was collected directly from prescription bottles, and the use of nonprescription drugs was ascertained by questionnaire.[415] Collection of blood samples at baseline was performed via venipuncture after a 12-hour fast. Multiple aliquots of plasma and serum were prepared and frozen at -70 °C at Field Centers, then shipped weekly on dry ice to the Central Blood Analysis Laboratory.[415,416] Fasting serum chemistry analyses were performed on the Kodak Ektachem 700 Analyzer (Eastman Kodak Corp., Rochester, NY, USA) and included creatinine, uric acid, and glucose. The plasma lipid profile was obtained on an Olympus Demand system (Olympus Corp., Lake Success, NY, USA) and included total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) derived using Friedewald equation.[415,417] Plasma IL-6

levels were measured by enzyme-linked immunosorbent assay (ELISA, High Sensitivity Quantikine kit, R&D Systems, Minneapolis, MN, USA). The plasma samples used for IL-6 ELISA were prepared using ethylenediaminetetraacetic acid (EDTA) and were run in duplicates. The detectable limit was 0.10 pg/mL, the intra-assay coefficient of variability was 6.3% and the inter-assay coefficient of variability was 7%.[418,419]

Hypertension was defined as blood pressure > 140/90 mmHg or medical history of hypertension or ongoing antihypertensive treatment. Diabetes mellitus was defined as fasting blood glucose > 7 mmol/L or history of diabetes mellitus or ongoing treatment with insulin or oral antidiabetic drugs. Dyslipidemia was defined by at least one of the followings: LDL-C > 100 mg/dL (2.6 mmol/L), HDL-C < 50 mg/dL (1.26 mmol/L), triglycerides > 150 mg/dL (1.7 mmol/L), total cholesterol > 200 mg/dL (5.2 mmol/L), treatment with lipid-lowering drugs. Hyperuricemia was defined as uric acid > 7 mg/dL or ongoing treatment with uric acid-lowering drugs (uricosurics or xanthine oxidase inhibitors).

5.2.3. Carotid ultrasound assessment

Duplex ultrasonography of both carotid arteries was performed at baseline and at 5 years with a Toshiba SSA-270A ultrasound device (Toshiba American Medical Systems, Tustin, CA, USA) equipped with 5.0 MHz transducer. Sonographers performing the 5-year carotid ultrasound examination were blinded to baseline images. Two-dimensional gray scale imaging was used to detect focal plaques. Pulsed wave, continuous wave, and color Doppler images were also obtained. All images were stored on optical disc and transferred to the CHS Ultrasound Reading Center for centralized reading and interpretation (Ultrasound Reading center, New England Medical Center, Boston MA). Two readings were obtained for the 5-year carotid ultrasound images: the first was blinded to baseline images and the second was not. The latter was used in this study since it reflects real-world practice. Periodic duplicate studies were carried out to assess the intra- and inter-observer agreement between Field Center and Reading Center technicians. Analytic measurements included the grade of stenosis based on the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria, a plaque irregularity score, and a description of plaque echogenicity focusing on the carotid bulb and proximal internal carotid arteries.[415] Plaque severity or grade of stenosis was scored 0 to 5 for each of the right and left internal carotid arteries with 0 corresponding to a normal carotid, 1 to 1-24% stenosis, 2 to 25-49% stenosis, 3 to 50-74% stenosis, 4 to 75-99% stenosis, and 5 to an occluded carotid artery. Plaque irregularity was scored 0 for smooth plaque, 1 for mildly irregular (height variations < 0.4

mm), 2 for markedly irregular (height variations of 0.4 - 2.0 mm), and 3 for ulcerated plaques (discrete depression of > 2 mm).[420] Plaque echogenicity was coded 0 for the absence of plaque (normal carotid), 1 for hypoechoic or echolucent plaque (echogenicity similar to or lower than that of the vessel lumen), and 2, 3, or 4 for isoechoic, hyperechoic, or calcified plaques.[92]

Mild, moderate, and severe carotid stenosis were defined as 1-49%, 50-74%, and 75-100% stenosis. Plaque vulnerability at baseline was defined as the presence of a markedly irregular plaque, an ulcerated plaque or an echolucent plaque on at least one carotid artery.[11] Plaque progression at 5 years was defined as an increase by one point or more on the plaque severity score for at least one carotid artery.

5.2.4. Statistical analysis

Categorical variables were summarized as frequency and percentage. Continuous variables were summarized as mean (95% CI) or median (IQR) as appropriate. The distribution of continuous variables was assessed by visual inspection of histograms and quantile-quantile plots and by performing skewness and Shapiro-Wilk tests. Logarithmic transformation was applied to continuous variables with non-Gaussian distribution, and the resulting log-transformed variables were used for subsequent analyses unless stated otherwise. Comparisons between included and excluded participants (missing IL-6 or ultrasound data) were performed using Student t test or Mann-Whitney U test for continuous variables and chi-squared or Fisher exact tests for categorical variables. The relationship of IL-6 with cardiovascular risk factors was assessed by comparing the mean log IL-6 between groups defined by the presence of each risk factor using the Student t test. The relationship of log IL-6 with age and biomarkers of cardiovascular disease such as log creatinine, log CRP, uric acid, LDL-C, and cystatin-based glomerular filtration rate was assessed using the Pearson correlation test.

The relationship of IL-6 with plaque characteristics was modeled using univariable and multivariable linear regression for maximum plaque severity at baseline and multivariable logistic regression for plaque vulnerability at baseline and plaque progression at 5 years. The following independent variables were considered during the modelling process based on available evidence of association with atherosclerosis[421,422]: age (years), sex, race, atrial fibrillation, hypertension, diabetes, dyslipidemia, smoking status, alcohol consumption (drinks of beer, wine, or liquor per week), history of stroke or TIA, history of coronary heart disease, history of peripheral artery disease, body mass index, baseline cystatin-based glomerular filtration rate, log IL-6, log CRP,

hyperuricemia, and treatment with anti-inflammatory (steroids or non-steroidal) or antiplatelet drugs. Lipid levels and treatment with statins or uric acid levels and treatment with uric acid-lowering drugs were not considered because they were already included in the definition of dyslipidemia and hyperuricemia. Considering our previous work suggesting that the presence of high-risk features is not dependent on plaque severity, we did not include plaque severity in the model to predict baseline plaque vulnerability.[301] However, we considered the baseline ipsilateral plaque severity score and the baseline ipsilateral plaque vulnerability in the logistic regression model to predict plaque progression at 5 years and explored the interaction with log IL6.

In all multivariable regression analyses, a stepwise backward elimination process was applied with $p>0.05$ for removal of variables based on Wald test for the significance of regression coefficients. In this process, variables are removed in decreasing order of p-values. All quantitative variables were included in regression models as continuous to optimize the statistical power. Comparison of the relative contribution of each independent variable to the regression models was based on standardized coefficients and odds ratios. The significance of regression models was assessed by the Fisher test for the percent explained variance (linear regression) and the Chi-squared test for the log likelihood ratio (logistic regression). The model performance was assessed by the percent explained variance (R^2); the model calibration by computing the calibration-in-the-large index (CITL), calculating the proportion of observations correctly classified, and inspecting calibration plots; the model discrimination by computing the area under the receiver operating characteristic curve (AUC). Detection of multicollinearity and influential observations was based on variance inflation factor >10 and Cook distance >1. Statistical assumptions governing multivariable linear and logistic regression modelling were verified for all models reported.

To assess the stability of all logistic regression models, we computed the E-value defined as the minimum strength of association on the odds ratio scale that an unmeasured confounder must have with both log IL-6 and the dependent variable (baseline vulnerability or progression at 5 years) to fully suppress the observed association, conditional on the measured covariates.[423] Furthermore, we computed optimism-adjusted odds ratios using the heuristic shrinkage method [424] and performed bootstrapping-based internal validation.[425] The reported optimism-adjusted AUC, CITL, and calibration slope were computed after applying a bootstrap shrinkage factor derived from an internal validation process with 100 bootstrap samples. Shrinkage and

bootstrapping are penalization methods to account for the propensity of prediction models to display a reduced performance when applied to other populations.

In a secondary analysis, we attempted to define a candidate clinical threshold for plasma IL-6 levels. For this, we computed the mean value of log IL-6 in patients with a >50% predicted probability (greater than chance) of plaque progression using the optimism-adjusted multivariable logistic regression model and derived the corresponding plasma concentration of IL-6 in pg/mL by applying the exponential function and rounding up to the nearest multiple of 0.5. Then, we dichotomized baseline plasma IL-6 levels using the derived cut-off to identify participants with high IL-6 levels at baseline. We then repeated all logistic regression analyses to verify if a high IL-6 level at baseline was independently associated with plaque severity, vulnerability, and progression. We also checked if the performance, calibration, discrimination, and stability of the logistic regression models would be significantly affected.

All statistical tests were two-sided and unpaired, with a significance threshold of $p \leq 0.05$. Data analyses were performed using Stata software, version 17 (StataCorp LLC, College Station, TX, USA). Data were analyzed from September 9, 2021, to October 28, 2021.

5.2.5. Ethical considerations and reporting

The CHS was approved by the Institutional Review Board of the University of Washington and each of the participating field centers. All participants provided written informed consent.[415]

This study is part of the Carotid Atherosclerosis and Stroke Collaboration (CASCO) research initiative. The CASCO is an international research consortium bringing together investigators from across the world to accelerate the resolution of current and future challenges regarding the diagnosis, assessment, and management of carotid atherosclerosis for optimal stroke prevention. The CASCO research protocol is approved by the University of Alberta Human Research Ethics Board (Pro00106520).

This report is compliant with the Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) and the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statements.[426,427]

5.3. Results

5.3.1. Characteristics of the participants

Of the 5888 participants of the CHS, 4334 (58.9% women) had complete data for baseline plasma IL-6 levels and carotid ultrasound assessment. The mean age was 72.7 ± 5.1 years (Table 5.1). Excluded participants were older, less often women or blacks, and had higher prevalence of diabetes, smoking, coronary or peripheral artery disease. They also had poorer kidney function and higher levels of uric acid and inflammatory markers. The distribution of baseline IL-6, CRP, uric acid and cystatin-based GFR is shown in Appendix3, Figures I, II, and III.

The prevalence of mild, moderate, and severe stenosis was 72%, 3%, and 0.7%. The baseline carotid ultrasound examination was reported as normal in 24.3%. There were 1267 (29.2%) participants with a vulnerable carotid plaque at baseline and 1474 (34.0%) diagnosed with plaque progression at 5 years. Participants with plaque progression at 5 years were less likely to have an ipsilateral vulnerable carotid plaque on the baseline carotid ultrasound examination (16.4 % versus 36.0%, p <0.001).

Median (IQR) plasma IL-6 level (pg/mL) at baseline was 1.4 (1.0-2.2) in participants without carotid plaque and 1.7 (1.2-2.5), 1.6 (1.2-2.8), and 2.3 (1.5-2.7) in participants with mild, moderate, and severe carotid stenosis. Plasma IL-6 levels were higher in participants with cardiovascular risk factors (Figure 5.1). Plasma IL6 levels had a moderate positive correlation with log CRP (r=0.5, p <0.001) and a moderate negative correlation with cystatin derived GFR (r = -0.3, p<0.001) (Appendix 3, Table I).

Table 5.1. Baseline clinical characteristics of the participants

Characteristics	Participants included (n = 4334)	Participants excluded (n = 1554)	p
Age (years, mean ± SD)	72.7 ± 5.1	75.2 ± 6.4	<0.001
Women	2553 (58.9)	776 (49.9)	<0.001
Blacks	744 (17.2)	157 (10.1)	<0.001
Body mass index (kg/m^2, mean ± SD)	26.6 ± 4.1	26.4 ± 4.2	0.04
Atrial fibrillation	160 (3.7)	67 (4.3)	0.28
Hypertension	2543 (58.7)	952 (61.3)	0.08
Diabetes mellitus	646 (14.9)	302 (19.4)	<0.001
Dyslipidemia	3967 (91.5)	1280 (82.4)	<0.001
Current smoker	496 (11.4)	202 (13.0)	0.10
Alcohol consumption (drinks per week, median with IQR)	0.02 (0-1.3)	0 (0-1)	<0.001
Hyperuricemia	860 (19.8)	373 (24.0)	< 0.001
Coronary heart disease	761 (17.6)	376 (24.2)	<0.001
Peripheral artery disease	127 (2.9)	86 (5.5)	<0.001
Prior stroke or TIA	227 (5.2)	120 (7.7)	<0.001
Treatment with statins	102 (2.4)	25 (1.6)	0.08
Treatment with antiplatelet drugs	142 (3.3)	52 (3.3)	0.90
Treatment with uric acid-lowering drugs*	118 (2.7)	51 (3.3)	0.26
Treatment with anti-inflammatory drugs†	571 (13.2)	260 (16.8)	<0.001
Cystatin-based GFR (ml/min, mean ± SD)	79.5 ± 19.1	71.0 ± 20.6	<0.001
Interleukin-6 (pg/mL, median with IQR)	1.6 (1.1 – 2.5)	2.1 (1.4 – 3.3)	<0.001
C-reactive protein (mg/L, median with IQR)	2.4 (1.2 – 4.2)	3.0 (1.5 – 6.8)	<0.001
Uric acid (mg/dL, mean ± SD)	5.6 ± 1.5	5.9 ± 1.6	<0.001

CRP: C-Reactive Protein; GFR: Glomerular Filtration Rate; TIA: Transient Ischemic Attack.

* Uric acid-lowering drugs refer to xanthine oxidase inhibitors and uricosurics.

† Anti-inflammatory drugs refer to steroids and non-steroidal anti-inflammatory drugs.

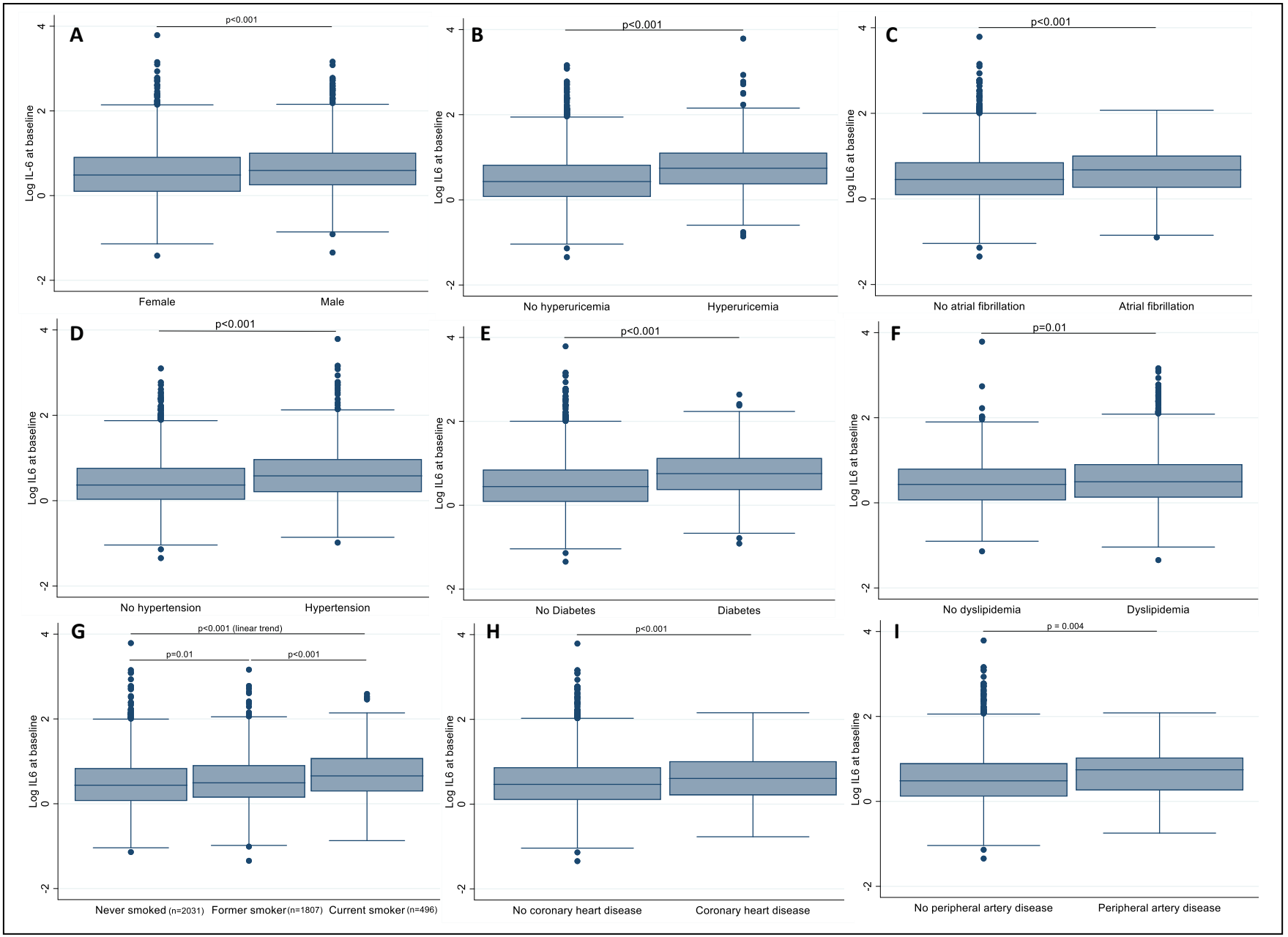
A
p<0.001
Log IL-6 at baseline
Female
Male
B
p<0.001
Log IL6 at baseline
No hyperuricemia
Hyperuricemia
C
p<0.001
Log IL6 at baseline
No atrial fibrillation
Atrial fibrillation
D
p<0.001
Log IL6 at baseline
No hypertension
Hypertension
E
p<0.001
Log IL6 at baseline
No Diabetes
Diabetes
F
p=0.01
Log IL6 at baseline
No dyslipidemia
Dyslipidemia
G
p<0.001 (linear trend)
p=0.01
p<0.001
Log IL6 at baseline
Never smoked (n=2031)
Former smoker (n=1807)
Current smoker (n=496)
H
p<0.001
Log IL6 at baseline
No coronary heart disease
Coronary heart disease
I
p = 0.004
Log IL6 at baseline
No peripheral artery disease
Peripheral artery disease

Figure 5.1. Relationship of IL-6 with cardiovascular risk factors

Panels illustrate the comparison of mean log IL-6 across categories of sex (**A**), hyperuricemia (**B**), atrial fibrillation (**C**), hypertension (**D**), diabetes mellitus (**E**), dyslipidemia (**F**), smoking status (**G**), coronary artery disease (**H**), and peripheral artery disease (**I**). The prevalence of each cardiovascular risk factor is available in Table 5.1. Counts are provided when they cannot be derived from the table.

5.3.2. Relationship of plasma IL-6 with plaque severity at baseline

In the univariable linear regression model, log-IL-6 increased by 0.08 for each point increment in the plaque severity score (Figures 5.2A and 5.2B).

In the multivariable linear regression analysis, log IL-6 was independently associated with plaque severity ($\beta = 0.09$, $p=0.001$, Table 5.2). Peripheral artery disease, smoking, dyslipidemia, prior stroke or TIA, and hypertension were the most important predictors of plaque severity with standardized beta coefficients of 0.33, 0.33, 0.24, 0.22, and 0.17 (Table 5.2). The distribution of the standardized residuals of the model is provided in Appendix 3, Figure IV.

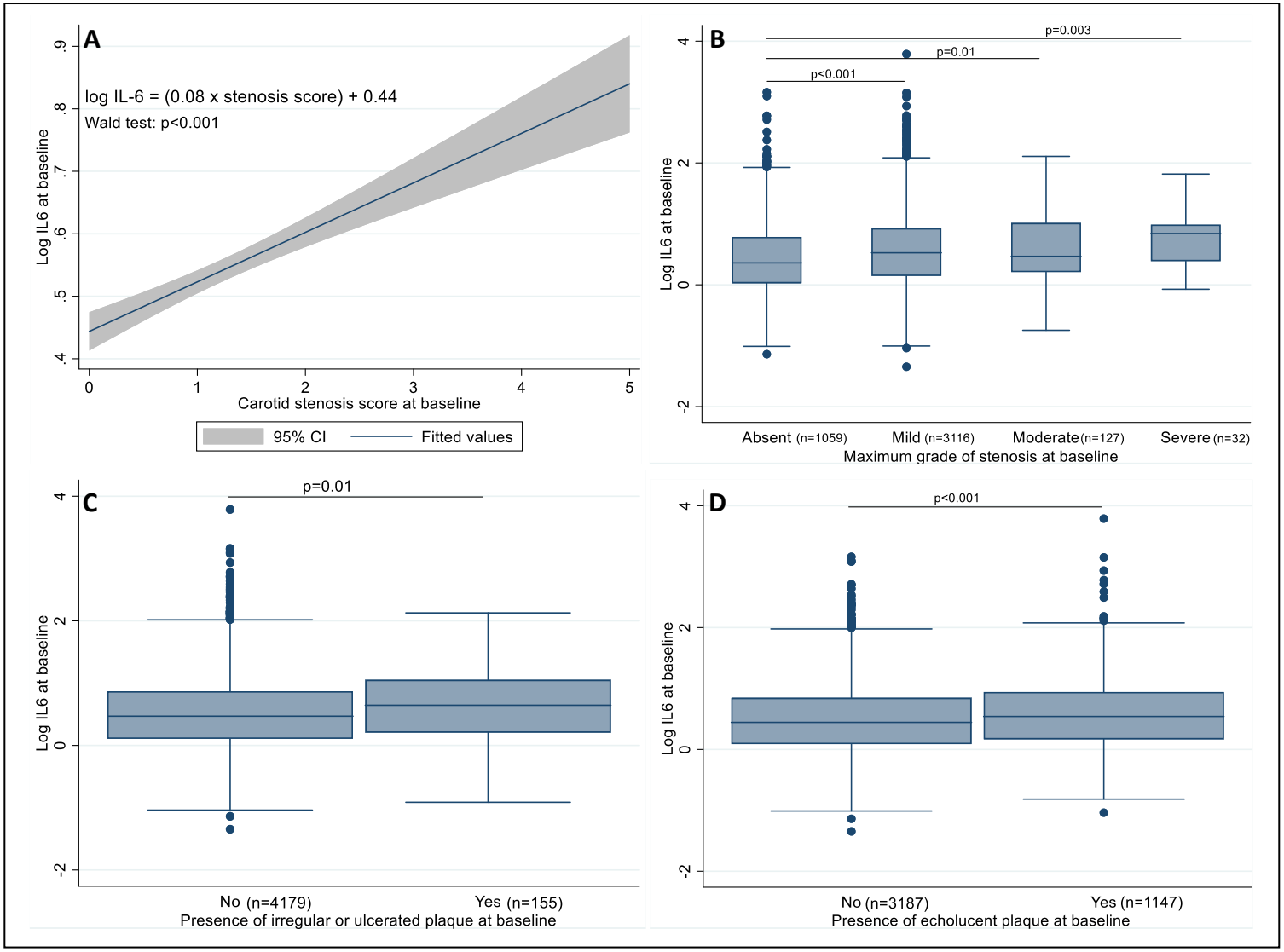
A
log IL-6 = (0.08 x stenosis score) + 0.44
Wald test: p<0.001
Log IL6 at baseline
Carotid stenosis score at baseline
95% CI
Fitted values
B
p=0.003
p=0.01
p<0.001
Log IL6 at baseline
Absent (n=1059)
Mild (n=3116)
Moderate (n=127)
Severe (n=32)
Maximum grade of stenosis at baseline
C
p=0.01
Log IL6 at baseline
No (n=4179)
Yes (n=155)
Presence of irregular or ulcerated plaque at baseline
D
p<0.001
Log IL6 at baseline
No (n=3187)
Yes (n=1147)
Presence of echolucent plaque at baseline

Figure 5.2. Relationship of IL-6 with the carotid plaque severity and vulnerability at baseline

A: Univariable linear regression of log IL-6 over the baseline carotid stenosis score ($\beta = 0.08$, p-value for the Wald test <0.001).

B: Distribution of log IL-6 across categories of stenosis severity.

C: Comparison of mean log IL-6 in patients with versus without markedly irregular or ulcerated carotid plaques.

D: Comparison of mean log IL-6 in patients with versus without echolucent carotid plaques.

Table 5.2. Multivariable linear regression model for the association of IL-6 with carotid plaque severity at baseline

Independent variables	β1*	95% CI	p-value	β2†
Log IL-6	0.09	0.03 – 0.14	<0.001	0.08
Age	0.02	0.02 – 0.03	<0.001	0.02
Male	0.11	0.05 – 0.18	0.001	0.14
Black (African American)	-0.21	(-0.29) – (-0.13)	<0.001	-0.20
Hypertension	0.16	0.10 – 0.22	<0.001	0.17
Diabetes mellitus	0.11	0.02 – 0.20	0.020	0.07
Dyslipidemia	0.24	0.13 – 0.35	<0.001	0.24
Current smoker	0.32	0.22 – 0.42	<0.001	0.33
Hyperuricemia	0.10	0.01 – 0.18	0.032	0.06
Coronary heart disease	0.16	0.07 – 0.25	0.001	0.13
Peripheral artery disease	0.31	0.10 – 0.51	0.003	0.33
Prior stroke or TIA	0.20	0.05 – 0.35	0.007	0.22
Intercept	-0.94	(-1.41) – (-0.48)	<0.001	-0.81

IL-6: interleukin-6; NA: not applicable; TIA: transient ischemic attack.

* Non-standardized coefficients (linked to change in stenosis severity score per 1 unit increase)

† Standardized coefficients (linked to change in stenosis severity score per 1 standard deviation increase)

Treatment with antiplatelet drugs (p=0.85), atrial fibrillation, cystatin-based glomerular filtration rate, alcohol consumption, body mass index, log C-Reactive Protein, treatment with anti-inflammatory drugs (p=0.06) were consecutively removed from the model automatically due to coefficients with p-value >0.05.

Fisher F test for significance of the model: $F = 24.1$, $df = 12$, $p < 0.001$. Maximum Cook distance = 0.03. Maximum variance inflation factor = 1.15.

5.3.3. Relationship of plasma IL-6 with plaque vulnerability at baseline

Plasma IL-6 levels were higher in participants with a vulnerable carotid plaque (mean log IL-6 of 0.59 versus 0.50, p<0.001). They were also higher in patients with markedly irregular or ulcerated carotid plaques (mean log IL-6 of 0.65 versus 0.52, p=0.01, Figure 5.2C) and in patients with an echolucent carotid plaque (mean log IL-6 of 0.59 versus 0.50, p<0.001, Figure 5.2D).

In the multivariable logistic regression (Table 5.3), log IL-6 was independently associated with the presence of a vulnerable carotid plaque at baseline (OR = 1.22, 95% CI: 1.06-1.40, p=0.006). There was a 12% increase in the probability of having a vulnerable carotid plaque per standard deviation increase in log IL-6 (Table 5.3 and Figure 5.3), thus making log IL6 one of the most important contributors to plaque vulnerability. The logistic regression model displayed good calibration (Figure 5.4A). The association of log IL-6 with plaque vulnerability remained significant after adjustment for optimism (OR = 1.18, 95% CI: 1.05-1.33, Table 5.3).

In the sensitivity analysis, the E-value was 1.71 suggesting that, to explain away the observed OR of 1.22, an unmeasured confounder would need to be associated with both log IL-6 and plaque vulnerability with an OR of at least 1.71 each, above and beyond the measured confounders (Appendix 3, Figure V-A).

Table 5.3. Multivariable logistic regression model for the association of IL-6 with carotid plaque vulnerability at baseline

Independent variables	OR1 (95% CI) *	p-value	OR2†	OR3 (95% CI) ‡
Log IL-6	1.22 (1.06 – 1.40)	0.006	1.12	1.18 (1.05 – 1.33)
Male	1.21 (1.02 – 1.44)	0.031	1.10	1.18 (1.01 – 1.37)
Dyslipidemia	1.55 (1.11 – 2.16)	0.010	1.13	1.45 (1.09 – 1.92)
Hyperuricemia	1.36 (1.09 – 1.69)	0.006	1.12	1.30 (1.07 – 1.56)
Intercept (baseline odds)	0.26 (0.19 – 0.37)	<0.001	NA	0.49 (0.36 – 0.70)

IL-6: interleukin-6; NA: not applicable.

* Non-standardized odds ratio (linked to change in odds per 1 unit increase)

† Standardized odds ratio (linked to change in odds per 1 standard deviation increase)

‡ Optimism-adjusted odds ratio using the heuristic shrinkage method.

Coronary heart disease (p=0.94), diabetes mellitus, race, history of stroke or transient ischemic attack, log C-reactive protein, cystatin-based glomerular filtration rate, atrial fibrillation, alcohol consumption, age, treatment with anti-inflammatory drugs, hypertension, peripheral artery disease, treatment with antiplatelet drugs, body mass index, smoking status (p=0.08) were consecutively removed from the model automatically due to coefficients with p-value >0.05.

Likelihood ratio chi-squared test for significance of the model: $\chi^2 = 37.3$, df = 4, $p < 0.001$. Area under the Receiver Operating Characteristic curve (AUC) = 0.57. Count $R^2 = 67\%$. Proportion of patients correctly classified = 66%. Maximum Cook distance = 0.03. Maximum variance inflation factor = 1.09.

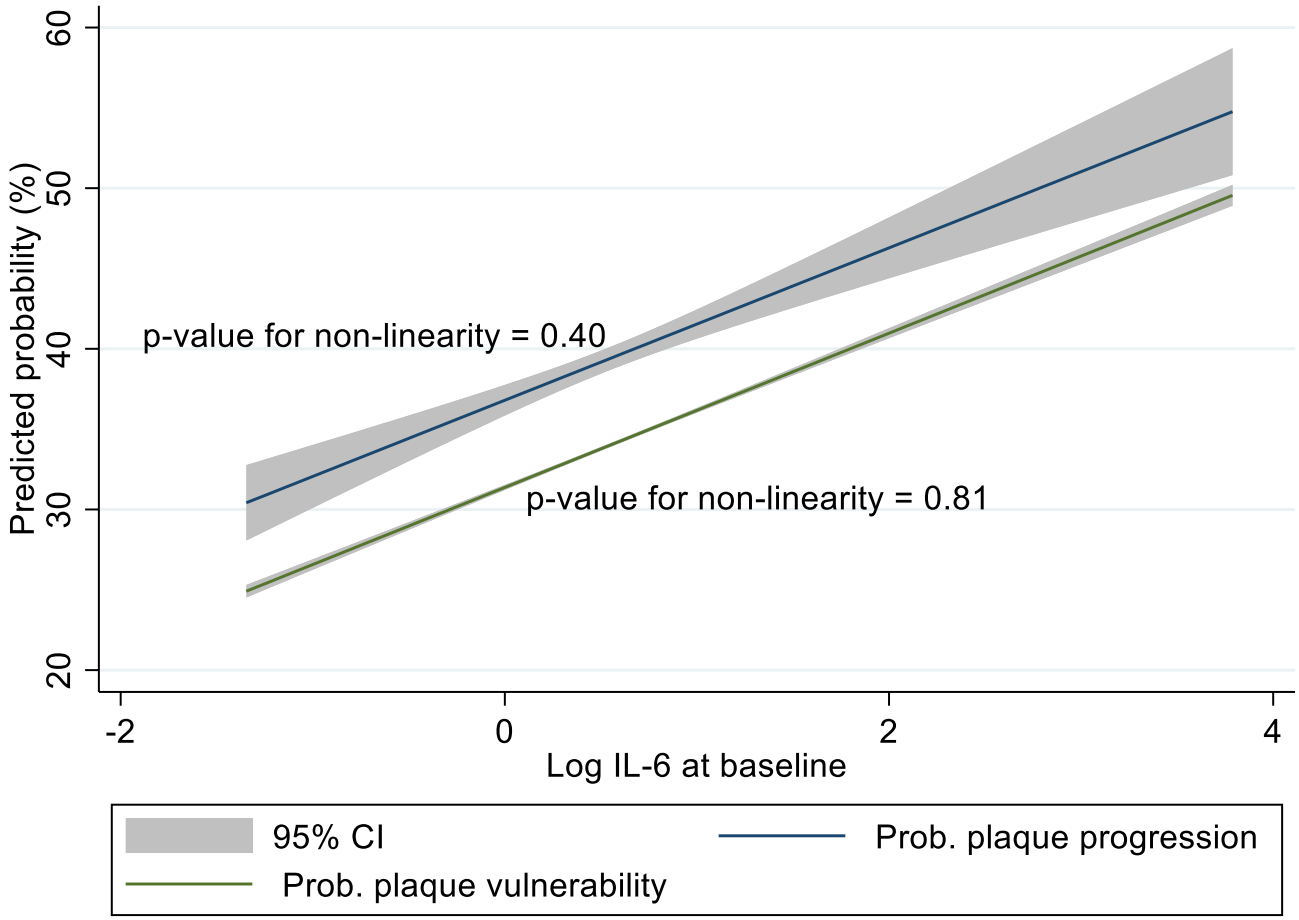

Figure 5.3. Linear relationship of IL-6 with the probability of carotid plaque vulnerability and progression

The curves are derived from the optimism-adjusted multivariable logistic regression models reported in Tables 3 and 4.

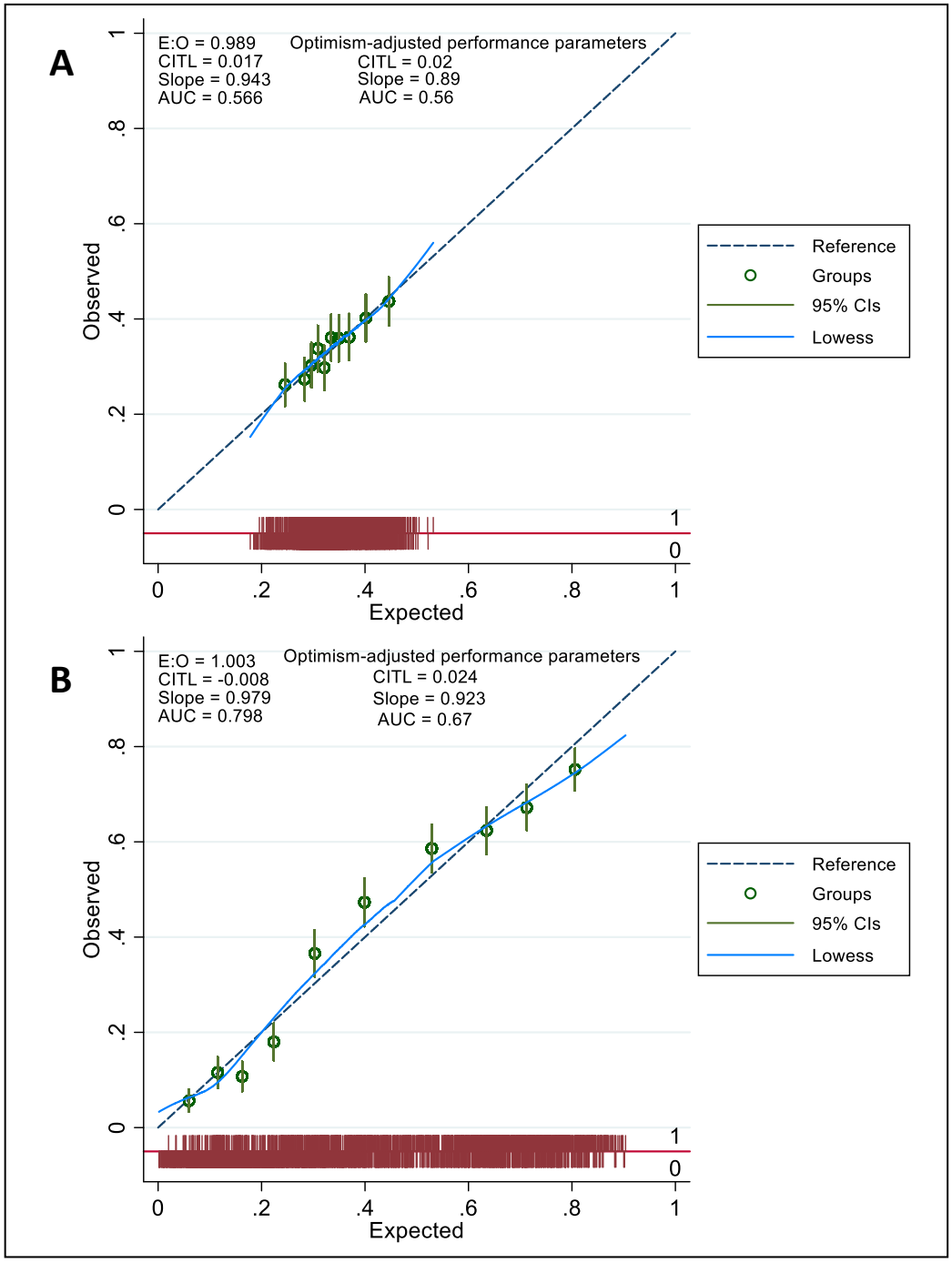

Figure 5.4. Calibration plots for the logistic regression models to predict plaque vulnerability and plaque progression

E:O = ratio of expected and observed probabilities
CITL = Calibration-in-the-large indicates whether predictions are systematically too low (CITL>0) or systematically too high (CITL<0).
AUC = Area under the receiver operating characteristic curve
The 45° reference line represents the line of perfect agreement between the model and the data (equality of observed and predicted probabilities). The groups are created using deciles of risk as cut points (10 risks groups). The Locally Weighted Scatterplot Smoothing (lowess) is the

smoothed calibration line across individuals displayed on the bar graph at the bottom of each plot.
Optimism-adjusted performance parameters are obtained after applying a bootstrap shrinkage factor derived from an internal validation process with 100 bootstrap samples.
A: Calibration plot for the logistic regression model to predict plaque vulnerability
B: Calibration plot for the logistic regression model to predict plaque progression

5.3.4. Relationship of plasma IL-6 with plaque progression at 5 years

In the multivariable logistic regression (Table 5.4), log IL-6 was independently associated with plaque progression at 5 years (OR = 1.44, 95% CI: 1.23-1.69, p<0.001). There was no interaction between log IL-6 and ipsilateral baseline plaque severity score (OR for the interaction term = 0.92, p=0.39) or ipsilateral baseline plaque vulnerability (OR for the interaction term = 1.01, p=0.95). There was a 24% increase in the probability of carotid plaque progression per standard deviation increase in log IL-6 (Table 5.4 and Figure 5.3), thus making log IL-6 the second most important contributor to plaque progression after dyslipidemia. The probability of carotid plaque progression decreased by 73% per standard deviation increase in baseline ipsilateral plaque severity score (Table 5.4). Progression was less often reported for carotid plaques with high-risk features at baseline (OR = 0.77, 95% CI: 0.61 – 0.97, Table 5.4). The logistic regression model displayed good calibration (Figure 5.4B). The association of log IL-6 with plaque progression remained significant after adjustment for optimism (OR = 1.34, 95% CI: 1.18-1.53, Table 5.4).

In the sensitivity analysis, the E-value was 2.24 suggesting that, to explain away the observed OR of 1.44, an unmeasured confounder would need to be associated with both log IL-6 and plaque progression with an OR of at least 2.24 each, above and beyond the measured confounders (Appendix 3, Figure V-B).

Table 5.4. Multivariable logistic regression model for the association of IL-6 with carotid plaque progression at 5 years

Independent variables	OR1 (95% CI) *	p-value	OR2†	OR3 (95% CI) ‡
Log IL-6	1.44 (1.23 – 1.69)	<0.001	1.24	1.34 (1.18 – 1.53)
Current smoker	1.64 (1.20 – 2.25)	0.002	1.16	1.50 (1.16 – 1.93)
Dyslipidemia	2.32 (1.64 – 3.30)	<0.001	1.26	1.98 (1.50 – 2.63)
Diabetes mellitus	1.46 (1.09 – 1.95)	0.011	1.13	1.36 (1.07 – 1.72)
Hypertension	1.37 (1.12 – 1.66)	0.002	1.17	1.29 (1.10 – 1.51)
Coronary heart disease	1.37 (1.03 – 1.83)	0.028	1.12	1.29 (1.03 – 1.63)
Male	1.28 (1.05 – 1.56)	0.014	1.13	1.22 (1.04 – 1.43)
Age (years)	1.03 (1.01 – 1.05)	0.007	1.14	1.02 (1.01 – 1.04)
Vulnerability at baseline (ipsilateral)	0.77 (0.61 – 0.97)	0.024	0.89	0.81 (0.67 – 0.97)
Stenosis score at baseline (ipsilateral)	0.24 (0.21 – 0.28)	<0.001	0.27	0. 32 (0.28 – 0.35)
Intercept (baseline odds)	0.08 (0.02 – 0.37)	<0.001	NA	0.52 (0.13-2.41)

IL-6: interleukin-6; NA: not applicable.

* Non-standardized odds ratio (linked to change in odds per 1 unit increase)

† Standardized odds ratio (linked to change in odds per 1 standard deviation increase)

‡ Optimism-adjusted odds ratio using the heuristic shrinkage method.

The first interaction term (Log IL-6 # ipsilateral vulnerability at baseline, p = 0.99), atrial fibrillation, treatment with anti-inflammatory drugs, race, history of stroke or transient ischemic attack, alcohol consumption, the second interaction term (Log IL-6 # ipsilateral baseline stenosis score), body mass index, log C-reactive protein, treatment with antiplatelet drugs, peripheral artery disease, hyperuricemia, and cystatin-based glomerular filtration rate (p=0.06) were consecutively removed from the model automatically due to coefficients with p-value >0.05.

Likelihood ratio chi-squared test for significance of the model: $\chi^2 = 712.21$, df = 10, $p < 0.001$. Area under the Receiver Operating Characteristic curve (AUC) = 0.80. Count R^2 = 73%. Proportion of patients correctly classified = 73.4%. Maximum Cook distance = 0.07. Maximum variance inflation factor = 1.15.

5.3.5. Selection of a clinical threshold for plasma IL-6 levels

In participants with a >50% predicted probability of plaque progression based on the optimism-adjusted multivariable logistic regression model (Table 5.4), the median (IQR) level of plasma IL-6 was 1. 63 (1.12 – 2.46) pg/mL and the mean (95% CI) of log IL-6 was 0.54 (0.52 – 0.56), corresponding to a plasma IL-6 level of 1.72 pg/mL. Therefore, the threshold of 2.0 pg/mL was used to define high plasma IL-6 levels. There were 1584 (36.6%) participants with high plasma IL-6 at baseline. High IL-6 levels at baseline were significantly associated with all cardiovascular risk factors (Appendix 3, Table II). At baseline, there was a higher prevalence of severe stenosis (1.3% versus 0.4%, p=0.001) and vulnerable plaques (32.0% versus 27.6%, p <0.001) in participants with high IL-6 levels (Appendix 3, Table II). The rate of plaque progression at 5 years was similar in patients with high or low IL-6 levels at baseline (34.6% versus 33.7%, p=0.54). The prevalence of treatment with anti-inflammatory drugs was similar between participants with high versus low IL-6 levels at baseline (Appendix 3, Table II).

In the multivariable logistic regression analysis, high IL-6 levels at baseline were independently associated with the presence of vulnerable carotid plaque (OR = 1.21, 95% CI: 1.02-1.45, p=0.03, E-value = 1.71, Appendix 3, Table III) and with carotid plaque progression at 5 years (OR = 1.25, 95% CI: 1.01-1.57, p=0.04, E-value = 1.81, Appendix 3, Table IV). Dichotomization did not significantly affect the coefficients, performance, calibration, discrimination, and stability of the logistic regression models (Appendix 3, Table III and IV).

5.4. Discussion

This study shows that circulating IL-6 is an independent predictor of carotid plaque severity, vulnerability, and progression. The standardized odds ratios for log IL-6 in the multivariable logistic regression models show that inflammation is a major contributor to the risk of plaque vulnerability and progression. This observation has three important implications. First, achieving an optimal level of plasma cholesterol and controlling existing cardiovascular risk factors might not be sufficient to suppress the risk of stroke associated with carotid atherosclerosis in the absence of a treatment specifically targeting inflammation. This requires further investigation in trials where information on the control status of various cardiovascular risk factors, and not just the prescription of drugs, is properly recorded and factored into the interpretation of trial results. Second, carotid plaque imaging biomarkers could represent valid surrogate endpoints in trials of anti-IL-6 drugs for stroke prevention. Third, it is important to accelerate the integration of IL-6 assays in routine clinical practice by defining and validating a cut-off to improve risk stratification in patients with carotid atherosclerosis and identify patients who could benefit from anti-IL6 drugs in addition to current best medical therapy.

In this regard, we propose a non-arbitrary risk-informed threshold allowing the dichotomization of IL-6 levels and their use in prediction models without log transformation and without loss of predictive performance. If validated, this threshold could be used to assess residual inflammatory risk as is the case with CRP. IL-6 is targeted by several specific compounds already approved or under development,[28] and has stronger genetic, experimental, clinical, and epidemiological evidence for a causal relationship to atherosclerotic cardiovascular disease than CRP.[30] Moreover, preliminary evidence suggest that specific anti-IL-6 drugs like Ziltivekimab have a better safety profile than other anti-inflammatory drugs with respect to myelosuppression (increased risk of infections and bleeding), dyslipidemia, and toxicity to the liver and the kidney.[29] The renal toxicity of colchicine limits its prescription to patients with chronic kidney disease who derive a greater absolute cardiovascular benefit from anti-inflammatory drugs than those with normal renal function.[29,428] Anti-IL-6 drugs would be of particular interest in patients with carotid plaques at perceived higher risk of stroke who are not eligible for surgical revascularization. This includes patients with multiple comorbidities and patients with mild or moderate carotid stenosis, especially if high-risk features are present.[301,422]

IL-6 is a soluble proinflammatory cytokine secreted by activated monocytes, macrophages, endothelial cells, adipocytes, fibroblasts, T helper 2 cells, typically after stimulation by

interleukin-1 or tumor necrosis factor. In the classical signaling pathway, IL-6 binds to membrane-bound IL-6 receptors on hepatocytes and stimulates the production of acute phase reactants such as fibrinogen, plasminogen activator inhibitor that inhibits fibrinolysis, and CRP. In the alternate signaling pathway, the alpha subunit of the transmembrane IL-6 receptor is cleaved by a disintegrin and metalloproteinase with thrombospondin motifs 17 (ADAMTS17) and forms a complex with IL-6. This complex can bind the gp130 protein on other cell types such as endothelial and smooth muscle cells to promote atherogenesis. These pro-atherogenic and pro-thrombotic effects of IL-6 likely explain why higher plasma concentrations are found in patients with severe carotid stenosis, carotid occlusion and in patients with vulnerable or progressive carotid atherosclerotic lesions. Surprisingly, in our study, the univariable analysis did not find significantly higher levels of IL-6 in patients diagnosed with plaque progression at 5 years. This is explained by the fact that plaques causing a higher grade of stenosis are less likely to progress.[429] Indeed, we report a 73% decrease in the probability of plaque progression per standard deviation increase in the ipsilateral baseline stenosis severity score. After adjusting for the ipsilateral baseline grade of stenosis, the significant association between IL-6 and carotid plaque progression became apparent. The inverse relationship between plaque vulnerability and plaque progression is another intriguing finding that warrants further investigations. It suggests that vulnerable plaques are less likely to progress because of a higher risk of rupture that might cause cerebrovascular events and potentially justify surgery. However, this hypothesis could not be tested in the absence of information on carotid procedures during follow-up.

The association of IL-6 with all cardiovascular risk factors reported in this study suggests that cardiovascular risk factors exert their pro-atherothrombotic effect at least in part by promoting IL-6-mediated arterial inflammation and remodeling. For instance, previous studies have shown that lowering blood pressure decreases circulating IL-6 levels[430] and that IL-6 secretion is induced by hyperglycemia, hyperuricemia, and smoking.[431-433] Our findings also suggest that the relationship of IL-6 with stroke risk reported in previous observational studies might be driven by the formation, progression, and destabilization of carotid atherosclerotic plaques. This hypothesis warrants further investigation in population-based cohorts. Furthermore, the conflicting results regarding stroke prevention using various anti-inflammatory drugs in recent trials support the need for more granular subgroup analyses by stroke subtypes in future trials. It is possible that drugs targeting the NLRP3-interleukin 1-interleukin 6-C-reactive protein signaling pathway are more efficient for the prevention of large artery atherosclerosis-related stroke than other stroke subtypes.

Altogether, this study provides new pieces of evidence to suggest a causal relationship between circulating IL-6 levels and carotid plaque severity, vulnerability, and progression according to Bradford-Hill criteria.[434] It shows that the association between circulating IL-6 levels and carotid plaque vulnerability and progression is strong (criterion 1) given the 12% and 24% increase in the odds per standard deviation increase in log IL-6. The association is also specific (criterion 3) and displays a linear dose-response gradient (criterion 5). Indeed, the association is independent of all known risk factors of atherosclerosis and unlikely to be offset by an unobserved confounder. When considering the computed E-values, the existence of an unmeasured confounder that would have a stronger association with plaque vulnerability and plaque progression than dyslipidemia seems unlikely. Additionally, the multivariable logistic regression model using high plasma IL-6 levels to predict plaque progression correctly classifies >70% of the participants. The other causality criteria could be inferred from previous publications, notably consistency (criterion 2),[435-438] temporality (criterion 4),[27,435] plausibility and coherence (criteria 6 and 7).[30] Furthermore, experimental studies in humans and animals have shown that inhibition of the IL-6 signaling pathway decreases the progression of systemic atherosclerotic disease and prevents cardiovascular events (criterion 8) [408,409,439]. Therefore, by analogy (criterion 9), one could hypothesize that the same would be true when looking specifically at carotid plaques in humans. Further experimental evidence could come from the Colchicine for Prevention of Vascular Inflammation in Non-cardio Embolic Stroke trial (CONVINCE, NCT02898610) and the Research Study to Look at How Ziltivekimab Works Compared to Placebo in People with Cardiovascular Disease, Chronic Kidney Disease, and Inflammation (ZEUS, NCT05021835).

5.5. Limitations

Our study has some limitations. First, despite the large sample size, the study population was restricted to elderly patients, which means that our results may not be generalizable to the entire population. However, patients aged >65 years old are likely the most relevant subgroup for the study of predictors of carotid atherosclerosis across the spectrum of stenosis severity given the relatively low prevalence of moderate and severe stenosis in younger adults.[440] Second, we could not incorporate information on whether participants underwent carotid revascularization procedures between baseline and 5-year follow-up ultrasound examinations. This might have led to an underestimation of the number of participants with plaque progression and reduced statistical power for the multivariable logistic regression analysis. Nevertheless, we believe the

number of participants undergoing surgical revascularization would only represent a small percentage of the study population comprised mostly of healthy and asymptomatic individuals. Furthermore, the procedures performed to adjust for optimism, especially bootstrapping, suggest that our results would not be affected by fluctuations in the number of patients diagnosed with plaque progression at 5 years. The absence of information on carotid procedures during follow-up also precluded a reliable investigation of the relationship between circulating IL-6 levels and plaque regression.

Third, it was not possible to incorporate data on the occurrence of cerebrovascular events during follow-up in this study to also investigate the relationship of circulating IL-6 with stroke risk and test the hypothesis that the risk is mediated through carotid atherosclerosis. Nevertheless, the association between circulating IL-6 levels and the risk of stroke is already well-established from previous population-based studies.[25,26] Fourth, our analyses were not adjusted for the concurrent presence of conditions that could affect IL-6 levels either at baseline or during follow-up. However, patients with cancer were excluded from the CHS and all multivariable models were adjusted for CRP levels and treatment with anti-inflammatory drugs that are surrogate indicators of conditions such as infection, cancer, or auto-immune diseases that modulate IL-6 secretion. Moreover, we computed E-values to demonstrate that unmeasured confounders are likely not able to offset the reported associations. Fifth, our model for plaque progression at 5-years is based on a single measurement of IL-6 levels at baseline which may not reflect fluctuations of IL-6 levels during follow-up. Nevertheless, available evidence suggest that IL-6 levels are largely genetically determined, and the population variance of IL-6 might be explained mostly by inter-personal rather than intra-personal fluctuations.[411,441] Last, the proportion of patients treated with statins in the CHS was low. Given that statins also have anti-inflammatory properties, further studies are needed to determine their effect on the relationship between circulating IL-6 levels and carotid plaque features.

5.6. Conclusion and future directions

This study provides new evidence for a causal relationship between circulating levels of IL-6 and three key features of high-risk carotid atherosclerosis: severity of stenosis, baseline plaque vulnerability, and long-term plaque progression. It also defines a threshold of 2.0 pg/mL to identify individuals with a higher probability of plaque vulnerability and progression. This

threshold could be used to select patients who might derive greater stroke prevention benefits from anti-IL-6 drugs in future studies.

Chapter 6: Monocyte transcriptomic analysis of high-risk carotid atherosclerosis[6]

6.1. Introduction

Carotid atherosclerosis is responsible for 15-25% of the nearly 8 million first-ever ischemic strokes that occur each year worldwide.[1-3] Advances in the management of carotid atherosclerosis have reduced the associated risk of stroke with strategies including surgical revascularization, antiplatelet therapy, and lipid lowering agents. However, a significant proportion of patients with carotid atherosclerosis remain at high of stroke despite receiving optimal medical treatment. Therefore, novel therapies to better prevent carotid plaque progression and rupture are needed to reduce the risk of stroke.

Monocytes play important roles in the pathogenesis of atherosclerosis and plaque rupture. They adhere to the damaged endothelium via specific receptors and migrate into the vessel wall where they differentiate into macrophages. Lipid-loaded macrophages, also referred to as foam cells, contribute to a pro-inflammatory environment by secreting cytokines and proteins that degrade the fibrous cap, ultimately leading to plaque rupture and thromboembolic events.[442] As such monocytes play a pivotal role in the development of key features of high-risk carotid plaques, including lipid-rich necrotic core, thin or ruptured fibrous cap, and inflammation.[301]

We have previously identified changes in leukocyte gene expression that are associated with large vessel ischemic stroke.[152] Furthermore, we have identified differences in circulating leukocyte gene expression in large vessel strokes related to histone deacetylase 9 (HDAC9) single nucleotide polymorphism.[443] However, specifics of human monocyte gene expression in relationship to carotid atherosclerosis and stroke remain to be determined. In this study we evaluated differences in monocyte gene expression in patients with symptomatic versus asymptomatic carotid plaque. Differences in peripheral monocyte gene expression are identified which provide further insight into the human biology of carotid atherosclerosis and plaque rupture that cause stroke.

[6] This chapter will be submitted as "Kamtchum-Tatuene J, Falcione S, Munsterman D, Sykes G, Joy T, Jickling GC. Monocyte transcriptomic analysis of high-risk carotid atherosclerosis."

6.2. Methods

6.2.1. Study participants and sample collection

Participants were recruited from the University of Alberta Hospital from August 2019 to September 2020. There were 36 participants who met study inclusion and exclusion criteria, including 15 with symptomatic carotid atherosclerosis and 21 with asymptomatic carotid atherosclerosis (Table 1). Symptomatic carotid atherosclerosis was defined as a moderate or severe carotid stenosis (>50% stenosis) with ipsilateral stroke or a transient ischemic attack. Participants with asymptomatic carotid atherosclerosis comprised 14 with stroke not due to atherosclerosis and 7 controls with or without carotid atherosclerosis. Stroke diagnosis was made by two stroke neurologists based on clinical assessment and brain imaging (CT, MRI, or both). Stroke etiology was determined according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification.[200] Subjects with cancer, auto-immune disease, immunomodulatory treatment, systemic inflammatory disease, or infection were excluded because of the effect on leukocyte gene expression.

6.2.2. Monocyte isolation

Whole blood samples were collected via venipuncture in EDTA tubes within 72 hours of stroke onset or during outpatient visits for controls. Blood samples were processed within 30 minutes of collection. Monocytes were isolated from 6 mL of whole blood by density gradient centrifugation followed by magnetic bead isolation. In brief, 6 mL of blood were used to isolate peripheral blood mononuclear cells (PBMCs) by density gradient centrifugation using 50 mL SepMate tubes preloaded with 15 mL of Lymphoprep (StemCell Technologies Inc, Vancouver, British Columbia, Canada). Then, immunomagnetic isolation was performed to separate monocytes from PMBCs using the EasySep human CD14 positive selection kit II (#17858, StemCell Technologies Inc, Vancouver, British Columbia, Canada). The average monocyte purity was 98.9 ± 1.14 % by flow cytometry (viability+/APC-CD45+/FITC-CD66b-/PE-CD14+) on a BD Fortessa-X20 (Becton Dickinson, New Jersey, USA). Monocytes were placed in RNAprotect (QIAGEN GmbH, Hilden, Germany) and stored at -80 °C. RNAprotect prevents RNA degradation and arrests gene expression.

6.2.3. RNA extraction and sequencing

RNA was isolated from all monocyte samples on the same day using the Direct-Zol RNA Miniprep plus kit (#R2073, Zymo Research Corp, Irvine, California, USA). The extracted RNA was cleaned up to remove guanidine salt residues and concentrated using the RNeasy MinElute

Cleanup kit (#74204, QIAGEN GmbH, Hilden, Germany). The concentration and the purity of the final RNA solution were checked on the Nanodrop One spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The concentration was double-checked with the Qubit 4 fluorometer (Thermo Fisher Scientific, Waltham, MA). RNA integrity was assessed by Agilent 2100 bioanalyzer (Agilent, Santa Clara, CA). The average A260/280 ratio was 1.9 (range 1.8-2.1) and the average RNA integrity ratio was 9.0 (range 8-10). All samples contained > 100 ng of total RNA. Extracted RNA was processed using a NEBNext library preparation kit for Illumina (#E7645L, New England Biolabs, Ipswich, MA) and sequenced on an Illumina NovaSeq 6000 sequencer (Illumina, San Diego, CA). The average sequencing depth was 84 million paired end reads per sample.

6.2.4. Protein extraction

To prepare the protein extract, we mixed the frozen monocyte pellet with 250 µL of Radio-Immunoprecipitation Assay buffer (#R0278, Sigma-Aldrich, Saint-Louis, MO), 10 µL/mL of Halt Protease inhibitor cocktail 100X (Thermofisher catalog #78429), and 10 µL/mL of ethylenediaminetetraacetic acid 100 X. After incubation for 30 minutes on ice, the mixture was centrifuged at 20,000g for 10 minutes at 4˚C. The supernatant, containing the extracted proteins was aliquoted and placed on ice. The protein concentration of the extract was measured by fluorometry using the Qubit 4 protein assay kit (#Q33211, Thermo Fisher Scientific, Waltham, MA) after dilution with RNase-free water if necessary. Recording the overall protein concentration was necessary to normalize the concentration of specific proteins as measured by enzyme-linked immunosorbent assay (ELISA). The undiluted aliquots were frozen at -80 ˚C until further use.

6.2.5. Enzyme-linked immunosorbent assays

The concentrations of programmed cell death 1 (PD-1 or CD279), programmed cell death-ligand 1 (PD-L1), and hypoxia-inducible factor 1α (HIF-1α) were measured in plasma and protein extracts using the Human PD-1, PD-L1, and HIF-1α SimpleStep ELISA kits (#ab252360, #ab214565, #ab171577, Abcam, Cambridge, United Kingdom) according to the manufacturer's instructions except for the washing steps that were performed with an automated washer (BioTek ELx405; Agilent, Santa Clara, CA) using a mixture of Tween 20 and phosphate-buffered saline (0.05% vol/vol). All samples were run in duplicates and plates were read at 450 nm (Cytation 5; Agilent, Santa Clara, CA). The standard curves were fitted using 4-parameter logistic regression. The mean intra-assay and inter-assay coefficients of variability were <10% and

<15%, respectively. Normalized PD-1, PD-L1, and HIF-1α concentrations were obtained by dividing the crude concentration by the overall protein concentration in each sample.

6.2.6. Verification cohort

To further examine monocyte gene expression in relationship to large vessel stroke, we analyzed monocyte isolated from 37 patients with acute ischemic stroke (9 with large vessel stroke and 28 with stroke due to other causes) and 26 controls. Patients were enrolled at the University of California Davis from 2013 to 2015. Stroke diagnosis was made by two neurologists based on clinical assessment and brain imaging. Ischemic strokes included three etiologies: cardioembolic, large vessel, and small vessel. Subjects with current or recent (two weeks) infection, anticoagulation, immunosuppressive therapy, or blood malignancies were excluded from the study. Control subjects had vascular risk factors but no history of stroke, myocardial infarction, or peripheral vascular disease.

Blood was collected by venipuncture in PAXgene whole blood RNA tubes and K2 EDTA tubes (BD Biosciences). Monocytes were isolated using the RoboSep Cell Isolation Protocol, with the EasySep human buffy coat CD14+ selection for monocytes (Stemcell Technologies Inc.). Cell purity was validated using flow cytometry on a BD LSR II (Becton Dickinson, New Jersey, USA) and fluorescent antibodies directed to cell-type specific markers not used for cell sorting. Monocytes were detected by anti-CD36-APC (Biolegend) with purity >93%. Monocytes were placed in RNAlater (QIAGEN GmbH, Hilden, Germany) and stored at -80 ˚C.

Total RNA was extracted from monocyte samples using Zymo Direct-zol RNA mini-prep kit (Zymo Research Corp, Irvine, California, USA) followed by DNase treatment (QIAGEN GmbH, Hilden, Germany). Libraries were prepared using NuGEN Ovation Universal RNA-Seq system (Tecan, Zurich, Switzerland). Ribosomal RNA and globin transcripts were depleted using InDA-C (Tecan, Zurich, Switzerland). RNA sequencing was performed on Illumina HiSeq 4000 platform with an average of 200 ±10 million paired end reads per sample.

6.2.7. Data analysis

Continuous variables were summarized as mean ± SD or median (IQR) as appropriate while categorical variables were reported as frequency (percentage). To make comparisons between participants with or without symptomatic carotid atherosclerosis or between various groups defined by grade of stenosis or stroke etiology, we used the Kruskal-Wallis test for continuous

variables and the Fisher's exact test for categorical variables. Significant difference was defined as $p < 0.05$. Analysis of clinical characteristics were performed using Stata software, version 17 (StataCorp LLC, College Station, TX, USA).

Differential gene expression analysis was performed with Partek Flow software, version 9.0.20.0913 (Partek, Saint Louis, MO). FASTQ files were imported, and pre-alignment quality control performed, followed by sequence alignment against the human genome (homo sapiens, hg38) using STAR 2.5.3a. The average read quality score was 36 and the average GC content was 50%. The alignment generated 92% unique paired reads on average. Quantification to the transcriptome was done using the expectation-maximization algorithm[444] with annotation model Ensembl 100 version 2 and transcript counts were normalized to transcripts per million. Differentially expressed transcripts were identified using limma trend which derives log linear models of expression with shrinkage.[445] Analyses were adjusted for statin use since there was a significantly higher proportion of statin users among participants with symptomatic carotid atherosclerosis. [446-448] Differential expression was considered significant for $|\text{fold change}| \geq 1.2$ and $p < 0.05$.

To identify differentially expressed transcripts related to high-risk carotid plaques, we performed two analyses yielding two lists. First, to identify transcripts related to plaque destabilization (rupture and atheroembolism), we compared participants with symptomatic carotid atherosclerosis (large vessel stroke) to those with asymptomatic carotid atherosclerosis (stroke due to other causes and controls). Second, to identify transcripts related to plaque progression, we compared participants with severe carotid stenosis to those with mild or moderate carotid stenosis. Transcripts present on both lists were identified and considered most related to high-risk carotid plaque since they are relevant to both plaque progression and plaque destabilization. The differences in transcript expression were visually demonstrated by principal component analysis and hierarchical clustering plots. Principal component analysis was performed on normalised values without standardisation. For clustering, the data were standardised to a mean of zero and standard deviation of 1 and average linkage with Euclidean distances was used to group participants according to their characteristics.

To identify signaling pathways relevant to the differentially expressed transcripts on list 1 and list 2, all genes on both lists were analyzed according to the enrichment approach based on Fisher's exact test and using the Kyoto Encyclopedia of Genes and Genomes database. The selection of

pathways for further investigation was based on Fisher's exact p-value <0.05 and relevance to atherosclerosis pathophysiology according to the scientific literature.

All analyses were repeated in the verification cohort using Partek Flow with the same parameters at each step.

6.2.8. Ethical considerations and data availability

The University of Alberta Stroke Genomics Study was approved by the University of Alberta Human Research Ethics Board (Pro00066577). The University of California-Davis Stroke Study was approved by the UC Davis Institutional Review Board (IRB-ID 248994-41). All participants provided written informed consent. The data supporting the findings in this study can be shared to qualified investigator upon reasonable request.

6.3. Results

6.3.1. Characteristics of patients in the derivation cohort

There were 36 participants (mean age: 69.5 ± 10.8 years, 33.3% women), including 15 with symptomatic carotid atherosclerosis and 21 with asymptomatic carotid atherosclerosis (Table 6.1). The frequency of severe stenosis and treatment with statins was significantly higher in participants with symptomatic carotid atherosclerosis. The distribution of stroke etiologies was as follows: 15 strokes due to carotid atherosclerosis and 14 non-large vessel strokes (5 embolic strokes of unknown source, 4 lacunar strokes, 3 cardioembolic strokes, and 2 cryptogenic strokes).

Table 6.1. Characteristics of the participants

Characteristic		Symptomatic carotid atherosclerosis	Asymptomatic carotid atherosclerosis		p
		Stroke due to carotid atherosclerosis (n=15)	Stroke not due to carotid atherosclerosis (n=14)	Controls (n=7)	
Age (mean ± SD)		69.7 ± 7.7	71.5 ± 12.9	65.3 ± 12.2	0.58
Female, n (%)		4 (26.7)	5 (35.7)	3 (42.9)	0.73
Hypertension, n (%)		11 (73.3)	9 (64.3)	5 (83.3)*	0.79
Diabetes, n (%)		6 (40.0)	1 (7.1)	2 (33.3)*	0.11
Dyslipidemia, n (%)		15 (100.0)	9 (64.3)	4 (66.7) *	0.03
Statin before admission, n (%)		12 (80.0)	4 (28.6)	4 (66.7)*	0.02
Smoking, n (%)		10 (66.7)	12 (85.7)	4 (66.7)*	0.51
Grade of stenosis	Mild (<50%)	0 (0.0)	13 (92.9)	5 (71.4)	<0.001
	Moderate (50-69%)	3 (20.0)	1 (7.1)	2 (28.6)	
	Severe (>70%)	12 (80.0)	0 (0.0)	0 (0.0)	
NIHSS (mean, range)		3.6 (0-17)	3.6 (0-9)	NA	0.23

NIHSS: National Institutes of Health Stroke Scale

* Missing data for 1 control

6.3.2. Differential transcript expression and functional pathways in the derivation cohort

There were 1029 transcripts (960 genes) differentially expressed between participants with symptomatic carotid atherosclerosis and those with asymptomatic carotid atherosclerosis (List 1, Appendix 4, Table I) and 147 transcripts (144 genes) differentially expressed between participants with severe stenosis and those with mild or moderate stenosis (List 2, Appendix 4, Table II). There were 40 transcripts common to both lists (Appendix 4, Table III). Based on the expression pattern of those 40 transcripts, 14 (93.3%) of the 15 participants with symptomatic carotid atherosclerosis were grouped together in the hierarchical clustering plots (Figure 6.1 and Appendix 4, Figures I and II). The expression of pattern of the 40 transcripts also allowed the separation of participants according to their grade of stenosis in the principal component analysis (Figure 6.2).

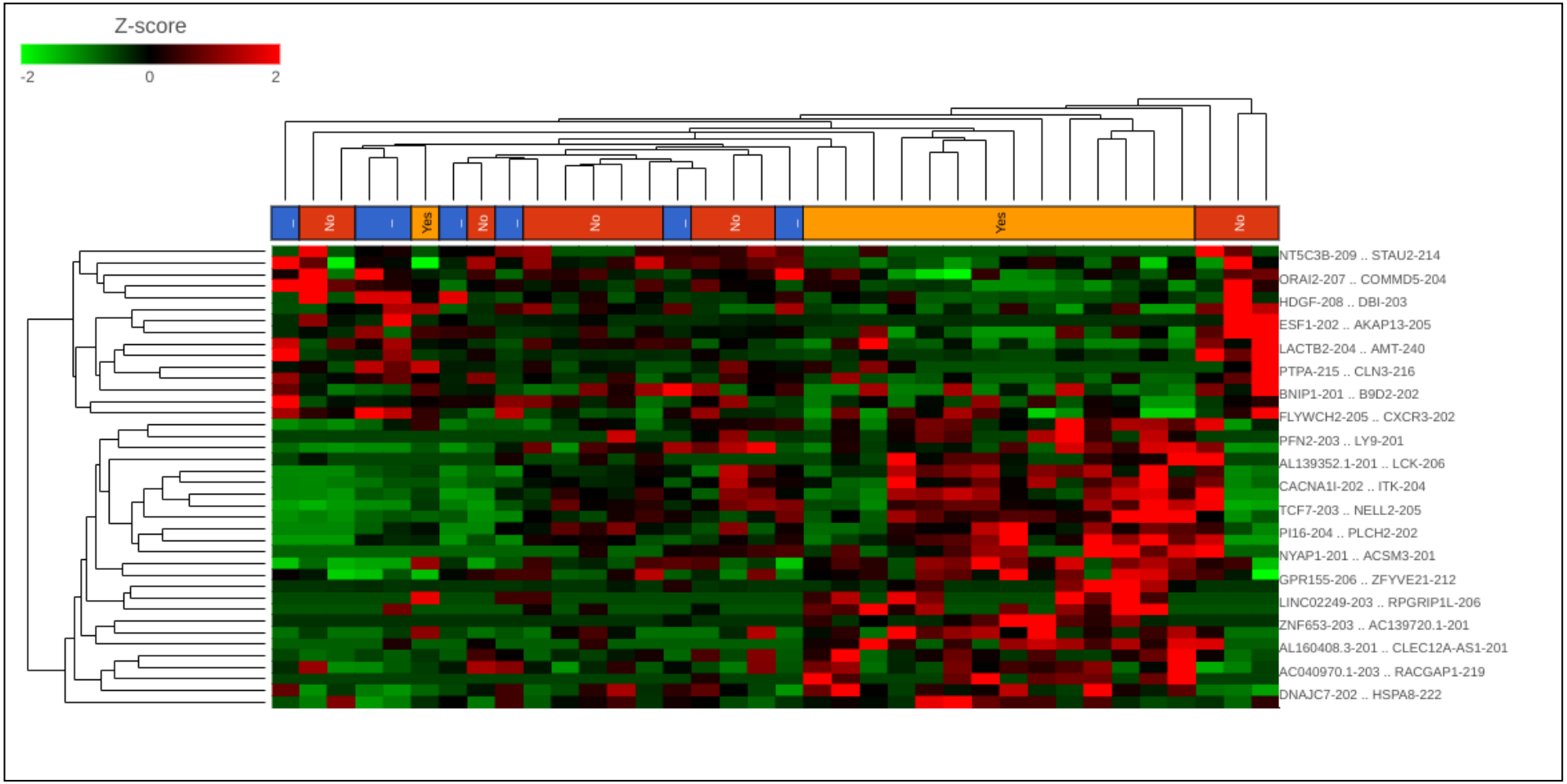

Figure 6.1. Hierarchical clustering plot of the 40 transcripts differentially expressed in patients with symptomatic versus asymptomatic carotid atherosclerosis – Color code for the X-axis: blue – controls (dash), red – stroke of other causes (no), orange – symptomatic carotid atherosclerosis (yes).

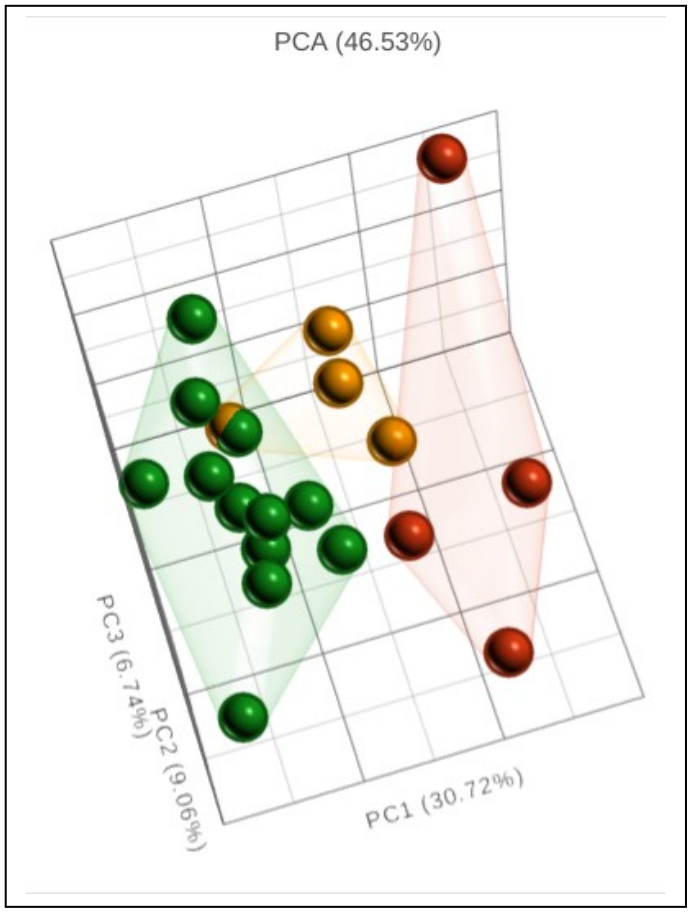

Figure 6.2. Principal component analysis plot of the 40 transcripts differentially expressed in patients with severe versus moderate or mild carotid stenosis

Color code: green – severe stenosis, orange – moderate stenosis, red – mild stenosis

The functional pathway analysis identified a predominance of transcripts relevant to programmed cell death-ligand 1/programmed cell death protein 1 checkpoint pathway (PD-L1/PD-1) and hypoxia-inducible factor 1 alpha signaling pathway (HIF-1α) (Table 6.2). There was a lower expression of HIF1A mRNA in participants with symptomatic carotid atherosclerosis than in participants with stroke due to other causes (FC = -1.35, $p<0.001$) and a higher expression of PD-L1 (CD274) mRNA in participants with severe stenosis than in those with mild stenosis (FC=+3.02, $p = 0.03$) (Figure 6.3).

6.3.3. Protein evaluation of selected targets

PD-L1 and HIF-1α were not detectable either in plasma or protein extracts. PD-1 was detectable in plasma but not in protein extracts. Plasma levels of PD-1 were not different between participants with symptomatic carotid atherosclerosis and those with asymptomatic carotid atherosclerosis (Appendix 4, Table IV).

6.3.4. Characteristics of patients in the validation sample

The frequency of treatment with statins was significantly higher in participants with large vessel stroke (Appendix 4, Table V). Participants with large vessel stroke were distributed as follows: 2 with severe stenosis (carotid and intracranial) and 7 with mild stenosis (2 vertebral and 5 intracranial). Participants with stroke due to other causes were distributed as follows: 13 cardioembolic strokes, 13 lacunar strokes, and 2 cryptogenic strokes.

Table 6.2. Functional pathways enriched with genes that differentiate patients with symptomatic carotid atherosclerosis

Canonical pathway	Enrichment score	p	Genes in list	Total number of genes
PD-L1 expression and PD-1 checkpoint pathway in cancer	7.34	6.4×10^{-4}	AKT2, CD3E, CD3G, CSNK2B, EML4, IKBKG, JAK2, LAT, LCK, MAP2K1, MAL, PIK3CB, PIK3CD, TLR4	14
HIF-1 signaling pathway	3.98	0.02	AKT2, ALDOA, ARNT, CAMK2D, EGLN1, ENO1, MAP2K1, MKNK1, PIK3CB, PIK3CD, PRKCB, TLR4	12
NF-kappa B signaling pathway	2.62	0.07	CD40LG, CSNK2B, ERC1, IKBKG, LAT, LCK, MAL, PRKCB, TLR4, XIAP	10
Th17 cell differentiation	2.01	0.13	CD3E, CD3G, GATA3, IKBKG, JAK2, LAT, LCK, RUNX1, SMAD4	9
Toll-like receptor signaling pathway	1.92	0.15	AKT2, IKBKG, MAP2K1, MAL, PIK3CB, PIK3CD, TLR4, TLR5	8
cAMP signaling pathway	1.63	0.2	AFDN, AKT2, ATP2A3, CAMK2D, CAMK4, CNGA1, MAP2K1, PIK3CB, PIK3CD, PPARA, PPP1CB, PTGER2, RAP1B, TIAM1	14
VEGF signaling pathway	1.49	0.23	AKT2, MAP2K1, PIK3CB, PIK3CD, PRKCB	5
AGE-RAGE signaling pathway in diabetic complications	1.18	0.31	AKT2, COL4A3, JAK2, PIK3CB, PIK3CD, PRKCB, SMAD4	7
PPAR signaling pathway	1.11	0.33	ACAA1, DBI, ILK, NR1H3, PPARA	5
Apelin signaling pathway	1.11	0.33	AKT2, BECN1, CAMK4, GNB1, ITPR1, MAP2K1, MEF2C, SLC8A1, SMAD4	9
PI3K-Akt signaling pathway	0.9	0.4	AKT2, COL4A3, GNB1, IKBKG, IL3RA, IL7R, ITGA3, ITGAV, JAK2, LPAR2, MAP2K1, MDM2, PIK3CB, PIK3CD, PPP2R5B, RBL2, TLR4, TSC2, YWHAZ	19
Fluid shear stress and atherosclerosis	0.75	0.47	AKT2, CTSL, IKBKG, ITGAV, EF2C, PECAM1, PIK3CB, PIK3CD	8
AMPK signaling pathway	0.73	0.48	AKT2, PIK3CB, PIK3CD, PPP2R5B, SIRT1, TBC1D1, TSC2	7
Platelet activation	0.63	0.53	AKT2, FYN, ITPR1, PIK3CB, PIK3CD, PPP1CB, RAP1B	7
MAPK signaling pathway	0.46	0.63	AKT2, CACNA1I, FLNA, FLNB, HSPA8, IKBKG, MAP2K1, MAP3K12, MAP3K4, MEF2C, MKNK1, PRKCB, RAP1B, RPS6KA3, TAOK3	15
mTOR signaling pathway	0.44	0.65	AKT2, MAP2K1, NPRL2, PIK3CB, PIK3CD, PRKCB, RPS6KA3, TSC2,	8
TNF signaling pathway	0.28	0.76	AKT2, IKBKG, MAP2K1, PIK3CB, PIK3CD	5
JAK-STAT signaling pathway	0.26	0.77	AKT2, IL3RA IL7R, JAK2, PIK3CB, PIK3CD	6

*Pathways are listed in decreasing order of enrichment score. Only the first two pathways fulfilled all the pre-specified selection criteria.

AGE-RAGE: advanced glycation end products-receptor for advanced glycation end products, AMPK: adenosine monophosphate-activated protein kinase, cAMP: cyclic adenosine monophosphate, HIF-1: Hypoxia inducible factor 1, JAK-STAT: Janus kinase-signal transducer and activator of transcription, MAPK: mitogen-activated protein kinases, mTOR: mammalian target of rapamycin, NF-kappa B: Nuclear factor kappa B, PD-L1/PD-1: programmed cell death-ligand 1/programmed cell death protein 1, PI3K-Akt: phosphatidylinositol 3-kinase Ak strain transforming, PPAR: peroxisome proliferator-activated receptor, Th17: T helper 17, TNF: tumor necrosis factor, VEGF: vascular endothelial growth factor.

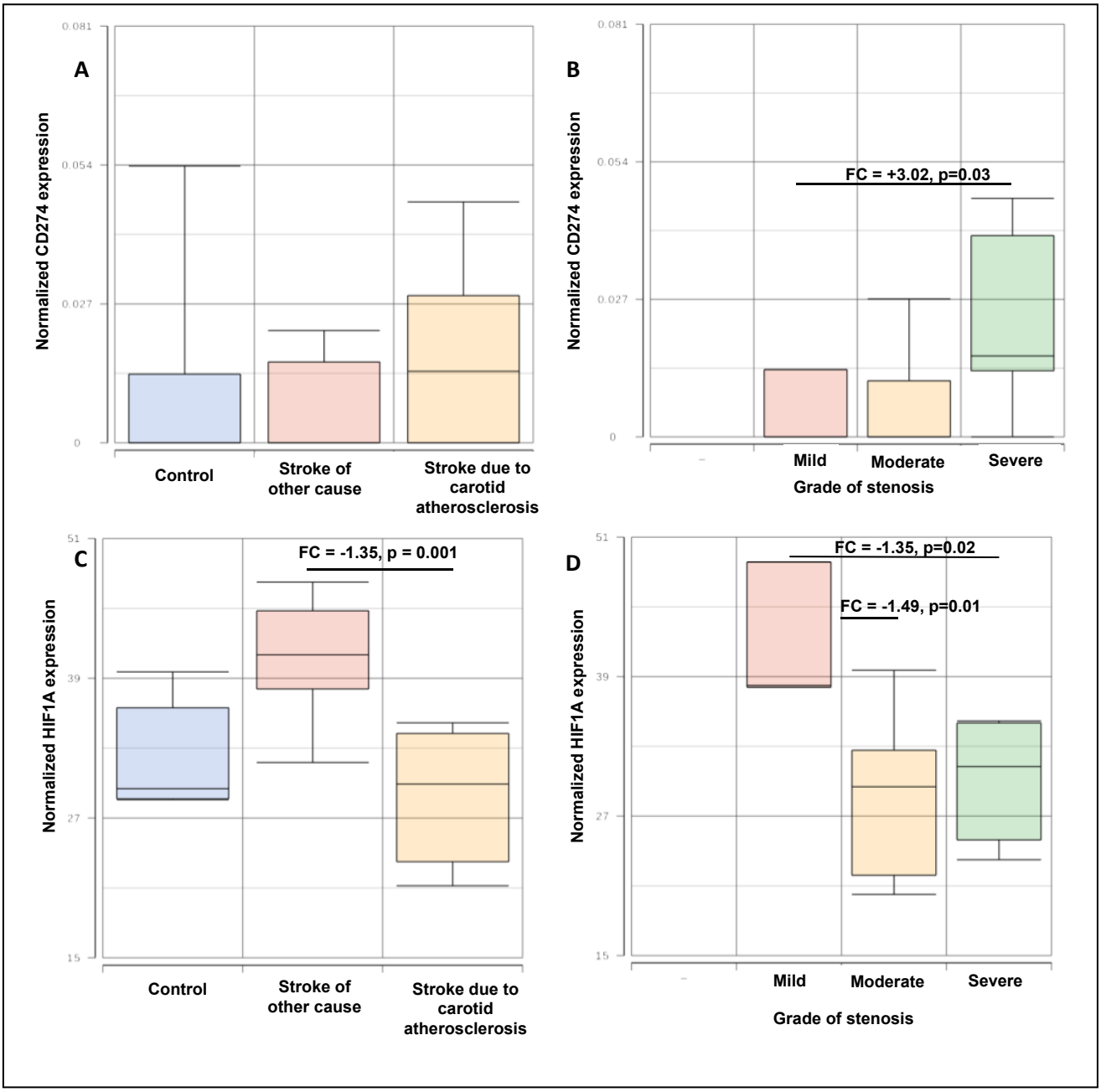

Figure 6.3. Expression of PD-L1 and HIF1A according to stroke etiology and grade of stenosis

A: Expression of *PD-L1 (CD274)* in patients with stroke due to carotid atherosclerosis versus stroke not due to atherosclerosis, and controls.
B: Expression of *PD-L1 (CD274)* according to grade of stenosis (only patients with visible plaque are displayed).
C: Expression of *HIF1A* in patients with stroke due to carotid atherosclerosis versus stroke not due to atherosclerosis, and controls.
D: Expression of *HIF1A* according to grade of stenosis (only patients with visible plaque are displayed).

Note: The Y axis shows normalized counts.

6.3.5. Differential gene expression in the validation sample

There were 185 transcripts (181 genes) differentially expressed in participants with stroke due to large artery atherosclerosis compared to participants with stroke due to other causes, and controls (Appendix 4, Table VI) and 156 (153 genes) transcripts differentially expressed in participants with severe stenosis compared to those with mild or moderate stenosis (Appendix 4, Table VII). There were 19 transcripts common to both lists (Appendix 4, Table VIII) but none was present on the 40-transcript panel obtained in the first cohort of participants. Five genes (NIPBL, NRDC, POML, SLC44A1, TNK2) in Table VI and Table VII were also identified by the differential transcript expression analyses in the first cohort of participants (Appendix 4, Table I and Table II) but none was present on either the 40-transcript or the 19-transcript panels.

6.4. Discussion

Monocytes play important roles in atherosclerosis. By comparing participants with symptomatic carotid atherosclerosis to those with asymptomatic carotid atherosclerosis, we identified differences in human monocyte transcript expression associated with plaque progression and plaque destabilization. The differentially expressed transcripts were relevant to the programmed cell death-ligand 1/programmed cell death protein 1 checkpoint pathway (PD-L1/PD-1) and hypoxia-inducible factor 1 alpha signaling pathway (HIF-1α). While further evaluation is needed, our findings provide insight into the biology of symptomatic human carotid atherosclerosis which may be useful in the search for new interventions to reduce stroke risk.

HIF-1 is a ubiquitous heterodimeric transcription factor that mediates the adaptive response to hypoxia in nucleated cells. It consists of two subunits: HIF-1β that is constitutively expressed in the nucleus and HIF-1α, a cytoplasmic protein that has a short half-life (5 minutes) and is highly regulated by oxygen.[447,449,450] Under hypoxic conditions, HIF-1α translocates into the nucleus where it binds to HIF-1β to form a heterodimeric transcription factor.[447,449] The accumulation and transcriptional activity of HIF-1α is increased by pro-inflammatory cytokines and, in turn, HIF-1α has several proinflammatory and proatherogenic effects including increased secretion of pro-angiogenic factors by endothelial and mast cells (e.g., vascular endothelial growth factor, endothelin-1, matrix metalloproteinase 2), promotion of macrophage maturation and formation of foam cells, promotion of neutrophil adhesion to the endothelium, increased intra-plaque accumulation of inflammatory dendritic cells, as well as migration and proliferation of vascular smooth muscle cells (vascular remodeling).[447] Thus HIF-1 may play an important role in

regulation of monocytes in relationship to carotid plaque progression and rupture. Unfortunately, it was not possible to measure HIF-1α in our monocyte protein extract to confirm this hypothesis. Several reason could explain the impossibility to measure the protein either in plasma or in protein extracts, notably its short half-life, its predominantly intracellular location, and the low number of monocytes used to prepare the protein extracts.

Our findings also suggest a role of the PD-1/PD-L1 pathway in atherosclerosis pathophysiology. Previous clinical studies have reported an association between PD-1 inhibition in patients with cancer and plaque progression or cerebrovascular events.[451,452] In a study enrolling 2842 patients with cancer receiving immune checkpoint inhibitors (>75% on PD-1 inhibitors) and 2842 controls matched for age, sex, history of cardiovascular events and cancer type, the use of immune checkpoint inhibitors was independently associated with a 3-fold higher risk of cardiovascular events and progression of aortic plaque.[452] Immune checkpoint inhibitors also increase FDG-PET uptake in atherosclerotic plaques.[451] Thus, the PD-1/PD-L1 signaling pathway might have effects on monocyte inflammatory activity in carotid plaque, contributing to plaque formation and destabilization. In our study, we could not demonstrate a decreased plasma concentration of PD-1 that could match the increased expression of PD-1/PD-L1 mRNA. Additionally, it was not possible to measure PD-1 in protein extracts from monocytes and PD-L1 was not detectable either in plasma or in protein extracts.

In the two cohorts studied, we observed changes in the expression of NIPBL, NRDC, POLM, SLC44A1, and TNK2 despite differences in phenotype and sample processing. These genes may also be involved in the pathophysiology of atherosclerosis and stroke. NIPBL codes for nipped B-like factor that regulates myeloid cell differentiation and might influence the differentiation of monocytes into inflammatory macrophages that promote plaque progression and rupture.[453] NRDC codes for nardilysin convertase, a zinc-dependent metalloproteinase that enhances the shedding of tumor necrosis factor through activation of ADAMTS17 and could contribute to plaque inflammation in atherosclerosis.[454] POLM codes for DNA Polymerase Mu involved in double-strand DNA break repair and leukocyte differentiation. Suboptimal DNA repair promotes atherosclerosis progression, likely via impaired response to oxidative stress.[455] SLC44A1 codes for Solute Carrier Family 44 Member 1, a protein that might contribute to monocyte differentiation into pro-inflammatory macrophages. Knockdown of the SLC44A1 gene in macrophages attenuates the production of proinflammatory cytokines, notably IL-1β and IL-18 that are promising therapeutic targets for the prevention of atherosclerotic cardiovascular

events.[355,456] Several genes of the solute carrier family have been previously associated with atherosclerosis in genome-wide association studies.[457] TNK2 codes for a non-receptor tyrosine kinase 2 that binds to cell division control protein 42 *Homo sapiens* (Cdc42Hs) and inhibits its activity. Cdc42Hs is a small GTPase of the Rho family that controls cell morphology, proliferation, migration, and endocytosis.[458] Inhibition of Cdc42Hs by a non-receptor tyrosine kinase 2 may impair cholesterol trafficking and promote the formation of foam cells.[459]

6.5. Strengths and limitations

Not all differentially expressed transcripts overlapped between the first and second cohort. The discrepancy could be explained by differences in the clinical phenotype of patients and in the sample processing. In the first cohort, all patients with large vessel stroke had carotid atherosclerosis whereas in the second cohort included patients with intracranial, vertebral, and carotid atherosclerosis. Thus, differences may exist in the biology of monocytes by subtype of large vessel atherosclerosis. The two cohorts also had differences in methods of monocyte isolation and RNA measurement (library preparation and sequencing).

Our study has several strengths, notably the careful phenotyping and selection of patients, the isolation of monocytes from patients with stroke, the standardized and rigorous processing of samples. However, sample size was small and further evaluation in larger cohorts is required. Moreover, not all features of high-risk plaques were evaluated, notably microembolic signals, intraplaque hemorrhage, neovascularization, and impaired cerebrovascular reserve.[301] Further our samples were also collected at a single time point. Evaluation of change in monocytes over time in relationship to plaque progression and atheroembolism would be of interest. Finally, genetic features such as HDAC9 single nucleotide polymorphism may affect monocyte gene expression and would be worth examining in patients with carotid atherosclerosis.

6.6. Conclusion and future directions

This study provides preliminary evidence that peripheral monocytes have a distinctive gene expression profile in patients with high-risk carotid atherosclerosis with increased expression of PD-L1 mRNA and decreased expression of HIF1A mRNA. Further studies are needed to validate these observations and determine if they could guide the design of new biomarkers and drugs for the management of carotid atherosclerosis.

Chapter 7: General conclusion and perspectives

Our doctoral thesis has provided new evidence to support the relevance of biomarkers for clinical decision making in patients with carotid atherosclerosis.

7.1. Summary of the key findings

In chapter 1, we have shown that nearly one third of patients with embolic stroke of unknown source have an ipsilateral high-risk carotid plaque.[298] We have also demonstrated that, in patients with embolic stroke of unknown source, high-risk plaques are 5 times more prevalent in the ipsilateral versus the contralateral carotid artery, thus suggesting a relationship to the risk of stroke. Moreover, some specific high-risk features might be decisive for the reclassification of embolic stroke of unknown source into large vessel stroke, notably plaque thrombus, intraplaque hemorrhage, and fibrous cap rupture that are 6, 9, and 18 times more frequent in the ipsilateral versus the contralateral carotid artery.[298] Before our study, it was commonly admitted that a < 50% stenosis cannot cause a stroke. Our work has spurred a new debate in the scientific community on how to best investigate and manage mild carotid stenosis in patients with embolic stroke of unknown source.[349,460,461]

In chapter 2, we have shown that at least one in four patients with asymptomatic carotid stenosis are potentially at high risk of stroke and the frequency of high-risk features is independent of the grade of stenosis, the prevalence of cardiovascular risk factors, and the treatment with statins.[301] Moreover, the risk of ipsilateral ischemic cerebrovascular events was 3.2 per 100 person-years overall and 4.3 per 100 person-years in those with at least one high-risk feature on vascular imaging. We also demonstrated that the risk of ipsilateral ischemic cerebrovascular events was three times higher in patients with high-risk features than in those without and the incidence was close to 10 per 100 person-years in patients with micro-embolic signals. This exceeds the risk attributable to carotid surgery. Before our work, it was commonly accepted that the risk of stroke attributable to any asymptomatic carotid stenosis is less than 1%.[47] Our results provide evidence that (1) there is a significant number of patients, identifiable with vascular imaging, that are misclassified as being at low risk of stroke; (2) the control of cardiovascular risk factors is likely not sufficient to prevent plaque destabilization and suppress the risk of stroke attributable to carotid stenosis.

In chapters 3 and 4, we identified several biomarkers that have been associated with carotid plaque vulnerability or progression and with cerebrovascular events in patients with carotid atherosclerosis. Such biomarkers included interleukin-1β, interleukin-6, C-reactive protein, uric acid, lipoprotein-associated phospholipase A2, and lectin-like oxidized LDL receptor among others.[78,422] However, none of the biomarkers had a validated threshold for use in the clinical management of patients with carotid atherosclerosis and only a few were targetable with existing drugs, notably interleukin-6 that is targeted by tocilizumab, sarilumab, and ziltivekimab.[28-30]

In chapter 5, we have provided novel evidence to further support the idea that there is an independent causal linear relationship between plasma interleukin-6 levels and various characteristics of carotid plaques that are typically associated with a higher risk of stroke: severity defined by the grade of stenosis; vulnerability defined by echolucency, marked irregularity or ulceration; and progression. Moreover, we have defined and internally validated a non-arbitrary risk-informed cut-off of 2.0 pg/mL to predict carotid plaque progression. This cut-off allows the use of interleukin-6 for prediction modelling without log-transformation and without loss of predictive performance. If externally validated, the proposed threshold could help to select individuals who would benefit from anti-interleukin-6 drugs in stroke prevention trials. The association between interleukin-6 and plaque vulnerability and progression also suggests that features of high-risk plaque could serve as surrogate endpoints in such trials.

In chapter 6, we provided preliminary evidence that omics tools are worth exploring to identify novel biomarkers and therapeutic targets to improve the management of carotid atherosclerosis. However, more work remains to be done to standardize experimental design, sample processing, and data analysis approaches for the safe and effective translation of omics tools from bench to bedside.

7.2. Future directions

Collectively, our results have at least 4 major implications for both clinical practice and research.

1. Routine assessment of carotid atherosclerosis beyond the grade of stenosis using multimodal neurovascular imaging should be implemented in clinical practice to support the etiological classification of embolic strokes of unknown source, improve stroke risk stratification in asymptomatic carotid stenosis, and optimize stroke prevention strategies.
2. Revascularization trials using multimodal neurovascular imaging for risk stratification before randomization in patients with asymptomatic carotid stenosis are warranted. Such trials would aim to demonstrate the added value of surgical revascularization for stroke prevention in patients with high-risk carotid plaques and in the context of current best medical therapy.
3. Biomarkers have a role to play in the management of carotid atherosclerosis, but more research is needed to accelerate their adoption in routine clinical practice, notably the definition and validation of thresholds, the combination into panels, and the integration into diagnostic and prognostic scores, algorithms, and calculators.
4. Considering that circulating interleukin-6 levels are associated with carotid plaque severity, vulnerability, and progression and to the risk of first-ever or recurrent ischemic stroke, trials of anti-interleukin-6 drugs as an adjuvant stroke prevention strategy in people with carotid atherosclerosis are warranted.

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522. Zhang C, Qu S, Li H, Li G, Chen G, Wang J, et al. Microembolic signals and carotid plaque characteristics in patients with asymptomatic carotid stenosis. *Scand Cardiovasc J* 2009; 43(5): 345-351.

523. Zhu Y, Deng YB, Liu YN, Bi XJ, Sun J, Tang QY, et al. Use of carotid plaque neovascularization at contrast-enhanced US to predict coronary events in patients with coronary artery disease. *Radiology* 2013; 268(1): 54-60.

Supplementary material

Appendix 1

Table I: Search strategy

PUBMED		**Number of records**
#1	Stroke OR "transient ischemic attack"	325873
#2	plaque OR atherosclerosis	227905
#3	cryptogenic	6548
#4	#1 AND #2	14250
#5	#3 AND #4	142
Ovid EMBASE		**Number of records**
#1	Stroke.mp. or exp cerebrovascular accident/	464331
#2	transient ischemic attack.mp. or exp transient ischemic attack/	37720
#3	#1 OR #2	475310
#4	cryptogenic.mp.	10600
#5	#3 AND #4	3292
#6	exp atherosclerotic plaque/	31772
#7	#5 AND #6	46
#8	limit #7 to (human and English language)	39

Table II: Risk of bias assessment tool

Risk of bias item	**Response:** Yes = 1, No = 0
External validity	
1. Was the study target population a close representation of the national population (adults, children, or both) in relation to relevant variables? (no restriction on sex/race/profession/marital status or other criteria that would limit the diversity of the sample and therefore its representativeness and the generalizability of the result)	
2. Was the sampling frame a true or close representation of the target population as suggested by the study title and objectives? (e.g. patients presenting with anterior circulation cryptogenic stroke or embolic stroke of undetermined source)	
3. Was some form of random selection used to select the sample, OR, was a census undertaken? (census or consecutive/exhaustive sampling)	
4. Was the likelihood of non-participation bias minimal? (probability that investigators have failed to include subjects that would normally be eligible)	
Internal Validity	
5. Were data collected prospectively directly from the participants (as opposed to mere review of medical records or retrospective data collection)?	
6. Was the process of identifying patients with cryptogenic stroke appropriate and clearly described?	
7. Was the diagnostic method (brain imaging) used to identify high-risk carotid plaque clearly described (type of imaging, *eventually with sequences and qualification of the reader*)?	
8. Was the same assessment protocol used for all the participants?	
9. Were the results of the plaque imaging clearly presented? (adequate reporting of data within each category of lesion/patients + discrete categories/no overlapping + no errors requiring a guess/adjustments)	
10. Were the numerator(s) and denominator(s) for the calculation of the prevalence of high-risk plaque appropriate?	
Interpretation of the score	
8 – 10: Low Risk of Bias / High-quality study. Further research is very unlikely to change our confidence in the estimate.	
5 – 7: Moderate Risk of Bias / Moderate-quality study. Further research is likely to have an important impact on our confidence in the estimate and may change the estimate.	
4 or less: High Risk of Bias / Low-quality study. Further research is very likely to have an important impact on our confidence in the estimate and is likely to change the estimate. Further research is mandatory.	

Adapted from Hoy D, Brooks P, Woolf A, Blyth F, March L, Bain C, et al. Assessing risk of bias in prevalence studies: modification of an existing tool and evidence of interrater agreement. J Clin Epidemiol. 2012;65:934-939, Copyright © 2012, with permission from Elsevier.

Table III: Specific prevalence of the high-risk features reported in the included studies

Risk feature	Number of studies*	Number of patients	Prevalence (95% CI) ‡		pooled OR (95% CI) †
			ipsilateral side	**contralateral side**	
intraplaque haemorrhage	5	162	24.4 (17.9 - 31.5)	0.6 (0.0 - 3.7)	9.4 (2.9 – 30.5)
echolucency	1	44	50.0 (35.8 - 64.2)	31.8 (20.0 - 46.6)	2.1 (0.9 - 5.1)
plaque thickness ≥ 3 mm	1	85	35.3 (26.0 - 45.9)	15.3 (9.2 - 24.4)	3.0 (1.4 - 6.3)
fibrous cap rupture	2	50	23.6 (12.4 - 36.7)	0.0 (0.0 - 3.7)	17.5 (2.2 – 140.1)
thrombus	3	94	6.9 (2.2 - 13.5)	0.0 (0.0 - 2.0)	5.8 (1.0 – 34.3)
ulceration	1	44	0.0 (0.0 - 8.0)	0.0 (0.0 - 8.0)	NA

*One study only provided aggregated data for the high-risk features considered: Bayer-Karpinska A, Schwarz F, Wollenweber FA, Poppert H, Boeckh-Behrens T, Becker A, et al. The carotid plaque imaging in acute stroke (CAPIAS) study: protocol and initial baseline data. BMC Neurol. 2013;13:201.

† The prevalence and odds ratios were pooled using a random effect meta-analysis.

Figure I: Study selection

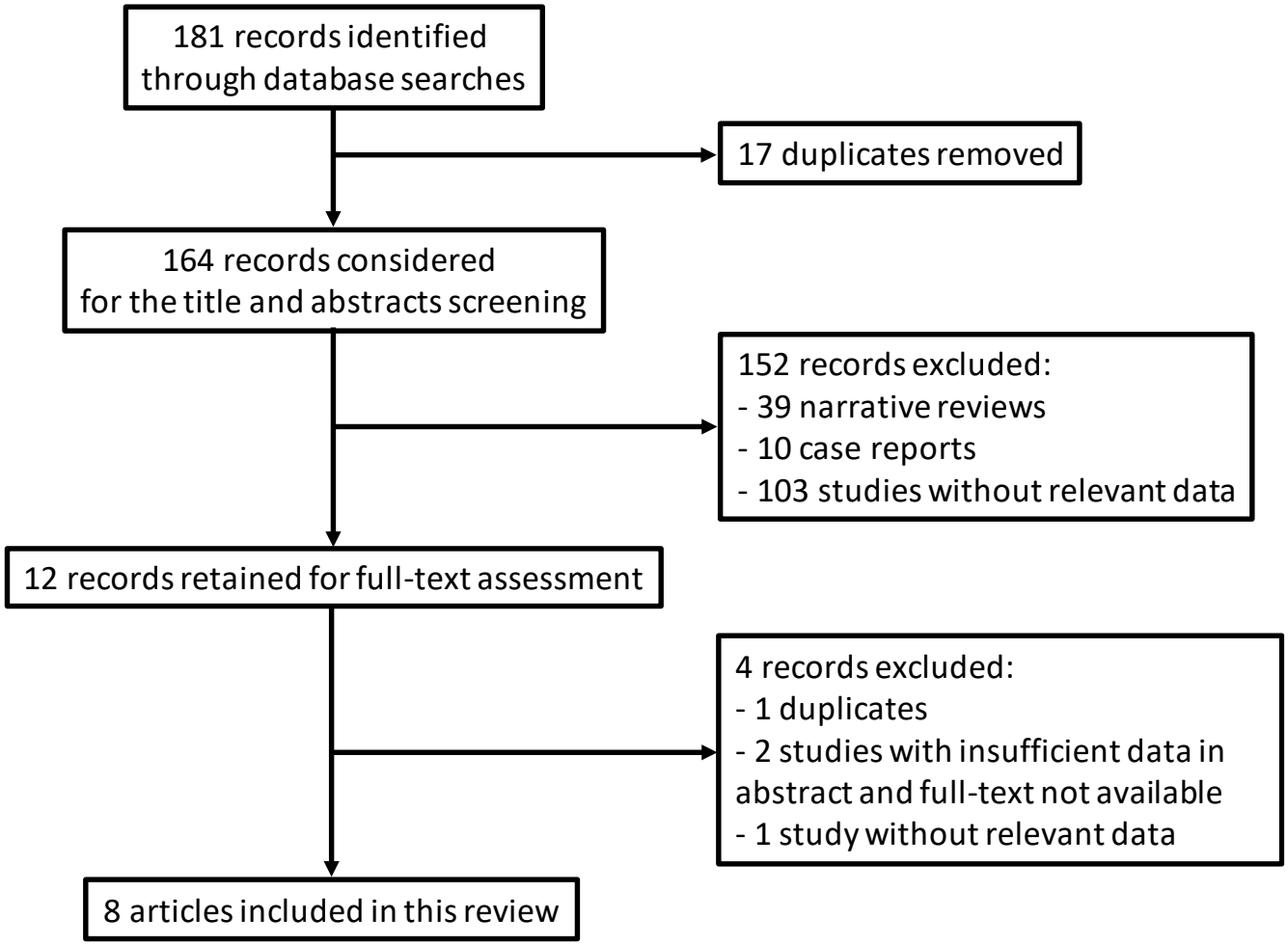
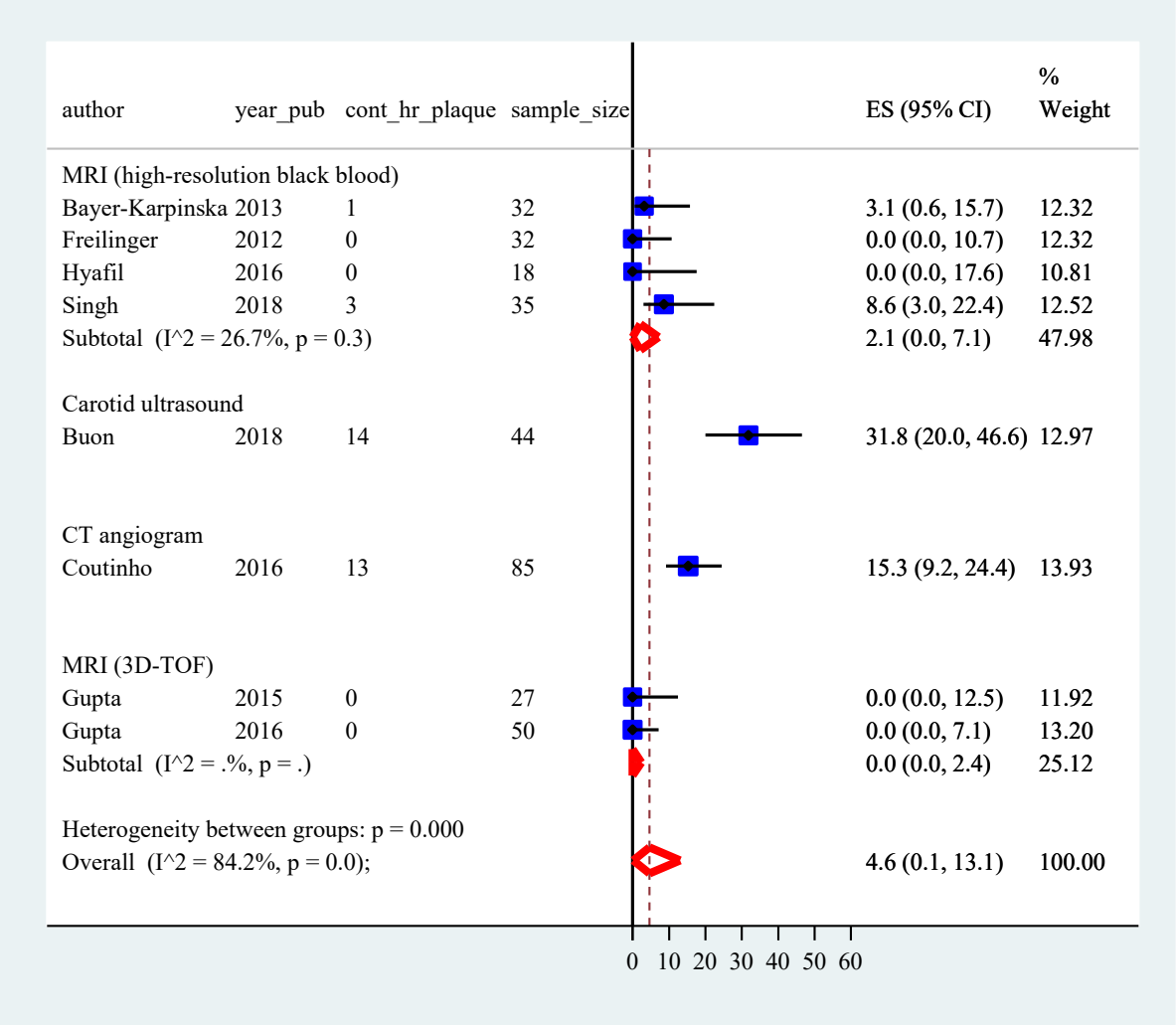
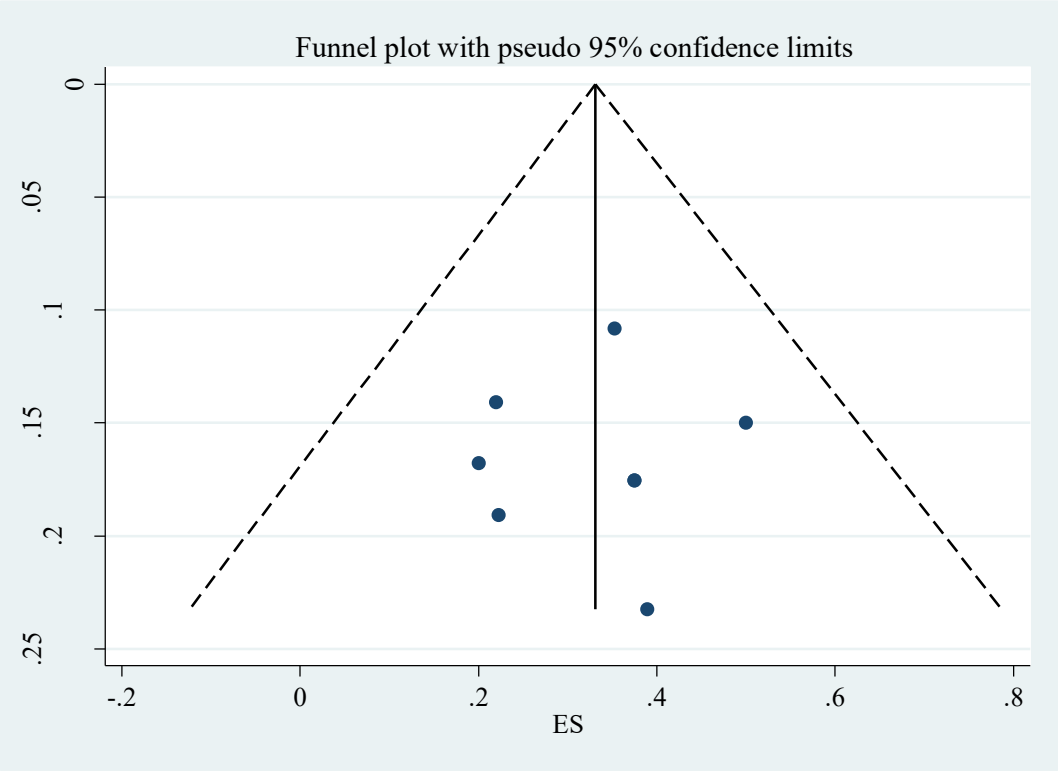


Figure II: Pooled prevalence of contralateral carotid plaque with high-risk features in ESUS



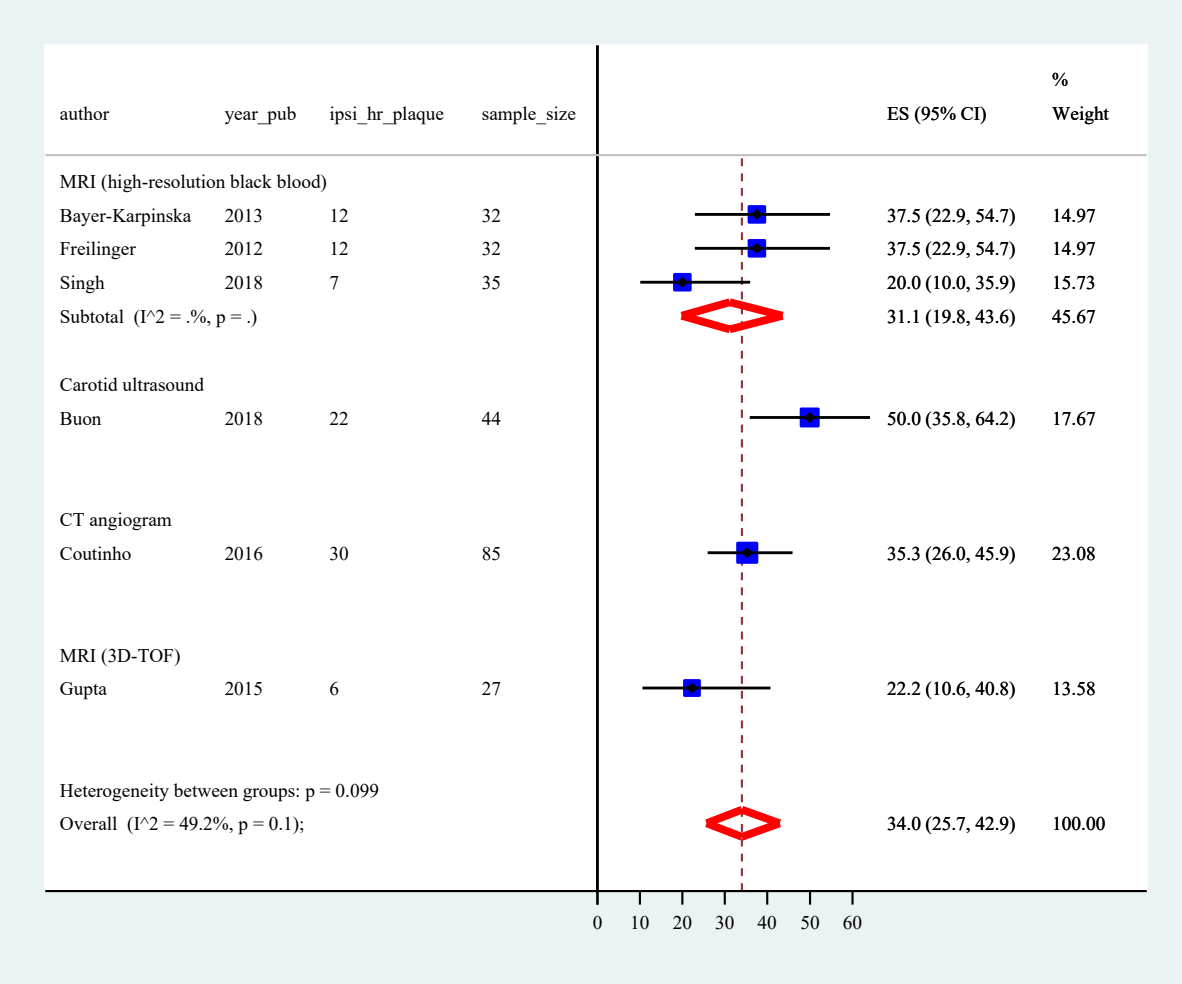
3D-TOF = 3-dimensional time of flight, CI = Confidence interval, CT = Computed tomography, cont_hr_plaque = contralateral carotid plaque with high-risk features, ES = Effect size, MRI = Magnetic resonance imaging, sample_size = number of participants in the study, year_pub = year of publication

Figure III: Funnel plot for the meta-analysis of prevalence of ipsilateral carotid plaque with high-risk features in ESUS



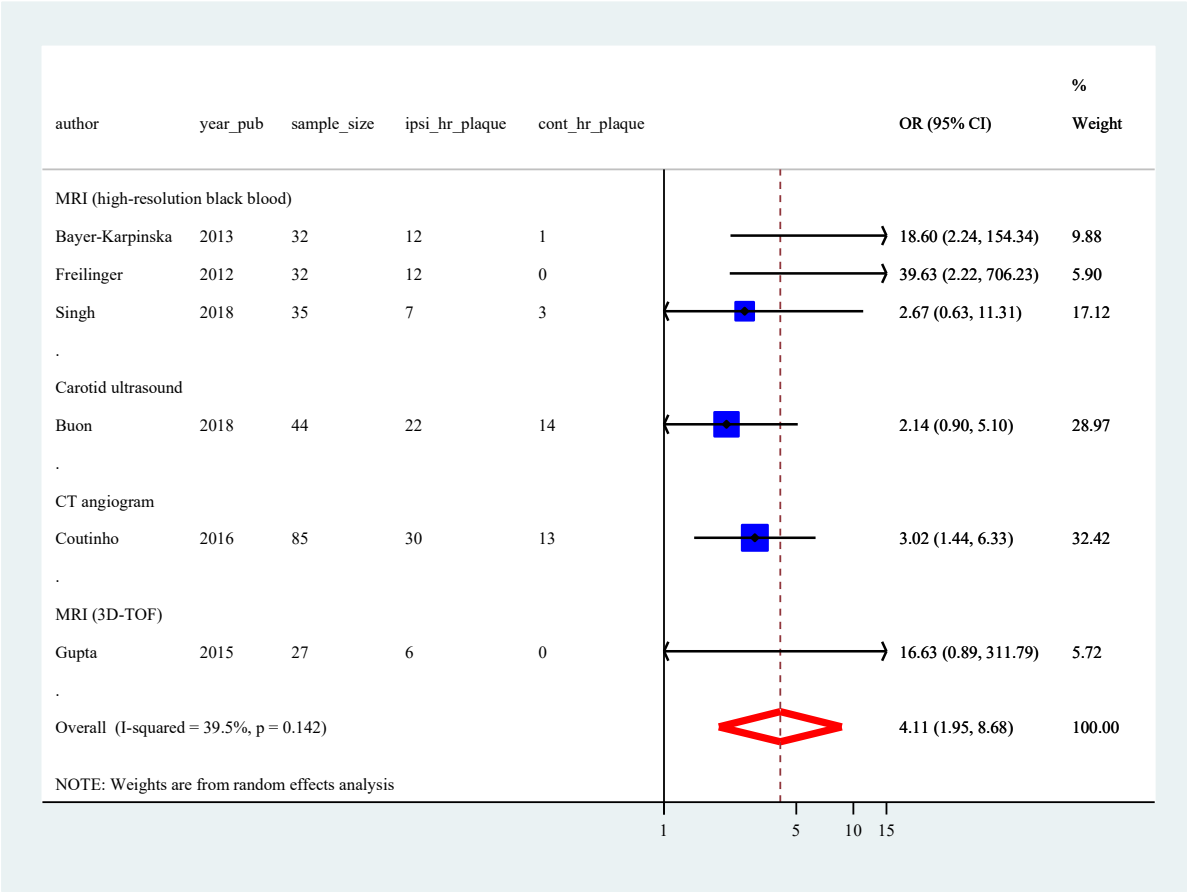
ES = Effect size (prevalence), se (ES) = standard error of effect size
The symmetric distribution of the studies (blue dots) around the average effect size (black vertical line, x = 0.325) supports the absence of small-study effect as confirmed by the Egger's test (p = 0.876). There are only 7 blue dots (instead of 8) because two studies have the same sample size and the same effect size (see Figure 1).

Figure IV: Pooled prevalence of ipsilateral carotid plaque with high-risk features in ESUS after excluding studies with sample size < 20 or with potential population overlap.



3D-TOF = 3-dimensional time of flight, CI = Confidence interval, CT = Computed tomography, ES = Effect size, ipsi_hr_plaque = ipsilateral carotid plaque with high-risk features, MRI = Magnetic resonance imaging, sample_size = number of participants in the study, year_pub = year of publication

Figure V: Odds-ratio of finding plaque with high-risk features in the ipsilateral versus the contralateral carotid in ESUS after excluding studies with sample size < 20 or with potential population overlap.



3D-TOF = 3-dimensional time of flight, CI = Confidence interval, CT = Computed tomography, cont_hr_plaque = contralateral carotid plaque with high-risk features, ipsi_hr_plaque = ipsilateral carotid plaque with high-risk features, MRI = Magnetic resonance imaging, OR = Odds ratio, sample_size = number of participants in the study, year_pub = year of publication

Appendix 2

Table I. Search Strategy

Search strategy in PubMed	
#1	"Carotid plaque" OR "carotid atherosclerosis" OR "carotid stenosis"
#2	asymptomatic
#3	"unstable" OR "vulnerable" OR "high-risk" OR "intraplaque hemorrhage" OR "intra-plaque hemorrhage" OR "lipid-rich necrotic core" OR "thin fibrous cap" OR "ruptured fibrous cap" OR "silent brain infarcts" OR "silent embolic infarcts" OR "ulceration" OR "irregularity" OR "juxta-luminal hypoechoic area" OR "microembolic signals" OR "high intermittent transient signals" OR "neovascularization" OR "echolucency" OR "hypoechogenicity" OR "impaired cerebrovascular reserve" OR "decreased cerebrovascular reserve" OR "impaired cerebrovascular reactivity" OR "decreased cerebrovascular reactivity" OR "inflammation" OR "plaque volume"
#4	#1 AND #2
#5	#3 AND #4
#6	Limit #5 to humans

Table II. Individual Characteristics of Included Studies

PMID	Author	Year	Country [a]	Design	Setting	Pre-CEA	Imaging	Grade	N	FU	LFU	ACAS	SCAS	ROB	HRF	Definition
15879327	Abbott[462]	2005	AU	cohort	HOSP	No	US	Moderate and severe	231	5	0	202	na	9	MES	consensus criteria + sound threshold of 6 dB (≥ 1 HITS)
27751505	Ammirati[463]	2016	IT	CS	HOSP	No	US	Mild and moderate	55	na	na	55	na	8	Neovascularization	extensive intraplaque enhancement (grade 2)
28874779	Ammirati[464]	2017	IT	CS	HOSP	No	US	Mild and moderate	62	na	na	62	na	9	Neovascularization	extensive intraplaque enhancement (grade 2)
															Echolucency	Gray-Weale classification: types I and II
8202968	Brott[465]	1994	US	CS	HOSP	No	CT	Moderate and severe	848	na	na	848	na	9	SBI (ipsilateral)	hypoattenuation compatible with infarction without history of stroke
29103477	Cai[466]	2017	CN	CS	POP	No	MRI (3.0T)	Any grade	140	na	na	140	na	9	IPH	hyperintensity (SNAP signal)
10359933	Cao[467]	1999	IT	CS	HOSP	Yes	CT	Any grade	301	na	na	301	na	10	SBI (ipsilateral)	hypodense lesion in the absence of previous history of hemispheric, ocular or vertebrobasilar symptom
27597156	Cattaneo[468]	2016	CH	CS	HOSP	No	US	Moderate and severe	40	na	na	40	na	9	Echolucency	Gray-Weale classification: types I and II
28651865	de Waard[469]	2017	Multiple	CS	HOSP	Yes	US	Moderate and severe	894	na	na	894	na	9	Echolucency	Gray-Weale classification: types I and II
20651015	DeMarco[470]	2010	US	CS	HOSP	No	MRI (3.0T)	Moderate and severe	90	na	na	44	na	9	LRNC	isointense on TOF/T1W, hypointense on CE-T1W, hypointense on T2W
															IPH	hyperintense on TOF and T1W
															Thin/ruptured fibrous cap	disrupted or invisible dark band adjacent to lumen on TOF, irregular luminal surface, hyperintense area adjacent to lumen on TOF
23519900	Deyama[471]	2013	JP	CS	HOSP	No	US	Any grade	304	na	na	304	na	10	Neovascularization	contrast enhancement of the plaque
18626232	Ding[472]	2008	CN	CS	HOSP	No	US	Moderate and severe	96	na	na	44	52	9	Echolucency	hypoechoic area within the plaque on B-mode (ref = bright area of the media-adventice interface)
															ulceration	discrete depression >2 mm extending into the media and exhibiting an area of low or reversed flow within the recess
															irregularity	height variations between 0.4 and 2 mm
30193730	D'Oria[473]	2018	IT	CS	HOSP	Yes	US	Severe only	58	na	na	58	na	8	Echolucency	Gray-Weale classification: types I and II
															Neovascularization	extensive intraplaque enhancement (grade 2)
10486404	Droste[474]	1999	DE	CS	HOSP	No	US	Any grade	105	na	na	41	64	10	MES	consensus criteria + sound threshold of 5 dB (≥ 1 HITS)
7643973	Eicke[475]	1995	DE	CS	HOSP	No	US	Any grade	76	na	na	37	39	10	MES	consensus criteria

31164272	Eilenberg[476]	2019	AT	CS	HOSP	Yes	US	Severe only	83	na	na	83	na	9	Echolucency	hypoechoic area within the plaque on B-mode (ref = bright area of the media-adventice interface)
17994312	Esposito[477]	2007	DE	CS	HOSP	Yes	MRI (1.5T)	Severe only	85	na	na	50	35	9	AHA type IV,V,VI	IV-V = presence of LRNC with thin fibrous cap, VI = ulceration, cap rupture, intraplaque hemorrhage, ruptured fibrous cap, or thrombus
23894291	Esposito-Bauer[478]	2013	DE	cohort	HOSP	No	MRI (1.5T)	Moderate and severe	77	3.4	0	77	na	9	AHA type IV,V,VI	IV-V = presence of LRNC with thin fibrous cap, VI = ulceration, cap rupture, intraplaque hemorrhage, ruptured fibrous cap, or thrombus
9183352	Georgiadis[479]	1997	DE	CS	HOSP	No	US	Any grade	100	na	na	54	46	10	MES	consensus criteria + sound threshold of 3 dB (≥ 1 HITS)
9448618	Golledge[480]	1997	UK	CS	HOSP	No	US	Moderate and severe	350	na	na	65	285	9	Echolucency	Gray-Weale classification: types I and II
															Ulceration	Plaque surface crater of ≥ 2 mm
11435340	Gronholdt[90]	2001	DK	cohort	HOSP	No	US	Moderate and severe	246	4.4	0	111	135	9	Echolucency	GSM < 74
8969778	Gur[481]	1996	UK	cohort	HOSP	No	US	Severe only	44	2	0	44	na	8	impaired CVR	< 40% increase of flow in the MCA ipsilateral to the carotid stenosis after the Diamox test
21160703	Huang[91]	2010	CN	CS	HOSP	No	US	Any grade	176	na	na	95	81	10	Neovascularization	Extensive internal plaque enhancement (grade IV)
26725253	Huibers[482]	2016	UK	cohort	HOSP	No	US	Severe only	814	6.5	0	814	na	9	Echolucency	Gray-Weale classification: types I and II
24075774	Irie[483]	2013	JP	cohort	HOSP	No	US	Any grade	287	4.6	0	287	na	8	Echolucency	GSM ≤ 37
19223148	Kakkos[484]	2009	Multiple	cohort	HOSP	No	CT	Moderate and severe	821	3.7	24	821	na	8	SBI (ipsilateral)	hypodense lesion in the absence of previous history of hemispheric, ocular or vertebrobasilar symptom
21474467	Kakkos[485]	2011	UK	CS	HOSP	No	US	Moderate and severe	180	na	na	43	137	9	Echolucency	Gray-Weale classification: types I and II
21527764	King[486]	2011	UK	cohort	HOSP	No	US	Severe only	106	1.9	0	106	na	9	Impaired CVR	< 10% increase of flow in the MCA ipsilateral to the carotid stenosis while breathing 6% CO2 or 20 minutes after receiving 1g acetazolamide IV
22498328	Lindsay[487]	2012	UK	CS	HOSP	No	MRI (3.0T)	Any grade	81	na	na	41	40	8	AHA type IV,V,VI	VI = ulceration, cap rupture, intraplaque hemorrhage, ruptured fibrous cap, or thrombus
															Thin/ruptured fibrous cap	disrupted or invisible dark band adjacent to lumen on TOF, hyperintense area adjacent to the lumen on TOF (thrombus?)
															IPH	hyperintense on T1W, T2W, 3D-TOF
															Mural thrombus	na
28716984	Liu[488]	2017	CN	cohort	HOSP	No	CT	Severe only	45	5.7	0	45	na	8	Impaired CVR	< 10% increase of flow in the MCA ipsilateral to the carotid stenosis while breathing 5% CO2
21849642	Madani[489]	2011	CA	cohort	HOSP	No	US	Moderate	253	3	0	253	na	9	Ulceration	Plaque surface depression of ≥ 1 mm

								and severe							MES	consensus criteria (≥ 2 HITS)
20554250	Markus[77]	2010	UK	cohort	HOSP	No	US	Severe only	467	2	0	467	na	9	MES	consensus criteria + sound threshold of 7 dB (≥ 1 HITS)
11222446	Markus[490]	2001	UK	cohort	HOSP	No	US	Severe only	59	1.8	0	59	na	9	impaired CVR	< 20% increase of flow in the MCA ipsilateral to the carotid stenosis while breathing 8% CO2
11331258	Mathiesen[491]	2001	NO	cohort	POP	No	US	Any grade	223	3	0	223	na	10	Echolucency	Gray-Weale classification: types I and II
22923447	Millon[492]	2012	FR	CS	HOSP	Yes	MRI (3.0T)	Any grade	59	Na	na	40	na	9	IPH	hyperintense on TOF and T1W
															Thin/ruptured fibrous cap	disrupted or invisible dark band adjacent to lumen on TOF, discontinuity of fibrous cap on post-contrast T1W
															Neovascularization	contrast enhancement of the plaque
															LRNC	> 50% of wall area
23375613	Millon[493]	2013	FR	CS	HOSP	No	MRI (3.0T)	Any grade	154	Na	na	102	52	10	IPH	hyperintense on TOF and T1W
															Thin/ruptured fibrous cap	disrupted or invisible dark band adjacent to lumen on TOF, discontinuity of fibrous cap on post-contrast T1W
															Neovascularization	contrast enhancement of the plaque
															LRNC	> 50% of wall area
10390320	Molloy[494]	1999	UK	cohort	HOSP	No	US	Moderate and severe	111	0.7	0	42	69	9	MES	consensus criteria + sound threshold of 7 dB
23154753	Mono[495]	2012	CH	cohort	HOSP	No	MRI (3.0T)	Moderate and severe	62	1.6	2	62	na	9	IPH	hyperintense on TOF and T1W
															Thin/ruptured fibrous cap	disrupted or invisible dark band adjacent to lumen on TOF, discontinuity of fibrous cap on post-contrast T1W
															LRNC	isointense on TOF, hyperintense on T1W, hypointense on CE-T1W
25370581	Muller[496]	2014	CH	CS	HOSP	No	US	Moderate and severe	110	na	na	55	55	9	MES	consensus criteria (≥ 1 HITS)
16229794	Nicolaides[497]	2005	Multiple	cohort	HOSP	No	US	Moderate and severe	1092	3.1	0	1092	na	9	Echolucency	Gray-Weale classification: types I and II
1561676	Norris[498]	1992	CA	CS	HOSP	No	CT	Any grade	318	na	na	115	203	9	SBI (ipsilateral)	na
10541601	Orlandi[499]	1999	IT	CS	HOSP	No	US	Mild and moderate	47	na	na	32	na	9	MES	consensus criteria + sound threshold of 7 dB (≥ 1 HITS)
23194832	Ota[500]	2013	US	CS	HOSP	No	MRI (3.0T)	Mild only	96	na	na	96	na	9	IPH	hyperintense on IR-FSPGR, TOF and T1W
															Thin/ruptured fibrous cap	disrupted or invisible dark band adjacent to lumen on TOF, discontinuity of fibrous cap on post-contrast T1W, hyperintense area adjacent to the lumen on TOF (thrombus?)
															LRNC	isointense on TOF and T1W,

																hypointense on CE-T1W and T2W
20616325	Ota[501]	2010	US	CS	HOSP	No	MRI (3.0T)	Moderate and severe	131	na	na	131	na	9	IPH	hyperintense on IR-FSPGR, TOF and T1W
															Thin/ruptured fibrous cap	disrupted or invisible dark band adjacent to lumen on TOF, discontinuity of fibrous cap on post-contrast T1W, hyperintense area adjacent to the lumen on TOF (thrombus?)
															LRNC	isointense on TOF and T1W, hypointense on CE-T1W and T2W
28300680	Pascot[502]	2017	FR	CS	HOSP	Yes	MRI (1.5T)	Severe only	41	na	na	41	na	9	ulceration	na
															IPH	na
															SBI (ipsilateral)	hyperintense on T2W and FLAIR, hypointense on T1W, involving gray and white matter, arterial distribution, no related symptoms
22210738	Pecoraro[503]	2012	IT	CS	HOSP	Yes	CT	Severe only	100	na	na	40	60	8	SBI (ipsilateral)	cortical or subcortical infarct in the territory of the carotid stenosis, no symptom
9722841	Polak[92]	1998	US	cohort	POP	No	US	Any grade	4886	3.3	0	4886	na	9	Echolucency	echogenicity equal of lower than that of the lumen
18768115	Ritter[504]	2009	DE	CS	HOSP	No	US	Moderate and severe	46	na	na	30	na	8	MES	consensus criteria + sound threshold of 5 dB (≥ 1 HITS)
20532633	Sadat[505]	2010	UK	CS	HOSP	No	MRI (1.5T)	Any grade	100	na	na	54	46	9	Ulceration	discontinuity of fibrous cap
															IPH	Hyperintense on T1W
27165952	Selwaness[506]	2016	NL	CS	POP	No	MRI (1.5T)	Any grade	1731	na	na	1731	na	10	IPH	hyperintense on 3D-T1GRE
															LRNC	hypointense on PDW-FSE/EPI and T2W
7482670	Siebler[507]	1995	DE	cohort	HOSP	No	US	Severe only	64	1.4	0	64	na	9	MES	consensus criteria (≥ 2 HITS)
7909360	Siebler[508]	1994	DE	CS	HOSP	No	US	Severe only	89	na	na	56	33	9	MES	consensus criteria + sound threshold of 7 dB (≥ 1 HITS)
10791504	Silvestrini[509]	2000	IT	cohort	HOSP	No	US	Severe only	94	2.4	na	94	na	9	Impaired CVR	BHI < 0.69
23361391	Silvestrini[93]	2013	IT	cohort	HOSP	No	US	Moderate and severe	621	2.3	0	621	na	9	Echolucency	Gray-Weale classification: types I and II
															Ulceration	na
20008646	Spence[510]	2010	CA	cohort	HOSP	No	US	Moderate and severe	468	2	0	468	na	9	MES	consensus criteria + sound threshold of 8 dB (≥ 2 HITS)
12154255	Stork[511]	2002	AU	CS	HOSP	Yes	US	Any grade	109	na	na	38	71	9	MES	consensus criteria + sound threshold of 6 dB (≥ 1 HITS)
30744544	Takasugi[512]	2019	JP	CS	HOSP	No	MRI (3.0T)	Any grade	89	na	na	60	na	9	IPH	hyperintense on T1W (maximal signal intensity within the plaque ≥ 150% of the adjacent sternocleidomastoid muscle)
															SBI (ipsilateral)	hyperintense on 3D-DIR and FLAIR, distinct from perivascular spaces and cerebral microbleeds on SWI)
16469957	Takaya[89]	2006	US	cohort	HOSP	No	MRI	Moderate	154	3.2	0	154	na	9	IPH	hyperintense on IR-FSPGR, TOF and

							(1.5T)	only								T1W
															Thin/ruptured fibrous cap	disrupted or invisible dark band adjacent to lumen on TOF, discontinuity of fibrous cap on post-contrast T1W, hyperintense area adjacent to the lumen on TOF (thrombus?)
															LRNC	isointense on TOF and T1W, hypointense on CE-T1W and T2W
21849657	Topakian[513]	2011	UK	cohort	HOSP	No	US	Severe only	435	1.8	0	435	na	9	Echolucency	Gray-Weale classification: types I and II
22075251	Turc[514]	2012	FR	CS	HOSP	No	MRI (1.5T)	Moderate only	234	na	na	120	114	9	IPH	hyperintense on T1W, T2W, 3D-TOF, and PDW
25966822	van den Bouwhuijsen[515]	2015	NL	CS	POP	No	MRI (1.5T)	Any grade	329	na	na	329	na	9	IPH	hyperintense on T1W
															LRNC	hypointense on PDW-FSE/EPI and T2W
24972806	van den Oord[516]	2014	NL	CS	HOSP	No	US	Any grade	51	na	na	51	na	8	Ulceration	disruption of the plaque-lumen boarder of ≥ 1x1mm
24125419	van den Oord[517]	2013	NL	CS	HOSP	No	US	Any grade	62	na	na	62	na	8	Neovascularization	extensive intraplaque enhancement (grade 2)
31002203	Wu[518]	2019	CN	CS	POP	No	US	Any grade	2888	na	na	2888	na	9	Echolucency	na
19304920	Xiong[519]	2009	CN	CS	HOSP	No	US	Any grade	104	na	na	69	35	10	Neovascularization	extensive intraplaque enhancement (grade 2)
															ulceration	recess on the surface of the plaque ≥ 2 mm deep and ≥ 2 mm long
24631510	Xu[520]	2014	US	cohort	HOSP	No	MRI (1.5T)	Moderate only	100	3	0	100	na	9	LRNC	> 40% of wall area
															IPH	IPH: hyperintense on T1W and TOF, isointense on T2W/PDW; ruptured cap: invisible dark juxtaluminal band on TOF with signs of juxtaluminal thrombus on TOF i.e. hyperintensity
24592924	Zavodni[521]	2014	US	cohort	POP	No	MRI (1.5T)	Any grade	939	5.5	0	939	na	10	LRNC	hypointense on CE-T1W
															Ulceration	na
19863395	Zhang[522]	2009	CN	cohort	HOSP	No	US	Moderate and severe	62	1	8	62	na	9	MES	consensus criteria + sound threshold of 7 dB (≥ 1 HITS)
23440321	Zhu[523]	2013	CN	CS	HOSP	No	US	Any grade	312	na	na	312	na	10	Neovascularization	extensive intraplaque enhancement (grade 2)

[a] The alpha-2 country code was used as described in the ISO 3166 international standard

ACAS = number with asymptomatic carotid stenosis, AHA = American Heart Association, CEA = Carotid endarterectomy, CS = cross-sectional, CT = computerized tomography, CVR = cerebrovascular reserve, FU = duration of follow-up (years), HOSP = hospital, HRF = high-risk feature reported, IPH = intraplaque hemorrhage, LFU = number lost to follow-up, LRNC = lipid-rich necrotic core, MES = microembolic signals, MRI = magnetic resonance imaging, N = sample size, na = not available or not applicable, PMID = PubMed accession number, POP = population, ROB = risk of bias score, SBI = silent brain infarct, SCAS = number with symptomatic carotid stenosis, US = ultrasound.

NOTE 1: For each included study, we extracted the following data: first author's name, year of publication, country, period of enrolment, circumstance of enrolment (routine screening or pre-endarterectomy), study design (cross-sectional or cohort), setting (hospital- or population-based), vascular imaging technique used to identify high-risk features, qualification of the image reader, grade

of carotid stenosis eligible and definition criteria, sample size (number of patients with asymptomatic carotid stenosis), mean or median age, proportion of men, proportion of patients with various cardiovascular risk factors or comorbidities (hypertension, diabetes, smoking, dyslipidemia, coronary artery disease, peripheral artery disease, and atrial fibrillation), proportion of patients on statins or antiplatelet therapy, mean or median duration of follow-up, number of patients lost to follow-up, high-risk features considered with the specific definition criteria used in the study, number of patients with each high-risk feature, number of ipsilateral ischemic CVE (stroke or transient ischemic attack) recorded during follow-up, method used to ascertain the CVE, hazard ratio associated with the occurrence of ipsilateral ischemic CVE, type and number of ipsilateral ischemic CVE occurring in patients with or without high-risk features on vascular imaging.

NOTE 2: Ultrasound was the most commonly used vascular imaging modality (48 studies, 71.6%). Echolucency (17 studies), intraplaque hemorrhage (16 studies), microembolic signals (15 studies), lipid-rich necrotic core (11 studies) and neovascularization (10 studies) were the most frequently reported high-risk features.

Table III. Risk of Bias Assessment Tool

Risk of bias item	**Response:** Yes = 1, No = 0
External validity	
1. Was the study target population selected without restriction on age/sex/race/profession/marital status or other socio-demographic criteria that would limit the diversity of the sample and therefore its representativeness and the generalizability of the result?	
2. Was the study target population a true or close representation of the population of patients with asymptomatic carotid stenosis (no restriction on degree of stenosis or comorbidities)?	
3. Was some form of random selection used to select the sample, OR, was a census or a consecutive/exhaustive sampling undertaken?	
4. Was the likelihood of loss to follow-up and non-participation bias minimal? (probability that investigators have failed to include subjects that would normally be eligible or could not follow-up specific subgroups of patients)	
Internal Validity	
5. Were data collected prospectively directly from the participants (as opposed to mere review of medical records or retrospective data collection)?	
6. Was the process of identifying patients with asymptomatic carotid stenosis appropriate and clearly described?	
7. Was the diagnostic method (brain imaging) used to identify high-risk carotid plaque and cerebrovascular events clearly described (type of imaging, *eventually with sequences and qualification of the reader*)?	
8. Was the same assessment protocol used for all the participants?	
9. Were the results of the plaque imaging clearly presented? (adequate reporting of data within each category of lesion/patients + discrete categories/no overlapping + no errors requiring a guess/adjustments)	
10. Were the numerator(s) and denominator(s) appropriate for the calculation of the prevalence of high-risk plaque or the incidence of cerebrovascular events?	
Interpretation of the score 8 – 10: Low Risk of Bias / High-quality study. Further research is very unlikely to change our confidence in the estimate. 5 – 7: Moderate Risk of Bias / Moderate-quality study. Further research is likely to have an important impact on our confidence in the estimate and may change the estimate. 4 or less: High Risk of Bias / Low-quality study. Further research is very likely to have an important impact on our confidence in the estimate and is likely to change the estimate. Further research is mandatory.	

Adapted from Hoy D, Brooks P, Woolf A, et al. Assessing risk of bias in prevalence studies: modification of an existing tool and evidence of interrater agreement. J Clin Epidemiol. 2012;65:934-39, Copyright © 2012, Elsevier.

Table IV. Subgroup Analysis for the Prevalence of High-Risk Features in Asymptomatic Carotid Stenosis

	Number of studies	Number of participants	Prevalence (95%CI)	I^2	p-values			
					Heterogeneity		Egger's test	Meta-regression
					Within subgroup	Between subgroups		
Decade of publication								
1990-1999	13	6585	18.7 (14.4-23.5)	88.4	<0.001	<0.001	0.52	0.28
2000-2009	13	2228	31.7 (24.2-39.6)	93.5	<0.001		0.25	
2010-2019	37	11938	27.1 (22.2-323)	98	<0.001		0.04	
Enrolment before endarterectomy								
Yes	9	1545	27.2 (19.0-36.2)	98	<0.001	0.9	0.001	0.82
No	54	19206	26.5 (22.5-30.8)	91.9	<0.001		0.7	
Settings								
Hospital	56	9615	26.2 (22.3-30.4)	95.9	<0.001	0.6	0.45	0.53
Population	7	11136	29.6 (18.1-42.6)	99.6	<0.001		0.84	
Grade of stenosis								
Mild only	1	96	21.4 (5.4-43.9)	NE	NE	0.4	0.16	0.89
Mild and moderate	3	154	41.3 (21.6-62.9)	89.6	<0.001		0.55	
Moderate only	3	374	31.6 (14.5-51.6)	97	<0.001		0.17	
Moderate and severe	20	5184	30.1 (22.8-37.9)	97.5	<0.001		0.43	
Severe only	15	2456	26.2 (18.9-34.2)	94	<0.001		0.07	
Any grade	21	12487	22.3 (16.5-28.6)	98.6	<0.001		0.41	
Mean age of participants (years)								

Below 70	25	6659	29.4 (21.5-38.0)	98.3	<0.001	0.6	0.11	0.25
At least 70	26	10087	25.1 (20.8-29.5)	96.5	<0.001		0.42	
Not indicated	12	4005	24.8 (15.9-34.9)	97.5	<0.001		0.26	
Proportion of male participants								
Below 68%	25	11663	30.1 (24.3-36.2)	98.4	<0.001	0.2	0.09	0.56
At least 68%	27	7521	25.4 (20.5-30.7)	95.9	<0.001		<0.001	
Not indicated	11	1567	20.0 (11.6-29.9)	91.8	<0.001		0.02	
Proportion of participants with hypertension								
Below 72%	20	12590	25.3 (17.7-33.8)	99	<0.001	0.3	0.96	0.64
At least 72%	25	4854	29.1 (24.7-33.6)	94.4	<0.001		0.28	
Not indicated	18	3307	22.8 (16.2-30.2)	16.2	<0.001		0.06	
Proportion of participants with diabetes mellitus								
Below 24%	25	15527	27.2 (22.2-32.6)	98.1	<0.001	0.5	0.62	0.89
At least 24%	22	3152	28.2 (20.5-36.6)	97.7	<0.001		0.12	
Not indicated	16	2072	21.7 (14.1-30.4)	93.4	<0.001		0.02	
Proportion of smokers								
Below 32%	23	5819	28.8 (22.0-36.2)	97.9	<0.001	0.2	0.03	0.67
At least 32%	20	6149	28.4 (23.6-33.4)	95.2	<0.001		<0.001	
Not indicated	20	8783	20.9 (14.7-27.8)	97.5	<0.001		0.96	
Proportion of participants with dyslipidemia								
Below 70%	14	14	27.6 (20.3-35.5)	95	<0.001	0.7	0.002	0.67
At least 70%	13	2153	29 (21.4-37.3)	95.8	<0.001		0.03	
Not indicated	36	16716	24.8 (19.8-30.2)	98.4	<0.001		0.41	

Proportion of participants with coronary artery disease								
Below 33%	16	6735	26.7 (19.4-34.7)	98.4	<0.001	0.7	0.09	0.69
At least 33%	19	4593	28.2 (22.4-34.5)	95.9	<0.001		0.24	
Not indicated	28	9423	24.8 (18.7-31.4)	97.8	<0.001		0.29	
Proportion of participants with peripheral artery disease								
Below 30%	8	1228	26.4 (17.0-37.1)	95.8	<0.001	0.6	0.64	0.57
At least 30%	8	1648	30.9 (22.4-40.1)	92.7	<0.001		0.05	
Not indicated	47	17875	25.9 (21.5-30.6)	98.2	<0.001		0.61	
Proportion of participants treated with statins								
Below 69%	12	4654	32.8 (21.1-45.7)	99.1	<0.001	0.4	0.54	0.15
At least 69%	12	966	23.6 (17.4-30.5)	91.8	<0.001		0.39	
Not indicated	39	15131	25.9 (21.6-30.3)	97.2	<0.001		0.45	
Proportion of participants treated with antiplatelets								
Below 78%	14	2526	17.8 (12.3-24.1)	93.9	< 0.001	< 0.001	0.04	0.002
At least 78%	12	2733	34.6 (27.9-41.6)	93.2	< 0.001		0.01	
Not indicated	37	15492	26.7 (22.2-31.6)	98	< 0.001		0.62	

CI = confidence interval

NE = not estimable because of the small number of studies ($n \leq 3$)

Table V. Prevalence of High-Risk Features in the 20 Studies Reporting Data on Symptomatic Carotid Stenosis

	Number of studies	Number of participants with symptomatic carotid stenosis	Number of cases	Prevalence in symptomatic patients (95% CI)	I^2	p-values		Prevalence in asymptomatic patients (95% CI)	OR (95% CI) [c]
						Heterogeneity	Egger's test		
Overall analysis									
Any high-risk feature	20	1652	NA [a]	43.3 (33.6-53.2)	95.7	<0.001	0.31	19.9 (14.5-25.8)	3.4 (2.5-4.6)
Specific high-risk features									
AHA type IV,V,VI†	2	75	47	62.8 (51.4-73.6)	NA	NA	NA	23.0 (14.8-32.4)	6.0 (3.0-12.0)
Echolucency	4	609	469	75.9 (61.1-88.1)	92.9	<0.001	0.67	51.6 (45.0-58.1)	3.1 (1.2-8.4)
Impaired cerebrovascular reserve†	0	NA	NA	NA	NA	NA	NA	NA	NA
Intraplaque haemorrhage	4	252	57	17.7 (4.7-36.2)	90.3	<0.001	0.42	13.2 (2.2-30.6)	1.2 (0.4-3.4)
Ipsilateral silent brain infarcts [b]	2	263	119	45.2 (39.2-51.3)	NA	NA	NA	14.6 (9.3-20.7)	5.0 (3.0-8.3)
Lipid-rich necrotic core [b]	1	52	20	38.5 (26.5-52.0)	NA	NA	NA	23.5 (16.4-32.6)	2.0 (0.9-4.2)
Microembolic signals	7	377	148	41.3 (28.8-54.5)	84.6	<0.001	0.16	11.6 (6.5-17.6)	5.7 (2.7-12.1)
Mural thrombus [b]	1	40	0	NA	NA	NA	NA	7.3 (2.5-19.4)	0.1 (0.0-2.7)
Neovascularization	3	168	101	65.8 (39.9-87.7)	90.9	<0.001	0.25	32.8 (11.9-58.1)	4.2 (2.1-8.3)
Plaque irregularity [b]	1	52	22	42.3 (29.9-55.8)	NA	NA	NA	34.1 (21.9-48.9)	1.4 (0.6-3.3)
Thin/ruptured fibrous cap [b]	2	92	25	27.1 (18.4-36.8)	NA	NA	NA	7.5 (3.5-12.6)	4.7 (2.1-10.3)
Ulceration	4	418	120	34.6 (17.8-53.6)	90.2	<0.001	0.43	14.9 (9.0-21.9)	3.0 (1.5-6.2)

[a] The number of patients with at least one high-risk feature was not provided in each study and cannot be computed because some participants had more than one high-risk feature. The overall prevalence is obtained by pooling the prevalence of specific high-risk features across studies.

[b] The I^2 index and the p-values for heterogeneity and risk of bias are provided for prevalence estimates in symptomatic patients. The parameters cannot be estimated when the number of studies is ≤ 3.

[c] Odds ratio of finding a high-risk plaque or a specific high-risk plaque feature in symptomatic versus asymptomatic patients.

Table VI. Subgroup Analysis for the Incidence of Ipsilateral Ischemic Stroke in Asymptomatic Carotid Stenosis With High-Risk Features

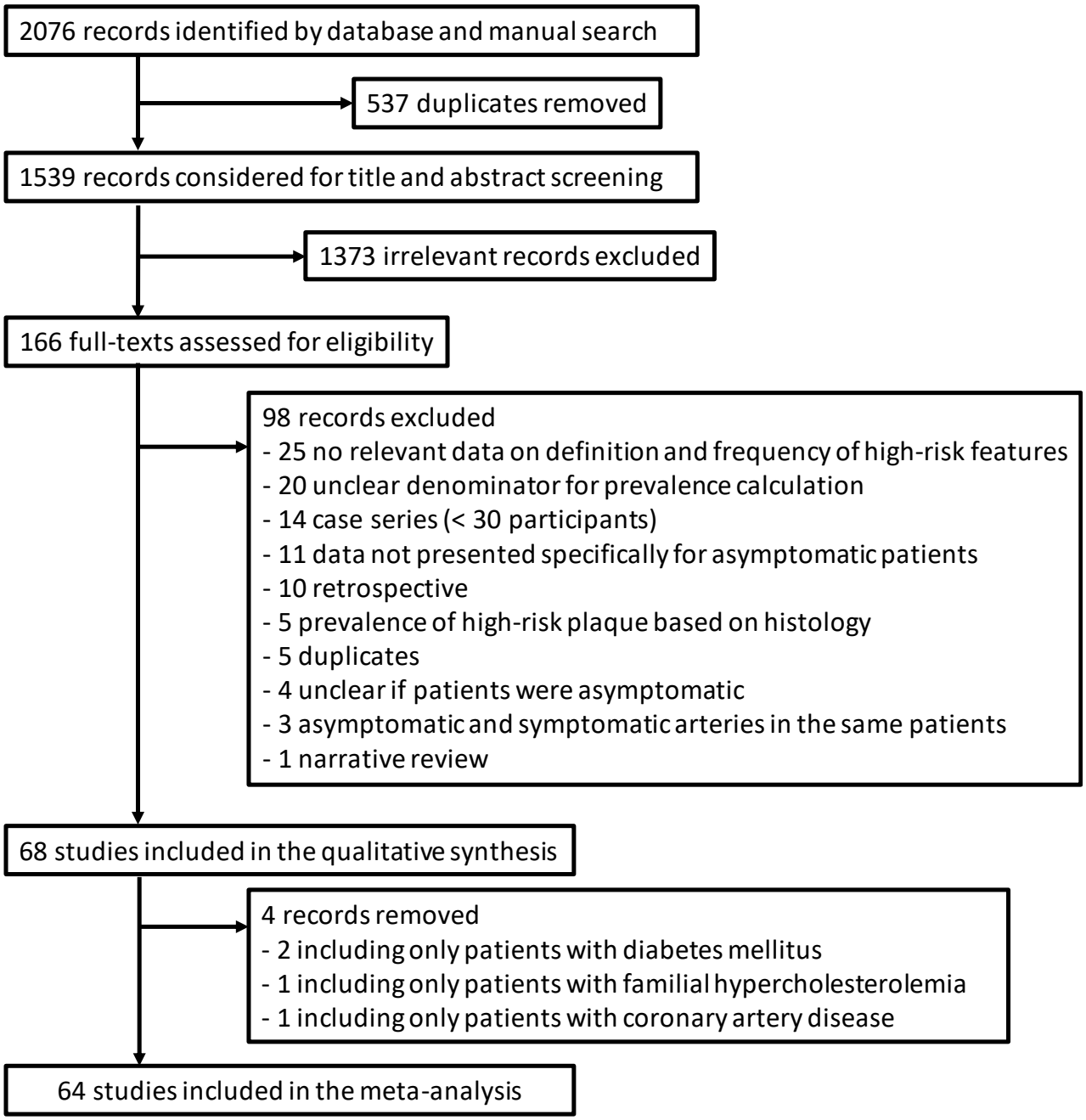
	Number of studies	Number of participants	Incidence per 100 person-years (95% CI)	I^2	p-values			
					Heterogeneity		Egger's test	Meta-regression
					Within subgroup	Between subgroups		
Mean age of participants (years)								
Below 70	7	1159	8.6 (3.2-15.9)	85.9	< 0.001	< 0.001	0.002	0.38
At least 70	7	5986	3.9 (1.6-7.0)	90.7	< 0.001		0.03	
Not indicated	3	1948	2.3 (0.0-8.1)	NE	NE		0.50	
Proportion of male participants								
Below 65%	8	6150	4.9 (2.3-8.2)	90.5	< 0.001	< 0.001	0.001	0.86
At least 65%	6	1747	4.1 (0.9-8.7)	89.3	< 0.001		0.04	
Not indicated	3	1196	14.3 (0.0-41.2)	NE	NE		0.12	
Proportion of participants with hypertension								
Below 70%	7	6200	3.5 (1.4-6.3)	90.6	< 0.001	< 0.001	0.002	0.43
At least 70%	6	1474	4.5 (2.3-7.3)	68.6	<0.001		0.21	
Not indicated	4	1419	4.2 (1.2-8.2)	68.3	< 0.001		0.07	
Proportion of participants with diabetes mellitus								
Below 21%	8	7260	1.9 (0.8-3.4)	83.8	< 0.001	< 0.001	0.02	0.22
At least 21%	5	1474	8.3 (3.9-14.0)	78.0	<0.001		0.007	
Not indicated	4	1419	4.2 (1.2-8.2)	68.3	< 0.001		0.07	
Proportion of smokers								
Below 27%	6	1540	4.7 (1.6-9.1)	83.7	< 0.001	< 0.001	0.06	0.67

At least 27%	6	657	6.5 (3.3-10.4)	72.4	<0.001		0.004	
Not indicated	5	6896	1.5 (0.0-4.3)	94.6	< 0.001		0.09	
Proportion of participants with dyslipidemia								
Below 64%	4	260	9.9 (6.7-13.6)	6.2	0.4	< 0.001	0.12	0.36
At least 64%	3	824	4.0 (1.2-8.1)	NE	NE		0.21	
Not indicated	10	8009	2.4 (0.7-4.6)	91.5	< 0.001		0.01	
Proportion of participants treated with statins								
Below 64%	5	1734	3.4 (1.2-6.7)	88.7	< 0.001	< 0.001	0.04	0.64
At least 64%	2	183	5.1 (2.2-8.9)	NE	NE		NE	
Not indicated	10	7176	5.8 (2.6-9.8)	92.2	< 0.001		0.001	
Proportion of participants treated with antiplatelets								
Below 88%	5	1593	5.0 (1.0-11.1)	90.1	< 0.001	< 0.001	0.01	0.42
At least 88%	3	227	7.1 (1.5-15.9)	NE	NE		0.76	
Not indicated	9	7273	3.8 (1.5-6.7)	91.8	< 0.001		0.01	

CI = confidence interval

NE = not estimable because of the small number of studies ($n \leq 3$)

Figure I. Study Selection

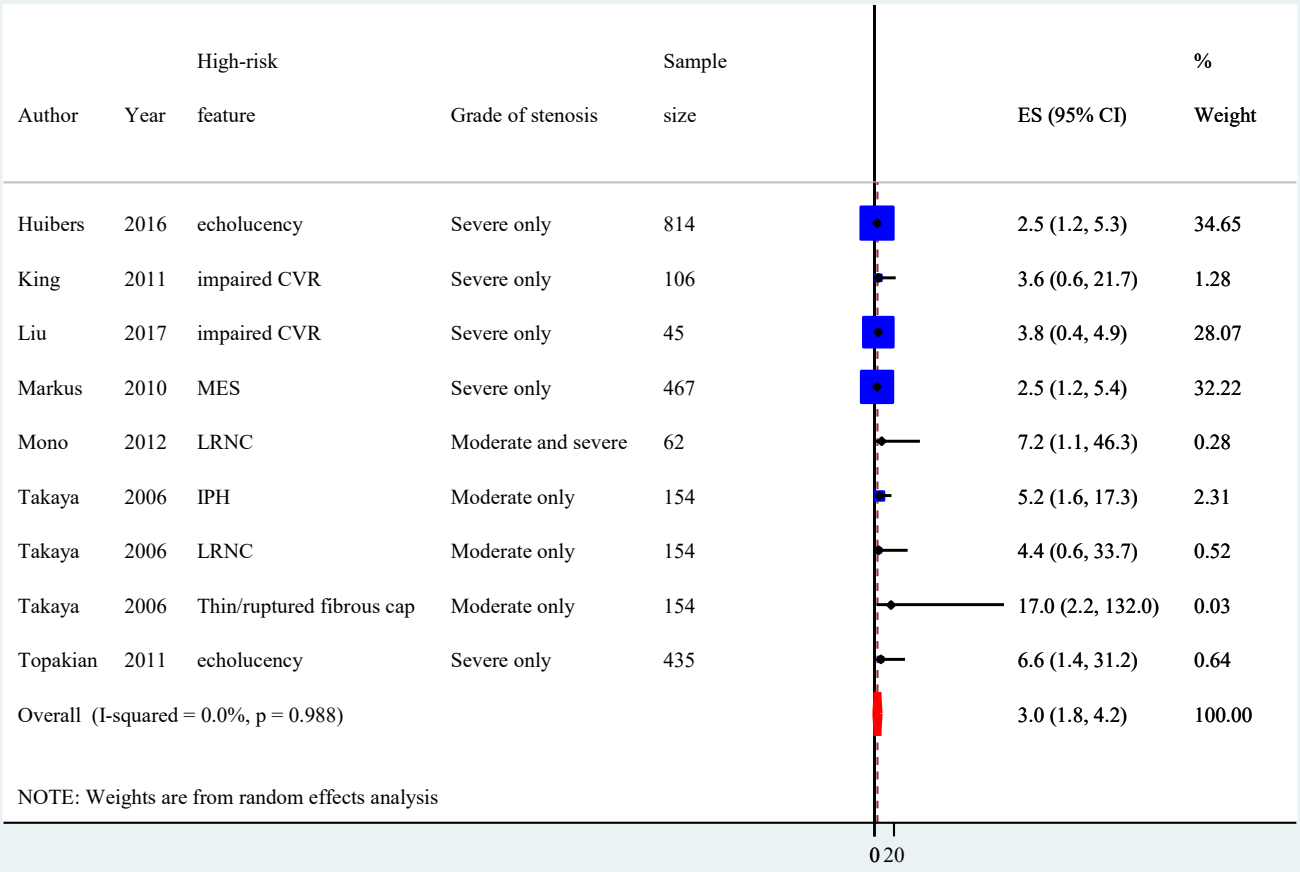


NOTE

1: We included prospective observational studies reporting the prevalence of high-risk plaques in subjects with asymptomatic carotid stenosis. We excluded: (i) studies with retrospective data collection; (ii) studies using definitions of carotid stenosis or high-risk plaque features that were not scientifically valid; (iii) studies focusing on plaque progression and studies reporting high-risk plaque features identified by histopathology after endarterectomy; (iv) studies using techniques that are not widely available, have not been well validated (videodensitometry, ultrasound elastography, microwave radiometry), or lack a consistent cut-off to discriminate high-risk from low-risk plaques (FDG-PET); (v) studies assessing an asymptomatic stenosis in patients with contralateral symptomatic stenosis, studies focusing on carotid occlusion, and case series with small sample size (< 30 participants).

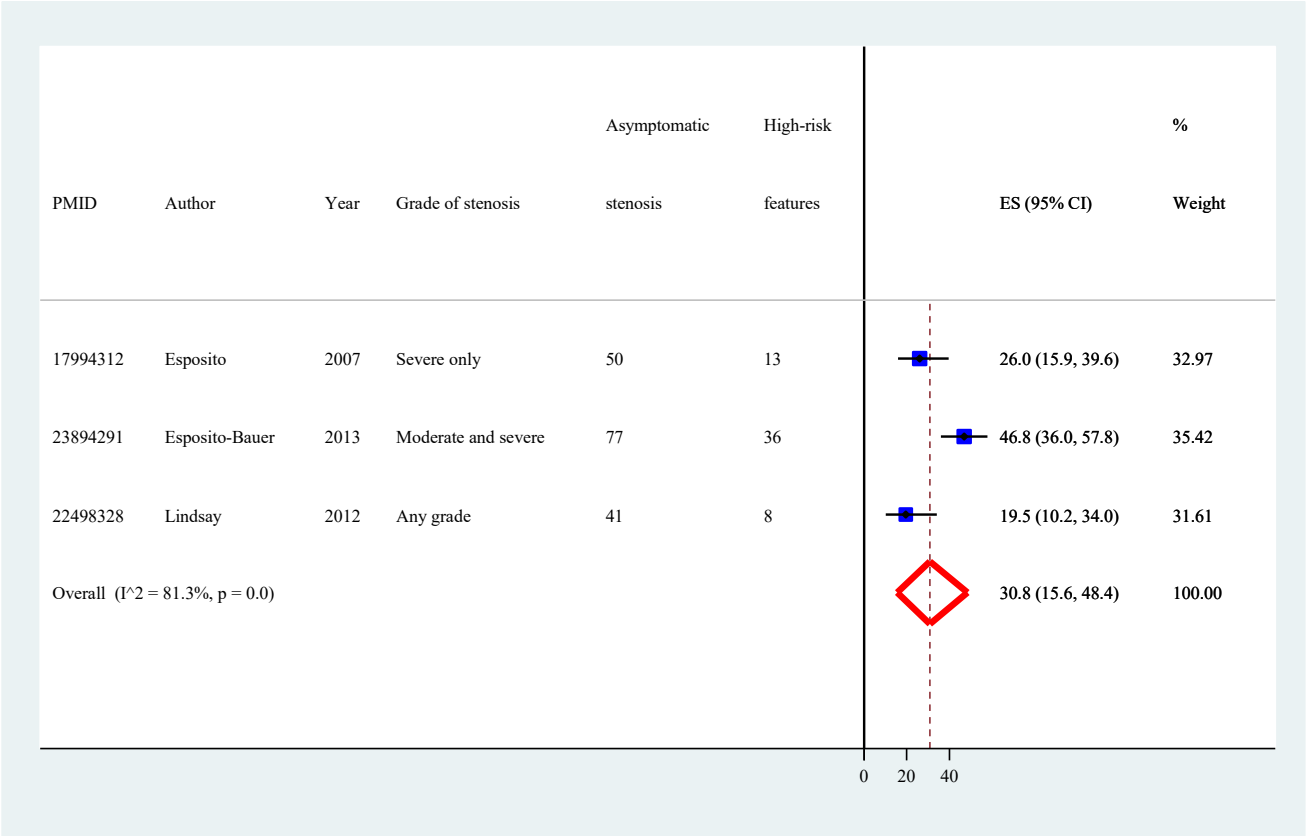
NOTE 2: Of the 26 cohorts eligible, only 22 studies were included in the meta-analysis. We did not consider one study focusing on patients with diabetes mellitus,[483] one study reporting plaque progression rather than ischemic events as outcome,[520] one study that did not indicate if the events were ipsilateral to the stenosis and how they were ascertained,[521] and one study[484] using data from a subset of the ACSRS cohort already included[497]. When reporting the occurrence of ipsilateral ischemic cerebrovascular events, 20 studies made the distinction between ischemic stroke and transient ischemic attack. The occurrence of ipsilateral ischemic cerebrovascular events was also reported separately for the subset of patients with high-risk features in 17 studies (9093 participants) and only 10 made the distinction between ischemic stroke and transient ischemic attack. Data on transient ischemic attacks were available in only 9 studies.

Figure II. Pooled Adjusted Hazard Ratio of Ipsilateral Ischemic Cerebrovascular Events in Patients With High-Risk Asymptomatic Carotid Stenosis



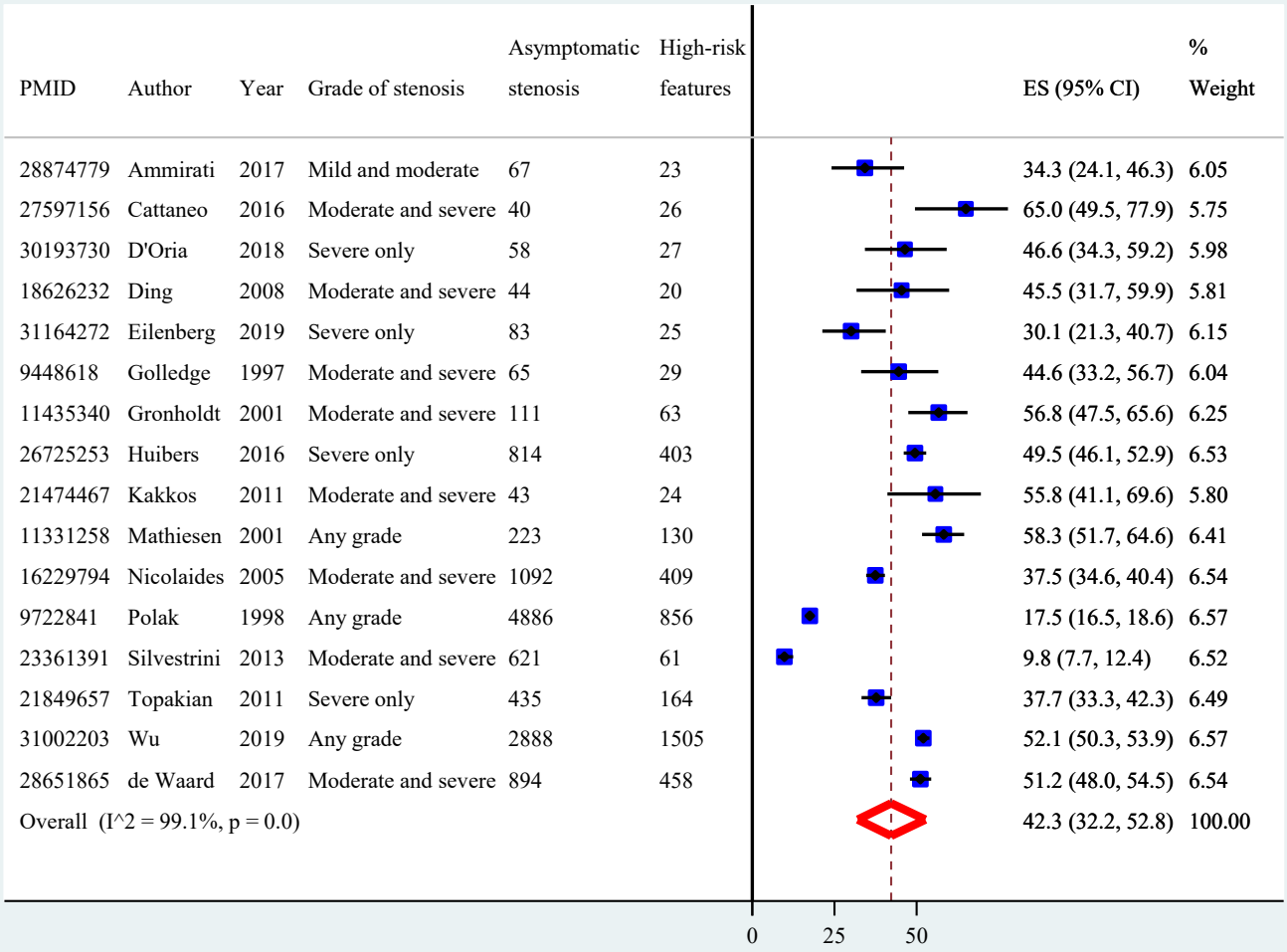
NOTE: Only seven studies reported the adjusted hazard ratio of ipsilateral ischemic cerebrovascular events for the high-risk features considered. ES = effect size (representing adjusted hazard ratio in this case)

Figure III. Prevalence of Plaque with High-Risk Features in Asymptomatic Carotid Stenosis in Studies Using the AHA Classification



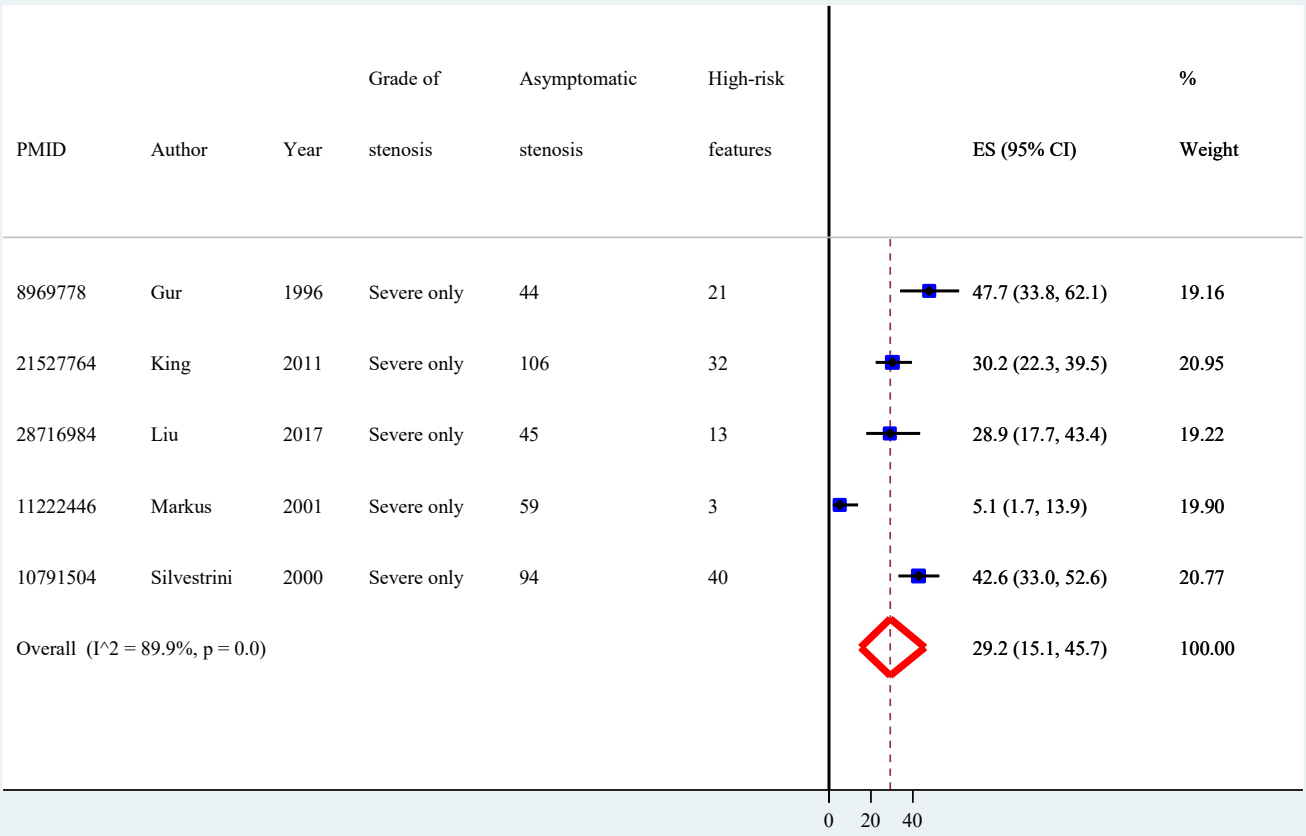
ES = effect size (representing prevalence in this case)

Figure IV. Prevalence of Echolucent Plaques in Asymptomatic Carotid Stenosis



ES = effect size (representing prevalence in this case)

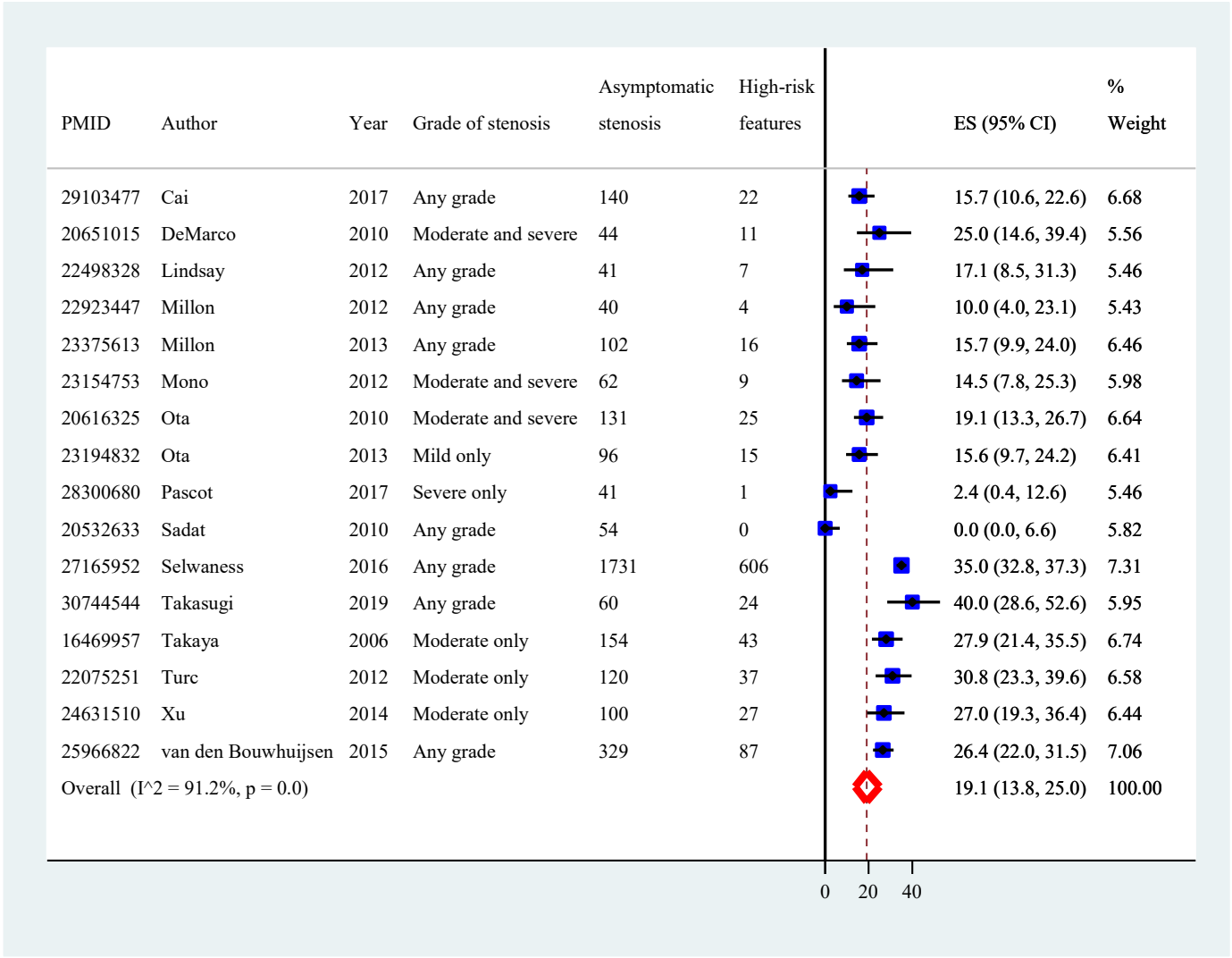
Figure V. Prevalence of Impaired Cerebrovascular Reserve in Asymptomatic Carotid Stenosis



ES = effect size (representing prevalence in this case)

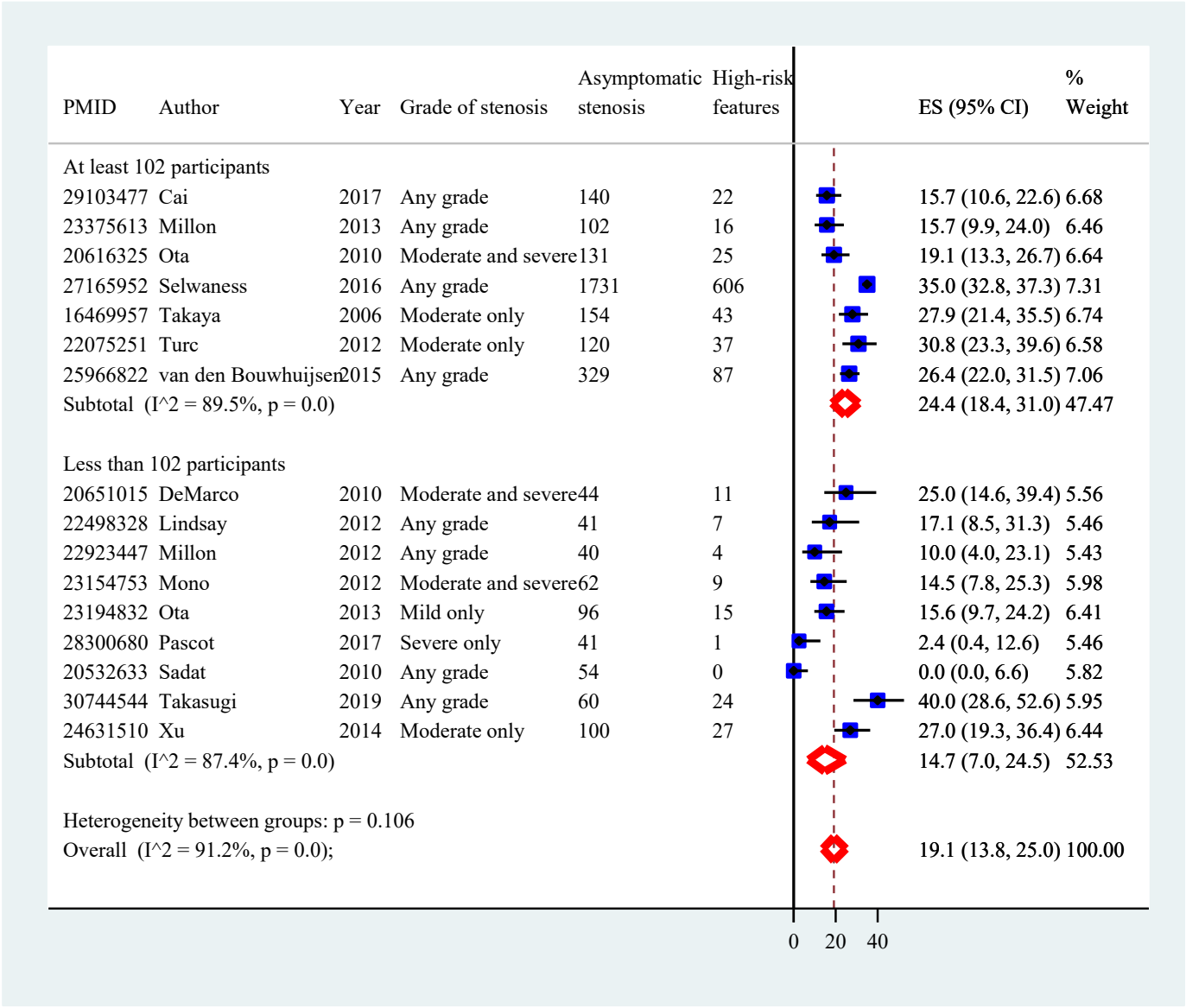
Figure VI. Prevalence of Intraplaque Hemorrhage in Asymptomatic Carotid Stenosis

A. Overall



ES = effect size (representing prevalence in this case)

B. According to the study sample size



There was a publication bias for studies of intraplaque hemorrhage (p <0.001, Table 2) with small studies reporting lower prevalence (14.7% versus 24.4%; also see supplemental figure 6C for the funnel plot).

ES = effect size (representing prevalence in this case)

C. Funnel plot for the meta-analysis of prevalence of intraplaque hemorrhage in asymptomatic carotid stenosis

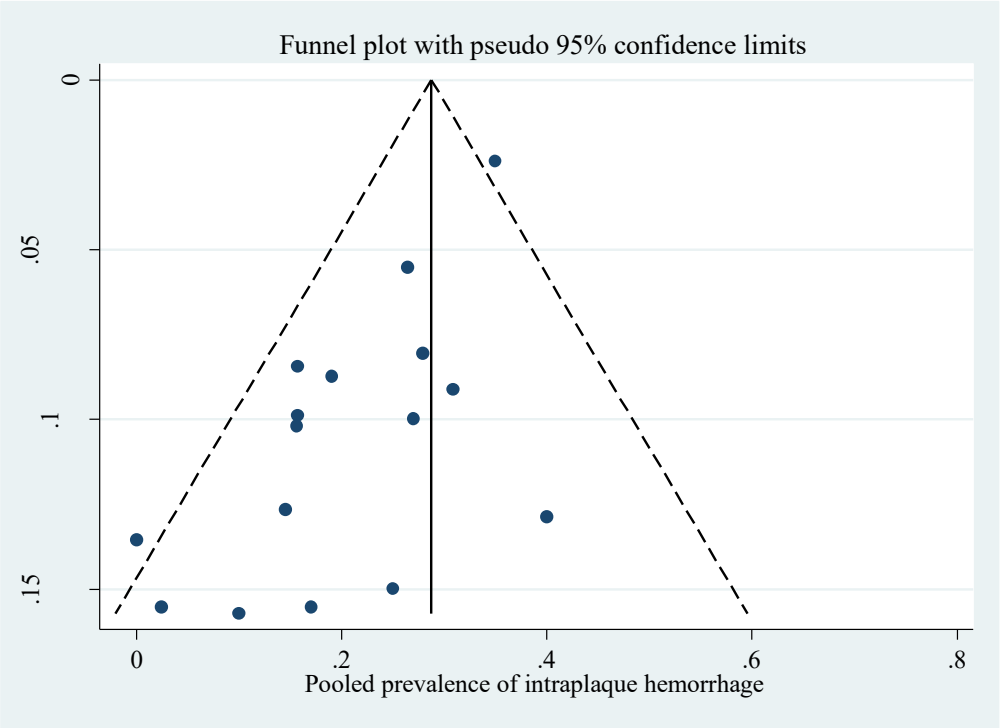
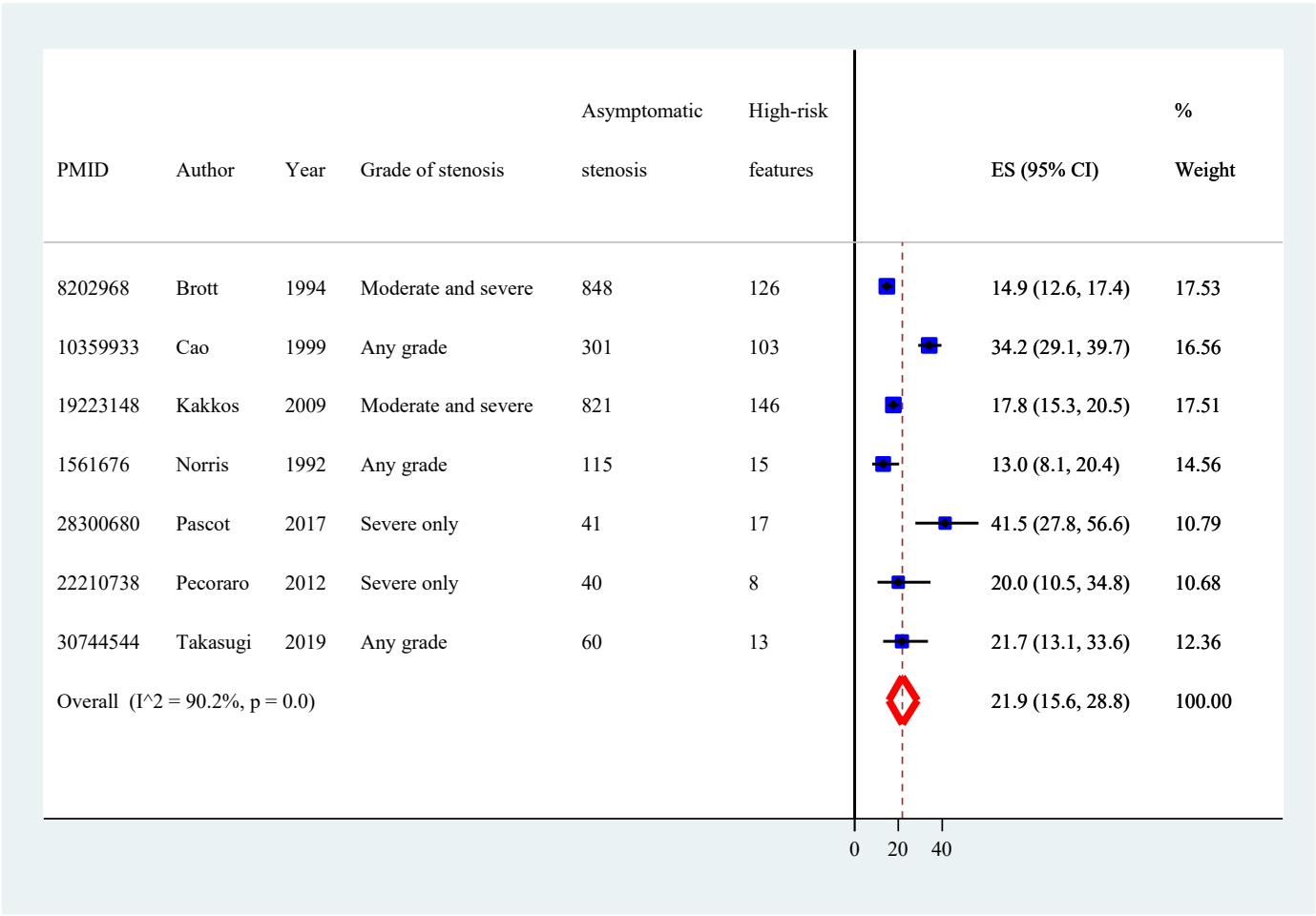


Figure VII. Prevalence of Ipsilateral Silent Brain Infarcts in Asymptomatic Carotid Stenosis



ES = effect size (representing prevalence in this case)

Figure VIII. Prevalence of Plaque With Lipid-Rich Necrotic Core in Asymptomatic Carotid Stenosis

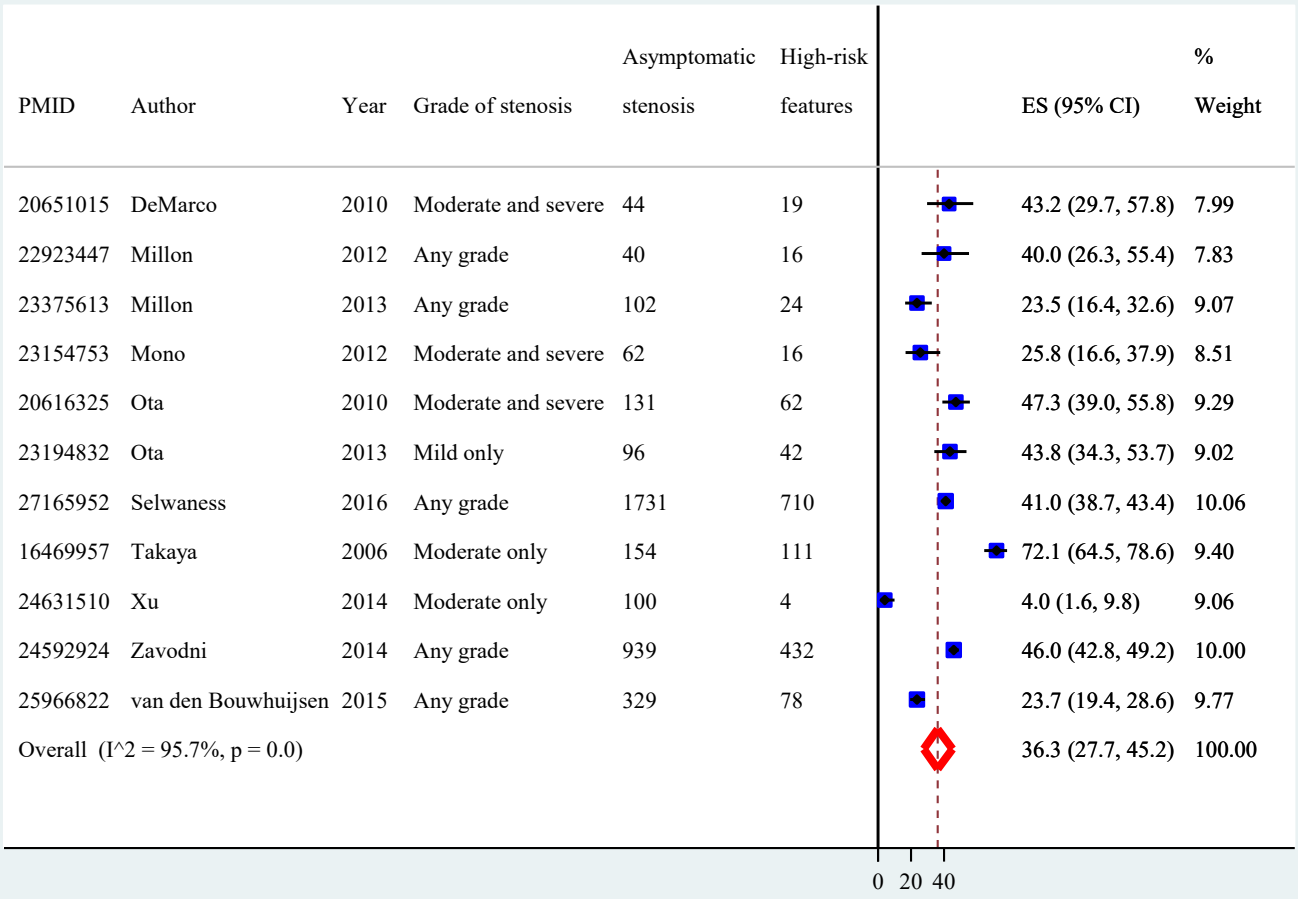
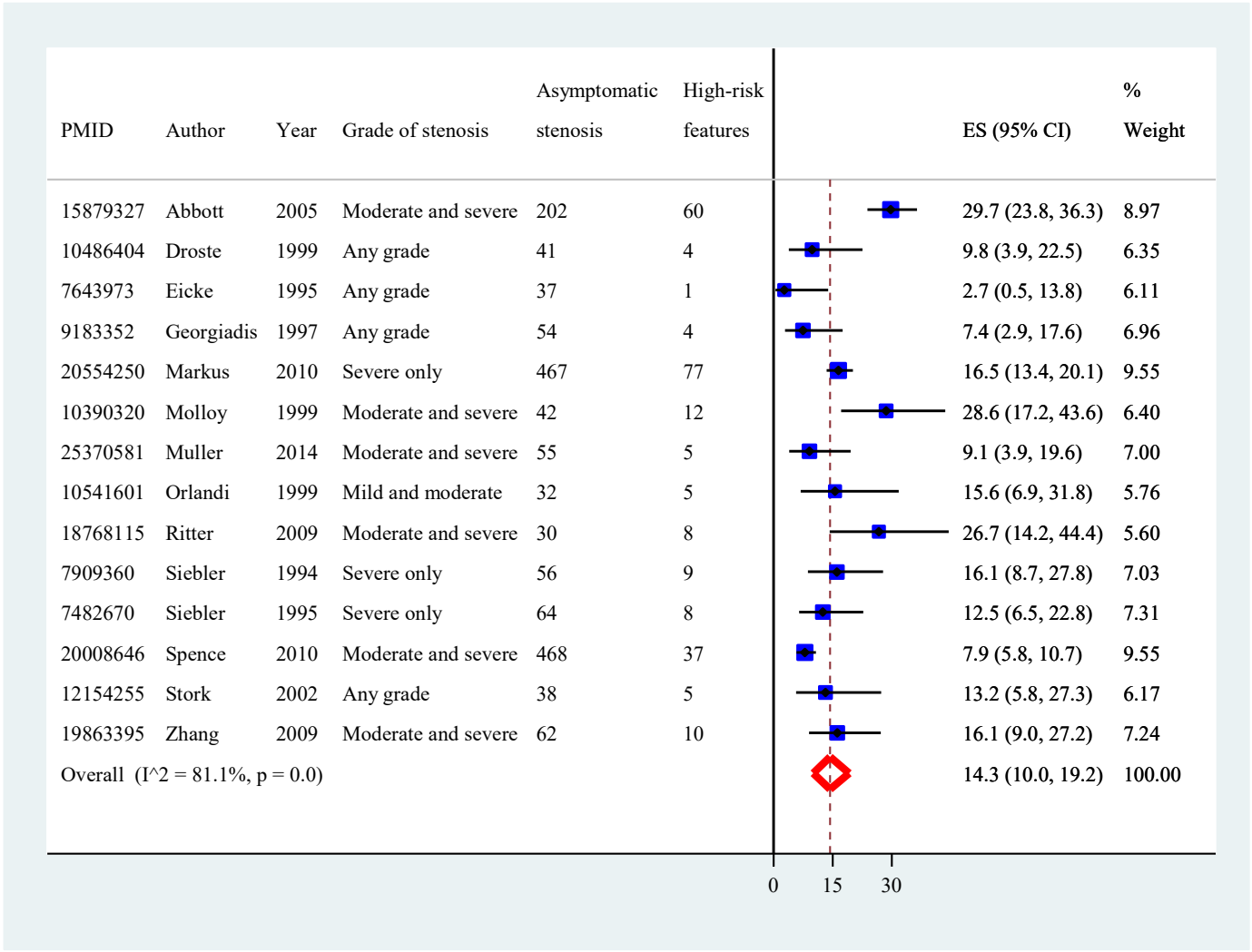


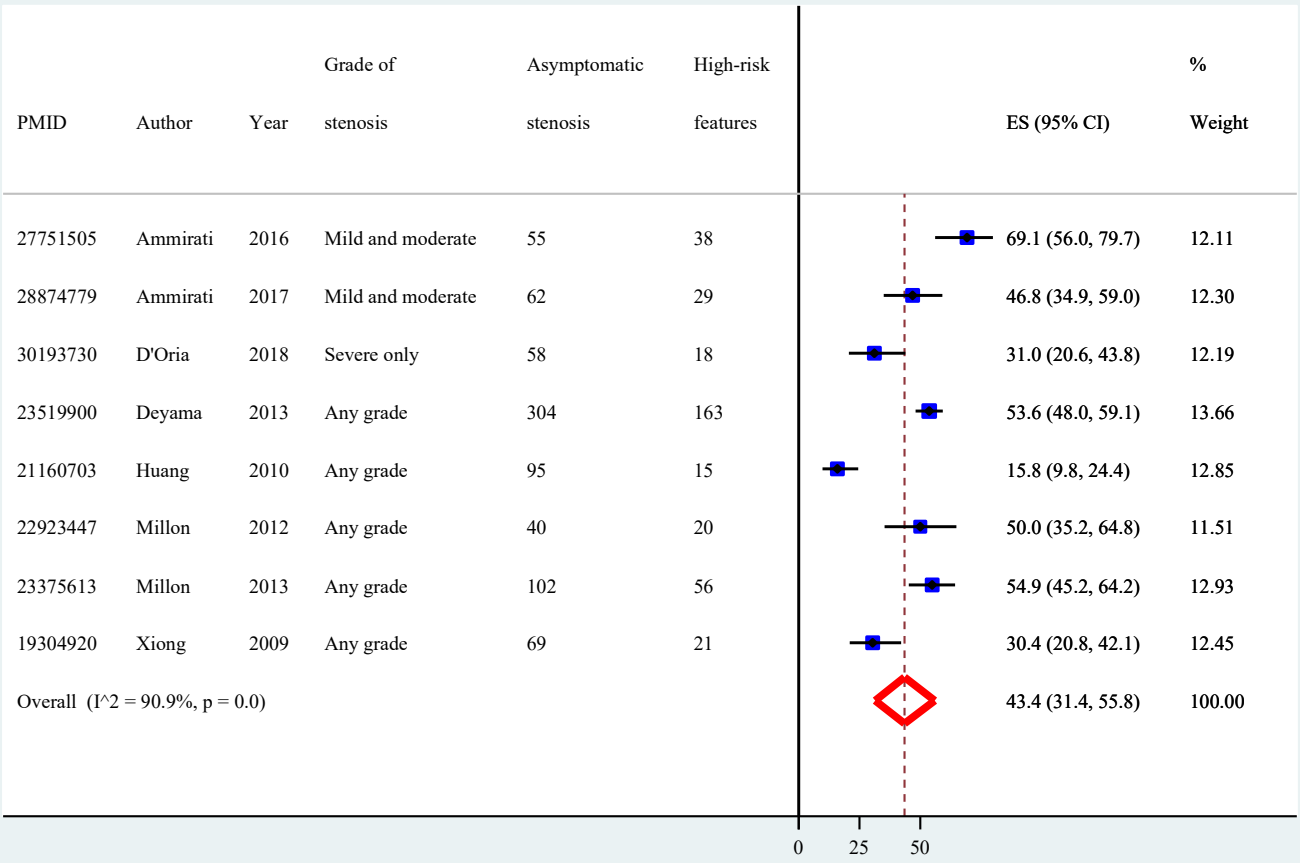
Figure IX. Prevalence of Microembolic Signals in Asymptomatic Carotid Stenosis



ES = effect size (representing prevalence in this case)

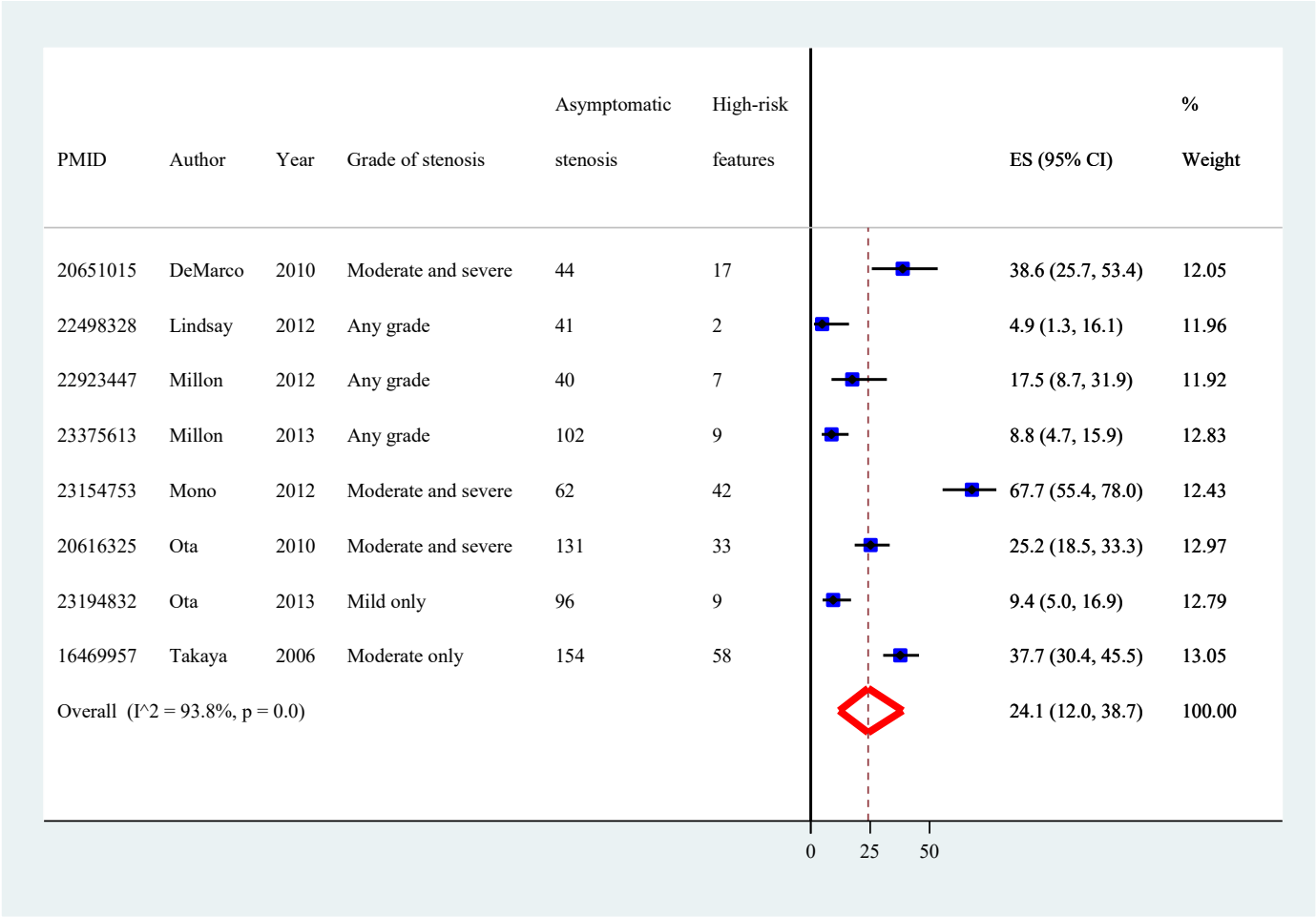
NOTE: Microembolic signals had a relatively low prevalence (14.3%), which might be expected in a population of asymptomatic patients. The low prevalence may also be explained by the transient nature of this marker. Indeed, it is unclear if the absence of microembolic signals on a classical one-hour monitoring equates to the absence of the same outside the monitoring period. Beyond the duration of the monitoring, there are several other parameters affecting the presence of microembolic signals, including the time of the day, and the treatment with statins and antiplatelet drugs.

Figure X. Prevalence of Plaque With Neovascularization in Asymptomatic Carotid Stenosis



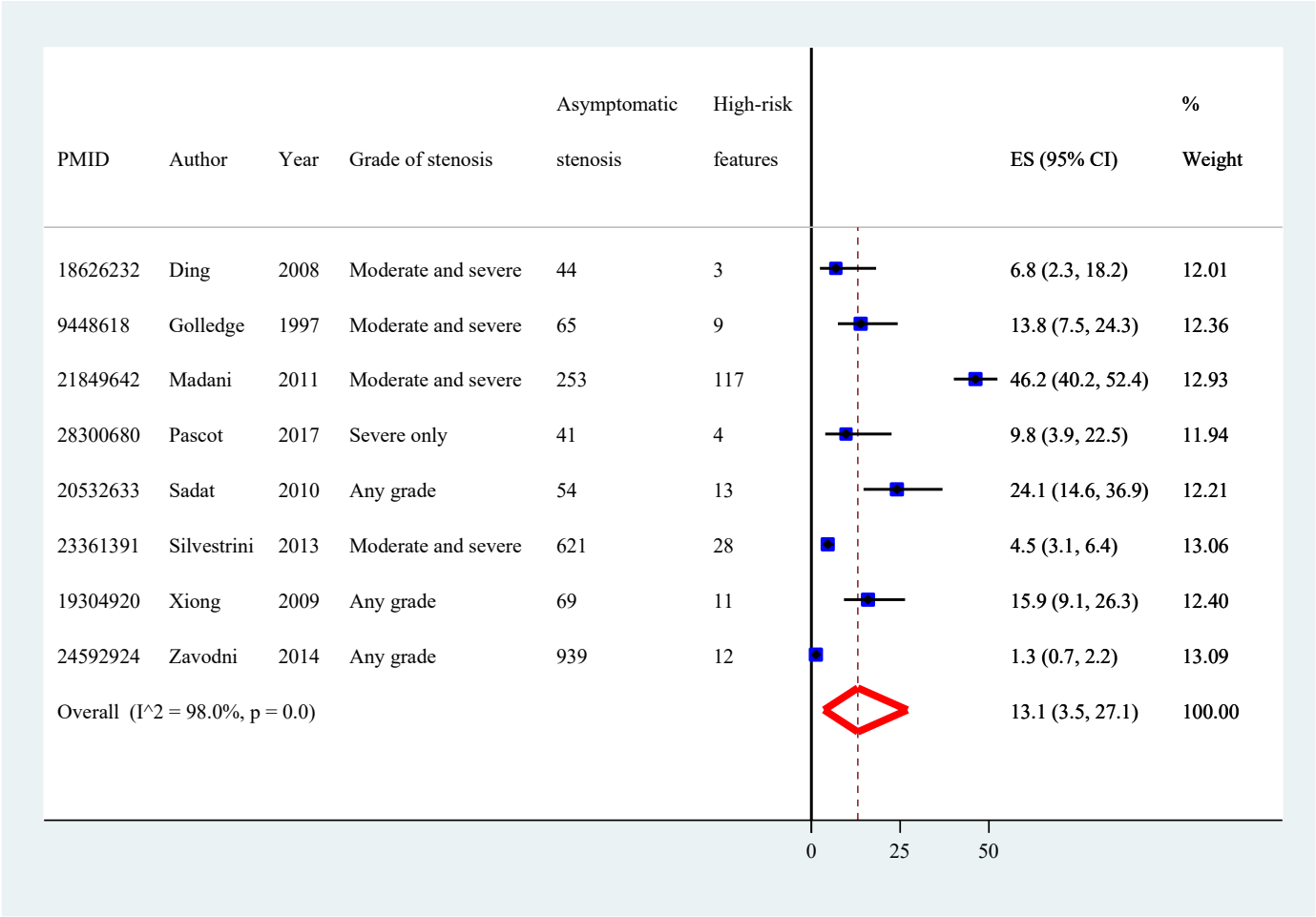
ES = effect size (representing prevalence in this case)

Figure XI. Prevalence of Plaque With Thin or Ruptured Fibrous Cap in Asymptomatic Carotid Stenosis



ES = effect size (representing prevalence in this case)

Figure XII. Prevalence of Plaque Ulceration in Asymptomatic Carotid Stenosis



ES = effect size (representing prevalence in this case)

Figure XIII. Funnel Plot for the Meta-analysis of Prevalence of Plaque With High-Risk Features in Asymptomatic Carotid Stenosis

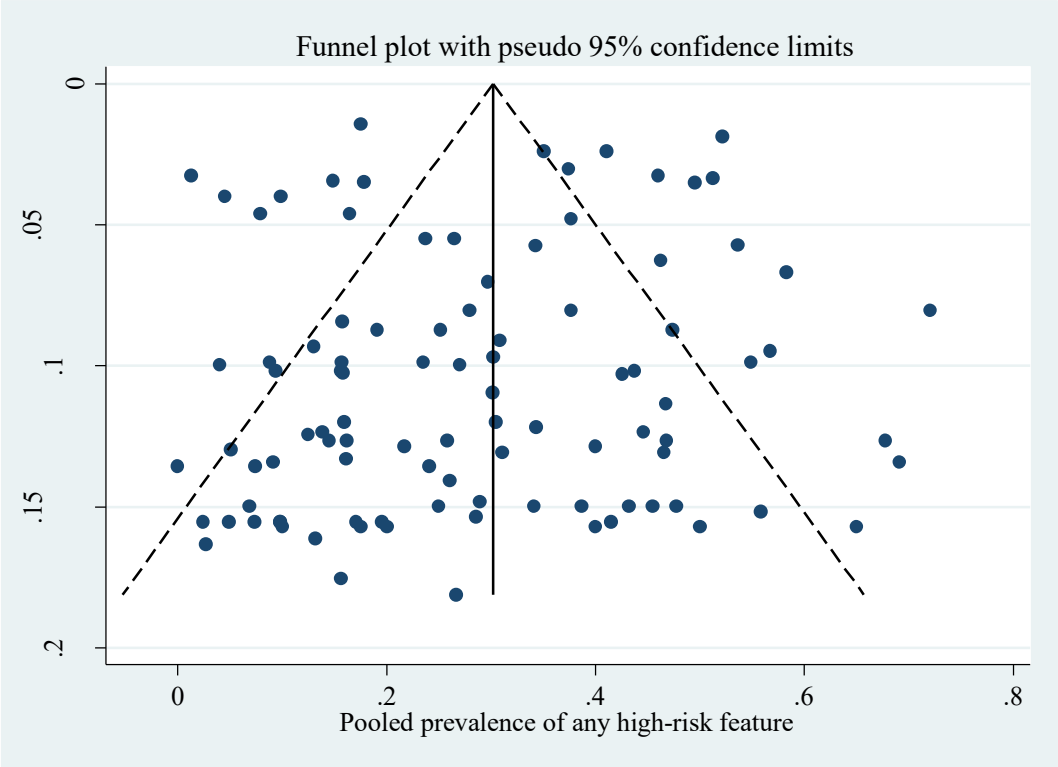
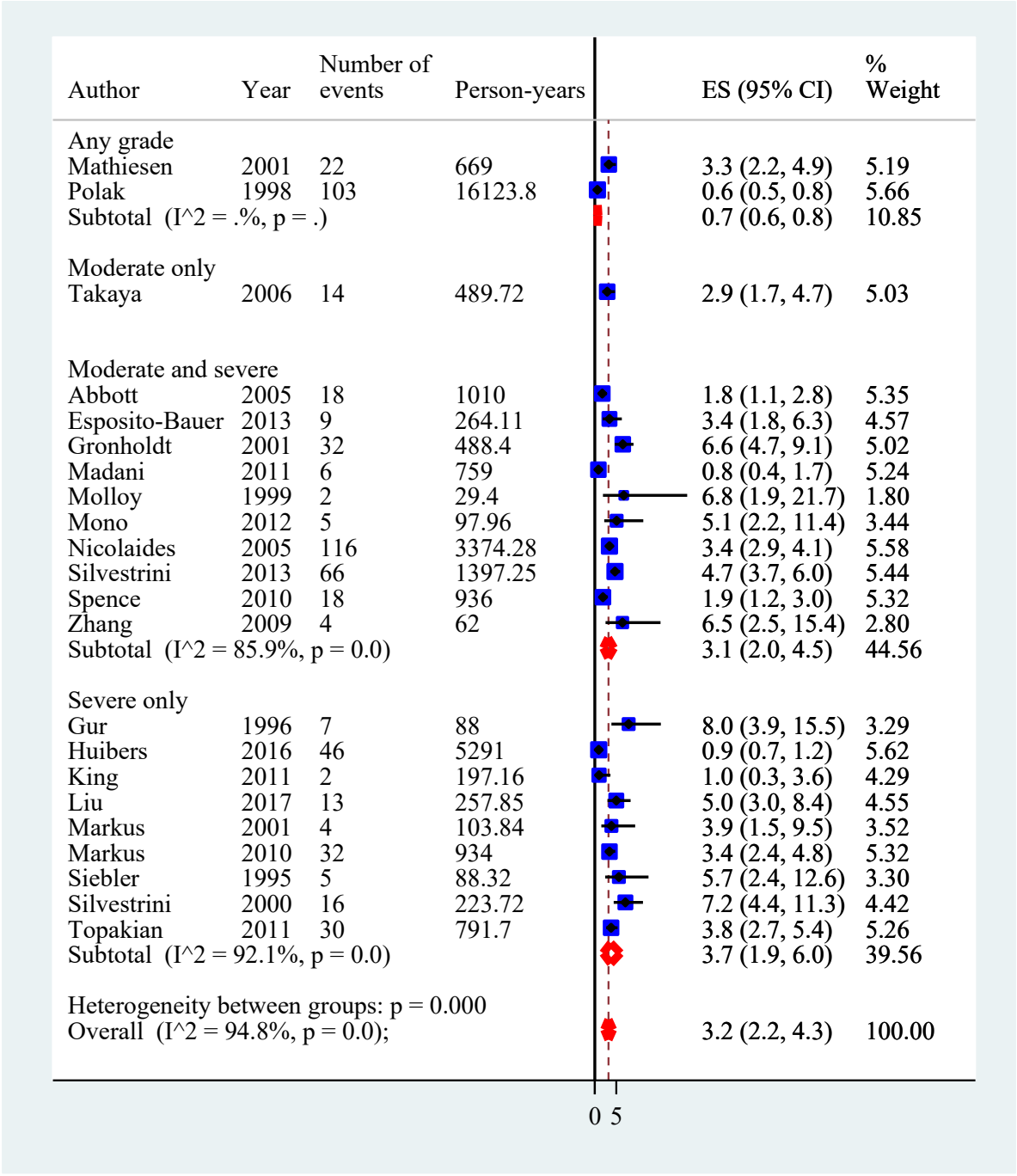
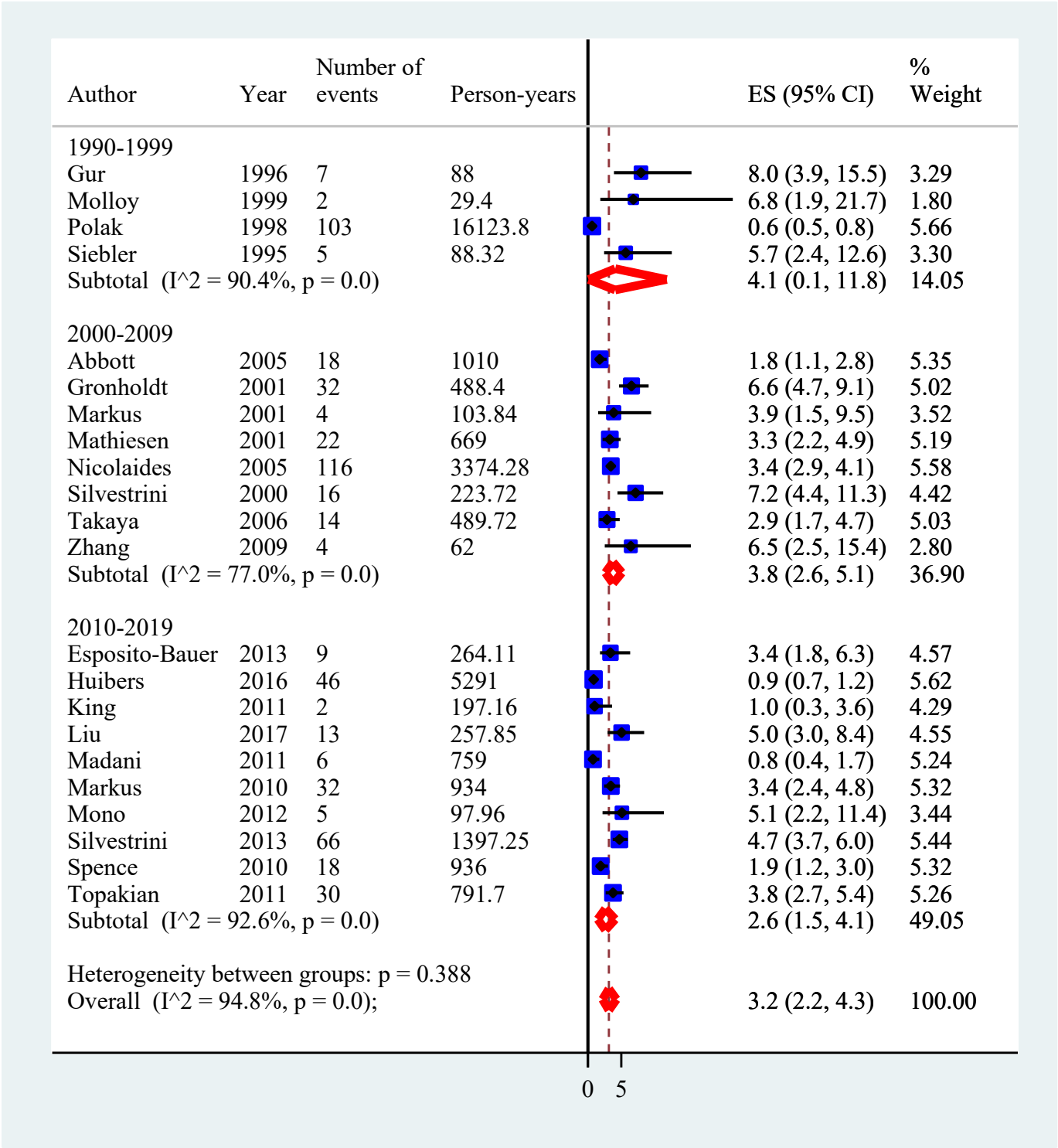


Figure XIV. Incidence of Ipsilateral Ischemic Cerebrovascular Events in Asymptomatic Carotid Stenosis by Grade



CI means confidence interval and ES means effect-size (representing the incidence in this case).

Figure XV. Incidence of Ipsilateral Ischemic Cerebrovascular Events in Asymptomatic Carotid Stenosis Over Time



ES = effect size (representing incidence in this case)

Figure XVI. Funnel Plot for the Meta-analysis of Incidence of Ipsilateral Ischemic Cerebrovascular Events in Asymptomatic Carotid Stenosis

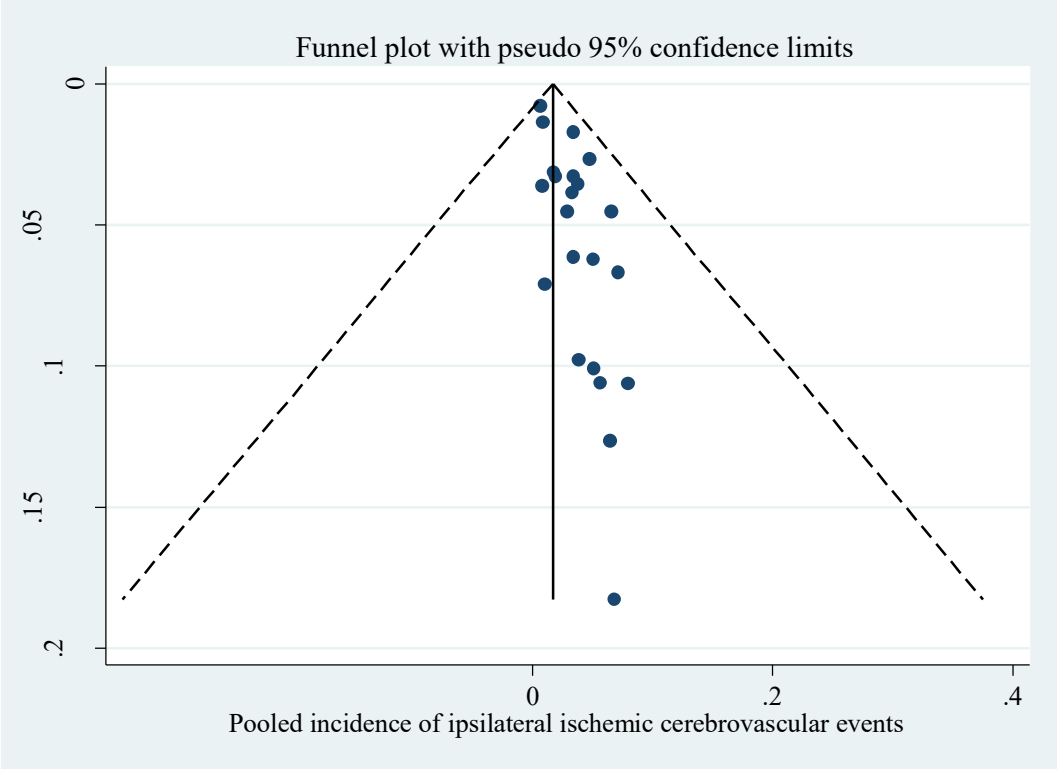
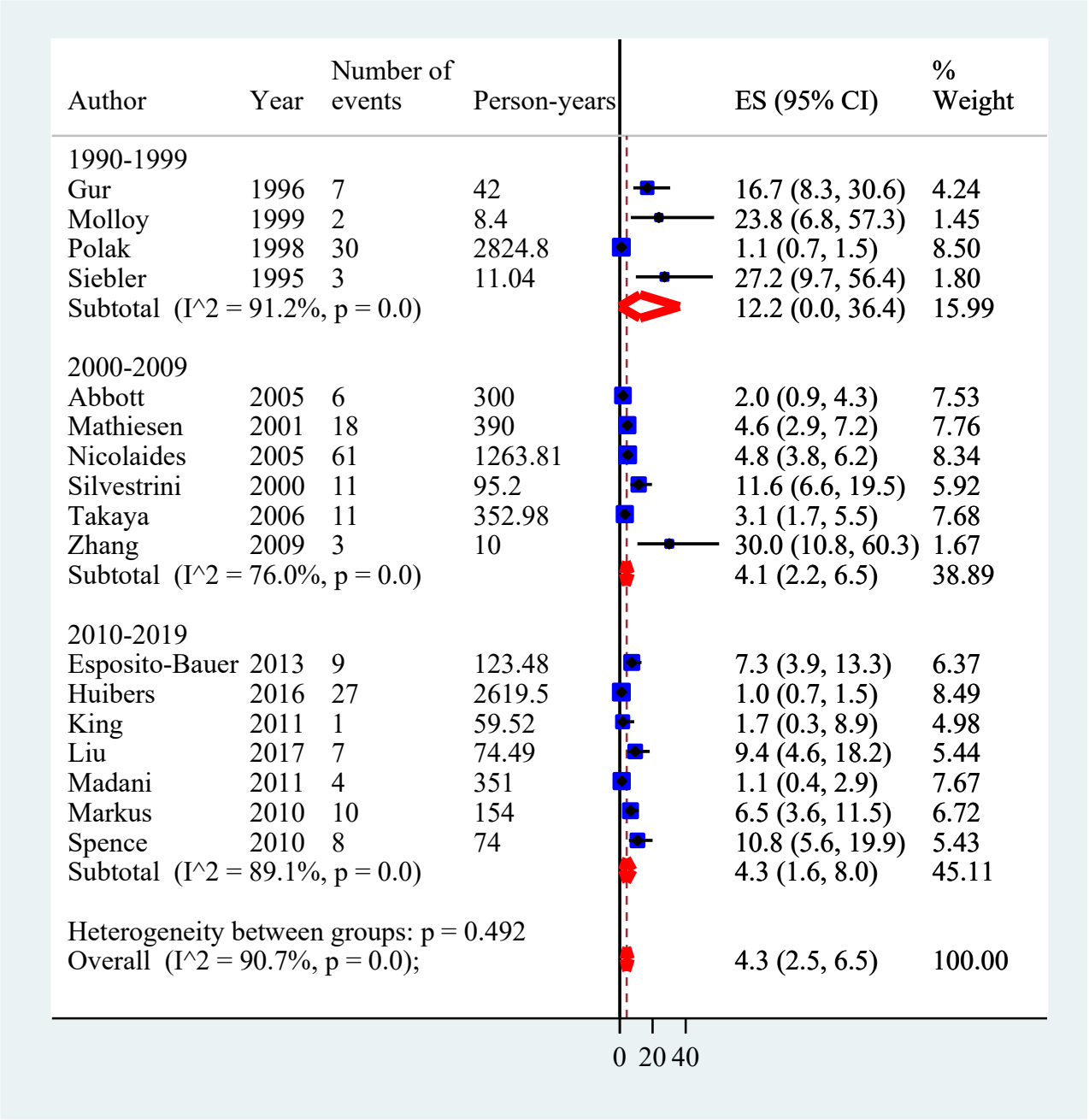
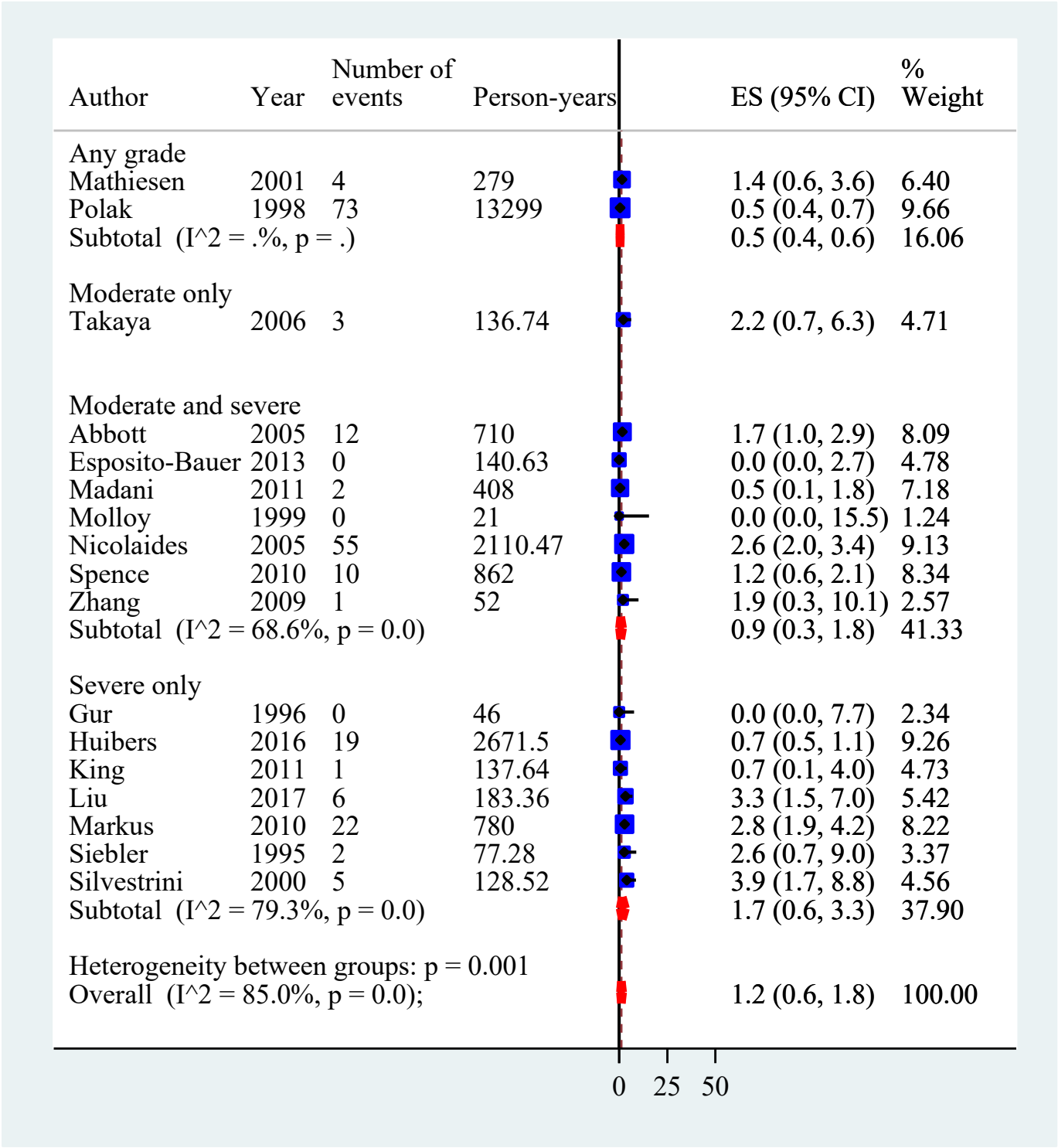


Figure VII. Incidence of Ipsilateral Ischemic Cerebrovascular Events in Asymptomatic Carotid Stenosis With High-Risk Features Over Time



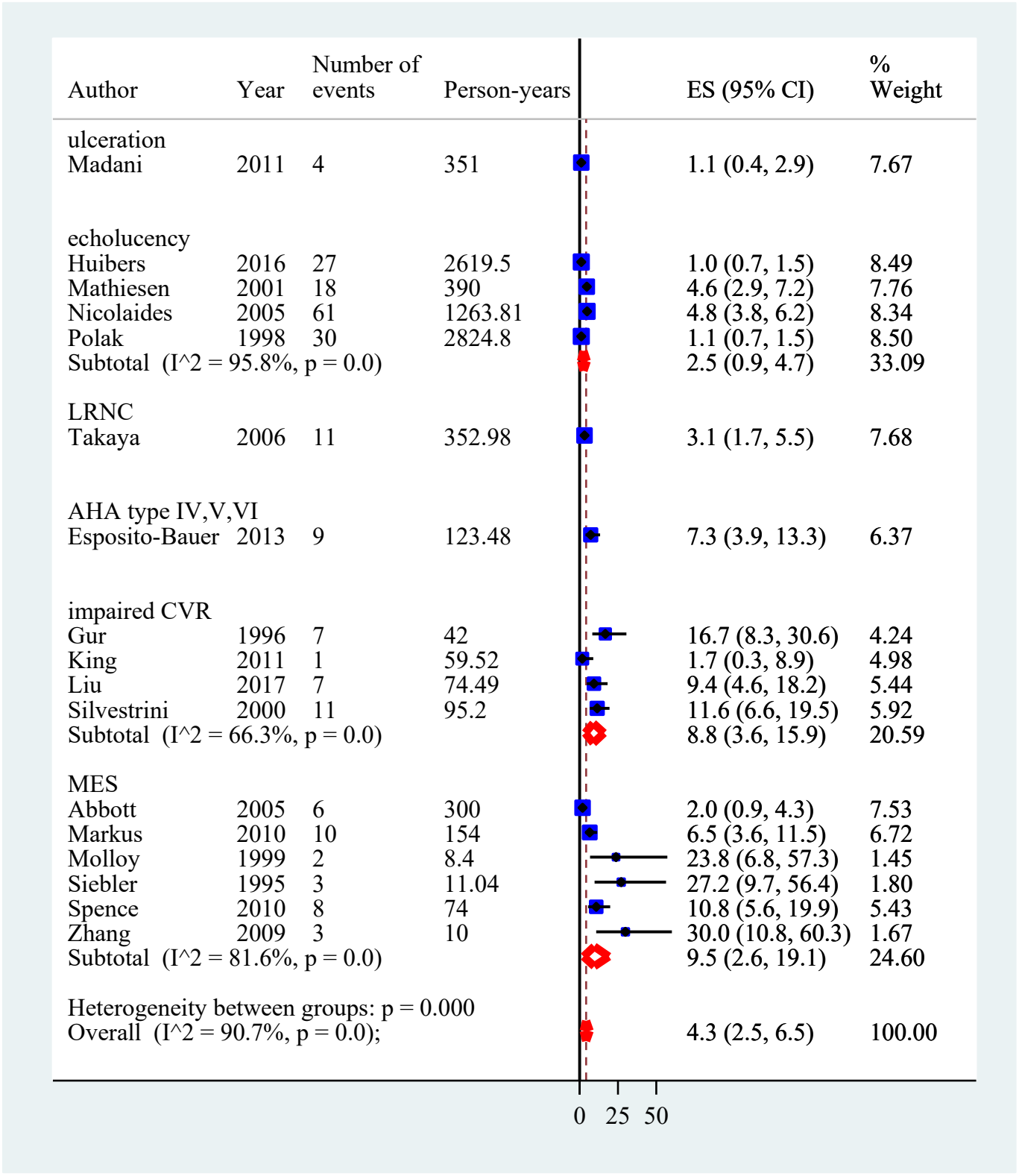
CI means confidence interval and ES means effect-size (representing the incidence in this case).

Figure XVIII. Incidence of Ipsilateral Ischemic Events in Asymptomatic Carotid Stenosis Without High-Risk Features



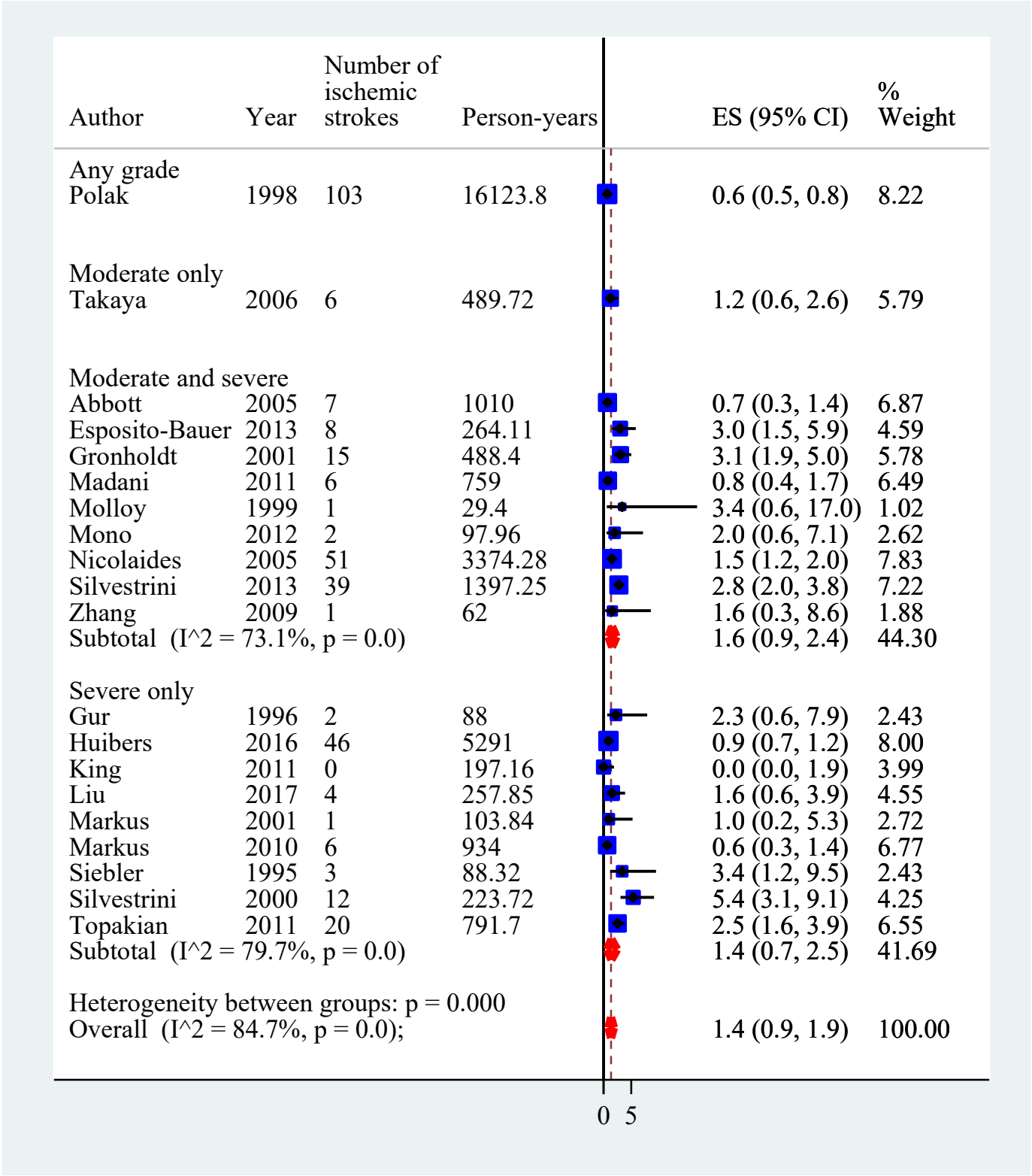
ES = effect size (representing incidence in this case)

Figure XIX. Incidence of Ipsilateral Ischemic Cerebrovascular Events in Asymptomatic Carotid Stenosis With High-Risk Features According to the Type of High-Risk Feature



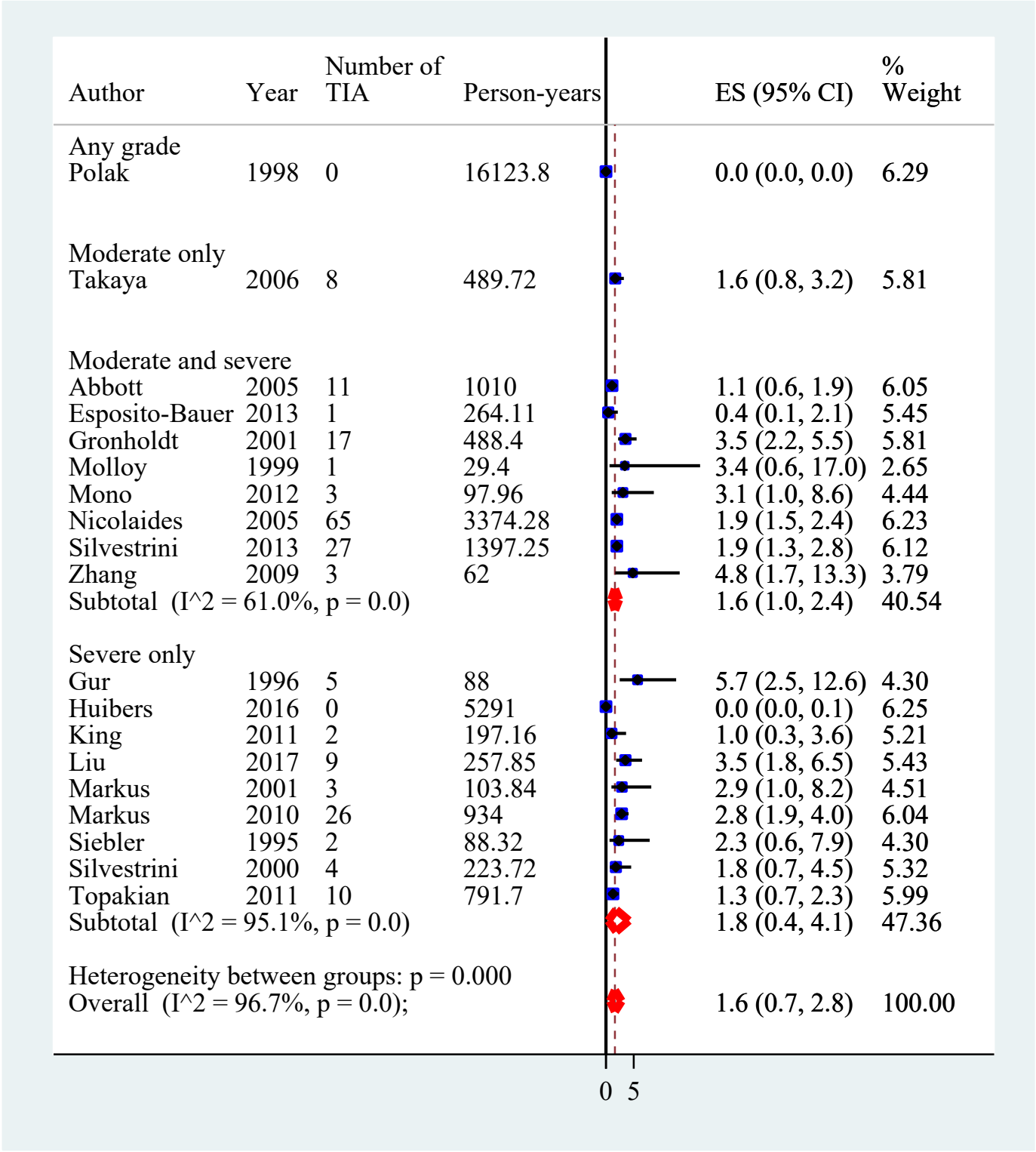
AHA means American Heart Association; CI, confidence interval; CVR, cerebrovascular reserve; ES, effect-size (representing the incidence in this case); LRNC, lipid-rich necrotic core; and MES, microembolic signal.

Figure XX. Incidence of Ipsilateral Ischemic Stroke in Asymptomatic Carotid Stenosis by Grade



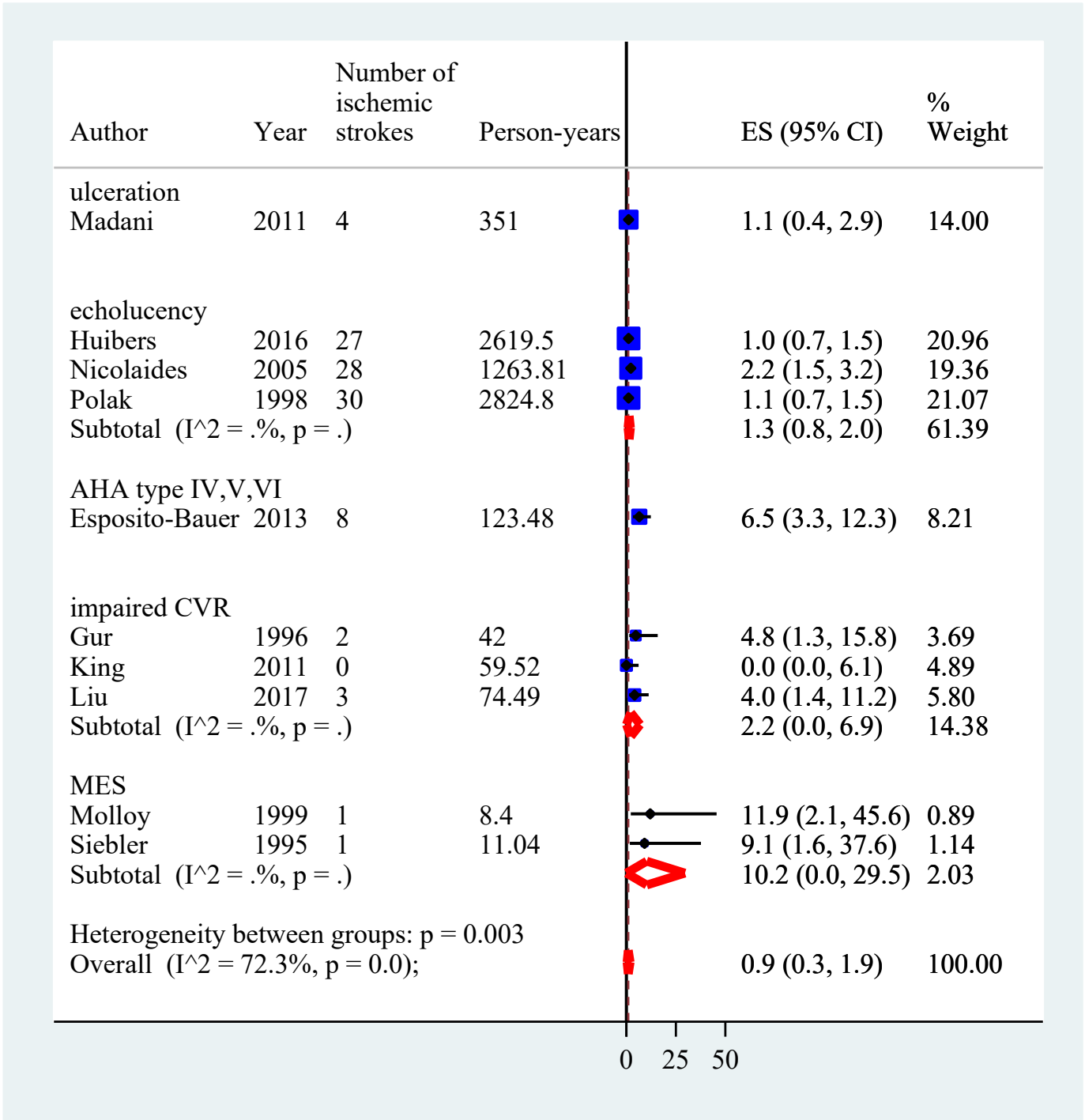
ES = effect size (representing incidence in this case)

Figure XXI. Incidence of Ipsilateral Transient Ischemic Attack in Asymptomatic Carotid Stenosis by Grade



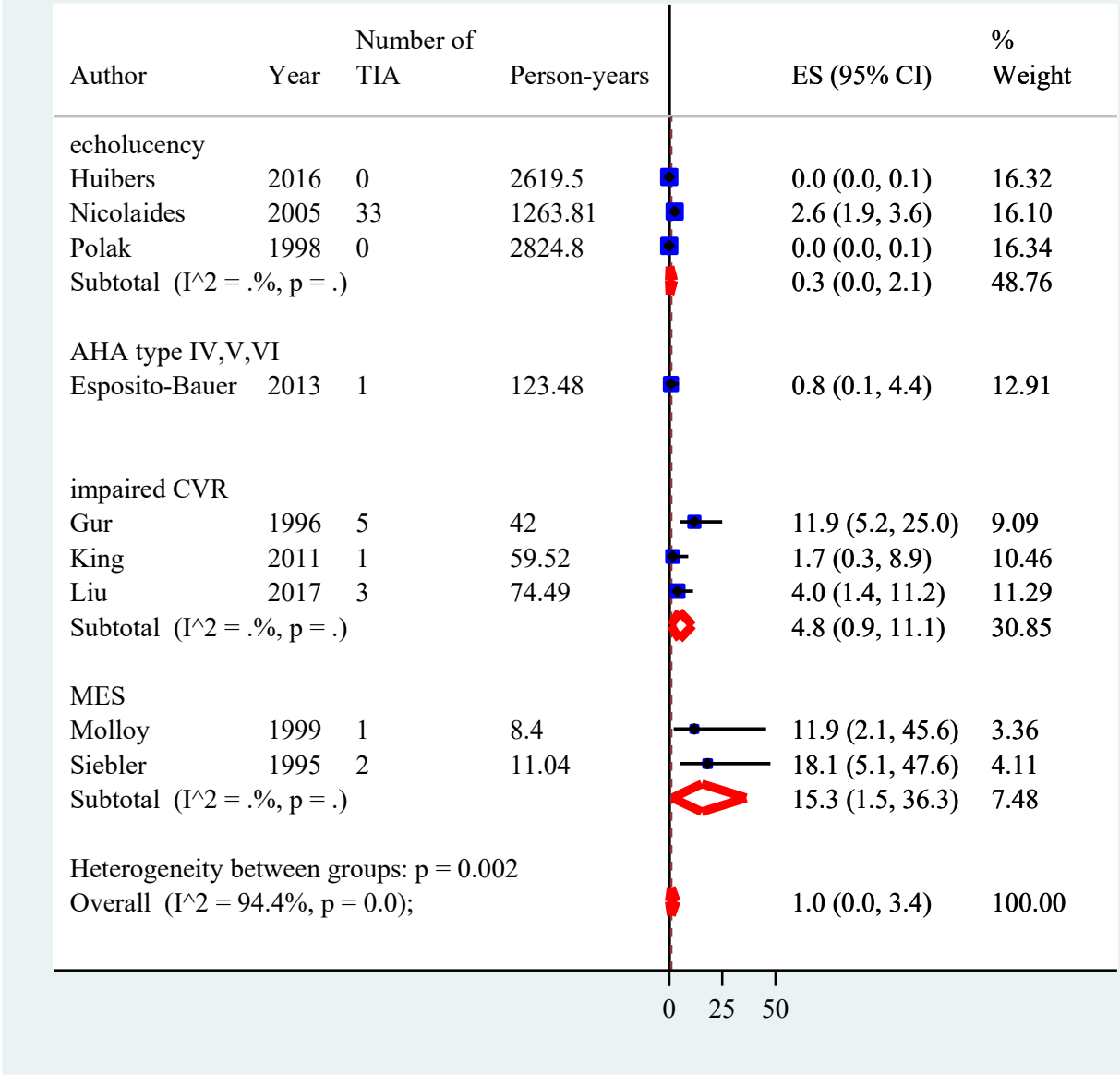
ES = effect size (representing incidence in this case)

Figure XXII. Incidence of Ipsilateral Ischemic Stroke in Asymptomatic Carotid Stenosis With High-Risk Features



ES = effect size (representing incidence in this case)

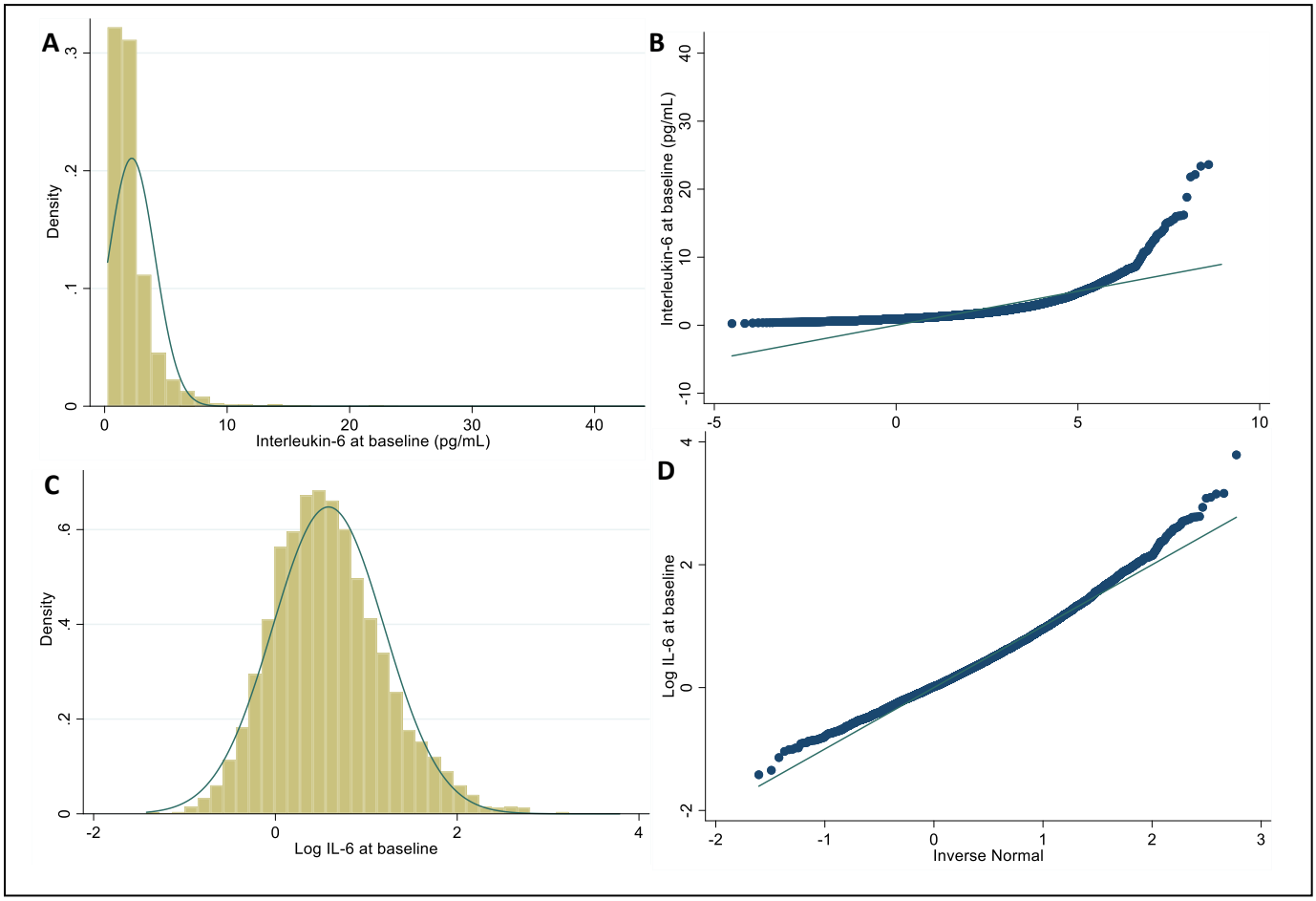
Figure XXIII. Incidence of Ipsilateral Transient Ischemic Attack in Asymptomatic Carotid Stenosis With High-Risk Features



ES = effect size (representing incidence in this case)

Appendix 3

Figure I: Distribution of IL-6 before and after logarithmic transformation



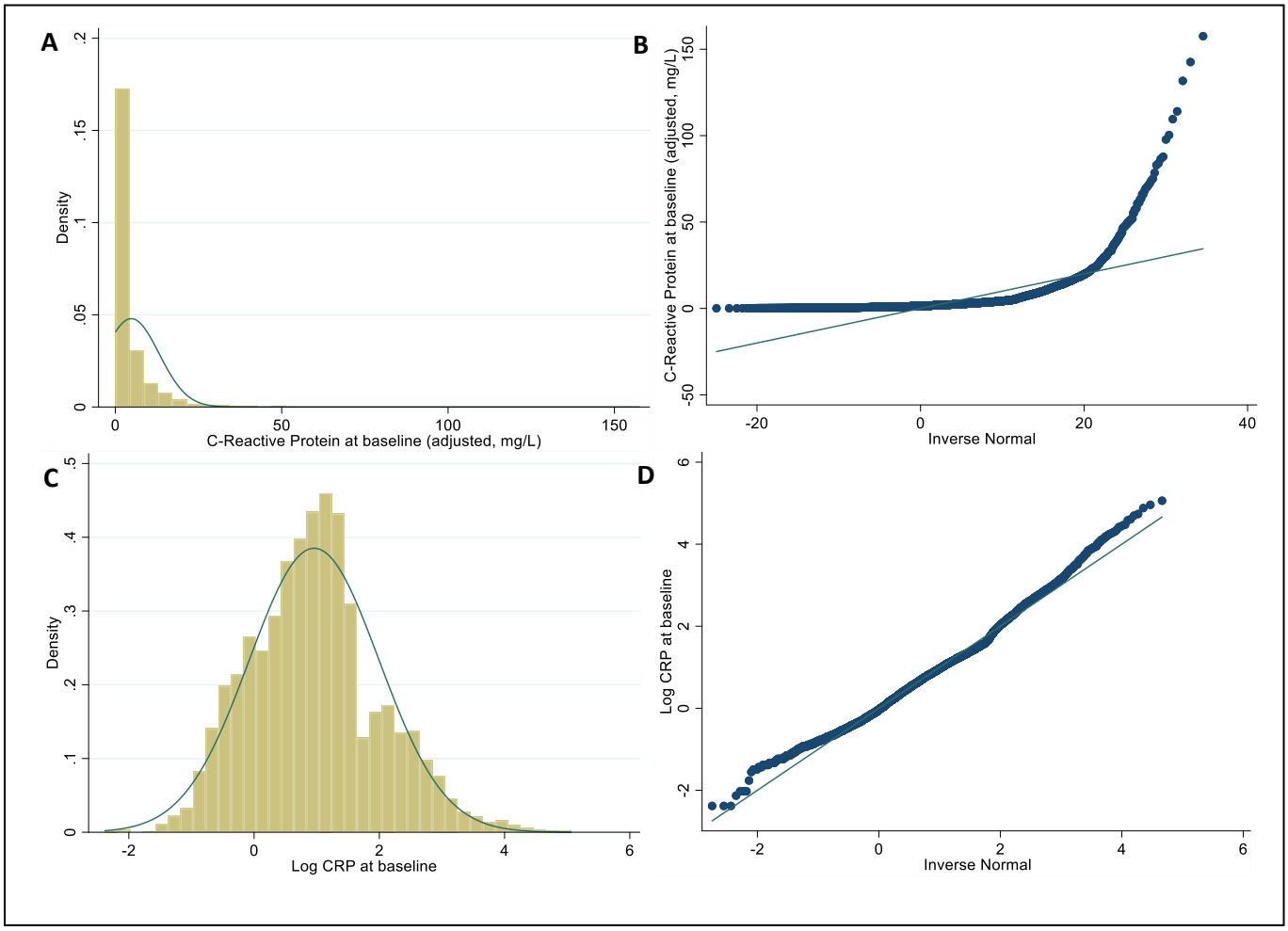
A: Histogram of the distribution of IL-6 levels with overlayed normal density curve

B: Quantile of IL-6 levels plotted against quantiles of the normal distribution

C: Histogram of the distribution of log IL-6 levels with overlayed normal density curve

D: Quantile of log IL-6 levels plotted against quantiles of the normal distribution

Figure II: Distribution of CRP before and after logarithmic transformation



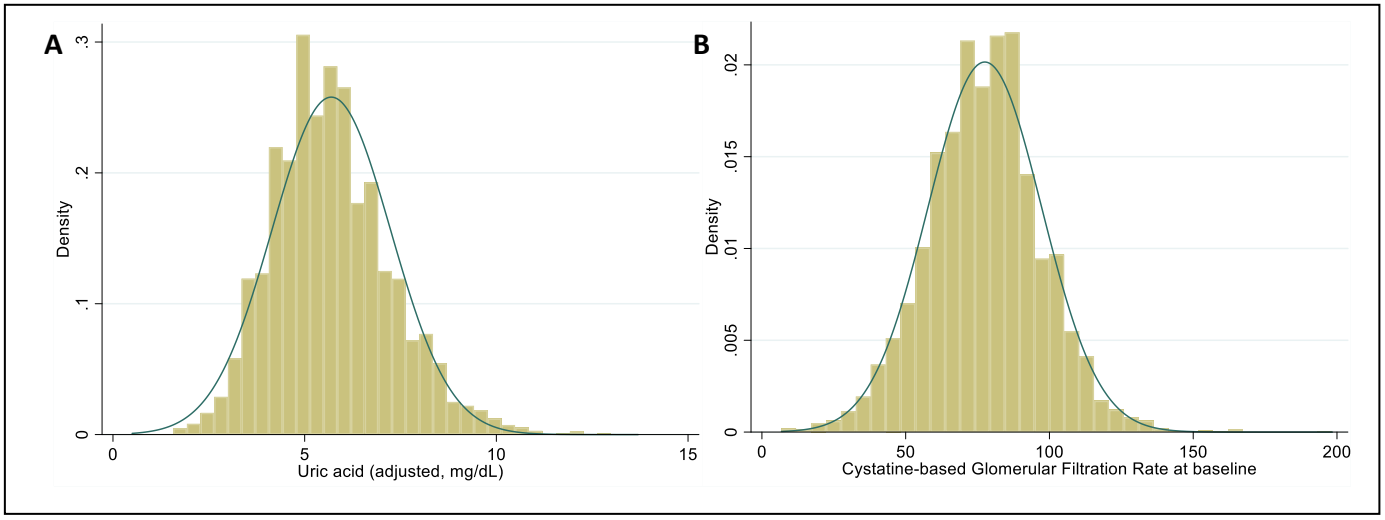
A: Histogram of the distribution of adjusted CRP levels with overlayed normal density curve (adjusted means corrected for instrument drift).

B: Quantile of CRP levels plotted against quantiles of the normal distribution

C: Histogram of the distribution of log CRP levels with overlayed normal density curve

D: Quantile of log CRP levels plotted against quantiles of the normal distribution

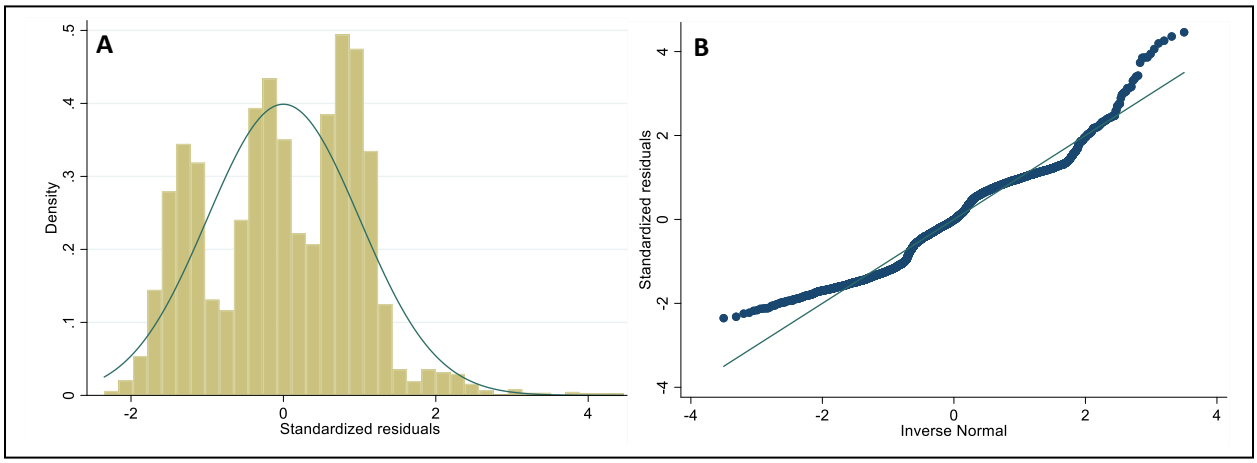
Figure III: Distribution of uric acid levels and cystatin-based glomerular filtration rate



A: Histogram of the distribution of uric acid levels with overlayed normal density curve (adjusted means corrected for instrument drift).

B: Histogram of the distribution of cystatin-based glomerular filtration rate with overlayed normal density curve.

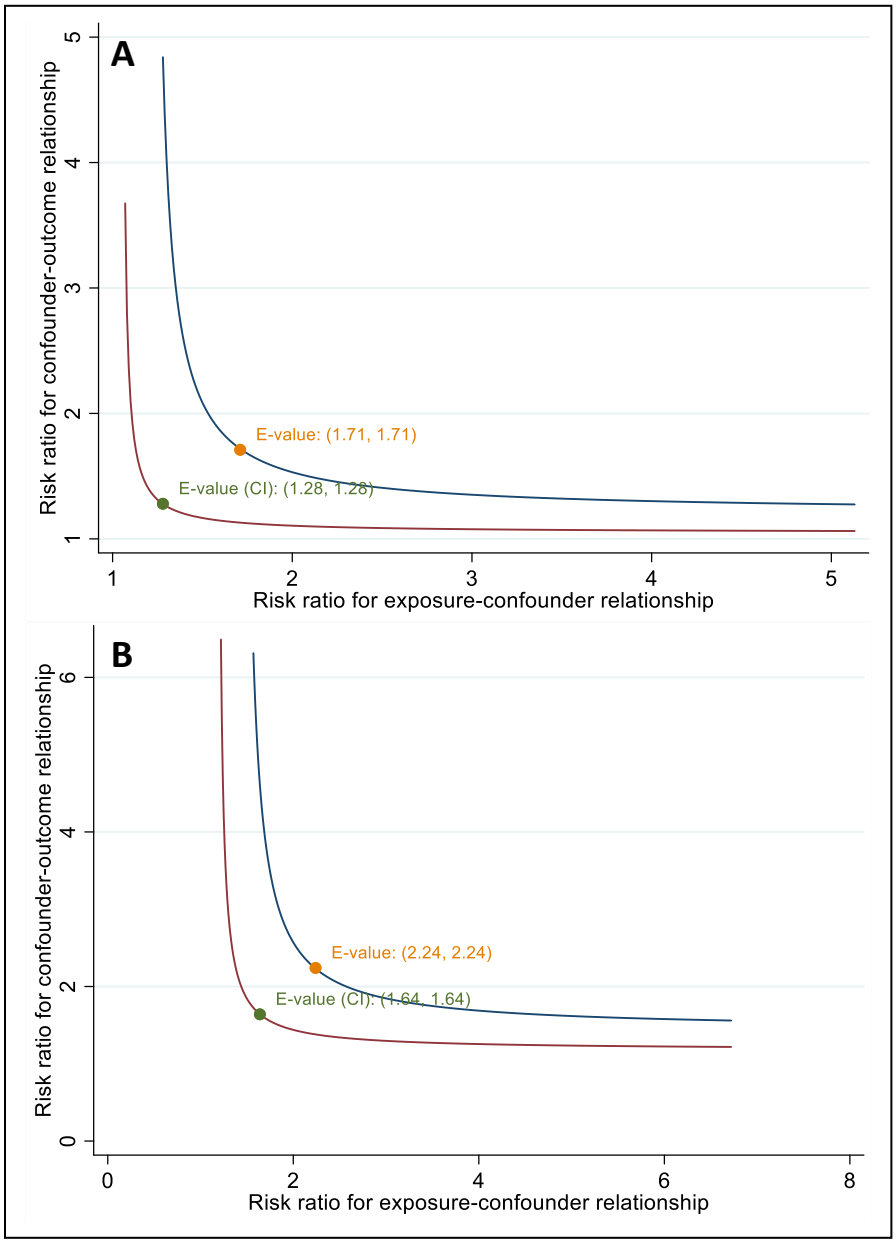
Figure IV: Distribution of standardized residuals for the multivariable regression of carotid stenosis score over log IL-6



A: Histogram of the distribution of standardized residuals with overlayed normal density curve

B: Quantile of standardized residuals plotted against quantiles of the normal distribution

Figure V: Curves of the sensitivity analysis for unobserved confounders with E-value highlighted



A: curve depicting the range of joint relationships (log IL6-confounder and confounder-plaque vulnerability) that may explain away the estimated effect and its confidence interval for the multivariable logistic regression model to predict plaque vulnerability.
B: curve depicting the range of joint relationships (log IL6-confounder and confounder-plaque vulnerability) that may explain away the estimated effect and its confidence interval for the multivariable logistic regression model to predict plaque progression.

Table I: Correlation of log IL-6 with various quantitative parameters

Variable	Pearson correlation coefficient	p
Age (years)	0.11	<0.001
Body mass index (kg/m^2)	0.25	<0.001
Fasting blood glucose (mg/dL)	0.16	<0.001
Log CRP	0.51	<0.001
Log creatinine	0.13	<0.001
Uric acid (mg/dL)	0.23	<0.001
Cystatin-based GFR (ml/min)	-0.30	<0.001

CRP = C-Reactive Protein

GFR = Glomerular filtration rate

Table II: Clinical characteristics of patients with low versus high plasma IL-6 levels at baseline

Characteristics	Low IL-6 levels (n = 2750)	High IL-6 levels (n = 1584)	p
Age (years, mean ± SD)	72.4 ± 5.0	73.2 ± 5.3	<0.001
Women	1670 (60.7)	883 (55.7)	0.001
Blacks	423 (15.4)	321 (20.3)	<0.001
Body mass index (kg/m², mean ± SD)	26.0 ± 3.7	27.8 ± 4.4	<0.001
Atrial fibrillation	82 (3.0)	78 (4.9)	0.001
Hypertension	1478 (53.7)	1065 (67.2)	<0.001
Diabetes mellitus	295 (10.7)	351 (22.2)	<0.001
Dyslipidemia	2490 (90.5)	1470 (92.8)	0.01
Current smoker	260 (9.5)	236 (14.9)	<0.001
Alcohol consumption (drinks per week, median with IQR)	0.02 (0.00 – 1.5)	0 (0.00-1.04)	< 0.01
Hyperuricemia	406 (14.8)	454 (28.7)	<0.001
Coronary heart disease	429 (15.6)	332 (21.0)	<0.001
Peripheral artery disease	58 (2.1)	69 (4.4)	<0.001
Prior stroke or TIA	116 (4.2)	111 (7.0)	<0.001
Treatment with statins	60 (2.2)	42 (2.7)	0.33
Treatment with antiplatelet drugs	77 (2.8)	65 (4.1)	0.02
Treatment with uric acid-lowering drugs*	57 (2.1)	61 (3.9)	<0.001
Treatment with anti-inflammatory drugs†	360 (13.1)	211 (13.3)	0.83
Cystatin-based GFR (ml/min , mean ± SD)	82.9 ± 18.1	73.4 ± 19.3	<0.001
Interleukin-6 (pg/mL, median with IQR)	1.3 (0.96 – 1.6)	2.9 (2.3 – 4.0)	<0.001
C-reactive protein (mg/L, median with IQR)	1.8 (0.9 – 3.1)	4.0 (2.3 – 8.7)	<0.001
Uric acid (mg/dL, mean ± SD)	5.4 ± 1.4	6.0 ± 1.6	<0.001
Stenosis severity score (mean ± SD)	1.2 ± 0.9	1.3 ± (0.9)	<0.001
Presence of severe stenosis at baseline	11 (0.4)	21 (1.3)	0.001
Presence of vulnerable plaque at baseline	760 (27.6)	507 (32.0)	<0.001
Plaque progression at 5 years	926 (33.7)	548 (34.6)	0.53

CRP: C-reactive protein; GFR: glomerular filtration rate; TIA: transient ischemic attack.

* Uric acid-lowering drugs refer to xanthine oxidase inhibitors and uricosurics.

† Anti-inflammatory drugs refer to steroids and non-steroidal anti-inflammatory drugs.

Table III: Multivariable logistic regression model for the association of high IL-6 levels with carotid plaque vulnerability

Independent variables	OR1 (95% CI) *	p-value	OR2†	OR3 (95% CI) ‡
High IL-6 level at baseline	1.21 (1.07 – 1.45)	0.03	1.10	1.19 (1.01 – 1.39)
Male	1.22 (1.03 – 1.46)	0.02	1.10	1.19 (1.02 – 1.39)
Dyslipidemia	1.56 (1.11 – 2.17)	<0.01	1.13	1.48 (1.10 – 1.98)
Hyperuricemia	1.38 (1.11 – 1.72)	<0.01	1.13	1.33 (1.09 – 1.61)
Intercept (baseline odds)	0.28 (0.20 – 0.38)	<0.001	NA	0.35 (0.25 – 0.48)

IL-6: interleukin-6; NA: not applicable.

* Non-standardized odds ratio (linked to change in the odds per 1 unit increase)

† Standardized odds ratio (linked to change in the odds per 1 standard deviation increase)

‡ Optimism-adjusted odds ratio using the heuristic shrinkage method.

Coronary heart disease (p=0.93), diabetes mellitus, race, history of stroke or transient ischemic attack, cystatin-based glomerular filtration rate, atrial fibrillation, alcohol consumption, treatment with anti-inflammatory drugs, hypertension, peripheral artery disease, log C-reactive protein, age, treatment with antiplatelet drugs, body mass index, and smoking status (p=0.08) were consecutively removed from the model automatically due to coefficients with p-value >0.05.

Likelihood ratio chi-squared test for significance of the model: $\chi^2 = 34.5$, df = 4, $p < 0.001$. Area under the Receiver Operating Characteristic curve (AUC) = 0.56. Count $R^2 = 67\%$. Proportion of patients correctly classified = 66%. Maximum Cook distance = 2.2. Maximum variance inflation factor = 1.09.

Table IV: Multivariable logistic regression model for the association of high IL-6 levels with carotid plaque progression

Independent variables	OR1 (95% CI) *	p-value	OR2†	OR3 (95% CI) ‡
High IL-6 level at baseline	1.25 (1.01 – 1.57)	0.04	1.11	1.25 (1.01 – 1.55)
Current smoker	1.66 (1.21 – 2.27)	<0.01	1.17	1.64 (1.21 – 2.24)
Dyslipidemia	2.29 (1.62 – 3.25)	<0.001	1.15	2.26 (1.60 – 3.19)
Diabetes mellitus	1.48 (1.11 – 1.97)	<0.01	1.14	1.47 (1.10 – 1.95)
Hypertension	1.38 (1.13 – 1.67)	<0.001	1.17	1.37 (1.13 – 1.66)
Coronary heart disease	1.35 (1.02 – 1.80)	0.04	1.11	1.34 (1.02 – 1.78)
Male	1.34 (1.10 – 1.63)	<0.01	1.15	1.33 (1.10 – 1.62)
Age (years)	1.03 (1.01 – 1.05)	<0.01	1.16	1.03 (1.01 – 1.05)
Vulnerability at baseline	0.77 (0.61 – 0.97)	0.03	0.89	0.77 (0.62 – 0.97)
Stenosis score at baseline	0.24 (0.21 – 0.28)	<0.001	0.27	0.25 (0.22 – 0.28)
Intercept (baseline odds)	0.06 (0.01 – 0.28)	<0.01	NA	0.07 (0.01 – 0.33)

IL-6: interleukin-6; NA: not applicable.

* Non-standardized odds ratio (linked to change in the odds per 1 unit increase)

† Standardized odds ratio (linked to change in the odds per 1 standard deviation increase)

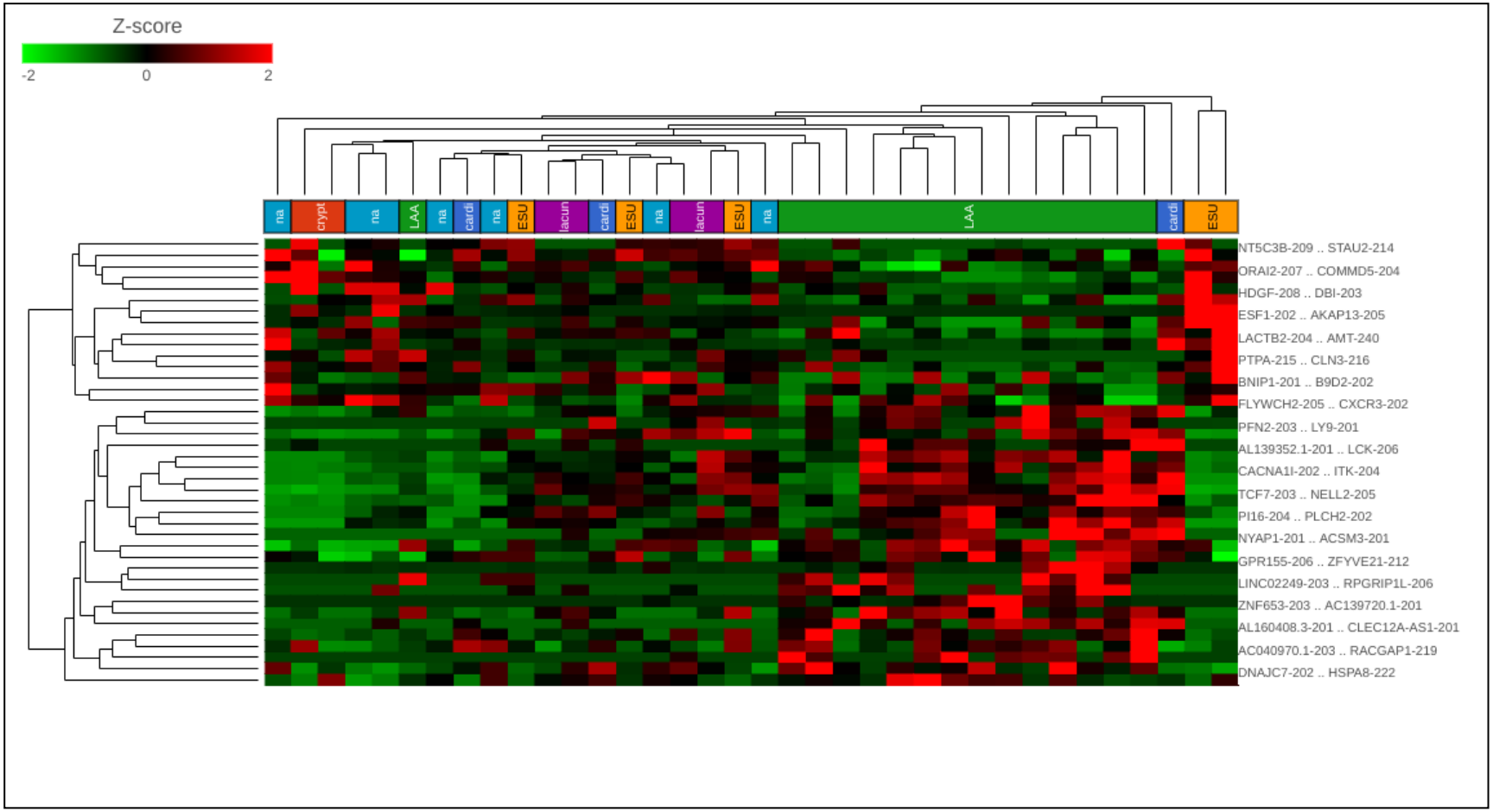
‡ Optimism-adjusted odds ratio using the heuristic shrinkage method.

Atrial fibrillation (p=0.88), treatment with anti-inflammatory drugs, history of stroke or transient ischemic attack, alcohol consumption, body mass index, race, treatment with antiplatelet drugs, peripheral artery disease, hyperuricemia, and cystatin-based glomerular filtration rate (p=0.11) were consecutively removed from the model automatically due to coefficients with p-value >0.05.

Likelihood ratio chi-squared test for significance of the model: $\chi^2 = 705.84$, df = 10, $p < 0.001$. Area under the Receiver Operating Characteristic curve (AUC) = 0.80. Count $R^2 = 72.4\%$. Proportion of patients correctly classified = 73%. Maximum Cook distance = 0.07. Maximum variance inflation factor = 1.04.

Appendix 4

Figure I: Hierarchical clustering plot of the 40 transcripts differentially expressed in patients with stroke due to carotid atherosclerosis versus stroke of other causes and controls



The 40 transcripts were differentially expressed in patients with stroke due to carotid atherosclerosis versus stroke of other causes and controls.

Color codes for the X-axis: light blue – controls (na), blue – cardioembolic stroke, green – stroke due to large artery atherosclerosis (carotid stenosis only), orange – embolic stroke of unknown source, purple – lacunar stroke, red – cryptogenic stroke.

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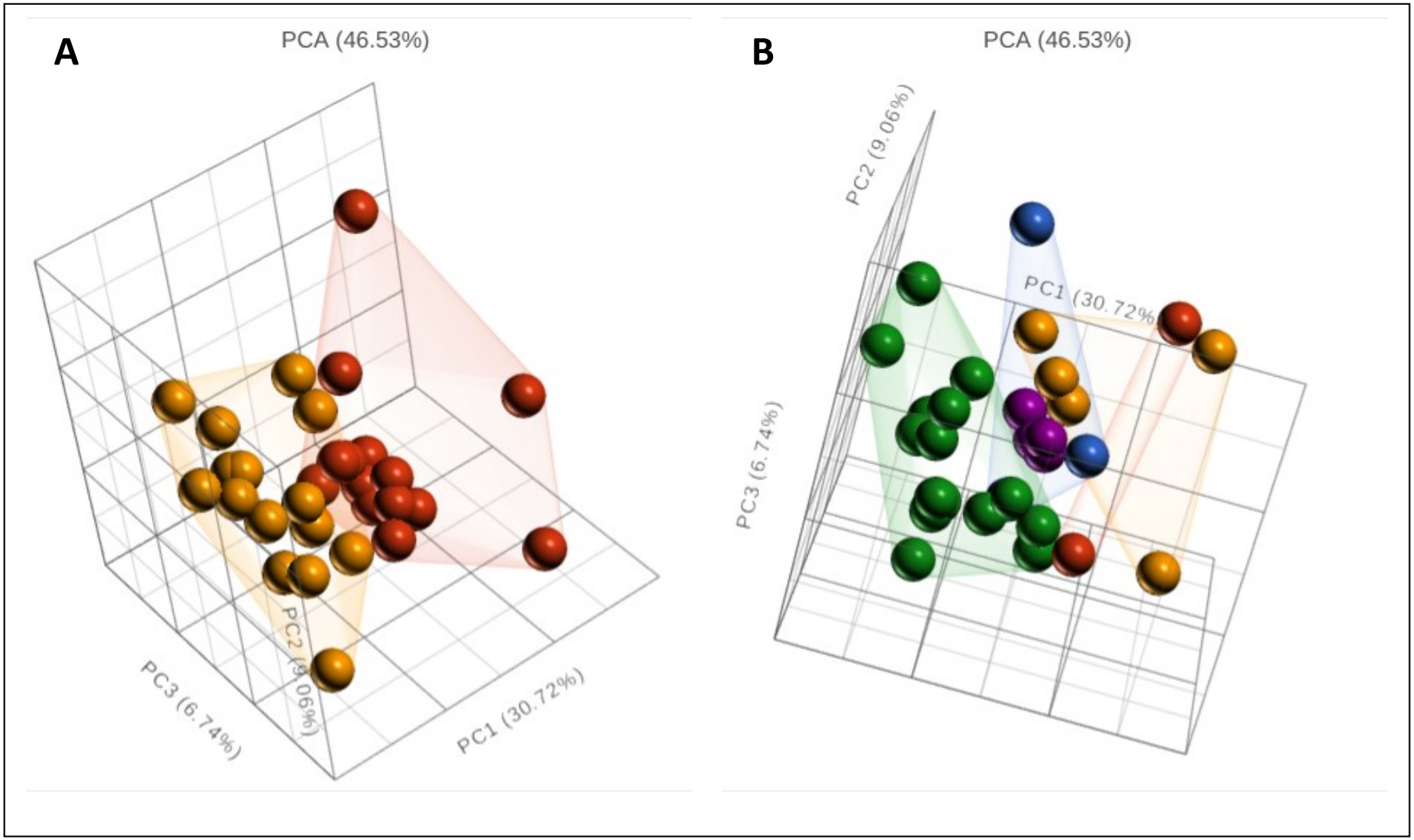
Figure II: Principal component analysis plot of the 40 transcripts differentially expressed in patients with stroke due to carotid atherosclerosis versus stroke of other causes and controls



A: symptomatic carotid atherosclerosis (orange) versus stroke of other causes (red)
B: Various stroke subtypes, cardioembolic stroke (blue), symptomatic carotid atherosclerosis (green), Embolic Stroke of Unknown Source (orange), lacunar stroke (purple), and cryptogenic stroke (red).

Table I: List of 1029 transcripts differentiating patients with stroke due to carotid atherosclerosis from patients with stroke not due to carotid atherosclerosis, and controls

Gene symbol	Transcript ID	Large vessel stroke versus stroke of other causes		Large vessel stroke versus controls		Large vessel stroke versus (stroke of other causes & controls)	
		p-value	FC	p-value	FC	p-value	FC
ABAT	ABAT-206	3.76*10-2	1.33	7.94*10-3	1.56	4.67*10-3	1.44
ABCA10	ABCA10-201	2.37*10-2	-4.97	5.00*10-2	-6.35	1.32*10-2	-5.66
ABCA2	ABCA2-206	4.94*10-3	-1.72	1.46*10-2	-1.75	2.06*10-3	-1.73
ABCA7	ABCA7-218	1.47*10-2	1.28	4.78*10-2	1.26	9.53*10-3	1.27
AC004801.2	AC004801.2-201	9.97*10-3	1.62	4.34*10-2	1.67	7.22*10-3	1.64
AC005520.4	AC005520.4-201	9.33*10-3	1.93	3.77*10-2	1.84	6.13*10-3	1.88
AC006206.1	AC006206.1-201	1.50*10-2	5.21	7.39*10-3	1.14*10+2	2.51*10-3	9.61
AC007598.2	AC007598.2-201	1.02*10-2	-2.47	3.49*10-2	-4.22	5.27*10-3	-3.37
AC008055.1	AC008055.1-201	4.94*10-2	1.21	1.86*10-2	8.59	1.00*10-2	2.12
AC008957.1	AC008957.1-202	8.77*10-3	1.92	3.28*10-2	2.44	5.35*10-3	2.15
AC008969.1	AC008969.1-209	3.06*10-2	8.39	2.90*10-2	8.46*10+1	1.02*10-2	1.14*10+1
AC009269.5	AC009269.5-201	1.66*10-3	-7.63*10+1	3.20*10-2	-5.51*10+1	2.16*10-3	-6.79*10+1
AC009831.1	AC009831.1-201	1.54*10-2	-1.24	3.47*10-3	-1.43	1.44*10-3	-1.34
AC010609.1	AC010609.1-201	1.21*10-2	3.30	1.12*10-3	5.69	8.01*10-4	4.11
AC011511.1	AC011511.1-201	2.84*10-2	1.49	4.01*10-2	1.36	2.52*10-2	1.38
AC011511.4	AC011511.4-201	4.31*10-2	1.47	3.49*10-2	1.37	1.47*10-2	1.41
AC012368.1	AC012368.1-205	3.63*10-2	-1.93	3.81*10-2	-1.81	9.62*10-3	-1.92
AC016727.1	AC016727.1-203	4.84*10-2	-1.48	1.82*10-2	-1.62	9.60*10-3	-1.55
AC018804.1	AC018804.1-201	1.78*10-2	-4.19	4.97*10-2	-5.36	1.07*10-2	-4.74
AC019131.3	AC019131.3-201	2.86*10-2	5.32	7.45*10-3	1.23*10+2	3.78*10-3	1.02*10+1
AC020893.1	AC020893.1-201	4.46*10-2	-1.49	4.62*10-2	-1.60	1.79*10-2	-1.54
AC020978.2	AC020978.2-201	3.72*10-2	-3.51	2.84*10-3	-6.76	2.34*10-3	-5.14
AC022154.1	AC022154.1-202	1.97*10-2	6.06	3.78*10-2	2.95	9.54*10-3	3.95
AC022706.1	AC022706.1-206	4.25*10-2	-1.33	4.92*10-2	-1.57	1.82*10-2	-1.45
AC026401.1	AC026401.1-201	2.34*10-2	-7.01	8.16*10-3	-7.83	3.55*10-3	-7.36

AC040970.1	AC040970.1-203	5.00*10-3	1.64	4.39*10-2	1.54	5.39*10-3	1.59
AC055839.2	AC055839.2-201	3.20*10-2	1.21	2.80*10-2	1.24	9.97*10-3	1.22
AC068446.1	AC068446.1-201	1.96*10-2	-1.21	2.31*10-2	-1.25	6.96*10-3	-1.23
AC079921.1	AC079921.1-201	4.08*10-2	-1.41	2.37*10-2	-1.77	1.06*10-2	-1.59
AC087430.1	AC087430.1-201	4.42*10-2	3.46	9.41*10-3	7.32*10+1	5.84*10-3	6.52
AC087501.4	AC087501.4-201	1.55*10-2	1.25	4.37*10-2	1.26	9.26*10-3	1.26
AC092140.1	AC092140.1-201	4.81*10-2	-1.57	7.65*10-3	-1.97	4.72*10-3	-1.78
AC092279.1	AC092279.1-204	5.28*10-3	-1.76	1.24*10-2	-2.17	1.99*10-3	-1.96
AC092418.3	AC092418.3-201	4.48*10-2	-2.27	2.21*10-2	-2.70	1.05*10-2	-2.48
AC093157.1	AC093157.1-219	1.43*10-2	-1.70	3.07*10-2	-1.27	4.55*10-3	-1.51
AC093668.1	AC093668.1-201	3.05*10-3	-1.22*10+2	2.41*10-2	-2.25*10+2	1.71*10-3	-2.38*10+2
AC096992.2	AC096992.2-201	2.53*10-2	1.25	3.59*10-4	1.59	4.44*10-4	1.40
AC100827.3	AC100827.3-201	9.44*10-3	3.40	2.03*10-2	2.03	4.81*10-3	2.42
AC104117.4	AC104117.4-201	3.93*10-2	-4.82	1.26*10-2	-5.60	6.53*10-3	-5.21
AC104332.1	AC104332.1-201	3.65*10-2	3.06	1.58*10-2	1.02*10+1	6.12*10-3	4.77
AC104452.1	AC104452.1-204	3.08*10-2	3.06	1.34*10-2	5.60	5.86*10-3	3.96
AC109587.1	AC109587.1-209	1.27*10-2	3.06	9.09*10-3	9.38	2.23*10-3	4.71
AC109587.1	AC109587.1-206	3.41*10-2	-2.35	4.75*10-2	-2.50	1.43*10-2	-2.43
AC110275.1	AC110275.1-201	1.73*10-2	-1.43*10+2	2.06*10-2	-1.26*10+2	5.73*10-3	-1.35*10+2
AC113208.3	AC113208.3-201	4.77*10-2	-3.10	7.18*10-3	-5.34	5.13*10-3	-4.23
AC135050.6	AC135050.6-202	1.28*10-2	1.26	3.32*10-2	1.25	6.91*10-3	1.25
AC138811.2	AC138811.2-201	3.48*10-4	-3.76	3.63*10-3	-2.96	1.76*10-4	-3.35
AC139256.3	AC139256.3-202	3.87*10-3	1.49	2.21*10-2	1.55	2.57*10-3	1.52
AC139720.1	AC139720.1-201	4.36*10-3	2.92	2.32*10-3	1.02*10+1	5.49*10-4	4.52
AC140076.1	AC140076.1-201	2.10*10-2	2.71	4.39*10-3	8.63	2.55*10-3	4.07
AC244197.3	AC244197.3-202	2.33*10-3	-4.48	1.03*10-2	-6.15	9.20*10-4	-5.86
AC246817.2	AC246817.2-211	3.51*10-2	-1.32*10+2	2.42*10-4	-1.84*10+2	4.45*10-4	-1.58*10+2
ACAA1	ACAA1-218	5.54*10-3	-5.13	1.18*10-2	-4.08	1.95*10-3	-4.62
ACAT1	ACAT1-222	1.39*10-2	-1.38	4.69*10-2	-1.40	9.21*10-3	-1.39
ACBD5	ACBD5-201	3.14*10-2	-1.74	4.67*10-2	-2.25	1.75*10-2	-2.00
ACER3	ACER3-201	1.02*10-2	-2.02	2.99*10-2	-3.34	5.17*10-3	-2.66

ACIN1	ACIN1-213	6.58*10-3	-1.58	4.44*10-2	-1.56	5.75*10-3	-1.57
ACSM3	ACSM3-201	4.10*10-3	1.91	4.90*10-5	2.71	5.83*10-5	2.24
ACSS1	ACSS1-205	4.39*10-2	1.72	4.29*10-2	1.59	8.63*10-3	1.66
ACTN2	ACTN2-201	2.35*10-3	1.34*10+1	1.71*10-2	5.21	2.29*10-3	7.71
ACTR10	ACTR10-205	9.13*10-3	1.82	8.58*10-3	1.64	1.99*10-3	1.68
ACTR10	ACTR10-204	1.54*10-2	-1.27	6.58*10-3	-1.43	2.32*10-3	-1.35
ACTR10	ACTR10-211	4.15*10-2	-1.40	3.10*10-2	-1.44	1.25*10-2	-1.42
ACTR6	ACTR6-208	3.15*10-2	-2.21	2.70*10-2	-2.02	1.16*10-2	-2.03
ACY1	ACY1-220	9.83*10-3	9.97	9.76*10-3	7.25*10+1	2.30*10-3	1.30*10+1
ACY1	ACY1-222	3.70*10-2	-2.38*10+2	2.69*10-3	-2.47*10+2	2.24*10-3	-2.42*10+2
AD000671.1	AD000671.1-201	8.43*10-3	-1.24	8.85*10-3	-1.91	2.01*10-3	-1.58
ADAMTSL5	ADAMTSL5-201	2.98*10-2	1.68	1.31*10-2	1.93	2.74*10-2	1.65
ADGRG1	ADGRG1-232	2.48*10-2	2.30	2.95*10-2	3.53	9.18*10-3	2.78
ADNP	ADNP-207	1.45*10-2	2.58	1.20*10-2	1.96	3.08*10-3	2.23
ADPGK	ADPGK-214	4.57*10-2	3.08	3.55*10-2	1.95	1.51*10-2	2.37
ADSL	ADSL-207	4.22*10-2	1.69	5.43*10-3	8.91*10+1	4.11*10-3	3.34
AFDN	AFDN-212	6.61*10-4	-1.09*10+1	2.22*10-2	-8.08	1.01*10-3	-9.47
AGPS	AGPS-204	3.76*10-2	-7.32	2.54*10-2	-3.95	1.05*10-2	-5.61
AGTRAP	AGTRAP-204	1.75*10-2	-1.40	4.65*10-2	-1.45	1.05*10-2	-1.43
AHCTF1	AHCTF1-203	1.16*10-2	1.53	1.17*10-2	1.50	2.58*10-3	1.51
AK2	AK2-210	1.55*10-2	-1.39*10+1	4.48*10-3	-1.24*10+1	1.82*10-3	-1.31*10+1
AKAP13	AKAP13-205	4.73*10-3	-2.13	4.08*10-2	-1.49	4.63*10-3	-1.81
AKAP9	AKAP9-214	1.27*10-2	1.58	1.47*10-3	1.99	3.85*10-3	1.70
AKNA	AKNA-201	1.06*10-2	1.32	1.05*10-2	1.36	2.72*10-3	1.34
AKNA	AKNA-205	4.72*10-2	1.40	1.34*10-2	1.38	8.71*10-3	1.39
AKT2	AKT2-240	1.47*10-2	-3.16	2.99*10-2	-4.06	6.94*10-3	-3.61
AKT2	AKT2-203	4.47*10-2	-1.29	3.96*10-2	-1.52	1.62*10-2	-1.40
AL021707.6	AL021707.6-201	4.57*10-2	1.31	2.58*10-2	1.40	1.16*10-2	1.35
AL022098.1	AL022098.1-201	4.74*10-2	1.37	3.50*10-3	1.67	3.13*10-3	1.50
AL031590.1	AL031590.1-202	1.89*10-2	2.69	3.95*10-3	1.31*10+2	1.90*10-3	5.12
AL109811.2	AL109811.2-202	1.01*10-2	2.29	2.32*10-3	2.03	1.26*10-3	2.12

AL138478.1	AL138478.1-201	4.46*10-3	1.42	1.10*10-2	1.43	1.24*10-3	1.43
AL139287.1	AL139287.1-201	2.73*10-2	1.32	2.96*10-2	1.42	9.68*10-3	1.37
AL139352.1	AL139352.1-201	8.95*10-3	2.44	3.78*10-3	4.56	1.47*10-3	3.13
AL157392.3	AL157392.3-203	6.02*10-5	2.79*10+1	4.30*10-2	1.24*10+1	4.32*10-2	2.28*10+1
AL157895.2	AL157895.2-203	1.74*10-2	-1.28	1.60*10-2	8.59	4.73*10-3	1.43
AL160408.3	AL160408.3-201	5.14*10-4	4.64	1.20*10-3	9.62	9.50*10-5	6.25
AL162231.1	AL162231.1-204	2.42*10-2	2.32	1.37*10-2	1.14*10+1	5.19*10-3	3.84
AL162377.3	AL162377.3-201	4.55*10-2	1.83	2.59*10-2	2.14	1.36*10-2	1.97
AL356585.4	AL356585.4-201	1.55*10-2	-7.17*10+1	5.00*10-4	-8.37*10+1	4.10*10-4	-7.82*10+1
AL360178.2	AL360178.2-201	2.38*10-2	-5.80	3.75*10-3	-5.50	2.07*10-3	-5.62
AL391807.1	AL391807.1-208	3.64*10-2	-1.21*10+1	2.70*10-2	-7.45	1.08*10-2	-9.75
AL391863.2	AL391863.2-201	3.51*10-2	-1.03*10+1	1.45*10-2	-1.19*10+1	5.54*10-3	-1.18*10+1
AL449423.1	AL449423.1-201	4.01*10-2	-3.80	4.38*10-3	-8.69	3.27*10-3	-6.25
AL450326.2	AL450326.2-201	4.17*10-2	-2.20	2.38*10-2	-2.17	1.07*10-2	-2.18
AL512310.9	AL512310.9-201	4.05*10-2	-2.26	2.90*10-2	-2.85	1.08*10-2	-2.55
AL590705.1	AL590705.1-203	2.76*10-2	6.11	7.22*10-3	1.79*10+2	3.61*10-3	1.18*10+1
AL671710.1	AL671710.1-201	1.86*10-2	1.23	4.18*10-2	1.24	9.70*10-3	1.23
ALDH3A2	ALDH3A2-240	1.25*10-3	2.35	3.58*10-2	1.79	2.33*10-3	2.03
ALDH3A2	ALDH3A2-219	2.05*10-2	1.67	4.75*10-2	1.51	1.36*10-2	1.58
ALDH3A2	ALDH3A2-236	2.53*10-2	5.01	2.96*10-2	3.83	9.14*10-3	4.35
ALDH5A1	ALDH5A1-209	5.64*10-3	7.53	9.53*10-3	4.78	1.66*10-3	5.86
ALDH6A1	ALDH6A1-205	1.52*10-2	-2.62	4.19*10-2	-1.86	1.06*10-2	-1.93
ALDOA	ALDOA-201	6.14*10-3	-1.21	2.79*10-5	-1.50	3.36*10-5	-1.35
ALG3	ALG3-204	3.32*10-2	-1.88	2.16*10-2	-2.09	7.08*10-3	-2.00
ALG8	ALG8-221	1.30*10-2	-2.96	3.66*10-2	-2.44	7.84*10-3	-2.69
ALKBH3	ALKBH3-208	2.25*10-2	4.23	3.08*10-2	4.69	8.87*10-3	4.45
AMBRA1	AMBRA1-209	3.17*10-2	-1.64	8.83*10-3	-2.65	4.56*10-3	-2.14
AMT	AMT-240	5.01*10-3	-5.10	2.41*10-2	-4.67	3.20*10-3	-4.89
AMZ2	AMZ2-210	1.50*10-2	1.53	2.09*10-2	1.42	5.36*10-3	1.47
ANAPC15	ANAPC15-207	2.08*10-2	-2.39	2.24*10-3	-4.26	1.38*10-3	-3.33
ANKRD13A	ANKRD13A-204	1.82*10-2	-1.54	2.78*10-2	-1.64	8.61*10-3	-1.59

ANKRD36	ANKRD36-202	3.28*10-2	1.48	2.09*10-2	1.51	1.01*10-2	1.49
ANKS1A	ANKS1A-202	2.70*10-2	-1.50	4.13*10-2	-1.32	1.22*10-2	-1.42
ANO10	ANO10-205	1.73*10-2	-1.55	3.66*10-2	-1.66	8.77*10-3	-1.60
ANO9	ANO9-201	1.25*10-2	3.14	1.28*10-2	4.22	3.43*10-3	3.57
ANP32E	ANP32E-205	1.49*10-2	-1.76	3.47*10-2	-1.34	7.66*10-3	-1.55
ANXA1	ANXA1-208	3.70*10-3	-1.25	3.17*10-3	-1.29	6.11*10-4	-1.27
AOAH	AOAH-212	3.12*10-3	1.69	3.44*10-2	1.45	3.29*10-3	1.56
AP000347.1	AP000347.1-201	4.28*10-2	1.30	2.89*10-2	1.39	1.22*10-2	1.34
AP000866.1	AP000866.1-212	1.99*10-2	4.11	3.47*10-2	3.57	9.08*10-3	3.82
AP001107.9	AP001107.9-204	4.11*10-2	2.42	4.95*10-2	3.97	2.04*10-2	2.98
AP002495.1	AP002495.1-207	4.58*10-2	-2.10	2.25*10-4	-4.38	5.15*10-4	-3.24
AP003120.1	AP003120.1-201	7.88*10-3	-1.70	3.36*10-2	-1.38	4.41*10-3	-1.54
AP4B1	AP4B1-203	3.16*10-2	1.64	3.89*10-2	1.66	1.21*10-2	1.66
APEX1	APEX1-202	2.09*10-2	1.99	5.43*10-3	2.07	3.29*10-3	2.02
APLP2	APLP2-210	1.46*10-2	-1.35	3.54*10-2	-1.41	8.11*10-3	-1.38
APLP2	APLP2-221	1.74*10-2	-1.39	1.89*10-2	-1.53	6.27*10-3	-1.46
APLP2	APLP2-203	2.65*10-2	-1.47	1.30*10-2	-2.17	5.25*10-3	-1.85
APTX	APTX-255	3.73*10-2	-1.74	3.90*10-2	-1.87	1.41*10-2	-1.80
AREL1	AREL1-211	9.37*10-3	1.35	1.93*10-2	1.26	3.38*10-3	1.30
AREL1	AREL1-208	1.66*10-2	1.17*10+1	2.11*10-2	1.23*10+2	5.82*10-3	2.30*10+1
ARHGAP17	ARHGAP17-216	2.36*10-2	-2.65	3.21*10-2	-2.59	9.41*10-3	-2.49
ARHGAP22	ARHGAP22-209	2.39*10-2	-9.66	2.32*10-3	-1.31*10+1	1.51*10-3	-1.14*10+1
ARHGAP26	ARHGAP26-220	3.40*10-2	-1.33	3.67*10-2	-1.49	1.30*10-2	-1.41
ARHGAP27P2	ARHGAP27P2-201	2.00*10-2	1.89	4.96*10-2	2.58	1.18*10-2	2.18
ARHGAP29	ARHGAP29-203	1.67*10-2	-1.82	2.70*10-2	-2.15	6.84*10-3	-1.99
ARHGAP4	ARHGAP4-217	9.28*10-3	1.88	2.60*10-2	1.72	4.61*10-3	1.79
ARHGEF18	ARHGEF18-206	2.33*10-2	1.30	1.82*10-2	1.39	5.86*10-3	1.34
ARHGEF18	ARHGEF18-205	4.67*10-2	-2.56	8.13*10-3	-4.31	4.33*10-3	-3.52
ARID3A	ARID3A-202	1.08*10-2	-2.62	3.50*10-2	-1.96	6.23*10-3	-2.29
ARL4A	ARL4A-205	2.63*10-2	-1.90	2.20*10-2	-1.89	7.56*10-3	-1.89
ARNT	ARNT-212	6.00*10-4	-1.92	1.75*10-2	-1.60	7.86*10-4	-1.76

ARPC1A	ARPC1A-205	9.92*10-3	1.42	3.80*10-2	1.37	6.81*10-3	1.40
ARPC1B	ARPC1B-201	8.11*10-3	1.73*10+2	2.83*10-2	1.24*10+2	4.55*10-3	1.71*10+2
ARRDC1-AS1	ARRDC1-AS1-201	1.12*10-3	1.38	7.25*10-3	1.31	5.73*10-4	1.34
ASAH1	ASAH1-239	8.61*10-3	-1.28	8.55*10-3	-1.36	1.97*10-3	-1.32
ASPSCR1	ASPSCR1-218	2.83*10-2	1.45	4.76*10-3	1.49	2.07*10-3	1.48
ASRGL1	ASRGL1-202	1.99*10-2	1.44	4.17*10-2	1.42	1.22*10-2	1.43
ATAD3A	ATAD3A-207	1.72*10-2	1.89	9.42*10-3	2.09	3.13*10-3	1.98
ATG13	ATG13-208	2.14*10-3	-2.28*10+2	1.67*10-2	-4.24*10+2	1.39*10-3	-3.80*10+2
ATG16L1	ATG16L1-202	2.23*10-2	3.26	5.94*10-3	2.73	3.59*10-3	2.95
ATG16L2	ATG16L2-215	2.82*10-2	1.77	4.40*10-2	1.52	1.29*10-2	1.63
ATG7	ATG7-221	2.04*10-2	-4.54	1.09*10-2	-6.34	4.00*10-3	-5.44
ATP2A3	ATP2A3-204	1.01*10-2	2.14	1.78*10-2	4.03	6.73*10-3	2.56
ATP2C1	ATP2C1-202	4.79*10-2	-1.23	1.85*10-2	-1.43	1.01*10-2	-1.33
ATP6V0A2	ATP6V0A2-205	9.85*10-3	-1.75	2.31*10-2	-2.30	4.55*10-3	-2.02
ATXN2	ATXN2-237	1.76*10-2	-1.79	1.34*10-2	-2.39	4.21*10-3	-2.09
B3GAT3P1	B3GAT3P1-201	1.46*10-2	-1.78	2.89*10-2	-1.79	6.62*10-3	-1.79
B9D2	B9D2-202	9.77*10-6	-4.06	4.47*10-2	-1.91	6.40*10-3	-1.64
BAIAP2L2	BAIAP2L2-201	3.84*10-2	2.02	2.63*10-2	1.59	1.09*10-2	1.77
BBS2	BBS2-213	1.94*10-2	-2.29	2.26*10-2	-1.64	5.39*10-3	-1.98
BCS1L	BCS1L-214	4.52*10-2	1.16*10+1	4.43*10-2	1.73*10+2	1.75*10-2	2.16*10+1
BECN1	BECN1-215	1.49*10-2	2.32	4.85*10-2	2.01	7.49*10-3	2.14
BEX5	BEX5-201	4.52*10-3	2.31	4.35*10-2	4.15	6.51*10-3	2.91
BISPR	BISPR-211	4.64*10-2	-2.27	1.45*10-2	-2.86	8.06*10-3	-2.57
BNIP1	BNIP1-201	3.72*10-3	-1.75	8.69*10-3	-1.74	1.21*10-3	-1.74
BNIP2	BNIP2-210	9.60*10-3	-1.26	4.44*10-3	-1.39	1.36*10-3	-1.33
BOLA2P2	BOLA2P2-202	4.95*10-2	1.46	7.98*10-3	2.12	2.00*10-2	1.73
BORCS5	BORCS5-203	2.36*10-2	-1.32	9.98*10-3	-1.50	4.10*10-3	-1.41
BPNT1	BPNT1-202	1.07*10-2	-3.43	4.15*10-2	-3.57	7.30*10-3	-3.51
BRD8	BRD8-226	1.30*10-2	-2.02	3.14*10-2	-2.05	6.47*10-3	-2.04
BX842570.1	BX842570.1-201	2.99*10-2	1.38	8.89*10-3	1.53	4.13*10-3	1.45
BX890604.2	BX890604.2-217	3.27*10-2	-1.44	2.73*10-2	-1.63	1.03*10-2	-1.53

C10orf88	C10orf88-201	3.74*10-2	1.83	1.54*10-2	2.10	8.29*10-3	1.95
C12orf4	C12orf4-208	2.17*10-2	-1.57*10+1	7.50*10-3	-2.14*10+1	3.12*10-3	-1.86*10+1
C16orf90	C16orf90-201	1.57*10-2	1.78*10+1	1.99*10-2	1.28*10+2	5.38*10-3	3.16*10+1
C19orf18	C19orf18-201	3.55*10-2	-1.47	3.51*10-2	-2.14	1.28*10-2	-1.80
C20orf203	C20orf203-202	1.26*10-2	2.90	4.13*10-2	3.60	7.88*10-3	3.20
C2CD2L	C2CD2L-205	2.87*10-2	1.24	2.16*10-2	1.29	7.63*10-3	1.27
C3	C3-212	1.52*10-2	2.38	4.33*10-2	2.61	9.25*10-3	2.49
C6orf136	C6orf136-207	2.83*10-2	1.35	4.84*10-2	1.38	1.41*10-2	1.36
C8orf33	C8orf33-202	1.53*10-2	-1.37	9.04*10-3	-1.48	2.88*10-3	-1.43
CACNA1I	CACNA1I-202	3.98*10-2	1.84	9.52*10-3	3.83	5.63*10-3	2.48
CALCOCO1	CALCOCO1-203	3.00*10-3	1.70	2.54*10-2	1.35	2.37*10-3	1.49
CALD1	CALD1-201	2.09*10-4	6.60	1.16*10-2	3.34	3.47*10-4	4.55
CAMK2D	CAMK2D-209	1.33*10-2	8.93*10+1	3.91*10-2	1.05*10+2	8.06*10-3	9.57*10+1
CANX	CANX-210	8.18*10-4	-1.41	2.18*10-2	-1.29	1.07*10-3	-1.35
CAPN3	CAPN3-220	1.05*10-2	-2.86	3.12*10-2	-3.62	5.80*10-3	-3.24
CAPN5	CAPN5-201	1.37*10-2	-6.18	3.69*10-2	-7.81	7.73*10-3	-7.00
CAPN5	CAPN5-204	4.91*10-2	-6.19*10+1	3.07*10-2	-6.12*10+1	1.33*10-2	-6.37*10+1
CAPN7	CAPN7-205	3.03*10-2	-1.78	3.93*10-2	-1.92	1.48*10-2	-1.86
CAPZA2	CAPZA2-205	2.63*10-3	-1.75	2.66*10-3	-2.12	4.66*10-4	-1.94
CAPZB	CAPZB-207	1.83*10-2	-3.16	3.00*10-2	-2.59	7.59*10-3	-2.89
CARMIL2	CARMIL2-205	7.89*10-3	2.46	1.55*10-3	1.45*10+2	5.68*10-4	4.81
CASC8	CASC8-201	4.13*10-2	2.30	4.28*10-2	9.98	1.78*10-2	3.69
CCDC126	CCDC126-202	1.49*10-2	-1.54	9.26*10-3	-1.78	2.92*10-3	-1.66
CCDC15	CCDC15-202	2.66*10-2	2.45	8.17*10-4	1.90*10+2	8.33*10-4	4.76
CCDC32	CCDC32-210	1.95*10-2	-6.40*10+1	2.71*10-2	-3.85*10+1	5.42*10-3	-5.58*10+1
CCDC6	CCDC6-202	2.34*10-2	-1.55	3.49*10-2	-1.69	9.67*10-3	-1.62
CCDC82	CCDC82-215	1.29*10-2	2.84	7.45*10-3	4.45	8.30*10-4	3.64
CCDC84	CCDC84-207	2.32*10-2	1.36	4.67*10-2	1.38	1.21*10-2	1.37
CCNG1	CCNG1-204	1.51*10-2	-1.40	6.35*10-3	-1.57	2.27*10-3	-1.48
CCSAP	CCSAP-202	1.39*10-2	-1.90	8.60*10-3	-2.29	2.66*10-3	-2.10
CCT3	CCT3-206	1.33*10-2	2.48	4.00*10-2	3.22	7.97*10-3	2.80

CCT5	CCT5-212	4.58*10-2	-4.70	3.68*10-2	-2.22	1.55*10-2	-3.46
CD160	CD160-203	1.83*10-2	3.73	7.85*10-3	1.23*10+1	2.87*10-3	5.64
CD33	CD33-206	3.09*10-3	2.00	3.47*10-2	1.70	3.09*10-3	1.83
CD3E	CD3E-204	4.44*10-2	1.51	4.94*10-3	2.48	4.17*10-3	1.87
CD44	CD44-210	1.66*10-2	-1.29	6.86*10-4	-1.59	4.72*10-4	-1.44
CD55	CD55-203	2.00*10-2	-1.45	6.20*10-3	-1.91	2.63*10-3	-1.68
CD96	CD96-207	1.97*10-4	2.09	3.61*10-2	2.37	1.00*10-2	2.09
CDC14B	CDC14B-201	1.87*10-2	1.73	2.29*10-2	3.42	6.24*10-3	2.30
CDC37P1	CDC37P1-201	6.44*10-3	-3.30	5.52*10-3	-1.93	1.22*10-3	-2.61
CDC73	CDC73-205	2.32*10-2	-1.34	3.96*10-2	-1.36	1.26*10-2	-1.35
CDCA5	CDCA5-202	4.98*10-2	-2.25	2.58*10-2	-1.64	1.26*10-2	-1.93
CDIPT	CDIPT-203	9.85*10-4	-1.30	1.81*10-2	-1.24	1.04*10-3	-1.27
CDK14	CDK14-203	7.48*10-3	2.73	2.90*10-2	3.84	4.64*10-3	3.18
CEACAM21	CEACAM21-206	3.72*10-2	3.27	9.52*10-3	7.25	5.26*10-3	4.52
CEMIP2	CEMIP2-210	3.93*10-2	-1.48	2.22*10-2	-1.68	5.86*10-3	-1.64
CENPU	CENPU-201	1.03*10-2	1.45	1.25*10-2	1.56	2.87*10-3	1.50
CEP104	CEP104-205	3.13*10-2	-2.10	1.26*10-2	-3.22	5.71*10-3	-2.64
CEP192	CEP192-215	5.65*10-3	2.21	1.76*10-3	1.89	6.54*10-4	1.98
CERCAM	CERCAM-209	7.95*10-3	-8.03*10+1	1.91*10-2	-1.43*10+2	3.34*10-3	-1.15*10+2
CERNA1	CERNA1-202	2.84*10-2	2.60	1.42*10-2	5.47	5.69*10-3	3.49
CEROX1	CEROX1-210	5.31*10-3	1.17*10+2	1.75*10-2	1.51*10+2	2.65*10-3	1.29*10+2
CEROX1	CEROX1-201	2.49*10-2	1.43	1.27*10-2	6.59	4.96*10-3	2.34
CERT1	CERT1-223	4.63*10-2	-3.25	4.67*10-2	-4.33	2.18*10-2	-3.85
CFAP298-TCP10L	CFAP298-TCP10L-211	2.10*10-2	-1.50	3.26*10-2	-1.51	8.73*10-3	-1.51
CFL1	CFL1-208	4.80*10-3	9.02	1.77*10-2	1.02*10+1	2.41*10-3	9.50
CHD4	CHD4-212	3.44*10-2	1.55	3.52*10-2	1.38	1.28*10-2	1.45
CHFR	CHFR-203	1.58*10-3	-1.45	2.83*10-3	-1.52	4.04*10-4	-1.48
CHFR	CHFR-213	1.67*10-2	1.23	2.54*10-2	1.26	6.47*10-3	1.25
CHN1	CHN1-230	1.03*10-2	1.06*10+1	1.51*10-2	2.26*10+2	3.72*10-3	2.42*10+1
CHTF18	CHTF18-213	1.83*10-2	2.19	3.79*10-3	2.78	1.69*10-3	2.45

CHTOP	CHTOP-207	2.90*10-2	5.07	1.37*10-2	7.23	5.70*10-3	5.94
CIART	CIART-203	2.58*10-2	7.67*10+1	4.52*10-2	1.41*10+2	1.28*10-2	9.36*10+1
CIP2A	CIP2A-201	2.80*10-2	-1.83	2.64*10-2	-2.79	1.08*10-2	-2.36
CIR1	CIR1-204	1.78*10-2	1.40	4.79*10-3	1.49	2.67*10-3	1.44
CITED2	CITED2-204	1.30*10-2	-5.41	4.53*10-3	-3.74	1.64*10-3	-3.67
CIZ1	CIZ1-207	1.24*10-2	1.73	3.20*10-2	1.65	7.75*10-3	1.69
CLASRP	CLASRP-216	3.36*10-2	1.28	1.21*10-2	1.38	5.40*10-3	1.33
CLEC12A-AS1	CLEC12A-AS1-201	1.05*10-2	2.50	3.02*10-2	3.55	7.21*10-3	2.91
CLEC16A	CLEC16A-201	7.61*10-4	1.26	1.71*10-2	1.21	7.62*10-4	1.23
CLIP4	CLIP4-204	2.62*10-2	-1.99	3.44*10-3	-3.13	2.86*10-3	-2.57
CLN3	CLN3-216	9.26*10-4	-3.07	3.14*10-3	-2.66	2.77*10-4	-2.86
CLNS1AP1	CLNS1AP1-201	1.47*10-2	2.06	3.10*10-2	1.92	7.31*10-3	1.98
CLPB	CLPB-203	1.13*10-2	-2.39	2.15*10-2	-3.03	4.55*10-3	-2.71
CNDP2	CNDP2-203	3.48*10-2	-1.51	1.96*10-2	-2.06	8.29*10-3	-1.78
CNGA1	CNGA1-203	2.76*10-2	2.40	1.52*10-2	1.25*10+1	6.02*10-3	4.06
CNN2	CNN2-206	1.18*10-2	1.29	3.20*10-2	1.31	6.63*10-3	1.30
CNOT1	CNOT1-212	1.55*10-2	-1.12*10+1	1.66*10-4	-1.81*10+1	2.01*10-4	-1.47*10+1
CNOT7	CNOT7-202	2.31*10-2	-1.89	4.45*10-2	-1.93	1.19*10-2	-1.91
CNTNAP3	CNTNAP3-212	1.51*10-2	2.05*10+1	2.00*10-2	1.63*10+2	5.15*10-3	3.54*10+1
COA8	COA8-215	4.41*10-2	-3.05	1.12*10-2	-1.95	6.51*10-3	-2.50
COG6	COG6-201	1.67*10-3	1.44	4.06*10-3	1.57	5.46*10-4	1.51
COL4A3	COL4A3-202	4.75*10-2	2.70	1.37*10-2	4.12	1.79*10-2	3.13
COMMD5	COMMD5-204	9.60*10-4	-3.10	1.50*10-2	-2.39	8.92*10-4	-2.74
COPA	COPA-215	3.39*10-2	-1.34	4.36*10-2	-1.47	1.60*10-2	-1.40
COPB2	COPB2-202	2.00*10-2	-1.27	4.84*10-2	-1.27	1.16*10-2	-1.27
COPZ1	COPZ1-206	9.22*10-3	-1.23	2.06*10-2	-1.23	3.99*10-3	-1.23
COQ8A	COQ8A-206	3.53*10-2	1.47	3.03*10-2	1.49	1.16*10-2	1.48
COX18	COX18-205	1.94*10-2	-1.40	2.21*10-2	-1.48	6.29*10-3	-1.44
COX4I1	COX4I1-218	2.94*10-2	-1.72	7.84*10-3	-2.34	3.96*10-3	-2.03
CPLANE1	CPLANE1-213	2.51*10-2	-7.46	2.29*10-2	-1.31*10+1	6.70*10-3	-1.08*10+1
CPNE3	CPNE3-213	3.00*10-2	-3.65	6.82*10-3	-3.20	3.61*10-3	-3.43

CPSF1	CPSF1-201	8.80*10-3	1.30	1.76*10-2	1.36	3.56*10-3	1.33
CPSF1	CPSF1-206	1.49*10-2	1.34	1.42*10-2	1.43	4.00*10-3	1.38
CREM	CREM-219	3.95*10-2	-2.00	2.44*10-2	-3.12	9.95*10-3	-2.56
CSDE1	CSDE1-214	4.86*10-2	-1.34	3.37*10-2	-1.48	1.63*10-2	-1.41
CSE1L	CSE1L-203	3.74*10-2	1.28	1.66*10-2	1.37	7.28*10-3	1.32
CSNK2B	CSNK2B-205	1.79*10-2	-1.32	3.99*10-2	-1.32	9.58*10-3	-1.32
CSPG5	CSPG5-202	7.72*10-3	7.12	1.02*10-2	1.16*10+2	2.13*10-3	1.33*10+1
CSRP1	CSRP1-210	9.84*10-3	1.31*10+2	3.12*10-2	1.19*10+2	5.62*10-3	1.22*10+2
CSRP1	CSRP1-216	1.87*10-2	-3.84	1.80*10-2	-7.45	5.38*10-3	-5.64
CTDSP1	CTDSP1-209	2.47*10-2	-1.32	4.41*10-2	-1.78	1.22*10-2	-1.55
CTSL	CTSL-205	7.40*10-4	-2.13	2.29*10-2	-1.81	1.13*10-3	-1.94
CTSW	CTSW-201	3.54*10-2	1.86	1.87*10-2	2.22	8.40*10-3	2.02
CUBN	CUBN-201	4.68*10-2	-2.50	2.03*10-2	-4.21	1.03*10-2	-3.35
CUL4A	CUL4A-203	4.68*10-2	-1.39	2.82*10-2	-1.59	1.38*10-2	-1.49
CXCR3	CXCR3-202	2.22*10-2	1.75	2.10*10-2	2.15	6.60*10-3	1.93
CXCR6	CXCR6-201	4.75*10-2	3.53	4.13*10-2	1.94	2.56*10-2	2.52
CXXC1	CXXC1-201	4.81*10-2	-1.50	3.92*10-2	-1.77	1.84*10-2	-1.62
CYB5B	CYB5B-204	3.86*10-2	-1.43	4.59*10-2	-1.48	1.59*10-2	-1.46
CYBC1	CYBC1-216	1.60*10-2	-1.45	4.03*10-2	-1.47	8.60*10-3	-1.46
CYBC1	CYBC1-206	4.81*10-2	-1.45*10+1	2.37*10-2	-2.45*10+2	4.75*10-3	-2.07*10+2
DAGLB	DAGLB-208	3.47*10-3	1.45	3.37*10-3	1.55	5.97*10-4	1.50
DAPK1	DAPK1-212	2.28*10-2	-3.63*10+1	5.77*10-3	-4.78*10+1	2.76*10-3	-4.21*10+1
DBI	DBI-212	6.32*10-3	-1.63	1.44*10-2	-1.93	2.44*10-3	-1.78
DBI	DBI-203	1.42*10-2	-2.34	3.77*10-2	-2.06	9.13*10-3	-2.10
DCAF11	DCAF11-210	1.26*10-2	-3.21	9.96*10-3	-2.67	2.71*10-3	-2.94
DCAF6	DCAF6-204	2.16*10-2	-1.64	3.66*10-2	-1.76	9.75*10-3	-1.70
DCBLD2	DCBLD2-201	1.93*10-2	1.24	1.46*10-2	1.30	4.64*10-3	1.27
DCTN2	DCTN2-206	4.45*10-2	9.07	4.18*10-2	1.38*10+2	1.67*10-2	1.72*10+1
DCXR	DCXR-207	1.52*10-2	1.52	2.59*10-2	1.79	6.94*10-3	1.64
DDB2	DDB2-213	4.82*10-2	1.31	8.15*10-3	2.31	2.22*10-2	1.68
DDX1	DDX1-207	2.68*10-2	1.45	2.33*10-2	1.32	8.52*10-3	1.38

DDX3X	DDX3X-253	4.37*10-2	-1.48	1.68*10-2	-2.11	7.76*10-3	-1.82
DDX5	DDX5-231	2.75*10-2	-1.24	2.74*10-2	-1.31	1.06*10-2	-1.28
DDX5	DDX5-210	4.42*10-2	-1.36	2.38*10-2	-1.59	1.08*10-2	-1.48
DDX51	DDX51-203	6.46*10-3	1.63	4.87*10-3	1.74	1.13*10-3	1.68
DEK	DEK-201	7.91*10-3	-3.18	3.75*10-2	-3.13	3.95*10-3	-3.19
DENND2D	DENND2D-204	2.48*10-2	1.29	2.95*10-2	1.27	1.08*10-2	1.28
DENND4B	DENND4B-208	1.99*10-2	1.20	2.73*10-2	1.24	6.94*10-3	1.22
DERL1	DERL1-203	2.56*10-2	-4.54	5.00*10-3	-5.64	2.68*10-3	-5.09
DES	DES-203	3.96*10-2	-4.65	2.08*10-2	-3.13	9.64*10-3	-3.90
DHRS7B	DHRS7B-205	2.89*10-2	-1.86	4.85*10-2	-2.16	1.44*10-2	-2.00
DHX16	DHX16-203	2.75*10-3	-5.33	1.10*10-3	-5.60	2.45*10-4	-5.47
DHX16	DHX16-201	4.20*10-3	1.59	3.06*10-3	1.65	6.67*10-4	1.62
DHX40	DHX40-208	2.06*10-2	-1.42	1.59*10-2	-1.52	5.30*10-3	-1.47
DKK3	DKK3-206	1.66*10-2	2.55	4.78*10-2	3.57	1.01*10-2	2.97
DLG1	DLG1-229	3.10*10-2	-3.88	4.67*10-2	-2.81	1.45*10-2	-3.35
DLGAP5	DLGAP5-203	1.55*10-2	-7.38*10+1	3.73*10-3	-8.32*10+1	1.61*10-3	-7.85*10+1
DMKN	DMKN-254	1.83*10-2	1.98*10+1	2.23*10-2	1.56*10+2	6.20*10-3	3.27*10+1
DNAH1	DNAH1-207	4.83*10-2	1.21	2.75*10-2	1.30	1.32*10-2	1.25
DNAJC13	DNAJC13-206	3.42*10-2	-5.83	4.12*10-2	-6.74	1.36*10-2	-6.32
DNAJC7	DNAJC7-226	1.48*10-3	1.67	3.29*10-2	1.26	1.18*10-3	1.43
DNAJC7	DNAJC7-202	2.45*10-2	1.62	4.15*10-2	1.59	1.29*10-2	1.61
DNMBP	DNMBP-203	2.52*10-2	1.46	3.01*10-2	1.32	9.79*10-3	1.38
DNMT3A	DNMT3A-204	3.97*10-3	-1.63	1.65*10-2	-1.64	2.06*10-3	-1.64
DNPEP	DNPEP-218	2.44*10-2	-4.20	1.52*10-2	-5.07	4.88*10-3	-4.74
DOCK1	DOCK1-206	5.09*10-3	-2.62	2.92*10-2	-2.78	3.53*10-3	-2.71
DOCK11	DOCK11-203	2.83*10-2	9.16	3.69*10-6	1.45	2.34*10-5	1.96
DOCK7	DOCK7-231	3.70*10-2	1.30	3.23*10-2	1.24	1.03*10-2	1.27
DPY19L1P1	DPY19L1P1-201	8.67*10-3	-3.16	1.75*10-2	-2.65	4.48*10-3	-2.84
DPY19L2P2	DPY19L2P2-202	3.93*10-2	2.31	1.85*10-2	5.79	8.51*10-3	3.30
DPY19L2P3	DPY19L2P3-201	8.63*10-3	-1.39	3.66*10-2	-1.39	5.89*10-3	-1.39
DPYSL3	DPYSL3-201	2.64*10-2	-1.79	1.98*10-2	-2.73	7.00*10-3	-2.27

DRAIC	DRAIC-265	4.42*10-2	-4.91*10+1	8.29*10-4	-7.88*10+1	1.16*10-3	-6.39*10+1
DROSHA	DROSHA-213	3.18*10-2	1.05*10+2	2.59*10-2	2.11	9.98*10-3	3.83
DSE	DSE-216	3.28*10-2	-1.22	1.13*10-2	-1.40	5.46*10-3	-1.31
DTL	DTL-201	1.47*10-2	4.28	1.33*10-2	5.64	3.61*10-3	4.60
DYNC1H1	DYNC1H1-209	4.91*10-2	-9.70*10+1	1.17*10-3	-3.07*10+2	1.57*10-3	-2.02*10+2
ECHDC1	ECHDC1-209	4.56*10-2	-1.32	2.28*10-2	-1.77	1.09*10-2	-1.55
EEF1D	EEF1D-254	1.36*10-2	2.38	4.25*10-2	2.47	7.88*10-3	2.41
EFTUD2	EFTUD2-205	1.46*10-3	1.26	1.66*10-2	1.22	1.20*10-3	1.24
EIF3E	EIF3E-208	1.69*10-2	-1.75	2.63*10-2	-1.74	5.98*10-3	-1.75
EIF4G1	EIF4G1-226	1.74*10-2	-2.11	1.62*10-2	-2.05	4.79*10-3	-2.07
EIF5A2	EIF5A2-201	2.25*10-3	-1.43	2.28*10-2	-1.38	1.96*10-3	-1.41
EMBP1	EMBP1-202	3.10*10-2	-1.56*10+1	2.80*10-2	-2.76*10+1	1.02*10-2	-2.16*10+1
EML3	EML3-207	8.62*10-3	1.28	3.44*10-2	1.26	5.06*10-3	1.27
EML4	EML4-207	1.11*10-2	1.57	2.43*10-3	1.93	1.06*10-3	1.73
EML4	EML4-203	3.15*10-2	-1.86*10+1	4.59*10-2	-8.17	1.46*10-2	-1.34*10+1
ENO1	ENO1-210	8.11*10-3	-1.35	4.59*10-2	-1.31	6.63*10-3	-1.33
ENOX2	ENOX2-207	5.52*10-3	-1.33	4.92*10-2	-1.26	5.58*10-3	-1.30
ENPP2	ENPP2-202	3.45*10-2	-1.52	3.69*10-2	-2.02	1.30*10-2	-1.77
ENTPD1	ENTPD1-203	1.34*10-2	-1.24	4.05*10-2	-1.25	8.07*10-3	-1.24
ENTPD6	ENTPD6-218	2.64*10-2	1.27	2.63*10-2	1.37	8.68*10-3	1.32
EP400	EP400-201	1.42*10-2	1.21	1.36*10-3	1.32	7.97*10-4	1.26
EPHB6	EPHB6-211	4.35*10-2	-1.26	2.72*10-2	-1.34	1.09*10-2	-1.30
EPHX2	EPHX2-201	1.30*10-2	1.69	1.59*10-2	7.77	3.95*10-3	2.79
ERAL1	ERAL1-204	2.65*10-2	1.27	3.07*10-3	1.55	1.97*10-3	1.40
ERC1	ERC1-201	9.15*10-4	-7.05	2.98*10-3	-5.01	2.52*10-4	-5.97
ERCC6	ERCC6-212	5.15*10-3	1.29	2.43*10-2	1.31	2.83*10-3	1.30
ERF	ERF-207	1.08*10-2	-3.56	6.06*10-4	-3.53	3.67*10-4	-3.55
ERLIN1	ERLIN1-202	2.85*10-2	-1.45	5.92*10-3	-1.80	3.35*10-3	-1.63
ESF1	ESF1-202	5.76*10-4	-4.90*10+1	9.49*10-3	-2.64*10+1	4.85*10-4	-3.77*10+1
ESR2	ESR2-208	4.96*10-2	1.66	1.59*10-2	4.03	1.17*10-2	2.29
ETAA1	ETAA1-203	2.23*10-2	2.10	2.14*10-2	4.72	6.78*10-3	2.91

EXOC3	EXOC3-202	1.55*10-2	1.38	2.07*10-2	1.51	4.91*10-3	1.45
EXOC3L4	EXOC3L4-205	4.83*10-2	-4.19*10+1	1.89*10-3	-9.00*10+1	2.12*10-3	-6.59*10+1
EXOSC10	EXOSC10-207	2.66*10-2	1.27	3.39*10-2	1.31	1.04*10-2	1.29
FAAP100	FAAP100-206	8.15*10-3	-3.18	3.86*10-2	-6.80	5.88*10-3	-5.00
FAM174B	FAM174B-209	1.85*10-2	-9.84*10+1	1.62*10-2	-1.42*10+2	5.01*10-3	-1.20*10+2
FAM193B	FAM193B-219	1.70*10-2	1.28	2.08*10-2	1.31	5.67*10-3	1.29
FAM201A	FAM201A-201	1.21*10-2	8.51	2.52*10-2	4.07*10+1	8.11*10-3	2.16*10+1
FAM214B	FAM214B-209	3.35*10-4	-1.58	1.74*10-2	-3.56	3.70*10-3	-2.73
FAM215A	FAM215A-202	1.04*10-2	2.19	4.24*10-2	1.31	7.51*10-3	1.63
FAM234A	FAM234A-206	1.21*10-2	-2.07	2.57*10-2	-2.05	5.32*10-3	-2.07
FARP2	FARP2-211	3.66*10-2	1.42	1.17*10-2	1.53	6.00*10-3	1.47
FBXO3	FBXO3-213	6.43*10-4	-1.50	1.71*10-3	-1.58	1.32*10-4	-1.54
FCAR	FCAR-203	3.22*10-2	-1.22	9.94*10-3	-1.33	4.80*10-3	-1.27
FCHSD2	FCHSD2-201	1.24*10-2	1.27	3.26*10-3	1.38	1.60*10-3	1.32
FERMT1	FERMT1-203	5.69*10-3	2.39*10+1	3.40*10-2	2.84*10+1	4.61*10-3	2.91*10+1
FES	FES-202	3.24*10-2	-1.80	2.85*10-2	-2.24	1.05*10-2	-2.02
FGD4	FGD4-206	1.47*10-2	-1.62	1.48*10-2	-1.77	4.69*10-3	-1.69
FGD4	FGD4-211	2.40*10-2	-1.59	1.25*10-2	-1.84	5.70*10-3	-1.72
FGFR1OP2	FGFR1OP2-203	4.30*10-2	-1.34	1.81*10-2	-1.43	9.14*10-3	-1.38
FKBP10	FKBP10-204	3.47*10-3	1.09*10+1	4.46*10-2	3.37	4.85*10-3	4.83
FKBP4P2	FKBP4P2-201	8.75*10-3	6.64	1.24*10-2	6.89	2.40*10-3	6.46
FLI1	FLI1-209	1.70*10-2	2.50	4.43*10-2	1.45	9.05*10-3	1.77
FLNB	FLNB-204	7.15*10-4	-2.31	1.29*10-2	-2.21	8.11*10-4	-2.29
FLNB	FLNB-213	7.49*10-3	-1.28	1.31*10-2	-1.64	2.52*10-3	-1.46
FLNB	FLNB-201	1.15*10-2	-1.60	2.49*10-2	-1.63	4.54*10-3	-1.62
FLNB	FLNB-214	4.70*10-2	-2.22	3.88*10-2	-2.68	1.65*10-2	-2.34
FLYWCH2	FLYWCH2-205	4.26*10-2	-1.27	3.66*10-2	-1.78	1.63*10-2	-1.50
FMNL2	FMNL2-202	3.24*10-2	1.43	7.69*10-3	1.96	4.68*10-3	1.66
FOXM1	FOXM1-210	2.65*10-2	-3.76	5.78*10-3	-5.63	3.00*10-3	-4.69
FTH1	FTH1-202	9.25*10-3	1.69	1.74*10-2	1.84	3.48*10-3	1.76
FTH1P25	FTH1P25-201	1.53*10-2	-8.04*10+1	3.26*10-2	-4.72*10+1	7.11*10-3	-6.64*10+1

FUNDC2P4	FUNDC2P4-201	3.80*10-2	3.20	4.73*10-2	5.94	1.65*10-2	4.17
FUS	FUS-211	3.42*10-2	4.59	2.85*10-2	2.20	1.16*10-2	2.51
FYN	FYN-223	7.86*10-3	-1.33	9.13*10-3	-1.40	1.96*10-3	-1.36
GANAB	GANAB-211	2.08*10-2	1.39	4.12*10-2	1.21	5.92*10-3	1.30
GAS7	GAS7-211	2.73*10-2	-1.80	3.51*10-2	-2.00	9.61*10-3	-1.89
GATA3	GATA3-202	1.69*10-2	1.61	4.37*10-2	2.21	3.40*10-2	1.74
GGT1	GGT1-223	3.30*10-2	-6.63	2.32*10-3	-5.69	1.93*10-3	-6.16
GGT1	GGT1-229	3.88*10-2	3.26	1.66*10-2	7.73*10+1	7.93*10-3	6.25
GIGYF2	GIGYF2-210	3.40*10-2	-3.19	1.09*10-2	-3.90	5.50*10-3	-3.55
GIT2	GIT2-220	4.05*10-3	-2.54*10+2	1.99*10-2	-8.54*10+1	2.46*10-3	-1.70*10+2
GLIPR2	GLIPR2-201	4.05*10-2	-1.30	2.32*10-2	-1.57	1.02*10-2	-1.44
GLT8D1	GLT8D1-212	1.04*10-2	-1.39	1.71*10-2	-1.41	3.49*10-3	-1.40
GNB1	GNB1-210	1.38*10-2	1.61	4.72*10-2	1.30	7.41*10-3	1.43
GNPDA1	GNPDA1-208	3.34*10-2	-1.64	2.72*10-2	-1.90	1.01*10-2	-1.77
GNS	GNS-206	2.74*10-2	-1.32	3.06*10-2	-1.46	9.91*10-3	-1.39
GORASP1	GORASP1-224	8.35*10-3	-1.75	2.90*10-2	-1.63	4.82*10-3	-1.69
GOSR2	GOSR2-201	1.63*10-3	-8.43	1.12*10-2	-2.65	2.77*10-3	-2.54
GPR141	GPR141-203	4.04*10-4	-1.49	1.49*10-2	-1.34	5.39*10-4	-1.41
GPR155	GPR155-206	2.43*10-3	1.36	1.35*10-2	1.38	1.37*10-3	1.37
GPRC5B	GPRC5B-201	4.17*10-2	2.01	4.72*10-2	3.07	1.75*10-2	2.43
GPT	GPT-206	1.46*10-2	1.63*10+1	1.94*10-2	1.21*10+2	4.93*10-3	2.85*10+1
GRIP1	GRIP1-213	4.23*10-2	-3.51	2.13*10-2	-5.21	9.92*10-3	-4.36
GRN	GRN-208	2.93*10-2	-1.21	4.72*10-2	-1.24	1.36*10-2	-1.23
GSPT1	GSPT1-203	1.73*10-2	-1.31	1.49*10-2	-1.40	4.70*10-3	-1.35
GTF2IRD2B	GTF2IRD2B-204	6.55*10-3	1.83	2.54*10-2	1.92	3.81*10-3	1.87
GTF3C2	GTF3C2-202	1.08*10-2	1.33	1.55*10-2	1.42	3.52*10-3	1.37
GUK1	GUK1-233	2.82*10-3	-2.18	2.23*10-2	-2.08	2.15*10-3	-2.13
HAX1	HAX1-210	3.97*10-2	1.39	7.03*10-3	1.51	4.44*10-3	1.45
HDGF	HDGF-208	4.01*10-2	-2.68	2.15*10-2	-3.90	9.80*10-3	-3.28
HECW2-AS1	HECW2-AS1-202	1.53*10-2	1.72	2.70*10-2	3.01	1.30*10-2	2.26
HERC2	HERC2-208	2.65*10-2	1.46	3.58*10-2	1.55	1.07*10-2	1.50

HERPUD1	HERPUD1-215	1.05*10-2	-1.32	3.82*10-2	-1.32	6.64*10-3	-1.32
HERPUD1	HERPUD1-202	2.46*10-2	1.92	2.62*10-2	2.27	1.01*10-2	2.02
HEXIM2	HEXIM2-208	1.97*10-2	3.75	2.55*10-2	4.64	7.56*10-3	4.19
HEY1	HEY1-203	2.05*10-2	3.20	1.41*10-2	3.97*10+1	4.78*10-3	5.91
HIPK1	HIPK1-207	2.50*10-3	-2.48	8.26*10-3	-3.62	1.18*10-3	-3.30
HMGB3	HMGB3-204	3.46*10-2	-3.04	2.58*10-2	-2.97	1.02*10-2	-3.01
HOOK2	HOOK2-214	2.33*10-2	1.93	4.92*10-2	2.29	1.31*10-2	2.09
HOOK3	HOOK3-205	3.60*10-3	-3.49	5.96*10-3	-3.40	9.05*10-4	-3.46
HSD17B4	HSD17B4-214	4.51*10-2	-1.40	2.91*10-2	-1.67	1.29*10-2	-1.54
HSPA8	HSPA8-222	4.34*10-2	1.37	3.42*10-2	1.45	1.40*10-2	1.41
ICAM3	ICAM3-207	3.21*10-2	1.31	1.51*10-2	1.43	6.35*10-3	1.37
ICOS	ICOS-201	4.58*10-2	1.54	2.23*10-2	2.66	4.95*10-2	1.74
IFFO1	IFFO1-206	1.12*10-2	-1.96	4.05*10-2	-1.66	7.35*10-3	-1.81
IFRD2	IFRD2-207	1.06*10-2	1.37	1.60*10-2	1.47	3.52*10-3	1.42
IFT22	IFT22-209	2.06*10-2	-2.69	9.09*10-3	-4.42	3.52*10-3	-3.55
IFT22	IFT22-206	4.01*10-2	-6.08*10+1	4.40*10-3	-5.46*10+1	2.89*10-3	-5.79*10+1
IGFBP7	IGFBP7-203	3.93*10-2	-1.33	3.58*10-3	-1.89	2.81*10-3	-1.61
IGFLR1	IGFLR1-207	2.34*10-2	1.43	1.31*10-2	1.61	3.27*10-2	1.41
IGHV3-13	IGHV3-13-201	4.11*10-2	2.72	4.83*10-2	3.10	2.00*10-2	2.87
IKBKG	IKBKG-209	3.07*10-2	1.32	4.66*10-2	1.39	1.12*10-2	1.36
IL18BP	IL18BP-201	4.31*10-2	1.73	1.20*10-2	1.35	5.58*10-3	1.52
IL32	IL32-222	2.88*10-2	1.44	4.50*10-2	2.40	1.36*10-2	1.80
IL3RA	IL3RA-201	1.72*10-2	1.50	8.34*10-3	1.58	2.38*10-3	1.54
ILK	ILK-204	4.29*10-2	-2.07	2.05*10-2	-2.17	9.79*10-3	-2.12
IMPDH1	IMPDH1-217	3.48*10-2	-1.27	5.06*10-4	-1.57	5.56*10-4	-1.42
INAFM1	INAFM1-201	4.00*10-3	1.23	3.11*10-2	1.21	3.50*10-3	1.22
INCENP	INCENP-203	8.80*10-3	8.84*10+1	2.57*10-2	1.13*10+2	4.69*10-3	9.72*10+1
ING4	ING4-203	2.97*10-2	-1.56	4.24*10-2	-1.47	1.27*10-2	-1.51
INO80C	INO80C-207	1.02*10-2	-3.35	1.00*10-2	-2.84	2.48*10-3	-3.09
INPP5K	INPP5K-202	2.99*10-2	-1.53	2.11*10-2	-1.66	7.95*10-3	-1.60
INTS3	INTS3-204	7.44*10-3	-2.36	3.44*10-2	-2.05	5.14*10-3	-2.21

IPO5	IPO5-223	4.51*10-2	1.41	2.62*10-2	1.46	1.24*10-2	1.44
IQCB1	IQCB1-207	2.14*10-2	-1.89	2.62*10-2	-1.60	7.69*10-3	-1.74
IQGAP2	IQGAP2-201	3.32*10-2	-1.24	1.71*10-2	-1.43	8.06*10-3	-1.34
ITFG2	ITFG2-213	2.27*10-2	1.41	4.01*10-2	1.46	1.05*10-2	1.43
ITGA3	ITGA3-202	4.19*10-2	1.75	1.79*10-2	2.16	1.25*10-2	1.91
ITGAV	ITGAV-201	5.47*10-3	-1.24	1.43*10-3	-1.39	4.29*10-4	-1.31
ITGB3BP	ITGB3BP-202	3.96*10-2	-2.25	2.63*10-2	-1.97	1.11*10-2	-2.11
ITK	ITK-204	4.13*10-2	1.62	2.49*10-2	2.20	1.29*10-2	1.86
ITPR1	ITPR1-229	1.26*10-2	1.73	3.84*10-3	1.95	1.86*10-3	1.84
ITSN1	ITSN1-210	2.92*10-2	-2.31	1.48*10-4	-7.31	2.86*10-4	-4.83
IVD	IVD-215	2.37*10-2	-1.43	3.69*10-2	-1.46	1.05*10-2	-1.44
JAK2	JAK2-202	3.50*10-2	-3.28	2.62*10-2	-3.01	1.13*10-2	-3.15
JKAMP	JKAMP-213	2.46*10-2	1.93	2.95*10-2	1.91	9.95*10-3	1.91
JPT1	JPT1-212	3.78*10-2	9.32	3.99*10-2	1.15*10+2	1.49*10-2	1.73*10+1
KANK1	KANK1-206	4.19*10-2	1.26*10+1	3.08*10-2	1.79*10+1	1.29*10-2	1.46*10+1
KARS1	KARS1-208	4.30*10-2	-1.69	3.20*10-2	-2.19	1.34*10-2	-1.94
KAT6A	KAT6A-215	3.20*10-3	-2.23	2.25*10-2	-2.13	1.99*10-3	-2.19
KCNAB1	KCNAB1-201	2.55*10-2	2.86*10+1	2.04*10-2	8.36*10+1	7.27*10-3	4.33*10+1
KCNH2	KCNH2-206	3.23*10-2	4.41	2.59*10-2	2.96*10+1	9.09*10-3	7.72
KCNJ10	KCNJ10-203	1.27*10-2	-5.24	1.43*10-2	-6.45	3.46*10-3	-5.77
KCNN4	KCNN4-209	1.84*10-3	1.11*10+1	1.23*10-3	1.91	2.04*10-4	3.25
KCNN4	KCNN4-206	2.72*10-2	1.31	9.59*10-3	1.76	4.36*10-3	1.50
KCNQ3	KCNQ3-205	1.50*10-3	8.25	7.17*10-3	1.23*10+1	6.51*10-4	9.87
KCTD21-AS1	KCTD21-AS1-203	3.92*10-2	4.55	1.70*10-2	1.60*10+2	8.13*10-3	8.84
KDM5B	KDM5B-237	4.35*10-2	1.36	3.83*10-2	2.45	1.50*10-2	1.75
KHNYN	KHNYN-201	2.17*10-2	1.20	1.06*10-2	1.27	3.99*10-3	1.24
KIAA0319L	KIAA0319L-209	1.24*10-2	-2.87	3.34*10-2	-2.78	6.89*10-3	-2.81
KIAA0895L	KIAA0895L-207	1.40*10-2	-2.77	9.45*10-3	-4.36	2.87*10-3	-3.59
KIAA0930	KIAA0930-212	1.78*10-2	-1.30	1.45*10-2	-1.44	3.35*10-3	-1.38
KIAA0930	KIAA0930-211	4.91*10-2	-1.34	1.77*10-2	-1.49	9.67*10-3	-1.41
KIF20B	KIF20B-204	2.17*10-3	1.57	6.11*10-4	1.92	1.32*10-4	1.74

KIF21A	KIF21A-214	3.74*10-2	1.60	6.89*10-3	1.91	4.17*10-3	1.73
KIRREL3	KIRREL3-205	1.35*10-2	2.73	1.95*10-2	3.18	4.69*10-3	2.93
KIZ	KIZ-214	3.06*10-2	1.46	1.09*10-2	1.80	5.00*10-3	1.61
KLC1	KLC1-213	8.52*10-3	1.03*10+1	1.89*10-2	2.37*10+1	3.66*10-3	1.44*10+1
KLC1	KLC1-218	1.34*10-2	-1.34	3.12*10-3	-1.57	1.27*10-3	-1.45
KLF6	KLF6-204	1.44*10-2	-2.03	3.26*10-2	-1.83	6.94*10-3	-1.93
KLF6	KLF6-201	2.29*10-2	-1.36	4.15*10-2	-1.58	1.12*10-2	-1.47
KLHDC4	KLHDC4-225	2.77*10-2	1.27	1.56*10-2	1.35	6.03*10-3	1.31
KLRF1	KLRF1-201	4.00*10-2	5.23	4.28*10-2	8.81	2.38*10-2	6.23
KMT2A	KMT2A-208	3.87*10-2	1.30	9.10*10-3	1.53	4.82*10-3	1.41
KMT2B	KMT2B-209	1.25*10-2	1.23	2.17*10-2	1.27	4.95*10-3	1.25
KMT2C	KMT2C-210	5.98*10-3	-2.29	7.25*10-3	-1.98	1.40*10-3	-2.14
KRIT1	KRIT1-221	7.13*10-3	1.21	1.54*10-2	1.22	3.16*10-3	1.21
KRT72	KRT72-202	4.47*10-2	7.33	2.07*10-2	1.16*10+1	1.38*10-2	8.18
KTN1	KTN1-203	4.46*10-2	-1.45	2.41*10-2	-1.65	1.14*10-2	-1.55
KYNU	KYNU-204	2.94*10-2	-1.28	3.54*10-2	-1.38	1.13*10-2	-1.33
LACTB2	LACTB2-204	1.88*10-3	-2.05	9.57*10-3	-2.12	9.22*10-4	-2.08
LAIR2	LAIR2-202	2.34*10-2	-6.26	2.43*10-2	-6.14	7.69*10-3	-6.21
LARS1	LARS1-201	1.75*10-2	1.32	5.38*10-3	1.38	2.46*10-3	1.34
LAT	LAT-202	8.59*10-3	2.25	4.06*10-2	4.21	6.35*10-3	2.93
LAT	LAT-201	1.10*10-2	3.04	4.87*10-3	5.31	2.91*10-3	3.36
LAT	LAT-213	1.36*10-2	1.45	2.79*10-3	1.68	1.21*10-3	1.56
LCK	LCK-206	2.64*10-2	2.18	4.93*10-3	3.92	2.61*10-3	2.78
LIG4	LIG4-202	3.67*10-2	-1.26	3.72*10-2	-1.32	6.99*10-3	-1.31
LIMS1	LIMS1-215	3.50*10-3	-1.22	9.04*10-4	-1.33	2.21*10-4	-1.27
LINC00243	LINC00243-201	3.36*10-2	1.49	2.02*10-2	1.70	8.60*10-3	1.59
LINC00384	LINC00384-201	2.42*10-2	1.81	1.34*10-2	9.88*10+1	4.71*10-3	3.61
LINC00426	LINC00426-203	2.72*10-2	2.30	4.48*10-2	6.84	1.30*10-2	3.44
LINC00662	LINC00662-242	8.42*10-4	1.66	1.49*10-3	3.17	4.77*10-4	2.18
LINC00662	LINC00662-270	2.42*10-3	-2.23	2.93*10-5	-4.50	1.97*10-5	-3.36
LINC00662	LINC00662-276	1.77*10-2	-6.52*10+1	9.38*10-3	-1.14*10+2	1.51*10-3	-1.39*10+2

LINC00667	LINC00667-213	4.11*10-2	-2.45	2.84*10-2	-2.86	1.39*10-2	-2.64
LINC00861	LINC00861-204	1.98*10-2	5.25	1.95*10-2	1.53*10+2	6.07*10-3	1.02*10+1
LINC00877	LINC00877-201	2.02*10-4	-4.90*10+1	2.63*10-2	-5.34*10+1	5.26*10-4	-5.50*10+1
LINC00900	LINC00900-210	1.95*10-2	-1.41	2.32*10-2	-2.58	6.57*10-3	-1.99
LINC00968	LINC00968-202	4.34*10-2	-1.94	7.85*10-3	-2.78	5.18*10-3	-2.33
LINC01138	LINC01138-204	4.34*10-2	1.20	2.53*10-2	1.29	1.14*10-2	1.24
LINC01278	LINC01278-228	4.08*10-3	2.35*10+1	8.24*10-3	3.53*10+1	1.16*10-3	1.95*10+1
LINC01331	LINC01331-202	3.54*10-2	2.40	2.97*10-2	3.21	1.15*10-2	2.75
LINC01426	LINC01426-202	1.01*10-2	-4.21	2.38*10-3	-4.69	8.75*10-4	-4.44
LINC01572	LINC01572-201	6.16*10-3	4.61	4.39*10-2	2.86	6.48*10-3	3.54
LINC01684	LINC01684-217	2.34*10-2	1.31*10+1	3.51*10-2	9.54*10+1	1.03*10-2	2.34*10+1
LINC01733	LINC01733-201	1.03*10-2	-1.73*10+1	2.63*10-2	-5.81	5.10*10-3	-1.15*10+1
LINC02021	LINC02021-206	2.80*10-2	-8.76	1.52*10-3	-1.86*10+1	1.20*10-3	-1.41*10+1
LINC02207	LINC02207-225	4.18*10-2	-3.96	9.13*10-3	-3.17	5.45*10-3	-3.56
LINC02249	LINC02249-203	2.44*10-3	6.47	9.27*10-3	6.04	7.15*10-4	6.16
LINC02714	LINC02714-201	9.03*10-3	1.06*10+2	3.06*10-2	7.87*10+1	5.27*10-3	8.24*10+1
LINC02804	LINC02804-204	9.27*10-3	-3.68	3.02*10-2	-6.31	5.35*10-3	-5.00
LINS1	LINS1-208	1.70*10-3	-2.18	9.92*10-3	-1.94	8.72*10-4	-2.06
LLGL1	LLGL1-203	2.23*10-3	-2.89	6.80*10-3	-2.51	7.81*10-4	-2.70
LMTK3	LMTK3-203	4.75*10-2	1.92	3.31*10-2	3.30	1.48*10-2	2.25
LONP2	LONP2-203	1.74*10-2	-2.51	1.74*10-2	-3.65	4.94*10-3	-3.07
LPAR2	LPAR2-203	4.74*10-2	1.23	1.22*10-2	1.44	6.80*10-3	1.33
LRP8	LRP8-204	3.99*10-2	-5.83*10+1	2.25*10-2	-2.36*10+2	1.00*10-2	-1.47*10+2
LRRC6	LRRC6-211	1.08*10-2	5.78	6.27*10-3	9.38	1.77*10-3	7.01
LRRC63	LRRC63-203	4.55*10-2	8.53	4.38*10-2	9.66*10+1	1.74*10-2	1.57*10+1
LRRFIP2	LRRFIP2-218	4.62*10-3	-1.71	1.76*10-3	-2.14	4.60*10-4	-1.93
LSM14A	LSM14A-202	2.48*10-2	-1.26	2.45*10-2	-1.44	7.89*10-3	-1.38
LSS	LSS-208	1.95*10-3	1.48	3.70*10-2	1.38	2.57*10-3	1.43
LUNAR1	LUNAR1-206	3.75*10-2	1.37*10+1	3.74*10-2	1.34*10+1	1.38*10-2	1.35*10+1
LY9	LY9-201	1.26*10-3	1.66	8.29*10-3	2.14	5.66*10-4	1.86
LYPLA2	LYPLA2-204	4.09*10-3	-1.71	2.36*10-3	-2.08	5.09*10-4	-1.89

MAGED2	MAGED2-212	1.68*10-2	-2.13	3.62*10-2	-1.95	1.18*10-2	-1.87
MAL	MAL-201	2.88*10-2	1.70	4.64*10-2	2.31	2.85*10-2	1.83
MAP2K1	MAP2K1-203	2.29*10-4	3.20	3.72*10-2	1.45	7.15*10-4	1.99
MAP3K12	MAP3K12-203	1.39*10-2	-1.42	5.25*10-3	-1.74	1.76*10-3	-1.59
MAP3K4	MAP3K4-208	4.81*10-2	1.55	2.86*10-2	1.61	1.22*10-2	1.58
MAPRE2	MAPRE2-211	3.17*10-2	1.55	4.32*10-2	1.58	1.55*10-2	1.56
MARF1	MARF1-203	3.27*10-2	-1.58	3.73*10-2	-1.88	1.26*10-2	-1.73
MARS1	MARS1-209	2.55*10-2	1.34	3.69*10-2	1.28	4.78*10-3	1.32
MCCC1	MCCC1-203	2.89*10-2	3.71	2.67*10-2	1.10*10+2	9.28*10-3	7.18
MCF2	MCF2-210	4.40*10-4	-1.92*10+1	2.27*10-2	-1.30*10+1	8.31*10-4	-1.61*10+1
MCF2L	MCF2L-201	6.81*10-3	2.99	3.92*10-4	7.43	1.56*10-3	4.11
MCF2L	MCF2L-205	1.43*10-2	5.65	5.85*10-3	5.32*10+1	2.05*10-3	1.02*10+1
MCM10	MCM10-201	1.03*10-2	-2.30	1.07*10-3	-2.67	4.55*10-4	-2.50
MCM7	MCM7-210	4.43*10-2	1.94	4.02*10-2	2.05	1.60*10-2	1.99
MCOLN1	MCOLN1-209	4.43*10-3	3.10	2.68*10-2	1.90	3.08*10-3	2.32
MCOLN2	MCOLN2-204	3.74*10-2	1.98	1.23*10-4	4.02	3.01*10-4	2.66
MCRIP2	MCRIP2-207	1.38*10-2	2.23	4.52*10-3	2.72	1.62*10-3	2.45
MED13L	MED13L-225	1.27*10-2	1.74	3.87*10-2	1.64	7.57*10-3	1.69
MED17	MED17-201	3.34*10-2	-1.47	1.80*10-2	-1.65	7.85*10-3	-1.56
MED30	MED30-203	1.23*10-3	-2.61	1.83*10-2	-1.55	1.19*10-3	-2.08
MEF2C	MEF2C-207	2.61*10-2	-7.50	2.13*10-2	-6.82	6.88*10-3	-7.49
MEG3	MEG3-224	2.01*10-2	5.69	4.43*10-2	3.04*10+1	1.10*10-2	9.69
MEGF6	MEGF6-202	4.89*10-2	1.39	2.66*10-2	1.48	1.28*10-2	1.43
METTL16	METTL16-204	1.75*10-2	1.36	1.14*10-2	1.49	3.68*10-3	1.42
MFSD4B	MFSD4B-202	1.92*10-2	2.14	2.59*10-2	1.40	7.45*10-3	1.69
MFSD4B	MFSD4B-210	4.64*10-2	-2.25	2.41*10-2	-2.14	1.16*10-2	-2.19
MGAT3	MGAT3-201	3.87*10-2	1.48	4.10*10-3	2.32	3.05*10-3	1.80
MGAT4B	MGAT4B-216	1.94*10-3	1.78	1.67*10-2	1.61	1.25*10-3	1.69
MIB2	MIB2-229	1.01*10-2	2.66	3.77*10-2	1.98	7.82*10-3	2.25
MIGA2	MIGA2-209	1.47*10-2	-1.68	4.82*10-2	-1.36	9.65*10-3	-1.49
MIR646HG	MIR646HG-241	3.99*10-2	-6.86	3.85*10-2	-7.18	1.47*10-2	-7.02

MIR9-3HG	MIR9-3HG-222	3.08*10-2	1.06*10+1	1.25*10-2	3.32*10+1	5.58*10-3	1.51*10+1
MKNK1	MKNK1-204	9.29*10-3	1.60	2.03*10-2	1.73	4.32*10-3	1.65
MKRN9P	MKRN9P-201	1.16*10-2	-1.33	4.28*10-3	-1.49	1.45*10-3	-1.41
MLLT11	MLLT11-201	3.76*10-2	1.23	2.65*10-2	1.32	1.04*10-2	1.27
MOGS	MOGS-207	1.37*10-2	-1.69	4.22*10-2	-1.79	8.72*10-3	-1.77
MORC2	MORC2-204	9.90*10-3	1.57	3.86*10-2	1.67	6.77*10-3	1.61
MPZL1	MPZL1-206	1.60*10-2	-1.75	1.65*10-2	-1.91	4.60*10-3	-1.83
MROH1	MROH1-211	2.48*10-2	1.26	2.56*10-2	1.30	7.49*10-3	1.28
MRPL28	MRPL28-205	1.36*10-2	-3.14	7.72*10-3	-2.77	2.44*10-3	-2.95
MRTFA	MRTFA-209	2.23*10-2	-1.28	1.58*10-2	-1.41	5.29*10-3	-1.34
MSC-AS1	MSC-AS1-208	2.80*10-2	1.13*10+1	2.90*10-2	1.07*10+2	9.90*10-3	2.06*10+1
MSL1	MSL1-210	1.91*10-2	-2.97	5.97*10-3	-2.69	2.53*10-3	-2.83
MT1X	MT1X-202	9.28*10-3	-2.03	4.69*10-3	-2.63	1.37*10-3	-2.33
MTA2	MTA2-202	1.97*10-2	1.78	3.39*10-2	1.50	5.05*10-3	1.66
MTA3	MTA3-209	8.76*10-3	-3.55	3.13*10-3	-4.35	9.81*10-4	-3.95
MTHFD1	MTHFD1-218	1.13*10-2	1.53	3.55*10-2	1.54	6.70*10-3	1.54
MTRF1	MTRF1-208	3.32*10-2	1.44	4.97*10-2	1.48	1.59*10-2	1.46
MTSS1	MTSS1-216	4.17*10-2	-1.65	4.43*10-2	-2.31	1.83*10-2	-1.98
MVD	MVD-209	1.91*10-2	1.43	2.70*10-2	1.50	7.10*10-3	1.47
MYADM	MYADM-205	1.68*10-2	-1.22	3.54*10-2	-1.23	8.22*10-3	-1.23
MYADM	MYADM-202	2.02*10-2	-1.75	2.24*10-2	-1.81	8.00*10-3	-1.78
MYBBP1A	MYBBP1A-212	4.62*10-2	1.28	4.22*10-2	1.33	1.72*10-2	1.30
MYBPH	MYBPH-201	1.73*10-2	2.08	3.41*10-3	3.10	2.56*10-3	2.47
MYH11	MYH11-205	2.18*10-2	-8.43	4.14*10-2	-6.96	1.10*10-2	-7.69
MYO1C	MYO1C-215	4.92*10-2	-3.02*10+1	2.11*10-3	-6.97*10+1	2.16*10-3	-5.00*10+1
NBEAL2	NBEAL2-210	3.56*10-2	1.31	2.91*10-2	1.37	9.88*10-3	1.34
NCOA2	NCOA2-203	2.23*10-3	-2.52	2.03*10-2	-3.10	3.83*10-3	-2.91
NCOA3	NCOA3-201	7.69*10-3	-1.96	1.39*10-2	-2.42	3.06*10-3	-2.23
NCOR2	NCOR2-220	7.39*10-3	-2.06	1.82*10-2	-1.86	3.19*10-3	-1.96
NDST1	NDST1-208	1.37*10-2	-2.36	3.73*10-2	-2.26	7.73*10-3	-2.31
NDUFS1	NDUFS1-207	2.13*10-2	-1.35	1.12*10-2	-1.47	3.94*10-3	-1.41

NDUFS2	NDUFS2-207	2.87*10-2	-1.25	1.98*10-2	-1.34	7.38*10-3	-1.29
NDUFS2	NDUFS2-203	4.53*10-2	1.93	1.02*10-3	2.87	1.24*10-3	2.33
NEK6	NEK6-208	1.53*10-2	-1.61	3.25*10-2	-1.64	7.35*10-3	-1.62
NEK9	NEK9-203	7.67*10-3	-1.42	4.76*10-2	-1.35	6.59*10-3	-1.39
NELL2	NELL2-205	7.56*10-3	3.85	1.91*10-2	2.72	4.21*10-3	2.83
NEURL3	NEURL3-204	4.95*10-2	1.97	6.60*10-3	1.17*10+1	4.93*10-3	3.37
NEUROG3	NEUROG3-201	1.47*10-2	-1.11*10+2	5.83*10-3	-7.70*10+1	2.12*10-3	-9.42*10+1
NFYC	NFYC-210	1.11*10-2	-1.43	1.38*10-2	-1.47	3.22*10-3	-1.45
NGEF	NGEF-202	3.87*10-2	-8.25*10+1	2.10*10-2	-8.29*10+1	9.40*10-3	-8.27*10+1
NIPA1	NIPA1-207	1.14*10-2	4.24	4.28*10-2	1.56	8.60*10-3	2.29
NIPBL	NIPBL-208	1.70*10-2	1.32	3.26*10-2	1.27	7.71*10-3	1.29
NKAIN1	NKAIN1-203	2.05*10-2	1.11*10+1	2.30*10-2	7.97*10+1	6.98*10-3	1.97*10+1
NKRF	NKRF-201	2.62*10-3	1.37	2.30*10-2	1.32	1.87*10-3	1.35
NLE1	NLE1-206	1.40*10-3	-2.61	6.03*10-3	-2.63	5.46*10-4	-2.62
NLRP1	NLRP1-202	2.33*10-2	1.34	1.05*10-2	1.41	4.46*10-3	1.37
NLRP12	NLRP12-203	1.75*10-2	-1.43	2.60*10-2	-1.49	6.86*10-3	-1.46
NLRX1	NLRX1-203	1.83*10-2	1.36	2.42*10-2	1.35	8.40*10-3	1.35
NOMO2	NOMO2-202	3.83*10-2	-1.93	1.47*10-2	-2.20	7.10*10-3	-2.07
NOP2	NOP2-203	1.69*10-2	1.41	1.50*10-2	1.65	4.51*10-3	1.52
NOP58	NOP58-206	3.86*10-3	1.44	4.72*10-3	1.53	7.52*10-4	1.49
NPIPB12	NPIPB12-201	1.27*10-3	1.50	1.42*10-3	1.60	1.68*10-4	1.55
NPIPB12	NPIPB12-208	2.51*10-3	2.15	1.42*10-2	1.92	1.40*10-3	2.03
NPRL2	NPRL2-213	1.02*10-2	1.34	2.08*10-2	1.39	4.18*10-3	1.37
NR1H3	NR1H3-211	4.07*10-2	1.43	2.63*10-2	1.76	1.06*10-2	1.59
NR2C2	NR2C2-205	3.74*10-2	-2.26	3.35*10-2	-1.74	1.19*10-2	-2.02
NT5C3B	NT5C3B-209	4.95*10-4	-1.12*10+1	5.35*10-4	-8.91	5.31*10-5	-1.02*10+1
NUDT22	NUDT22-210	2.66*10-2	-1.47	2.11*10-2	-1.78	7.15*10-3	-1.61
NUDT4P2	NUDT4P2-201	2.50*10-2	-1.24	4.65*10-2	-1.23	1.27*10-2	-1.23
NUGGC	NUGGC-201	4.19*10-2	1.81	2.19*10-2	2.70	1.01*10-2	2.17
NUMB	NUMB-223	4.52*10-2	-1.37	4.88*10-2	-1.43	1.87*10-2	-1.40
NUP133	NUP133-202	4.27*10-2	-2.71	1.24*10-2	-6.71	6.91*10-3	-4.71

NUP205	NUP205-210	3.06*10-2	1.29	4.07*10-2	1.35	1.34*10-2	1.32
NYAP1	NYAP1-201	3.74*10-2	2.70	4.49*10-2	3.85	1.51*10-2	3.15
ONECUT1	ONECUT1-201	4.22*10-2	1.79	3.29*10-2	7.08*10+1	1.35*10-2	3.47
OPA1	OPA1-202	1.73*10-2	-1.32	1.47*10-2	-1.40	4.42*10-3	-1.36
OPA1	OPA1-225	2.10*10-2	-2.82	4.27*10-2	-2.43	1.07*10-2	-2.57
OPA1	OPA1-229	2.39*10-2	-1.35	3.66*10-2	-1.30	8.03*10-3	-1.33
OR1L8	OR1L8-202	1.84*10-2	-5.96*10+1	2.11*10-2	-9.28*10+1	6.05*10-3	-7.62*10+1
OR2A9P	OR2A9P-204	8.21*10-3	3.53	3.67*10-2	1.95	6.54*10-3	2.39
OR2L13	OR2L13-203	2.22*10-2	2.56	1.45*10-2	3.68	6.34*10-3	3.03
ORAI2	ORAI2-207	2.63*10-2	-1.54	4.73*10-2	-1.75	1.43*10-2	-1.65
OSCAR	OSCAR-204	9.84*10-3	-1.33	2.74*10-2	-1.30	1.25*10-2	-1.25
OSCAR	OSCAR-206	1.62*10-2	-1.47	1.25*10-2	-1.76	3.80*10-3	-1.61
OSER1-DT	OSER1-DT-201	5.01*10-3	1.58	2.29*10-2	2.29	1.41*10-2	1.81
P3H1	P3H1-202	3.34*10-3	-1.37	1.99*10-2	-1.37	2.07*10-3	-1.37
PABPC1P10	PABPC1P10-201	1.40*10-2	-1.64	9.90*10-3	-1.39	2.93*10-3	-1.51
PACS2	PACS2-201	6.09*10-3	-1.58	2.72*10-3	-1.93	7.17*10-4	-1.75
PAK6	PAK6-206	4.34*10-2	-6.52	3.20*10-2	-5.09	1.33*10-2	-5.83
PARP11	PARP11-204	2.67*10-2	-1.58	2.10*10-2	-1.55	9.60*10-3	-1.55
PARP11-AS1	PARP11-AS1-204	3.60*10-2	-1.80	5.00*10-2	-1.45	1.66*10-2	-1.62
PATL2	PATL2-207	3.10*10-2	1.63	8.81*10-3	1.69	4.93*10-3	1.66
PAWR	PAWR-201	1.15*10-2	1.72	3.04*10-2	2.09	9.64*10-3	1.82
PCCB	PCCB-210	3.68*10-2	1.34	3.45*10-2	1.51	1.79*10-2	1.42
PCED1A	PCED1A-201	1.48*10-2	1.63	1.19*10-2	1.71	3.53*10-3	1.67
PCED1B-AS1	PCED1B-AS1-207	1.80*10-2	2.34	1.84*10-2	1.75	4.80*10-3	1.99
PCMTD1	PCMTD1-208	1.83*10-2	1.40	5.75*10-3	1.49	2.09*10-3	1.45
PCYT2	PCYT2-201	1.52*10-2	1.67	9.14*10-3	2.40	3.06*10-3	1.97
PDCD4	PDCD4-211	3.50*10-2	1.28	2.40*10-2	1.37	1.14*10-2	1.33
PDHX	PDHX-204	2.37*10-2	-1.70	3.32*10-2	-2.43	9.21*10-3	-2.07
PDLIM5	PDLIM5-223	4.56*10-2	-2.65	1.02*10-2	-6.07	6.26*10-3	-4.40
PDLIM7	PDLIM7-215	6.90*10-3	1.17*10+1	9.56*10-3	9.89*10+1	1.90*10-3	1.71*10+1
PELP1	PELP1-201	3.91*10-2	1.23	2.33*10-2	1.36	1.01*10-2	1.29

PEX5	PEX5-205	4.18*10-2	-1.44	2.04*10-2	-1.92	9.63*10-3	-1.68
PFAS	PFAS-202	2.22*10-3	1.65	9.30*10-3	1.63	1.02*10-3	1.64
PFN2	PFN2-203	2.41*10-2	3.15	1.76*10-2	3.24*10+1	6.16*10-3	5.73
PGD	PGD-205	3.75*10-2	-1.06*10+1	4.19*10-2	-2.15*10+1	1.51*10-2	-1.61*10+1
PGLS	PGLS-202	2.06*10-2	1.28	3.32*10-2	1.33	9.17*10-3	1.31
PGM3	PGM3-217	4.96*10-3	-2.47*10+1	3.74*10-2	-1.83*10+1	4.01*10-3	-2.45*10+1
PHB	PHB-212	1.55*10-2	-1.56	1.03*10-3	-2.02	6.71*10-4	-1.79
PHF12	PHF12-215	3.36*10-2	-4.44	2.25*10-2	-3.49	8.99*10-3	-3.97
PHF20	PHF20-211	4.37*10-2	-2.61	1.08*10-2	-3.34	6.39*10-3	-2.98
PHF21A	PHF21A-217	3.76*10-2	1.66	1.45*10-2	2.62	1.02*10-2	2.02
PHF8	PHF8-204	1.31*10-3	1.46	2.80*10-3	1.45	4.09*10-4	1.45
PHGDH	PHGDH-202	1.48*10-2	-2.57	1.98*10-3	-3.13	1.00*10-3	-2.85
PHYHD1	PHYHD1-213	1.47*10-2	5.93	1.21*10-2	9.38*10+1	3.52*10-3	1.11*10+1
PI16	PI16-204	2.75*10-2	1.73	4.64*10-2	2.52	1.38*10-2	2.05
PIGQ	PIGQ-207	2.52*10-3	-2.07	1.70*10-2	-2.38	1.55*10-3	-2.24
PIGT	PIGT-250	3.81*10-2	-1.21	3.78*10-3	-1.39	2.85*10-3	-1.30
PIH1D1	PIH1D1-222	1.90*10-4	-6.57	2.89*10-3	-6.31	1.09*10-4	-6.44
PIH1D1	PIH1D1-217	2.66*10-3	-2.34	4.38*10-2	-1.26	3.59*10-3	-1.80
PIK3CB	PIK3CB-214	1.56*10-3	-1.33	2.72*10-2	-1.29	1.86*10-3	-1.31
PIK3CD	PIK3CD-206	1.25*10-2	1.32	1.29*10-2	1.32	4.26*10-3	1.32
PITPNM2	PITPNM2-204	3.06*10-3	4.70	4.38*10-2	2.37	4.21*10-3	3.06
PIWIL2	PIWIL2-201	4.66*10-2	1.84	4.21*10-2	1.82	1.87*10-2	1.82
PKD2	PKD2-205	2.81*10-3	-1.93	4.01*10-2	-2.79	3.24*10-3	-2.37
PKM	PKM-219	2.50*10-2	-1.84	1.56*10-2	-1.99	7.20*10-3	-1.91
PLAA	PLAA-205	2.95*10-2	-1.26	2.13*10-2	-1.38	8.21*10-3	-1.32
PLCH2	PLCH2-202	2.77*10-4	2.72	4.63*10-2	3.23	7.60*10-3	2.71
PLCL2	PLCL2-204	2.27*10-3	-1.25	3.01*10-2	-1.21	2.36*10-3	-1.23
PLCL2	PLCL2-202	6.01*10-3	1.39	7.43*10-3	1.39	1.05*10-3	1.40
PLEKHA3	PLEKHA3-204	1.38*10-2	2.00*10+1	1.89*10-2	1.03*10+2	4.73*10-3	3.39*10+1
PLOD3	PLOD3-206	7.42*10-3	1.89	2.68*10-2	1.95	4.15*10-3	1.92
PLPBP	PLPBP-202	3.93*10-2	-1.23	4.08*10-2	-1.27	1.61*10-2	-1.25

PLXDC1	PLXDC1-214	4.77*10-2	7.41	4.49*10-2	7.74*10+1	1.82*10-2	1.28*10+1
PLXNA3	PLXNA3-206	3.57*10-2	2.79	1.03*10-2	5.06	6.43*10-3	3.55
PLXND1	PLXND1-214	2.18*10-2	1.42	1.75*10-3	1.39	5.00*10-4	1.41
PMS2	PMS2-209	6.70*10-3	-1.85	3.07*10-3	-2.58	8.09*10-4	-2.22
PNMA3	PNMA3-201	6.66*10-3	1.68	4.27*10-2	2.93	1.48*10-2	2.22
PNPLA8	PNPLA8-203	7.94*10-4	-1.41	6.87*10-3	-1.35	4.46*10-4	-1.38
POLB	POLB-211	2.78*10-3	1.41	1.32*10-2	1.41	1.43*10-3	1.41
POLE2	POLE2-201	4.90*10-3	3.07	2.38*10-2	3.87	2.96*10-3	3.43
POLM	POLM-208	1.43*10-3	1.48	1.47*10-2	1.38	1.12*10-3	1.42
POLM	POLM-216	3.79*10-2	1.38	1.84*10-2	1.43	9.61*10-3	1.40
POLR2J3	POLR2J3-208	2.18*10-2	-2.18	3.92*10-2	-2.88	1.04*10-2	-2.43
POMGNT1	POMGNT1-203	3.14*10-2	1.36	4.97*10-2	1.48	1.51*10-2	1.42
POMGNT2	POMGNT2-202	1.63*10-2	2.17	1.99*10-2	2.70	5.92*10-3	2.42
POR	POR-218	2.75*10-2	-1.28	3.87*10-3	-1.58	2.34*10-3	-1.43
PPARA	PPARA-204	2.89*10-2	-1.63	9.16*10-3	-2.30	4.11*10-3	-1.98
PPIAL4G	PPIAL4G-201	4.02*10-4	1.67	2.95*10-3	2.76	3.71*10-3	1.97
PPIL2	PPIL2-212	6.23*10-3	-1.98	1.00*10-2	-2.00	1.78*10-3	-1.99
PPIL3	PPIL3-215	4.63*10-2	3.25	3.00*10-2	1.05*10+1	1.34*10-2	4.97
PPM1L	PPM1L-204	1.24*10-2	-1.60	9.08*10-3	-1.87	2.61*10-3	-1.74
PPP1CB	PPP1CB-209	1.50*10-2	-1.32	2.12*10-2	-1.39	5.17*10-3	-1.36
PPP1CB	PPP1CB-204	3.26*10-2	-1.37	1.07*10-2	-1.57	5.40*10-3	-1.47
PPP1R2P1	PPP1R2P1-210	2.78*10-2	-2.52	2.44*10-2	-2.55	8.55*10-3	-2.54
PPP1R7	PPP1R7-212	5.45*10-3	-1.50	1.35*10-2	-1.47	1.98*10-3	-1.49
PPP2R5B	PPP2R5B-205	3.55*10-3	-2.42	5.12*10-3	-2.03	7.65*10-4	-2.14
PRKAR1A	PRKAR1A-212	2.31*10-2	-2.89	2.58*10-2	-1.80	7.94*10-3	-2.34
PRKAR1A	PRKAR1A-221	4.59*10-2	-1.22	3.56*10-2	-1.29	1.49*10-2	-1.25
PRKCB	PRKCB-209	4.10*10-2	-3.98*10+1	3.66*10-3	-4.86*10+1	2.53*10-3	-4.58*10+1
PRKDC	PRKDC-204	1.88*10-2	1.27	3.22*10-2	1.30	8.45*10-3	1.29
PRMT5	PRMT5-213	4.43*10-4	-4.09	2.80*10-2	-2.72	9.63*10-4	-3.41
PRMT7	PRMT7-205	1.18*10-2	-1.72	4.20*10-2	-1.72	7.93*10-3	-1.72
PRORSD1P	PRORSD1P-202	4.64*10-2	1.22	2.10*10-2	1.36	1.04*10-2	1.28

PROSER3	PROSER3-206	1.03*10-2	1.73	4.58*10-2	1.68	7.74*10-3	1.70
PRPF8	PRPF8-206	1.90*10-2	1.46	3.50*10-2	1.46	9.72*10-3	1.46
PRRT3-AS1	PRRT3-AS1-201	3.53*10-2	1.56	2.24*10-2	2.14	2.12*10-2	1.68
PRRT4	PRRT4-201	3.15*10-2	-1.94	9.75*10-3	-2.74	4.78*10-3	-2.34
PRSS16	PRSS16-216	4.51*10-2	4.36	4.94*10-2	9.16	1.88*10-2	5.90
PSMC3IP	PSMC3IP-208	1.63*10-2	1.75	2.28*10-2	4.22	5.31*10-3	2.49
PSMC5	PSMC5-213	2.50*10-2	-1.86	3.91*10-2	-1.34	1.11*10-2	-1.60
PSMC6	PSMC6-208	5.81*10-3	-3.09	3.10*10-2	-2.11	3.72*10-3	-2.60
PSME3IP1	PSME3IP1-218	5.45*10-3	-1.39	1.53*10-2	-1.37	2.27*10-3	-1.38
PTBP3	PTBP3-203	1.60*10-2	-1.73	2.85*10-2	-2.09	7.81*10-3	-1.92
PTGER2	PTGER2-201	1.24*10-2	-1.30	2.35*10-2	-1.34	5.13*10-3	-1.32
PTGER2	PTGER2-202	2.38*10-2	-1.48	4.98*10-3	-1.75	2.72*10-3	-1.61
PTMA	PTMA-204	1.73*10-2	4.33	3.78*10-3	2.40*10+2	1.73*10-3	8.49
PTPA	PTPA-215	3.98*10-2	-2.15	2.84*10-2	-1.94	1.18*10-2	-2.04
PTPA	PTPA-209	4.37*10-2	-1.79	3.30*10-2	-1.95	1.38*10-2	-1.87
PTPN18	PTPN18-211	1.23*10-2	-1.43	2.36*10-2	-1.47	5.31*10-3	-1.45
PTPRH	PTPRH-207	1.04*10-2	-3.90	1.70*10-2	-5.08	3.57*10-3	-4.45
PTPRVP	PTPRVP-204	3.48*10-4	1.18*10+2	2.96*10-3	1.14*10+2	1.61*10-4	1.25*10+2
PUM1	PUM1-205	2.42*10-3	2.34	2.02*10-2	1.45	1.57*10-3	1.72
PUS7L	PUS7L-207	1.02*10-2	-1.87	3.76*10-2	-2.08	6.49*10-3	-1.97
PVRIG	PVRIG-201	9.88*10-3	1.69	1.25*10-2	4.30	1.37*10-2	2.10
PVT1	PVT1-324	1.65*10-2	1.59*10+1	2.40*10-2	9.30*10+1	6.17*10-3	2.78*10+1
PXDN	PXDN-201	6.46*10-5	-4.59	1.44*10-2	-7.00	2.11*10-4	-5.80
QARS1	QARS1-207	6.30*10-3	1.93	8.15*10-3	1.65	1.90*10-3	1.77
R3HDM2	R3HDM2-202	2.84*10-2	1.37	3.89*10-2	1.30	1.06*10-2	1.34
RAB8B	RAB8B-202	9.02*10-3	-4.73	6.93*10-3	-3.72	1.79*10-3	-4.22
RABGGTA	RABGGTA-211	2.16*10-2	1.63	5.61*10-4	2.13	4.86*10-4	1.85
RACGAP1	RACGAP1-219	3.56*10-4	1.97*10+1	9.94*10-4	1.04*10+2	7.70*10-5	4.57*10+1
RACGAP1	RACGAP1-210	7.97*10-3	-6.42	2.40*10-2	-4.33	3.54*10-3	-5.35
RALY	RALY-204	2.97*10-3	-1.11*10+2	7.12*10-3	-7.39*10+1	9.53*10-4	-9.32*10+1
RAP1B	RAP1B-214	8.68*10-3	-1.65	7.23*10-3	-2.79	1.81*10-3	-2.22

RAP1B	RAP1B-216	4.78*10-2	-1.35	4.96*10-2	-1.38	1.96*10-2	-1.36
RAP1GDS1	RAP1GDS1-215	1.83*10-2	-4.46	2.83*10-2	-4.47	6.48*10-3	-4.49
RASGRF2-AS1	RASGRF2-AS1-204	2.47*10-2	3.32	9.81*10-3	8.36	4.49*10-3	4.71
RBBP7	RBBP7-201	1.36*10-2	1.56	4.59*10-3	1.69	1.14*10-3	1.61
RBBP8	RBBP8-202	2.89*10-2	-2.12	5.11*10-3	-2.88	2.96*10-3	-2.49
RBL2	RBL2-202	4.67*10-2	1.36	9.71*10-3	1.38	3.32*10-3	1.38
RCBTB2	RCBTB2-205	2.42*10-2	1.54	1.64*10-2	1.57	6.24*10-3	1.56
RCN3	RCN3-202	4.89*10-3	5.65	9.39*10-3	-2.05	1.25*10-3	1.24
RDH11	RDH11-211	1.67*10-2	-1.45	3.64*10-2	-1.56	8.49*10-3	-1.51
RDX	RDX-208	2.78*10-2	-6.27	3.60*10-3	-7.00	1.99*10-3	-7.16
REC8	REC8-203	1.32*10-2	1.57	3.95*10-2	1.44	8.30*10-3	1.50
RFX2	RFX2-222	3.84*10-2	-1.54	3.02*10-3	-2.19	2.51*10-3	-1.87
RGL2	RGL2-245	3.19*10-2	2.24	3.94*10-2	1.44	1.19*10-2	1.73
RGMB	RGMB-207	4.25*10-2	1.50	4.17*10-2	2.12	3.22*10-2	1.69
RGS3	RGS3-227	2.76*10-2	4.04	1.90*10-3	4.38*10+2	1.52*10-3	8.05
RIN3	RIN3-201	4.45*10-3	-1.23	1.03*10-2	-1.27	1.46*10-3	-1.25
RIN3	RIN3-206	1.20*10-2	-1.59	2.68*10-2	-1.66	5.56*10-3	-1.63
RIPOR1	RIPOR1-210	2.75*10-2	-1.38	3.64*10-2	-1.48	1.11*10-2	-1.43
RITA1	RITA1-203	4.29*10-2	1.53	3.70*10-2	1.35	1.74*10-2	1.43
RMDN1	RMDN1-212	9.76*10-3	-1.74	2.22*10-2	-1.72	4.17*10-3	-1.73
RNASEH2B-AS1	RNASEH2B-AS1-203	2.25*10-2	-7.24	4.35*10-3	-6.16	2.25*10-3	-6.70
RNASEH2C	RNASEH2C-201	1.06*10-2	1.42	3.81*10-2	1.40	6.86*10-3	1.41
RNF10	RNF10-211	4.20*10-2	-1.65	2.28*10-2	-2.26	1.04*10-2	-1.95
RNF114	RNF114-203	1.12*10-3	1.54	1.20*10-2	1.52	7.36*10-4	1.52
RNF13	RNF13-211	2.19*10-2	-1.83*10+1	3.17*10-4	-1.04*10+2	2.89*10-4	-8.65*10+1
RNF144B	RNF144B-202	1.99*10-2	1.43	1.98*10-2	3.48	5.97*10-3	2.02
RNF167	RNF167-204	1.23*10-2	-4.84	3.47*10-2	-3.66	6.83*10-3	-4.26
RNF170	RNF170-202	2.47*10-3	-2.51	9.80*10-3	-2.64	9.15*10-4	-2.60
RNF20	RNF20-203	5.76*10-3	7.77	2.09*10-2	8.61	3.08*10-3	8.17
RNF217	RNF217-211	3.79*10-2	-3.40	1.29*10-2	-3.85	6.86*10-3	-3.64

RNF34	RNF34-202	2.96*10-2	1.22	4.07*10-2	1.24	1.28*10-2	1.23
RNF40	RNF40-212	1.57*10-2	9.50	1.50*10-2	1.29	4.04*10-3	1.90
RP9	RP9-202	1.86*10-2	4.29	3.22*10-3	2.43*10+2	1.72*10-3	8.57
RPGRIP1L	RPGRIP1L-206	4.66*10-3	8.31	1.08*10-2	5.86	1.64*10-3	6.86
RPL31	RPL31-203	3.90*10-3	1.29	9.44*10-3	1.29	1.37*10-3	1.29
RPLP0	RPLP0-220	1.55*10-2	1.36	2.82*10-2	1.41	6.76*10-3	1.38
RPS3	RPS3-211	1.38*10-3	-7.52	5.59*10-3	-5.00	5.23*10-4	-6.26
RPS6KA3	RPS6KA3-211	1.74*10-2	-2.72	1.04*10-2	-4.78	3.32*10-3	-3.76
RRP7BP	RRP7BP-205	4.70*10-2	2.44	1.26*10-2	2.62*10+1	7.31*10-3	4.46
RSL24D1P11	RSL24D1P11-201	2.16*10-2	-1.25	2.80*10-2	-1.30	8.10*10-3	-1.27
RSPRY1	RSPRY1-202	5.28*10-3	-1.52	1.53*10-2	-1.55	2.39*10-3	-1.53
RSRC2	RSRC2-207	2.56*10-2	-1.44	4.05*10-2	-1.55	1.17*10-2	-1.49
RSRP1	RSRP1-213	2.68*10-2	1.50	1.78*10-2	1.46	7.40*10-3	1.47
RUFY1	RUFY1-202	7.74*10-3	2.45	1.71*10-2	2.23	3.71*10-3	2.34
RUNX1	RUNX1-202	4.22*10-2	-1.24	9.90*10-3	-1.49	5.73*10-3	-1.36
SAMD12	SAMD12-205	9.08*10-4	3.88	6.64*10-3	2.31*10+1	4.66*10-4	6.64
SAMD14	SAMD14-203	3.94*10-2	1.41	1.66*10-2	1.98	9.84*10-3	1.65
SATB1	SATB1-209	2.03*10-2	1.20	2.84*10-4	1.35	2.97*10-4	1.27
SATB1-AS1	SATB1-AS1-271	3.10*10-2	1.38	4.50*10-2	3.09	1.45*10-2	1.90
SBF1	SBF1-205	4.67*10-2	1.23	2.43*10-2	1.31	1.35*10-2	1.27
SCAF8	SCAF8-202	8.22*10-3	1.30	9.60*10-4	1.46	4.27*10-4	1.38
SCAMP5	SCAMP5-216	4.15*10-2	-3.18*10+1	2.39*10-2	-9.21*10+1	6.68*10-3	-8.66*10+1
SCARB2	SCARB2-215	7.26*10-3	-1.51	3.30*10-2	-1.52	4.82*10-3	-1.52
SCARB2	SCARB2-223	3.17*10-2	-1.31	1.11*10-2	-1.49	5.23*10-3	-1.40
SCPEP1	SCPEP1-214	3.16*10-2	-1.54	1.01*10-2	-1.74	4.85*10-3	-1.64
SCRN2	SCRN2-203	4.21*10-2	-2.57	4.39*10-2	-1.85	1.66*10-2	-2.21
SCYL1	SCYL1-205	1.21*10-2	1.22	5.56*10-3	1.30	2.29*10-3	1.26
SEC31A	SEC31A-227	3.10*10-3	-1.60	3.03*10-2	-1.55	2.72*10-3	-1.58
SELL	SELL-207	1.22*10-2	-1.28	4.40*10-4	-1.51	3.25*10-4	-1.40
SELL	SELL-201	3.91*10-2	-1.28	4.71*10-4	-1.61	7.43*10-4	-1.44
SENP2	SENP2-202	4.42*10-2	-1.27	2.01*10-2	-1.47	1.06*10-2	-1.37

SEPTIN2	SEPTIN2-208	1.40*10-3	1.27	2.73*10-2	1.20	1.64*10-3	1.23
SEPTIN2	SEPTIN2-206	8.20*10-3	1.20	1.17*10-2	1.22	2.42*10-3	1.21
SERF1A	SERF1A-204	1.09*10-2	-7.71	1.52*10-2	-5.34	2.93*10-3	-6.55
SERF1B	SERF1B-203	1.09*10-2	-7.71	1.52*10-2	-5.34	2.93*10-3	-6.55
SERPINB1	SERPINB1-204	2.49*10-3	-1.43	5.06*10-3	-1.47	6.58*10-4	-1.45
SERPINB1	SERPINB1-205	1.61*10-2	-1.43	3.97*10-3	-1.65	1.72*10-3	-1.54
SERPINH1P1	SERPINH1P1-201	1.90*10-2	-4.44	4.20*10-2	-4.86	9.80*10-3	-4.49
SETD3	SETD3-204	2.78*10-2	-1.40	3.95*10-2	-1.69	1.19*10-2	-1.54
SETMAR	SETMAR-201	1.49*10-2	1.27	4.72*10-2	1.27	9.22*10-3	1.27
SFXN5	SFXN5-216	3.18*10-2	-1.86	2.82*10-2	-2.37	1.03*10-2	-2.11
SGCE	SGCE-247	3.29*10-2	-3.62	2.34*10-2	-3.30	9.18*10-3	-3.46
SGO2	SGO2-202	3.05*10-3	-2.74*10+1	1.17*10-2	-2.44*10+1	1.41*10-3	-2.59*10+1
SH3TC1	SH3TC1-221	8.53*10-3	-1.59	4.11*10-2	-1.57	6.42*10-3	-1.58
SIRT1	SIRT1-206	2.41*10-2	-5.40	4.52*10-2	-6.12	5.42*10-3	-7.39
SLC16A10	SLC16A10-203	7.34*10-3	6.74*10+1	1.24*10-2	1.05*10+2	2.22*10-3	8.00*10+1
SLC16A4	SLC16A4-205	6.12*10-3	-2.87	4.87*10-2	-1.74	5.99*10-3	-2.26
SLC22A5	SLC22A5-209	6.33*10-3	-2.72	4.98*10-2	-1.80	6.13*10-3	-2.23
SLC24A3	SLC24A3-202	2.61*10-2	2.26	4.83*10-2	2.78	1.38*10-2	2.50
SLC25A24	SLC25A24-203	4.90*10-2	-1.21	4.01*10-2	-1.31	1.67*10-2	-1.27
SLC29A3	SLC29A3-202	3.23*10-2	-1.54	2.55*10-2	-1.72	9.51*10-3	-1.64
SLC2A8	SLC2A8-208	4.43*10-4	2.28*10+2	2.15*10-2	1.01*10+1	7.83*10-4	1.92*10+1
SLC2A8	SLC2A8-215	3.62*10-2	1.36	3.50*10-2	1.64	1.49*10-2	1.49
SLC30A9	SLC30A9-205	3.46*10-2	-1.23	9.51*10-3	-1.37	4.93*10-3	-1.30
SLC35A2	SLC35A2-210	2.97*10-2	-5.15	3.29*10-3	-4.86*10+1	2.21*10-3	-2.70*10+1
SLC35A3	SLC35A3-222	1.81*10-2	-1.86	3.06*10-2	-1.96	7.79*10-3	-1.91
SLC35A4	SLC35A4-204	2.98*10-2	-3.09*10+1	3.76*10-2	-1.30*10+2	1.22*10-2	-8.03*10+1
SLC35B1	SLC35B1-215	1.31*10-2	-2.64	7.29*10-3	-2.71	2.25*10-3	-2.68
SLC35B1	SLC35B1-202	3.89*10-2	-1.61	1.04*10-2	-1.77	5.75*10-3	-1.69
SLC35E2A	SLC35E2A-202	4.54*10-2	-1.85	3.47*10-2	-2.02	1.45*10-2	-1.93
SLC41A1	SLC41A1-202	4.25*10-2	1.44	1.93*10-2	1.53	6.89*10-3	1.49
SLC43A3	SLC43A3-225	3.76*10-2	-1.32	4.48*10-2	-1.42	1.69*10-2	-1.37

SLC44A1	SLC44A1-205	3.43*10-2	1.25	7.65*10-3	1.35	4.98*10-3	1.30
SLC6A4	SLC6A4-203	5.56*10-3	2.95	2.77*10-2	3.78	3.71*10-3	3.31
SLC7A9	SLC7A9-206	2.56*10-2	-1.07*10+1	2.67*10-2	-1.07*10+1	9.27*10-3	-8.73
SLC8A1	SLC8A1-204	3.72*10-2	-1.48	2.48*10-2	-1.88	1.69*10-2	-1.69
SLX1A	SLX1A-205	3.90*10-2	-1.44	1.92*10-2	-1.66	8.89*10-3	-1.55
SMAD4	SMAD4-205	1.90*10-2	-3.79	6.40*10-3	-2.98	2.59*10-3	-3.38
SMARCE1	SMARCE1-236	2.91*10-2	-1.48	4.17*10-2	-1.53	1.39*10-2	-1.50
SMG1P5	SMG1P5-201	2.83*10-2	-1.37	2.88*10-2	-1.37	9.98*10-3	-1.37
SMIM5	SMIM5-201	3.32*10-2	1.54	3.82*10-2	1.67	1.30*10-2	1.60
SMPDL3B	SMPDL3B-201	6.43*10-3	-7.50	1.67*10-3	-6.99	5.41*10-4	-7.24
SMTN	SMTN-224	4.09*10-2	3.03	3.41*10-2	2.68	8.29*10-3	2.94
SNHG12	SNHG12-202	5.48*10-3	1.39	9.52*10-3	1.37	1.83*10-3	1.38
SNHG20	SNHG20-202	2.92*10-2	1.33	3.85*10-2	1.30	1.28*10-2	1.31
SNHG5	SNHG5-249	4.60*10-2	-1.83	4.79*10-2	-1.76	1.87*10-2	-1.79
SNORA2C	SNORA2C-201	3.68*10-2	-3.56	1.58*10-2	-3.59	7.38*10-3	-3.56
SNORA51	SNORA51-201	2.82*10-2	-1.37*10+1	1.11*10-2	-1.33*10+1	8.27*10-3	-1.07*10+1
SNRNP200	SNRNP200-208	1.38*10-2	1.24	4.30*10-2	1.20	1.01*10-2	1.22
SPAG1	SPAG1-201	4.45*10-2	-1.31	1.11*10-2	-1.55	6.64*10-3	-1.43
SPATA6L	SPATA6L-213	3.05*10-2	-7.07	2.63*10-2	-9.88	9.53*10-3	-8.47
SPECC1	SPECC1-204	9.75*10-4	-5.92	4.85*10-2	-5.09	2.35*10-3	-5.50
SPG7	SPG7-268	4.59*10-2	-1.21	4.95*10-2	-1.25	1.90*10-2	-1.23
SPINT1	SPINT1-201	3.79*10-2	-1.23	3.12*10-2	-1.30	1.21*10-2	-1.27
SPON2	SPON2-202	2.80*10-2	1.62	4.41*10-2	1.87	1.32*10-2	1.73
SRP9	SRP9-207	4.25*10-2	-3.52	8.73*10-3	-3.43	5.62*10-3	-3.48
ST6GAL1	ST6GAL1-211	1.70*10-2	1.34	1.57*10-2	1.49	6.00*10-3	1.41
STARD3	STARD3-202	9.28*10-3	-1.33	2.89*10-2	-1.40	5.12*10-3	-1.37
STAU2	STAU2-214	3.76*10-2	-1.63	2.84*10-2	-1.67	1.12*10-2	-1.64
STIMATE-MUSTN1	STIMATE-MUSTN1-202	4.58*10-2	-1.36	4.63*10-2	-1.35	2.30*10-2	-1.35
STRN4	STRN4-211	1.19*10-2	-1.98	1.96*10-3	-3.07	7.47*10-4	-2.63
SUCLG2	SUCLG2-204	2.59*10-2	-6.40	9.94*10-3	-1.05*10+1	4.34*10-3	-8.44

SULT1A1	SULT1A1-201	2.64*10-2	-1.38	2.18*10-2	-1.50	7.65*10-3	-1.44
SULT1A1	SULT1A1-208	3.43*10-2	-1.80	1.63*10-2	-1.99	7.27*10-3	-1.90
SUPT7L	SUPT7L-205	4.52*10-2	1.36	1.08*10-2	1.64	6.60*10-3	1.49
SURF4	SURF4-204	4.01*10-2	-3.39	5.80*10-3	-7.33	3.92*10-3	-5.37
SVIL	SVIL-212	1.40*10-2	-4.17	2.60*10-2	-4.26	5.81*10-3	-4.24
SYCE3	SYCE3-201	4.42*10-2	-2.92	3.89*10-2	-5.29	1.61*10-2	-4.11
SYNE1	SYNE1-237	2.19*10-2	1.63	1.04*10-2	1.97	4.99*10-3	1.78
SYNE4	SYNE4-201	4.35*10-2	-1.77	3.23*10-2	-2.05	1.34*10-2	-1.92
TAF1	TAF1-219	1.26*10-2	-1.93	3.50*10-3	-3.24	1.34*10-3	-2.59
TAF1D	TAF1D-208	2.57*10-2	-3.34	1.92*10-2	-3.03	6.81*10-3	-3.17
TAF4B	TAF4B-202	4.46*10-2	1.53	5.73*10-3	2.22	4.00*10-3	1.83
TARBP1	TARBP1-204	8.00*10-3	4.07	2.19*10-2	8.67	3.84*10-3	5.54
TBC1D1	TBC1D1-217	7.45*10-3	3.36*10+2	2.65*10-2	2.83*10+2	4.17*10-3	3.41*10+2
TBC1D10C	TBC1D10C-206	4.62*10-3	1.42	3.49*10-2	1.38	3.97*10-3	1.40
TBC1D10C	TBC1D10C-201	1.00*10-2	2.63	4.25*10-2	1.48	7.13*10-3	1.86
TBC1D3D	TBC1D3D-201	1.27*10-2	2.23	4.63*10-2	2.31*10+1	9.21*10-3	4.08
TBC1D9B	TBC1D9B-208	4.55*10-2	1.60	5.60*10-3	1.55	3.51*10-3	1.57
TBCEL	TBCEL-206	2.85*10-2	-1.78	1.56*10-2	-2.58	6.43*10-3	-2.17
TBL1X	TBL1X-202	4.82*10-4	1.26	8.98*10-3	1.24	2.81*10-4	1.26
TCF12	TCF12-212	2.39*10-2	-2.30	3.99*10-2	-1.97	1.12*10-2	-2.13
TCF7	TCF7-203	3.52*10-2	1.54	2.04*10-2	2.19	9.45*10-3	1.79
TCHP	TCHP-207	3.16*10-2	1.37	4.14*10-2	1.50	1.43*10-2	1.45
TCL6	TCL6-203	2.22*10-2	-5.17*10+1	4.15*10-2	-4.17*10+1	1.11*10-2	-4.67*10+1
TCP1	TCP1-203	1.87*10-2	-1.77	3.42*10-2	-2.13	8.78*10-3	-1.95
TEPP	TEPP-202	3.38*10-2	2.35	2.10*10-2	1.07*10+1	8.11*10-3	3.87
TESMIN	TESMIN-202	2.15*10-2	-1.90	2.81*10-2	-1.71	8.07*10-3	-1.80
TGFBRAP1	TGFBRAP1-201	3.10*10-2	-3.01*10+1	8.69*10-3	-1.45*10+2	4.42*10-3	-8.77*10+1
THAP2	THAP2-204	4.69*10-3	6.73*10+1	1.16*10-2	1.14*10+2	1.84*10-3	8.52*10+1
THEMIS2	THEMIS2-208	3.51*10-2	-1.58	2.36*10-2	-2.20	9.57*10-3	-1.89
THOC1	THOC1-219	1.78*10-2	-2.02	4.74*10-2	-1.65	1.07*10-2	-1.81
THTPA	THTPA-202	6.31*10-3	-1.38	1.18*10-3	-1.66	4.13*10-4	-1.52

TIAM1	TIAM1-207	4.94*10-2	1.39	4.51*10-2	1.45	2.19*10-2	1.42
TIMM9	TIMM9-206	2.60*10-2	1.96	1.64*10-2	1.57	3.00*10-3	1.66
TIMMDC1	TIMMDC1-209	2.95*10-3	6.01*10+1	1.78*10-2	1.02*10+1	1.97*10-3	1.81*10+1
TLR5	TLR5-202	9.26*10-3	1.58	4.29*10-2	1.87	2.03*10-2	1.70
TM9SF1	TM9SF1-209	5.48*10-4	-1.59	1.51*10-3	-1.63	1.17*10-4	-1.61
TMC6	TMC6-215	4.52*10-2	1.42	2.12*10-2	1.59	9.56*10-3	1.50
TMED5	TMED5-204	1.45*10-3	-2.40	8.40*10-3	-2.84	9.77*10-4	-2.74
TMEM123	TMEM123-208	1.19*10-2	-3.65	4.10*10-3	-4.32	1.34*10-3	-3.83
TMEM191C	TMEM191C-205	3.34*10-2	-1.32	1.78*10-2	-2.17	7.57*10-3	-1.75
TMEM217	TMEM217-212	7.76*10-3	-4.41	9.78*10-4	-5.82	4.16*10-4	-5.12
TMEM229B	TMEM229B-204	4.29*10-2	2.33	8.42*10-3	3.74	7.97*10-3	2.89
TMEM232	TMEM232-212	1.85*10-3	-6.60*10+1	3.15*10-2	-5.65*10+1	2.30*10-3	-6.12*10+1
TMEM259	TMEM259-209	2.34*10-2	1.22	2.87*10-2	1.27	7.79*10-3	1.25
TMEM30A	TMEM30A-205	3.82*10-2	1.39	4.41*10-2	1.28	1.43*10-2	1.33
TMEM39A	TMEM39A-203	2.93*10-2	-6.28	2.49*10-2	-4.80	8.93*10-3	-5.52
TMEM41B	TMEM41B-205	8.12*10-4	-3.53	3.24*10-2	-1.64	1.43*10-3	-2.54
TMEM64	TMEM64-201	1.90*10-3	2.06	7.23*10-3	1.92	7.91*10-4	1.98
TMEM87B	TMEM87B-207	1.15*10-2	-1.56	1.63*10-2	-1.60	3.66*10-3	-1.58
TNFRSF13B	TNFRSF13B-207	7.69*10-3	1.61*10+2	2.87*10-2	1.51*10+2	4.52*10-3	1.61*10+2
TNK2	TNK2-216	2.91*10-2	1.31	3.07*10-2	1.33	9.29*10-3	1.32
TP53BP1	TP53BP1-211	2.59*10-2	-1.41	2.02*10-2	-1.73	7.13*10-3	-1.57
TPD52L1	TPD52L1-206	2.85*10-2	2.54	2.52*10-2	1.17*10+1	8.86*10-3	4.17
TPM1	TPM1-204	1.42*10-2	-2.49	3.87*10-2	-2.23	1.31*10-2	-2.22
TPM3	TPM3-213	5.09*10-3	1.39	1.20*10-2	1.44	1.90*10-3	1.42
TPM4	TPM4-216	1.08*10-2	1.81	2.09*10-2	1.61	5.31*10-3	1.70
TRABD2A	TRABD2A-203	9.90*10-3	1.72	4.61*10-2	1.92	7.97*10-3	1.80
TRAF3IP1	TRAF3IP1-205	4.23*10-2	-1.22	1.87*10-2	-1.68	9.07*10-3	-1.45
TRAF3IP3	TRAF3IP3-213	1.19*10-2	-8.07	2.66*10-2	-1.04*10+1	6.69*10-3	-9.18
TRAK1	TRAK1-203	3.41*10-2	1.49	3.62*10-2	1.61	1.47*10-2	1.55
TRAV8-4	TRAV8-4-201	5.24*10-3	2.45	1.92*10-2	2.62	2.96*10-3	2.50
TRBV6-2	TRBV6-2-202	3.97*10-4	2.71	1.47*10-2	3.49	6.83*10-4	3.08

TRGV5	TRGV5-201	4.57*10-5	-1.37	2.23*10-2	-2.43	1.21*10-2	-1.79
TRHDE-AS1	TRHDE-AS1-218	4.77*10-2	1.07*10+1	4.17*10-2	1.34*10+2	1.78*10-2	2.00*10+1
TRIM16	TRIM16-226	1.56*10-2	-1.80	2.39*10-2	-1.82	5.65*10-3	-1.81
TRIM28	TRIM28-207	3.64*10-2	1.38	3.28*10-2	1.40	1.28*10-2	1.39
TRIM36	TRIM36-209	1.35*10-2	-5.42	3.13*10-2	-5.54	5.27*10-3	-5.42
TRIM47	TRIM47-204	5.97*10-3	1.89	4.78*10-3	2.73	2.05*10-3	2.18
TRIQK	TRIQK-220	3.27*10-3	-3.27	3.01*10-4	-5.64	1.12*10-4	-4.45
TSC2	TSC2-204	3.22*10-2	-1.27	3.93*10-2	-1.34	1.34*10-2	-1.30
TSTD2	TSTD2-205	4.85*10-2	1.32	2.86*10-2	1.47	1.39*10-2	1.39
TTC12	TTC12-210	4.26*10-2	1.62	2.77*10-3	2.41	1.90*10-3	1.93
TUG1	TUG1-215	4.88*10-2	1.22	1.19*10-3	1.38	8.62*10-4	1.31
UBA5	UBA5-204	6.76*10-3	-2.36	2.36*10-3	-3.93	6.90*10-4	-3.14
UBAP2L	UBAP2L-210	1.61*10-2	-1.79	2.57*10-2	-1.99	7.61*10-3	-1.91
UBAP2L	UBAP2L-211	4.70*10-2	-2.27	2.46*10-2	-2.71	1.23*10-2	-2.49
UBE2D3	UBE2D3-229	3.49*10-2	-2.00	1.28*10-2	-3.27	6.16*10-3	-2.63
UBE2Q2	UBE2Q2-208	2.75*10-3	-1.28	3.82*10-2	-1.21	3.24*10-3	-1.25
UBXN1	UBXN1-212	4.53*10-2	1.92	3.61*10-2	2.53	1.49*10-2	2.19
UCP2	UCP2-207	3.53*10-2	-1.46	5.69*10-3	-1.81	3.02*10-3	-1.64
UGP2	UGP2-204	1.31*10-2	2.28	2.93*10-2	2.94	7.41*10-3	2.58
ULK3	ULK3-202	6.85*10-3	1.47	1.29*10-2	1.50	2.49*10-3	1.48
UNC119	UNC119-204	4.62*10-2	1.23	3.74*10-2	1.34	1.48*10-2	1.28
UQCRB	UQCRB-202	1.10*10-3	-1.72	2.37*10-2	-1.57	1.34*10-3	-1.63
UQCRC1	UQCRC1-208	6.02*10-3	-1.38	4.58*10-2	-1.28	5.68*10-3	-1.33
UROD	UROD-203	3.90*10-2	-2.70	3.28*10-2	-4.90	1.32*10-2	-3.82
UROS	UROS-221	4.22*10-4	3.28	3.18*10-2	1.61	9.14*10-4	2.16
UROS	UROS-205	1.18*10-2	-3.68	2.19*10-2	-3.01	4.63*10-3	-3.36
USP20	USP20-204	3.22*10-2	1.25	2.11*10-2	1.32	8.39*10-3	1.28
UTP4	UTP4-211	2.65*10-2	8.52	1.99*10-2	1.67*10+2	6.89*10-3	1.67*10+1
VASH1-AS1	VASH1-AS1-202	4.93*10-2	1.26	1.37*10-2	1.38	7.98*10-3	1.32
VBP1	VBP1-204	2.50*10-3	1.88	1.93*10-3	1.71	3.91*10-4	1.76
VDR	VDR-203	2.67*10-2	-2.68	3.52*10-2	-2.78	1.07*10-2	-2.70

VIM	VIM-205	4.06*10-2	-1.32	1.28*10-2	-1.49	8.44*10-3	-1.40
VIM	VIM-207	4.62*10-2	-1.21	8.90*10-3	-1.34	5.91*10-3	-1.27
VPS35L	VPS35L-203	1.46*10-2	-1.27	6.37*10-3	-1.43	2.18*10-3	-1.35
VPS35L	VPS35L-201	2.65*10-2	-1.25	1.51*10-2	-1.39	5.80*10-3	-1.32
VPS50	VPS50-201	4.08*10-2	-1.58	3.55*10-2	-2.10	1.40*10-2	-1.84
VRK3	VRK3-209	9.88*10-3	-3.94	3.00*10-2	-2.67	5.51*10-3	-3.30
VWA5A	VWA5A-203	3.80*10-2	-1.26	3.88*10-2	-1.35	1.43*10-2	-1.30
WDR20	WDR20-206	3.24*10-2	-2.61	3.31*10-2	-3.65	1.17*10-2	-3.14
XIAP	XIAP-210	2.24*10-2	-3.17	2.95*10-3	-4.74	1.71*10-3	-3.89
YEATS2-AS1	YEATS2-AS1-204	1.10*10-2	2.91	2.17*10-2	4.96	5.32*10-3	3.67
YIPF3	YIPF3-207	1.91*10-2	9.47	2.13*10-2	9.96*10+1	6.33*10-3	1.74*10+1
YWHAZ	YWHAZ-216	7.29*10-3	-2.25	3.24*10-2	-2.66	5.70*10-3	-2.47
Z84492.1	Z84492.1-204	4.18*10-3	4.23	1.75*10-2	2.97*10+1	2.25*10-3	7.40
Z98883.1	Z98883.1-202	4.43*10-2	-1.78	4.06*10-2	-1.71	1.58*10-2	-1.71
ZBED3-AS1	ZBED3-AS1-229	2.53*10-2	-9.50*10+1	2.22*10-3	-1.41*10+2	1.54*10-3	-1.18*10+2
ZBTB17	ZBTB17-203	2.31*10-2	2.54	2.03*10-2	1.64	7.75*10-3	1.94
ZCCHC7	ZCCHC7-205	3.85*10-2	-5.99	2.20*10-2	-1.43*10+1	9.65*10-3	-1.02*10+1
ZCCHC7	ZCCHC7-201	4.70*10-2	1.45	4.68*10-2	1.56	1.85*10-2	1.50
ZDHHC20	ZDHHC20-206	4.43*10-2	-1.44	1.16*10-2	-1.68	8.16*10-3	-1.56
ZEB2	ZEB2-239	1.14*10-2	-2.10	5.64*10-3	-2.38	1.76*10-3	-2.24
ZFAND2B	ZFAND2B-202	9.82*10-3	1.37	2.88*10-2	1.44	5.08*10-3	1.41
ZFP36L1	ZFP36L1-204	1.86*10-3	1.38	6.41*10-3	1.39	6.77*10-4	1.39
ZFYVE19	ZFYVE19-212	4.76*10-2	1.56	1.39*10-2	5.48	7.80*10-3	2.43
ZFYVE21	ZFYVE21-212	4.30*10-3	3.41*10+1	4.31*10-2	8.74	4.72*10-3	1.44*10+1
ZFYVE28	ZFYVE28-203	6.77*10-3	-3.48	4.08*10-4	-4.96	2.12*10-4	-4.22
ZGRF1	ZGRF1-209	4.70*10-2	1.38	1.40*10-3	1.93	1.67*10-3	1.61
ZMPSTE24	ZMPSTE24-204	2.25*10-2	-3.49	9.86*10-3	-6.28	3.59*10-3	-4.99
ZMYND11	ZMYND11-202	4.85*10-2	-3.71	1.80*10-2	-4.32	9.69*10-3	-4.02
ZMYND12	ZMYND12-203	4.64*10-2	-2.00	3.95*10-4	-3.81	7.49*10-4	-2.91
ZNF10	ZNF10-203	2.71*10-2	1.83	4.40*10-2	2.23	2.64*10-2	1.89
ZNF302	ZNF302-202	5.70*10-3	3.71	3.28*10-2	2.34	4.34*10-3	2.87

ZNF304	ZNF304-204	3.00*10-2	-1.81	4.02*10-2	-1.92	1.28*10-2	-1.86
ZNF320	ZNF320-203	4.54*10-2	-2.25	6.59*10-3	-2.35	4.77*10-3	-2.30
ZNF33B	ZNF33B-208	4.98*10-2	1.33	5.63*10-3	1.81	4.41*10-3	1.53
ZNF34	ZNF34-201	2.50*10-2	1.65	2.12*10-2	1.97	7.06*10-3	1.80
ZNF410	ZNF410-221	9.95*10-3	-1.54	1.33*10-2	-1.84	3.04*10-3	-1.69
ZNF432	ZNF432-207	3.98*10-3	-1.65	2.35*10-2	-1.41	2.74*10-3	-1.52
ZNF510	ZNF510-203	4.48*10-3	-1.73	4.68*10-2	-1.70	4.88*10-3	-1.71
ZNF582	ZNF582-201	3.68*10-2	1.26	2.09*10-2	1.38	9.15*10-3	1.32
ZNF589	ZNF589-207	1.88*10-2	1.56	1.82*10-2	1.71	5.39*10-3	1.63
ZNF653	ZNF653-203	8.40*10-7	1.84*10+2	6.34*10-4	2.26*10+1	1.94*10-6	3.51*10+1
ZNF778	ZNF778-206	3.13*10-2	-1.82	1.97*10-2	-2.41	7.82*10-3	-2.06
ZNF791	ZNF791-202	3.42*10-3	1.46	3.76*10-2	1.39	3.63*10-3	1.43
ZNF792	ZNF792-202	1.87*10-2	-2.00	1.71*10-2	-2.38	5.19*10-3	-2.19
ZNF808	ZNF808-202	4.27*10-2	-1.37	3.49*10-3	-2.33	2.91*10-3	-1.85
ZNF826P	ZNF826P-203	1.30*10-3	1.90	2.23*10-2	2.58	1.47*10-3	2.19

Note 1: The linear model contained the variables "Large vessel stroke (LVS)", "statin use" and the interaction term "LVS*statin use".

Note 2: Genes were included in the list if they met the criteria for at least one of the comparisons: $|FC| > 1.2$ and $p<0.05$.

Table II: List of 147 transcripts differentiating patients with severe stenosis from those with mild or moderate stenosis

Gene symbol	Transcript ID	Severe stenosis versus (no/mild stenosis)		Severe stenosis versus moderate stenosis		Severe/moderate stenosis versus no/mild stenosis	
		p-value	FC	p-value	FC	p-value	FC
ABLIM1	ABLIM1-206	6.01*10-4	2.71	1.32*10-2	1.96	1.62*10-2	2.04
AC010175.1	AC010175.1-201	8.56*10-5	3.00	2.84*10-2	1.79	2.19*10-3	2.32
AC040970.1	AC040970.1-203	2.55*10-3	1.57	3.19*10-2	1.39	4.15*10-2	1.35
AC068580.1	AC068580.1-201	1.82*10-2	-5.08	8.51*10-7	1.94	1.29*10-3	-5.13
AC068888.1	AC068888.1-212	4.41*10-2	1.47	4.18*10-2	2.04	1.82*10-2	1.59
AC090695.2	AC090695.2-201	6.50*10-3	1.38	4.29*10-2	2.58	1.23*10-2	1.30
AC139720.1	AC139720.1-201	5.09*10-4	5.02	1.74*10-2	2.21	1.91*10-2	3.65
AC243960.1	AC243960.1-202	1.26*10-3	1.94	2.73*10-2	1.80	2.79*10-2	1.51
ACSM3	ACSM3-201	3.03*10-3	2.17	4.08*10-2	1.53	4.32*10-2	1.79
AKAP13	AKAP13-205	5.78*10-4	-3.31	8.83*10-3	-2.18	3.28*10-2	-2.07
AL096816.1	AL096816.1-201	1.05*10-3	2.20	1.71*10-2	2.26	3.69*10-2	1.59
AL139352.1	AL139352.1-201	1.67*10-3	4.17	1.14*10-2	2.43	4.30*10-2	2.96
AL160408.3	AL160408.3-201	1.58*10-3	5.32	3.86*10-2	1.60	3.02*10-2	4.32
AMIGO1	AMIGO1-202	2.92*10-4	2.60	2.23*10-2	2.06	7.18*10-3	1.95
AMT	AMT-240	7.18*10-4	-7.44	4.18*10-2	-1.54	1.48*10-2	-5.86
AP002478.1	AP002478.1-203	1.48*10-4	-7.19*10+1	4.58*10-3	-5.45*10+1	1.70*10-2	-2.60
AQP3	AQP3-201	6.35*10-5	2.19	1.83*10-2	1.95	2.48*10-3	1.66
ARHGAP12	ARHGAP12-205	3.96*10-2	-1.73	1.05*10-2	3.14	2.39*10-4	-2.45
ARNT	ARNT-204	2.93*10-2	-1.42	6.73*10-3	1.94	1.16*10-4	-1.88
ARNTL	ARNTL-222	6.56*10-4	1.83	1.05*10-2	2.58	3.51*10-2	1.30

ASCC1	ASCC1-205	7.65*10-4	1.23*10+1	2.67*10-2	9.55	1.96*10-2	6.88
ATP10A	ATP10A-207	4.53*10-4	1.61*10+2	1.81*10-2	9.98	1.59*10-2	9.15*10+1
ATP11A	ATP11A-215	2.39*10-2	-1.55	3.58*10-2	1.88	1.01*10-2	-1.44
B9D2	B9D2-202	5.32*10-4	-1.77	1.13*10-7	-1.60	6.57*10-3	-1.57
BCL11B	BCL11B-201	9.06*10-4	2.57	1.48*10-2	2.03	2.58*10-2	1.91
BNIP1	BNIP1-201	1.77*10-5	-4.07	3.52*10-4	-4.28	1.04*10-2	-1.54
BNIP3P5	BNIP3P5-201	7.13*10-3	2.23	5.53*10-3	2.85	3.50*10-2	1.75
C11orf58	C11orf58-209	3.04*10-2	-1.96	3.18*10-3	2.08	1.47*10-2	-1.95
C2CD4D-AS1	C2CD4D-AS1-201	1.69*10-3	5.90	3.37*10-2	2.26	2.63*10-2	4.26
CACNA1I	CACNA1I-202	6.81*10-5	3.39	1.07*10-2	3.15	4.22*10-3	2.23
CAMK4	CAMK4-201	1.50*10-3	2.19	1.73*10-2	1.98	4.56*10-2	1.65
CANT1	CANT1-207	2.24*10-2	1.59	5.43*10-3	2.37	4.21*10-2	1.72
CANX	CANX-209	4.05*10-2	-2.73*10+1	1.99*10-6	1.46*10+1	3.40*10-3	-1.35*10+1
CCNJL	CCNJL-210	4.82*10-4	1.56	6.06*10-3	3.34	4.04*10-2	1.32
CD3E	CD3E-201	6.00*10-4	2.25	1.70*10-2	1.86	1.66*10-2	1.73
CD3E	CD3E-202	8.32*10-4	2.23	2.50*10-2	1.86	2.26*10-2	1.72
CD3G	CD3G-206	5.62*10-4	2.14	6.24*10-3	1.95	3.54*10-2	1.62
CD40LG	CD40LG-202	7.99*10-5	2.55	1.97*10-2	2.41	5.01*10-3	1.77
CD6	CD6-203	1.62*10-3	4.47	1.87*10-2	2.54	4.44*10-2	3.12
CDC42BPG	CDC42BPG-201	2.49*10-3	1.83	2.68*10-2	1.69	4.31*10-2	1.46
CDC42SE2	CDC42SE2-202	6.17*10-3	1.45	1.87*10-2	-1.46	4.38*10-3	1.48
CERS6-AS1	CERS6-AS1-212	5.90*10-3	1.32	3.22*10-2	-1.47	1.70*10-3	1.39
CHD4	CHD4-218	3.64*10-2	-2.01	1.37*10-2	2.46	1.47*10-2	-1.86
CLEC12A-AS1	CLEC12A-AS1-201	4.79*10-4	3.25	3.21*10-2	2.37	7.59*10-3	2.32
CLN3	CLN3-216	2.13*10-5	-5.00	1.38*10-3	-2.12	6.37*10-3	-4.53
COMMD5	COMMD5-204	9.55*10-5	-4.27	1.26*10-2	-1.72	5.36*10-3	-3.14
CTNND1	CTNND1-213	1.18*10-2	1.41	3.43*10-2	-1.60	1.80*10-3	1.57
CXCR3	CXCR3-202	2.78*10-3	2.26	3.77*10-2	1.75	4.99*10-2	1.77
DBI	DBI-203	3.07*10-3	-3.49	7.53*10-4	-2.48	3.31*10-2	-2.21

DGKA	DGKA-234	2.59*10-4	1.53	3.37*10-2	1.33	6.73*10-3	1.34
DMXL2	DMXL2-201	2.05*10-2	-1.62	4.54*10-3	1.60	1.50*10-2	-1.52
DNAJC7	DNAJC7-202	1.62*10-3	1.98	1.84*10-2	1.97	4.23*10-2	1.49
DNAJC8	DNAJC8-206	6.63*10-3	-2.54	3.45*10-2	-1.30	2.39*10-2	-2.40
DOCK9	DOCK9-219	1.49*10-3	3.32	2.31*10-2	2.95	4.12*10-2	2.22
EGLN1	EGLN1-206	2.73*10-2	1.44	3.77*10-2	-1.65	3.46*10-2	1.44
EIF4G2	EIF4G2-205	4.03*10-2	1.32	1.15*10-2	-1.53	4.24*10-2	1.35
EMB	EMB-204	2.43*10-2	-1.49	1.79*10-2	1.73	3.12*10-4	-1.88
ERCC8	ERCC8-212	3.25*10-4	1.53*10+1	6.92*10-3	3.72	2.35*10-2	9.71
ESF1	ESF1-202	8.62*10-5	-9.57*10+1	2.28*10-2	-9.65	3.25*10-3	-1.80*10+1
EXOG	EXOG-205	9.91*10-3	-5.82*10+2	1.98*10-3	-2.18	2.29*10-2	-2.41*10+1
FAAH2	FAAH2-201	3.86*10-4	2.50	3.48*10-3	5.12	3.99*10-2	1.51
FAM153A	FAM153A-201	4.63*10-4	2.29	5.46*10-3	2.42	3.93*10-2	1.64
FAM30A	FAM30A-203	2.02*10-3	7.61	3.41*10-2	1.10*10+1	4.18*10-2	4.23
FHIT	FHIT-208	1.73*10-4	2.17	4.48*10-2	1.52	2.37*10-3	1.80
FLNA	FLNA-218	3.90*10-2	-6.30	9.48*10-3	1.95	3.15*10-2	-3.46
FLYWCH2	FLYWCH2-205	6.35*10-3	-1.80	3.20*10-4	-1.90	4.95*10-2	-1.57
GPR155	GPR155-206	5.55*10-4	1.47	1.72*10-2	1.32	1.45*10-2	1.29
GTF2H3	GTF2H3-214	2.65*10-3	1.91	3.91*10-2	1.57	3.26*10-2	1.59
HDAC4-AS1	HDAC4-AS1-204	7.03*10-4	-2.37	1.82*10-2	-1.32	2.53*10-2	-2.05
HDGF	HDGF-208	2.77*10-2	-4.23	5.82*10-3	-1.76	3.03*10-2	-3.34
HSPA8	HSPA8-222	1.79*10-2	1.40	2.83*10-4	-1.79	3.11*10-3	1.57
IGHV5-51	IGHV5-51-201	9.27*10-4	3.07	3.16*10-2	2.84	1.47*10-2	2.08
IGKV1-16	IGKV1-16-201	2.08*10-3	2.30	2.30*10-2	2.55	4.61*10-2	1.60
IL7R	IL7R-201	3.56*10-4	2.22	7.83*10-3	2.17	2.05*10-2	1.62
IL7R	IL7R-206	2.16*10-3	2.22	4.90*10-2	2.15	3.46*10-2	1.63
IPO8	IPO8-203	1.82*10-2	-1.36	2.29*10-2	1.46	2.70*10-2	-1.28
ITIH2	ITIH2-201	4.28*10-4	-1.72*10+1	2.20*10-2	-8.11	1.38*10-2	-3.72
ITK	ITK-204	1.07*10-3	2.37	8.68*10-3	1.85	4.40*10-2	1.82
KLHDC4	KLHDC4-220	2.19*10-3	3.79	3.74*10-2	4.56	4.15*10-2	2.32

KRI1	KRI1-203	7.50*10-4	-1.75	3.42*10-2	-1.41	1.71*10-2	-1.45
LACTB2	LACTB2-204	8.43*10-5	-4.31	7.75*10-4	-4.89	2.75*10-2	-1.53
LCK	LCK-206	1.33*10-3	4.07	3.95*10-2	2.39	2.58*10-2	2.89
LIG1	LIG1-207	5.89*10-4	2.22	2.61*10-2	2.63	8.77*10-3	1.63
LINC02249	LINC02249-203	8.65*10-4	9.62	1.76*10-2	2.99	2.80*10-2	6.43
LRRK1	LRRK1-205	2.22*10-3	1.62	2.08*10-2	-1.88	6.70*10-5	2.00
LY9	LY9-201	5.70*10-4	2.76	6.53*10-3	2.74	2.10*10-2	1.89
MAFK	MAFK-202	2.10*10-2	-1.87	7.59*10-3	1.67	1.59*10-2	-1.78
MDM2	MDM2-225	2.48*10-2	-4.18	3.64*10-4	2.97	1.97*10-2	-1.98
MEFV	MEFV-214	4.77*10-2	-1.47	2.12*10-2	1.56	1.64*10-2	-1.46
MEG3	MEG3-201	4.05*10-2	1.90	2.53*10-2	-3.40	5.35*10-4	4.18
MEX3D	MEX3D-202	2.13*10-3	-2.28	2.41*10-2	-1.54	4.86*10-2	-1.78
MIR22HG	MIR22HG-210	1.38*10-3	7.65	1.15*10-2	7.63	4.49*10-2	4.67
MORN2	MORN2-204	3.98*10-2	-1.51	3.06*10-3	-1.28	2.92*10-2	-1.66
NBL1	NBL1-209	7.17*10-4	5.24	1.37*10-2	7.24	3.00*10-2	3.02
NELL2	NELL2-205	5.86*10-4	4.88	2.51*10-2	2.64	1.68*10-2	3.36
NRDC	NRDC-202	3.34*10-2	-1.42	9.32*10-3	1.67	1.12*10-2	-1.42
NT5C3B	NT5C3B-209	5.49*10-5	-5.19*10+1	1.63*10-2	-2.45*10+1	2.57*10-3	-3.94
NYAP1	NYAP1-201	1.62*10-3	6.38	3.14*10-2	3.19	3.32*10-2	4.19
ORAI2	ORAI2-207	4.48*10-2	-1.66	1.89*10-3	1.79	1.79*10-2	-1.51
OSBPL5	OSBPL5-208	9.61*10-3	1.57	2.98*10-2	-1.63	9.21*10-5	2.07
PATJ	PATJ-203	4.97*10-5	2.81	1.52*10-5	7.33	1.72*10-2	1.96
PCED1B-AS1	PCED1B-AS1-203	2.71*10-4	2.17	3.27*10-3	1.89	1.74*10-2	1.66
PECAM1	PECAM1-204	3.56*10-2	-1.41	1.88*10-2	1.60	1.23*10-2	-1.43
PFN2	PFN2-203	9.79*10-4	7.78	3.62*10-2	5.28	2.09*10-2	4.62
PI16	PI16-204	5.74*10-4	2.89	1.56*10-2	2.72	2.37*10-2	1.97
PLCH2	PLCH2-202	1.27*10-4	3.70	4.87*10-2	2.26	2.49*10-3	2.66
PLEKHB1	PLEKHB1-201	9.15*10-5	2.82	6.31*10-3	1.75	5.23*10-3	2.21
PPT1	PPT1-208	4.53*10-2	-4.43	1.12*10-2	2.38	1.35*10-2	-3.70
PTPA	PTPA-215	1.18*10-3	-7.77	4.94*10-2	-5.17	2.06*10-2	-2.52

PTPN18	PTPN18-202	4.66*10-4	-5.59	1.00*10-2	-4.23	1.65*10-2	-2.12
RACGAP1	RACGAP1-219	1.04*10-4	3.96*10+1	8.53*10-3	5.25	8.34*10-3	2.30*10+1
RACK1	RACK1-234	8.64*10-3	1.71	6.24*10-4	3.15	4.46*10-2	1.40
RPGRIP1L	RPGRIP1L-206	9.64*10-4	1.07*10+1	1.01*10-2	3.15	4.56*10-2	7.07
SERBP1	SERBP1-207	2.79*10-2	2.04	6.46*10-3	2.03	1.92*10-2	2.10
SESN1	SESN1-202	1.49*10-5	1.87	4.72*10-2	1.54	2.51*10-4	1.54
SIGIRR	SIGIRR-215	9.21*10-5	-4.24	1.48*10-3	-3.81	1.97*10-2	-1.76
SLC15A4	SLC15A4-204	2.54*10-3	1.47	3.26*10-2	1.31	3.82*10-2	1.29
SLC8A1	SLC8A1-202	1.57*10-2	-2.07	1.07*10-2	2.13	2.52*10-3	-2.28
SPOCD1	SPOCD1-201	1.25*10-3	2.78	4.48*10-2	1.32	1.87*10-2	2.48
STAU2	STAU2-214	1.80*10-2	-1.65	1.91*10-2	1.38	1.37*10-4	-1.92
STX16	STX16-204	2.53*10-2	3.22	2.44*10-2	-2.47	4.86*10-2	3.65
TAOK3	TAOK3-202	9.72*10-3	1.44	2.66*10-2	-1.54	6.50*10-3	1.51
TBCK	TBCK-213	3.71*10-2	-1.78	3.48*10-4	3.36	4.68*10-3	-2.10
TCF7	TCF7-203	6.54*10-6	3.10	1.73*10-2	1.69	1.86*10-4	2.46
TCF7	TCF7-214	3.19*10-5	2.82	1.28*10-2	2.40	1.21*10-3	2.00
TESPA1	TESPA1-202	4.67*10-4	2.44	7.55*10-3	1.86	1.75*10-2	1.88
THUMPD2	THUMPD2-205	5.37*10-3	2.00	3.03*10-4	3.00	4.92*10-2	1.52
TLR4	TLR4-204	2.15*10-2	-1.85	4.48*10-2	1.41	1.30*10-2	-1.69
TMEM185A	TMEM185A-202	6.54*10-4	-2.57*10+2	2.87*10-2	-1.38*10+2	2.09*10-2	-4.46
TNFRSF9	TNFRSF9-204	1.51*10-3	1.66	4.53*10-2	1.41	1.95*10-2	1.42
TRAC	TRAC-201	1.88*10-4	2.37	6.36*10-3	1.84	1.09*10-2	1.82
TRAK1	TRAK1-211	1.67*10-2	-4.86	3.09*10-4	1.56	8.29*10-3	-2.79
TRAV19	TRAV19-201	1.76*10-3	1.69	4.67*10-2	2.24	2.75*10-2	1.22
TRBC1	TRBC1-201	9.22*10-4	2.34	1.11*10-2	1.88	4.15*10-2	1.78
TRBC2	TRBC2-201	8.14*10-4	2.11	1.80*10-2	1.58	2.86*10-2	1.72
TRBV3-1	TRBV3-1-201	1.25*10-3	2.27	1.55*10-2	2.11	4.31*10-2	1.67
TRIM41	TRIM41-202	3.85*10-3	-7.58	2.38*10-2	1.23	3.02*10-3	-3.95
TXNRD2	TXNRD2-202	3.52*10-4	-2.65	2.09*10-2	-2.16	1.63*10-2	-1.79
UBAP2L	UBAP2L-214	9.68*10-5	1.66	4.70*10-2	-1.32	1.41*10-6	1.92

UBXN11	UBXN11-204	2.06*10-4	-1.52	1.98*10-3	-1.46	3.23*10-2	-1.23
UNC119	UNC119-205	3.93*10-2	-1.41	1.12*10-2	1.34	4.79*10-2	-1.31
VEZT	VEZT-207	9.76*10-4	-5.51*10+1	3.89*10-2	-2.22*10+1	1.63*10-2	-4.45
WASHC2A	WASHC2A-204	2.08*10-3	1.46	1.90*10-2	-1.43	3.95*10-4	1.53
ZFAND5	ZFAND5-203	5.35*10-3	-2.39	3.32*10-2	1.89	1.38*10-2	-1.87
ZFYVE21	ZFYVE21-212	4.23*10-4	2.99*10+1	3.37*10-3	9.54*10+1	4.33*10-2	1.51*10+1
ZNF264	ZNF264-204	1.29*10-3	-1.21*10+1	2.64*10-2	-1.02*10+1	3.15*10-2	-2.17
ZNF653	ZNF653-203	8.17*10-6	7.25*10+1	1.02*10-2	7.50	6.48*10-4	4.12*10+1

Note 1: The linear model contained the variables "grade of stenosis", "statin use" and the interaction term "grade of stenosis*statin use".

Note 2: Genes were included in the list if they met the criteria for at least one of the comparisons: $|FC| > 1.2$ and $p<0.05$.

Table III: List of 40 transcripts common to list 1 (Table I) and list 2 (Table II)

Gene symbol	Transcript ID
AC040970.1	AC040970.1-203
AC139720.1	AC139720.1-201
ACSM3	ACSM3-201
AKAP13	AKAP13-205
AL139352.1	AL139352.1-201
AL160408.3	AL160408.3-201
AMT	AMT-240
B9D2	B9D2-202
BNIP1	BNIP1-201
CACNA1I	CACNA1I-202
CLEC12A-AS1	CLEC12A-AS1-201
CLN3	CLN3-216
COMMD5	COMMD5-204
CXCR3	CXCR3-202
DBI	DBI-203
DNAJC7	DNAJC7-202
ESF1	ESF1-202
FLYWCH2	FLYWCH2-205
GPR155	GPR155-206
HDGF	HDGF-208
HSPA8	HSPA8-222
ITK	ITK-204
LACTB2	LACTB2-204
LCK	LCK-206
LINC02249	LINC02249-203
LY9	LY9-201
NELL2	NELL2-205

NT5C3B	NT5C3B-209
NYAP1	NYAP1-201
ORAI2	ORAI2-207
PFN2	PFN2-203
PI16	PI16-204
PLCH2	PLCH2-202
PTPA	PTPA-215
RACGAP1	RACGAP1-219
RPGRIP1L	RPGRIP1L-206
STAU2	STAU2-214
TCF7	TCF7-203
ZFYVE21	ZFYVE21-212
ZNF653	ZNF653-203

Table IV: Normalized plasma levels of PD-1 in patients grouped by stroke status and grade of stenosis

Groups		Median*	IQR	P-value
Phenotype	Control (n=7)	11.1	10.5-13.4	0.55
	Stroke not due to carotid atherosclerosis (n=14)	12.5	10.8-15.7	
	Stroke due to carotid atherosclerosis (n=14)**	11.4	7.6-14.8	
Grade of stenosis	Mild (n=18)	11.8	10.8-14.6	0.67
	Moderate (n=6)	11.4	11.1-15.7	
	Severe (n=11)**	11.4	7.3-14.8	

* Expressed in standard units

** Missing plasma sample for 1 patient with stroke due to carotid atherosclerosis

Table V: Characteristics of the participants in the verification cohort

Characteristic		Stroke due large artery atherosclerosis (n=9)*	Stroke not due to large artery atherosclerosis (n=28)	Controls (n=26)	p
Age (mean ± SD)		67.0±11.2	65.6±12.6	61.4±6.3	0.51
Female, n (%)		4 (44.4)	15 (53.6)	14 (53.9)	0.88
Hypertension, n (%)		8 (88.9)	22 (78.6)	16 (64.0)	0.27
Diabetes, n (%)		3 (33.3)	4 (14.3)	11 (42.3)	0.07
Dyslipidemia, n (%)		6 (66.7)	12 (42.9)	18 (69.2)	0.12
Statin before admission, n (%)		5 (55.6)	8 (28.6)	17 (65.4)	**0.02**
Smoking, n (%)**		3 (33.3)	10 (35.87)	7 (28.0)	0.83
Grade of stenosis***	Mild (<50%)	7 (77.8)	21 (80.7)	6 (42.9)	0.02
	Moderate (50-69%)	0 (0.00)	5 (19.2)	5 (35.7)	
	Severe (>70%)	2 (22.2)	0 (0.0)	3 (21.4)	
NIHSS (mean, range)†		3 (0-11)	4 (0-17)	NA	0.14

NIHSS: National Institutes of Health Stroke Scale

* Six of the nine cases were due to intracranial atherosclerosis

** Missing information for 1 control

*** Missing value for 2 patients with stroke not due to large artery atherosclerosis and 12 controls.

† Missing value for 1 patient with stroke due to large artery atherosclerosis

Table VI: List of 185 transcripts differentiating patients with stroke due to large artery atherosclerosis from patients with stroke of other causes, and controls in the validation sample

Gene symbol	Transcript ID	Large vessel stroke versus stroke of other causes		Large vessel stroke versus controls		Large vessel stroke versus (stroke of other causes and controls)	
		p-value	FC	p-value	FC	p-value	FC
ARHGAP45	ARHGAP45-215	5.18*10-5	-1.88	9.05*10-3	-1.52	3.33*10-4	-1.70
RBCK1	RBCK1-215	9.15*10-5	-2.36	1.82*10-3	-1.96	1.58*10-4	-2.16
SLC16A5	SLC16A5-201	1.99*10-4	-1.60	3.91*10-3	-1.27	3.99*10-4	-1.43
U2AF1L5	U2AF1L5-206	4.24*10-4	-3.14	1.46*10-4	-3.85	9.32*10-5	-3.50
EEF1D	EEF1D-246	4.50*10-4	-1.49	4.48*10-3	-1.28	6.61*10-4	-1.39
RHBDD2	RHBDD2-202	4.87*10-4	-1.93	5.96*10-3	-1.21	8.70*10-4	-1.57
LCP2	LCP2-202	4.95*10-4	-2.04	2.88*10-4	-2.12	1.54*10-4	-2.08
SH3BP2	SH3BP2-219	6.51*10-4	-1.62	5.98*10-3	-1.40	9.13*10-4	-1.51
SEC24A	SEC24A-201	6.78*10-4	-1.38	2.64*10-4	-1.44	1.65*10-4	-1.41
SLC17A9	SLC17A9-207	7.57*10-4	-1.72	3.37*10-4	-1.84	2.10*10-4	-1.78
HLA-C	HLA-C-209	9.48*10-4	-1.67	1.52*10-2	-1.42	1.99*10-3	-1.54
PTBP3	PTBP3-203	9.61*10-4	-1.64	3.18*10-3	-1.40	8.18*10-4	-1.52
UBE4B	UBE4B-201	1.01*10-3	1.59	1.85*10-2	1.40	2.55*10-3	1.49
LRRC41	LRRC41-203	1.19*10-3	-1.35	8.61*10-4	-1.29	4.38*10-4	-1.32
TSC1	TSC1-222	1.30*10-3	-2.22	1.03*10-3	-2.18	5.20*10-4	-2.20
MLX	MLX-206	1.31*10-3	-2.80	1.26*10-2	-2.48	2.21*10-3	-2.64
CUEDC1	CUEDC1-212	1.38*10-3	-1.51	2.95*10-3	-1.42	9.46*10-4	-1.47
CD55	CD55-208	1.64*10-3	-1.79	5.16*10-3	-1.49	1.42*10-3	-1.64
LRRC37A2	LRRC37A2-203	1.87*10-3	-1.54	2.05*10-2	-1.44	3.54*10-3	-1.49
PPP1CB	PPP1CB-202	2.05*10-3	-2.53	8.57*10-3	-2.17	2.30*10-3	-2.35
AC117382.1	AC117382.1-201	2.16*10-3	-1.44	4.67*10-4	-1.73	4.44*10-4	-1.59
NAA60	NAA60-203	2.41*10-3	-1.72	1.02*10-3	-1.88	6.88*10-4	-1.80
GOLGA2	GOLGA2-201	2.68*10-3	-1.66	9.84*10-3	-1.47	2.89*10-3	-1.56
CYRIB	CYRIB-204	2.88*10-3	-1.59	9.94*10-3	-1.35	2.90*10-3	-1.47
PLEKHM1P1	PLEKHM1P1-203	2.94*10-3	-1.80	2.12*10-3	-1.65	1.24*10-3	-1.72

DOK1	DOK1-201	3.12*10-3	-2.42	1.78*10-3	-2.25	1.15*10-3	-2.33
AMZ2	AMZ2-202	3.30*10-3	-1.69	7.60*10-4	-1.87	7.71*10-4	-1.78
RAPGEF6	RAPGEF6-208	3.34*10-3	-1.40	6.08*10-3	-1.33	2.46*10-3	-1.37
DCAF5	DCAF5-205	3.52*10-3	-1.42	2.52*10-2	-1.21	5.86*10-3	-1.32
STK10	STK10-213	3.53*10-3	-1.84	3.97*10-2	-1.50	7.43*10-3	-1.67
TET2	TET2-209	3.64*10-3	-1.43	3.68*10-3	-1.43	1.81*10-3	-1.43
TET2	TET2-207	3.64*10-3	-1.43	3.68*10-3	-1.43	1.81*10-3	-1.43
FOLR3	FOLR3-204	3.76*10-3	5.54	4.10*10-2	5.74	8.23*10-3	5.64
NSMF	NSMF-207	4.15*10-3	-1.73	4.89*10-2	-1.62	9.59*10-3	-1.67
TPRN	TPRN-202	4.28*10-3	-1.41	1.13*10-2	-1.28	4.03*10-3	-1.34
SIGLEC10	SIGLEC10-203	4.51*10-3	-1.97	2.00*10-3	-2.05	1.46*10-3	-2.01
KIAA1109	KIAA1109-213	4.53*10-3	-1.42	3.63*10-2	-1.30	8.35*10-3	-1.36
PI4KA	PI4KA-217	4.94*10-3	1.33	2.04*10-2	1.26	6.15*10-3	1.29
APBB3	APBB3-215	5.34*10-3	-1.54	5.33*10-3	-1.64	2.86*10-3	-1.59
PPFIA4	PPFIA4-205	5.46*10-3	2.56	6.21*10-3	2.31	3.24*10-3	2.43
SGSM2	SGSM2-213	5.72*10-3	-1.44	7.26*10-3	-1.36	3.54*10-3	-1.40
AC114878.2	AC114878.2-201	5.94*10-3	-1.46	1.45*10-2	-1.36	5.45*10-3	-1.41
PRKCD	PRKCD-202	6.38*10-3	1.48	1.69*10-2	1.40	5.81*10-3	1.44
SIDT2	SIDT2-206	6.54*10-3	-1.97	4.78*10-3	-1.89	3.05*10-3	-1.93
ZEB2-AS1	ZEB2-AS1-207	6.68*10-3	-1.51	1.23*10-3	-1.58	1.45*10-3	-1.55
NSUN6	NSUN6-201	7.80*10-3	-1.49	5.35*10-4	-1.73	1.05*10-3	-1.61
PRKD3	PRKD3-210	8.36*10-3	1.51	2.73*10-2	1.48	9.26*10-3	1.50
EIF4EBP3	EIF4EBP3-201	8.62*10-3	1.54	2.73*10-2	1.39	9.60*10-3	1.46
SEC31A	SEC31A-208	8.76*10-3	-1.50	2.90*10-2	-1.21	9.90*10-3	-1.36
BNIP3	BNIP3-201	8.88*10-3	-1.56	1.17*10-2	-1.56	6.10*10-3	-1.56
USP34	USP34-218	8.91*10-3	-1.64	2.17*10-2	-1.27	8.49*10-3	-1.46
SIK3	SIK3-210	9.03*10-3	-1.25	1.31*10-2	-1.21	6.36*10-3	-1.23
AC090114.2	AC090114.2-201	9.12*10-3	1.42	2.25*10-3	1.50	2.49*10-3	1.46
IPO5	IPO5-201	9.37*10-3	-1.45	3.71*10-2	-1.29	1.20*10-2	-1.37
KIAA0753	KIAA0753-201	9.52*10-3	-1.31	4.78*10-3	-1.36	3.69*10-3	-1.33
ATP2C1	ATP2C1-218	9.58*10-3	-1.88	1.11*10-2	-1.93	5.99*10-3	-1.91

SIN3B	SIN3B-201	9.61*10-3	1.31	9.37*10-3	1.34	5.59*10-3	1.33
LCLAT1	LCLAT1-203	9.65*10-3	-1.40	4.33*10-3	-1.46	3.40*10-3	-1.43
GATAD2A	GATAD2A-203	9.80*10-3	-1.78	6.10*10-3	-1.71	4.28*10-3	-1.74
CD63	CD63-206	1.01*10-2	1.92	4.71*10-2	1.59	1.39*10-2	1.74
RNF220	RNF220-214	1.02*10-2	1.38	2.26*10-2	1.36	9.58*10-3	1.37
CC2D1B	CC2D1B-201	1.03*10-2	-1.85	2.34*10-2	-1.46	9.83*10-3	-1.66
MAVS	MAVS-202	1.03*10-2	1.21	1.21*10-2	1.21	6.73*10-3	1.21
PRRC2A	PRRC2A-202	1.03*10-2	1.28	2.47*10-2	1.23	1.01*10-2	1.26
NQO2	NQO2-205	1.04*10-2	1.46	2.44*10-3	1.46	2.83*10-3	1.46
NUP98	NUP98-205	1.04*10-2	-1.66	1.27*10-2	-1.55	6.65*10-3	-1.61
SETD2	SETD2-204	1.10*10-2	-1.66	2.33*10-2	-1.47	1.02*10-2	-1.56
RELA	RELA-209	1.10*10-2	-1.42	9.01*10-3	-1.57	5.92*10-3	-1.50
H3-5	H3-5-201	1.12*10-2	-1.40	5.82*10-3	-1.49	4.74*10-3	-1.45
DUSP2	DUSP2-202	1.13*10-2	-1.59	6.51*10-3	-1.43	4.86*10-3	-1.51
NKD1	NKD1-201	1.13*10-2	-1.61	4.01*10-2	-1.36	1.37*10-2	-1.48
DAPK3	DAPK3-201	1.14*10-2	-1.21	3.93*10-3	-1.36	3.84*10-3	-1.28
TPM4	TPM4-229	1.16*10-2	-1.50	1.53*10-2	-1.58	8.18*10-3	-1.54
ACIN1	ACIN1-223	1.16*10-2	1.29	2.72*10-3	1.36	3.20*10-3	1.32
AC026401.3	AC026401.3-201	1.19*10-2	1.43	1.89*10-2	1.41	9.43*10-3	1.42
PPP1R12B	PPP1R12B-217	1.22*10-2	-1.38	1.48*10-2	-1.29	7.97*10-3	-1.34
SOCS4	SOCS4-202	1.28*10-2	-1.37	4.54*10-3	-1.51	4.44*10-3	-1.44
POU2F2	POU2F2-218	1.29*10-2	-1.62	1.28*10-2	-1.53	7.77*10-3	-1.57
DISC1	DISC1-223	1.29*10-2	-1.26	1.56*10-2	-1.21	8.67*10-3	-1.23
AC026271.1	AC026271.1-201	1.31*10-2	-1.38	1.48*10-2	-1.30	8.62*10-3	-1.34
MAP1S	MAP1S-201	1.33*10-2	1.21	3.08*10-3	1.27	3.65*10-3	1.24
DDX3X	DDX3X-265	1.33*10-2	-1.45	7.24*10-3	-1.55	5.87*10-3	-1.50
PAK1	PAK1-214	1.38*10-2	-1.49	2.39*10-3	-1.69	3.31*10-3	-1.59
B3GNT2	B3GNT2-201	1.38*10-2	-1.27	8.23*10-3	-1.31	6.30*10-3	-1.29
FNBP1	FNBP1-205	1.49*10-2	-1.33	6.61*10-3	-1.39	5.97*10-3	-1.36
MKNK2	MKNK2-206	1.50*10-2	-1.52	1.55*10-2	-1.20	9.45*10-3	-1.36
KCNK6	KCNK6-201	1.54*10-2	1.30	2.87*10-2	1.28	1.38*10-2	1.29

TKT	TKT-208	1.55*10-2	1.29	1.94*10-2	1.29	1.08*10-2	1.29
NR4A2	NR4A2-201	1.57*10-2	-1.39	8.65*10-3	-1.68	7.08*10-3	-1.53
OSER1	OSER1-201	1.61*10-2	-1.91	2.86*10-2	-2.05	1.37*10-2	-1.98
RPL9	RPL9-214	1.62*10-2	-1.69	3.66*10-3	-1.81	4.56*10-3	-1.75
RAD54L2	RAD54L2-203	1.62*10-2	-1.20	1.43*10-2	-1.37	9.43*10-3	-1.29
TMED7-TICAM2	TMED7-TICAM2-202	1.64*10-2	2.29	4.60*10-2	1.72	1.85*10-2	1.96
SLC2A3	SLC2A3-205	1.67*10-2	-2.34	1.31*10-2	-1.94	9.27*10-3	-2.14
QRICH1	QRICH1-204	1.68*10-2	-2.36	1.05*10-2	-2.40	7.90*10-3	-2.38
TNFAIP3	TNFAIP3-205	1.70*10-2	-1.83	3.93*10-2	-1.41	1.75*10-2	-1.62
RNF130	RNF130-207	1.71*10-2	-1.65	4.52*10-2	-1.37	1.84*10-2	-1.51
GSTP1	GSTP1-205	1.71*10-2	1.40	3.58*10-2	1.42	1.66*10-2	1.41
TMTC2	TMTC2-205	1.72*10-2	-2.10	7.31*10-3	-1.97	6.65*10-3	-2.03
CD300LB	CD300LB-202	1.73*10-2	1.24	3.86*10-2	1.21	1.75*10-2	1.22
RBM5	RBM5-212	1.73*10-2	1.31	4.01*10-2	1.26	1.68*10-2	1.29
TMEM121B	TMEM121B-201	1.77*10-2	1.52	1.18*10-2	1.59	8.95*10-3	1.55
STX4	STX4-213	1.82*10-2	-1.40	7.02*10-3	-1.45	6.87*10-3	-1.43
PPP4C	PPP4C-205	1.83*10-2	-1.61	7.87*10-3	-1.47	7.08*10-3	-1.54
PPM1D	PPM1D-202	1.84*10-2	1.52	1.94*10-2	1.48	1.22*10-2	1.50
EGR3	EGR3-201	1.89*10-2	-1.83	2.39*10-2	-1.96	1.33*10-2	-1.90
AC009948.4	AC009948.4-201	1.90*10-2	-1.35	1.02*10-3	-1.56	2.41*10-3	-1.46
MYCL	MYCL-203	1.92*10-2	1.47	4.63*10-2	1.41	1.99*10-2	1.44
ITSN2	ITSN2-211	1.94*10-2	1.46	7.68*10-3	1.52	7.29*10-3	1.49
NLRP3	NLRP3-202	1.94*10-2	-1.76	4.27*10-2	-1.36	1.87*10-2	-1.56
CHD8	CHD8-202	1.98*10-2	-2.89	1.60*10-2	-2.99	1.13*10-2	-2.94
SRRM2	SRRM2-218	1.99*10-2	1.29	1.43*10-2	1.33	9.99*10-3	1.31
ZNF696	ZNF696-201	2.00*10-2	-1.37	4.51*10-2	-1.32	2.08*10-2	-1.34
JRK	JRK-210	2.02*10-2	1.91	1.60*10-2	1.93	1.15*10-2	1.92
DDX5	DDX5-218	2.26*10-2	-1.54	1.20*10-2	-1.48	1.05*10-2	-1.51
C17orf107	C17orf107-201	2.27*10-2	-1.55	2.30*10-2	-1.23	1.48*10-2	-1.39
CCNT2	CCNT2-201	2.32*10-2	1.46	2.83*10-2	1.49	1.64*10-2	1.48

RNASE6	RNASE6-201	2.33*10-2	1.35	3.73*10-2	1.34	1.98*10-2	1.34
SRC	SRC-204	2.42*10-2	-1.76	1.45*10-2	-1.80	1.20*10-2	-1.78
ATG9A	ATG9A-202	2.56*10-2	1.91	4.21*10-2	1.92	2.26*10-2	1.92
RNF38	RNF38-206	2.64*10-2	1.66	1.24*10-2	1.81	1.16*10-2	1.73
PARP6	PARP6-220	2.68*10-2	1.72	1.56*10-2	1.90	1.25*10-2	1.81
C15orf39	C15orf39-207	2.72*10-2	-1.32	1.04*10-2	-1.37	1.08*10-2	-1.34
KLHL36	KLHL36-204	2.78*10-2	1.29	3.57*10-2	1.28	2.04*10-2	1.29
RIPOR2	RIPOR2-214	2.79*10-2	-1.39	8.49*10-3	-1.46	9.84*10-3	-1.42
TYSND1	TYSND1-201	2.81*10-2	-1.58	7.23*10-3	-1.42	8.83*10-3	-1.50
TMEM11	TMEM11-201	2.83*10-2	1.39	3.64*10-2	1.41	2.20*10-2	1.40
LFNG	LFNG-203	2.83*10-2	-1.42	2.16*10-2	-1.45	1.60*10-2	-1.43
MAP2K5	MAP2K5-201	2.87*10-2	1.27	2.25*10-2	1.32	1.68*10-2	1.30
LINC-PINT	LINC-PINT-222	2.89*10-2	-1.46	2.37*10-2	-1.39	1.75*10-2	-1.42
GAA	GAA-202	2.95*10-2	1.29	1.57*10-2	1.37	1.40*10-2	1.33
DENND1C	DENND1C-211	2.96*10-2	-1.85	1.63*10-2	-2.23	1.39*10-2	-2.04
TTYH3	TTYH3-204	2.96*10-2	-1.73	2.69*10-2	-1.92	1.91*10-2	-1.83
CD74	CD74-210	3.07*10-2	-1.28	6.78*10-3	-1.45	9.23*10-3	-1.36
NPEPPS	NPEPPS-219	3.08*10-2	1.51	6.92*10-4	1.93	2.77*10-3	1.70
MBNL1	MBNL1-205	3.10*10-2	1.39	3.99*10-2	1.28	2.31*10-2	1.33
NGLY1	NGLY1-215	3.12*10-2	-1.61	4.94*10-2	-1.61	2.71*10-2	-1.61
HK3	HK3-203	3.12*10-2	1.27	1.19*10-2	1.34	1.22*10-2	1.30
NCK1	NCK1-201	3.13*10-2	1.33	4.22*10-2	1.35	2.53*10-2	1.34
JDP2	JDP2-208	3.17*10-2	-1.72	2.30*10-2	-1.49	1.80*10-2	-1.61
SYTL1	SYTL1-206	3.17*10-2	-1.41	4.80*10-2	-1.36	2.74*10-2	-1.39
MED13L	MED13L-206	3.19*10-2	-1.29	6.47*10-3	-1.55	9.12*10-3	-1.42
IKZF1	IKZF1-210	3.22*10-2	1.52	1.33*10-2	1.55	1.36*10-2	1.53
OLIG1	OLIG1-201	3.40*10-2	1.49	2.63*10-2	1.50	2.02*10-2	1.49
CD101	CD101-202	3.42*10-2	-1.73	2.52*10-2	-1.41	1.99*10-2	-1.57
SLC37A2	SLC37A2-205	3.42*10-2	1.36	4.82*10-2	1.35	2.84*10-2	1.36
CAMKK2	CAMKK2-211	3.44*10-2	-1.37	4.28*10-2	-1.65	2.68*10-2	-1.51
POLM	POLM-216	3.44*10-2	-1.52	2.50*10-2	-1.67	1.97*10-2	-1.60

SGMS1-AS1	SGMS1-AS1-203	3.49*10-2	-1.20	1.43*10-2	-1.38	1.44*10-2	-1.29
ALG12	ALG12-201	3.53*10-2	1.25	7.31*10-4	1.44	3.15*10-3	1.34
MTERF1	MTERF1-201	3.56*10-2	-1.84	3.89*10-2	-1.53	2.54*10-2	-1.69
LILRB2	LILRB2-202	3.56*10-2	-2.56	2.04*10-2	-2.01	1.81*10-2	-2.28
CELF2	CELF2-202	3.57*10-2	-1.43	3.13*10-2	-1.36	2.30*10-2	-1.40
EPHB3	EPHB3-201	3.59*10-2	1.78	4.47*10-2	1.77	2.82*10-2	1.78
SLC44A1	SLC44A1-205	3.59*10-2	2.28	8.58*10-3	3.31	1.13*10-2	2.70
SYK	SYK-203	3.60*10-2	1.28	2.74*10-2	1.30	2.15*10-2	1.29
HIF1A	HIF1A-208	3.66*10-2	-1.21	4.31*10-2	-1.22	2.79*10-2	-1.22
NIPSNAP2	NIPSNAP2-211	3.71*10-2	1.45	3.73*10-2	1.50	2.59*10-2	1.47
NAGK	NAGK-201	3.76*10-2	-1.22	2.81*10-2	-1.26	2.20*10-2	-1.24
HELLPAR	HELLPAR-201	3.79*10-2	-1.39	2.57*10-2	-1.40	2.13*10-2	-1.40
NOP14-AS1	NOP14-AS1-205	3.81*10-2	-1.21	5.51*10-3	-1.35	9.44*10-3	-1.28
SULT1A3	SULT1A3-209	3.84*10-2	1.53	2.36*10-2	1.58	2.05*10-2	1.55
TMED3	TMED3-202	3.90*10-2	-1.27	9.84*10-3	-1.33	1.27*10-2	-1.30
HCST	HCST-202	4.00*10-2	1.60	3.59*10-2	1.73	2.65*10-2	1.67
ARHGEF1	ARHGEF1-220	4.08*10-2	1.33	3.56*10-2	1.28	2.60*10-2	1.30
ST3GAL1	ST3GAL1-207	4.09*10-2	-1.24	1.77*10-2	-1.27	1.78*10-2	-1.25
MGAT4B	MGAT4B-202	4.10*10-2	1.37	9.27*10-3	1.58	1.30*10-2	1.47
USP7	USP7-201	4.19*10-2	-1.30	4.21*10-2	-1.29	2.97*10-2	-1.29
DERL1	DERL1-206	4.25*10-2	-1.27	3.15*10-3	-1.44	7.58*10-3	-1.36
PLOD1	PLOD1-201	4.28*10-2	1.24	3.13*10-2	1.27	2.54*10-2	1.25
BRAF	BRAF-212	4.34*10-2	-1.31	2.78*10-2	-1.33	2.32*10-2	-1.32
PPP1R37	PPP1R37-202	4.37*10-2	-1.52	3.59*10-3	-1.80	8.24*10-3	-1.66
MED13L	MED13L-213	4.43*10-2	-1.49	4.74*10-2	-1.51	3.23*10-2	-1.50
ZNF680	ZNF680-201	4.48*10-2	-1.47	2.19*10-3	-1.75	6.39*10-3	-1.61
GAPVD1	GAPVD1-203	4.49*10-2	2.09	4.12*10-3	2.23	8.80*10-3	2.16
BAHD1	BAHD1-201	4.62*10-2	1.58	4.63*10-2	1.51	3.27*10-2	1.54
ACIN1	ACIN1-218	4.64*10-2	1.50	8.44*10-3	1.64	1.32*10-2	1.57
GCH1	GCH1-204	4.67*10-2	-1.63	1.58*10-2	-1.69	1.80*10-2	-1.66
NSUN2	NSUN2-205	4.71*10-2	-1.61	2.83*10-2	-1.56	2.55*10-2	-1.59

TNFAIP3	TNFAIP3-208	4.72*10-2	-1.69	1.85*10-2	-1.42	1.98*10-2	-1.56
ATP1A1-AS1	ATP1A1-AS1-204	4.79*10-2	-1.69	4.42*10-2	-1.56	3.14*10-2	-1.63
ANPEP	ANPEP-201	4.81*10-2	1.34	2.96*10-2	1.38	2.60*10-2	1.36
BORCS6	BORCS6-201	4.86*10-2	1.24	3.00*10-2	1.28	2.64*10-2	1.26
RNF167	RNF167-211	4.88*10-2	-1.47	4.93*10-2	-1.52	3.51*10-2	-1.50
TBC1D2B	TBC1D2B-201	4.96*10-2	1.30	2.63*10-3	1.46	7.55*10-3	1.38

Note 1: The linear model contained the variables "Large vessel stroke (LVS)", "statin use" and the interaction term "LVS*statin use".

Note 2: Genes were included in the list if they met the criteria for at least one of the comparisons: $|FC| > 1.2$ and $p<0.05$.

Table VII: List of 156 transcripts differentiating patients with severe stenosis from those with mild or moderate stenosis in the verification cohort

Gene symbol	Transcript ID	Severe stenosis versus (no/mild stenosis)		Severe stenosis versus moderate stenosis		Severe/moderate stenosis versus no/mild stenosis	
		p-value	FC	p-value	FC	p-value	FC
ZNF76	ZNF76-209	2.09E-38	-2.01	4.37E-35	-1.78	3.84E-34	-1.45
CYLD	CYLD-205	1.70E-30	-2.05	1.13E-27	-1.93	3.44E-26	-1.40
TNRC6C-AS1	TNRC6C-AS1-202	1.79E-29	-3.01	1.60E-27	-3.14	2.12E-24	-1.45
CD55	CD55-208	5.75E-29	-1.45	1.24E-26	-1.39	2.61E-24	-1.21
EXOC5	EXOC5-201	8.20E-23	-2.52	9.65E-21	-2.58	1.52E-18	-1.41
NAGK	NAGK-201	4.48E-19	-3.05	4.81E-17	-2.73	2.56E-15	-1.64
PISD	PISD-211	1.65E-18	-3.49	1.18E-16	-3.55	1.10E-14	-1.53
ZBTB44	ZBTB44-208	1.75E-17	-3.93	1.01E-13	-2.74	1.49E-15	-2.10
SLC24A1	SLC24A1-205	1.87E-16	-2.19	9.72E-14	-2.13	8.54E-14	-1.39
WDR27	WDR27-219	1.49E-13	-1.97	7.08E-11	-2.19	1.74E-11	-1.24
BRAF	BRAF-220	9.27E-13	-1.61	2.71E-10	-1.49	1.07E-10	-1.29
DGAT1	DGAT1-203	4.82E-12	-1.84	1.12E-10	-1.74	3.54*10-9	-1.35
MTERF1	MTERF1-201	5.23E-12	-3.05	1.55*10-9	-1.63	3.84E-10	-2.32
ZNF654	ZNF654-201	5.76E-12	-3.31	1.17*10-9	-3.85	5.78E-10	-1.37
EML4	EML4-204	1.04E-11	-2.18	1.03E-10	-2.32	1.33*10-8	-1.32
MIER3	MIER3-204	5.42E-11	-2.20	3.24*10-9	-1.81	8.84*10-9	-1.56
AC087623.2	AC087623.2-201	6.42E-11	-1.94	1.68*10-8	-1.57	2.65*10-9	-1.51
RBM19	RBM19-203	6.62E-11	-1.86	2.01*10-7	-1.54	2.91E-10	-1.47
ZCCHC8	ZCCHC8-203	1.11E-10	-2.19	1.00E-10	-2.63	5.91*10-7	-1.20
GINM1	GINM1-201	2.55E-10	-2.04	1.05*10-8	-1.90	3.74*10-8	-1.41
ANKRD44	ANKRD44-202	3.06E-10	-2.14	1.39*10-7	-1.88	4.84*10-9	-1.49
NRDC	NRDC-202	3.50E-10	-2.52	7.56*10-9	-2.22	8.27*10-8	-1.57
NR1H2	NR1H2-204	5.31E-10	-2.22	1.34*10-9	-2.54	7.24*10-7	-1.25
LRRC41	LRRC41-203	9.13E-10	-1.65	2.83*10-8	-1.68	1.17*10-7	-1.23
TTLL5	TTLL5-202	1.34*10-9	-1.88	4.20*10-9	-1.86	1.14*10-6	-1.32

DDHD2	DDHD2-201	4.90*10-9	-1.99	1.17*10-6	-1.46	5.89*10-8	-1.61
ZNF696	ZNF696-201	4.93*10-9	-2.84	2.81*10-7	-2.44	2.17*10-7	-1.65
SDF2L1	SDF2L1-201	5.22*10-9	-2.38	1.99*10-8	-2.80	2.43*10-6	-1.25
WASH6P	WASH6P-204	5.54*10-9	-2.23	9.63*10-8	-2.08	6.77*10-7	-1.45
DYNC1H1	DYNC1H1-220	7.20*10-9	-2.86	8.77*10-6	-2.18	1.57*10-8	-1.80
TATDN2P2	TATDN2P2-201	2.26*10-8	-2.75	3.98*10-6	-2.28	2.15*10-7	-1.68
TMED3	TMED3-202	2.45*10-8	-2.49	1.32*10-5	-1.73	7.91*10-8	-1.83
ZNF747	ZNF747-202	3.06*10-8	-1.91	1.79*10-6	-1.74	7.26*10-7	-1.39
ORMDL1	ORMDL1-207	4.61*10-8	-2.80	6.83*10-5	-2.08	4.34*10-8	-1.82
FBXO48	FBXO48-201	1.44*10-7	-2.54	5.47*10-6	-2.23	2.88*10-6	-1.57
SFI1	SFI1-214	2.31*10-7	-2.09	9.85*10-7	-2.34	2.74*10-5	-1.25
P4HA1	P4HA1-203	2.35*10-7	-2.92	1.11*10-6	-2.82	2.54*10-5	-1.53
TJP2	TJP2-216	3.23*10-7	-2.49	1.18*10-3	-1.57	5.02*10-8	-1.94
NIPBL	NIPBL-208	3.57*10-7	-2.20	1.37*10-5	-2.00	5.28*10-6	-1.46
SUPT5H	SUPT5H-203	4.57*10-7	-1.53	1.55*10-5	-1.34	6.95*10-6	-1.31
MAP4K4	MAP4K4-220	5.13*10-7	-1.99	3.83*10-5	-2.05	3.53*10-6	-1.30
AL050343.2	AL050343.2-203	5.63*10-7	-1.76	1.15*10-5	-1.67	1.25*10-5	-1.32
CRYBG1	CRYBG1-204	6.31*10-7	-2.23	5.65*10-6	-2.00	2.79*10-5	-1.49
CREB3	CREB3-202	7.47*10-7	-2.74	1.14*10-4	-2.20	2.14*10-6	-1.71
NOP14	NOP14-203	7.60*10-7	-2.04	3.19*10-4	-1.62	8.29*10-7	-1.56
PLAGL1	PLAGL1-202	9.05*10-7	-1.62	2.04*10-5	-1.32	1.55*10-5	-1.40
CC2D1B	CC2D1B-201	9.63*10-7	-3.53	2.64*10-4	-2.38	9.50*10-7	-2.09
CBLB	CBLB-208	9.81*10-7	-2.38	7.61*10-5	-2.28	5.18*10-6	-1.45
AC097658.2	AC097658.2-201	1.09*10-6	-1.83	1.47*10-5	-1.64	2.76*10-5	-1.39
POM121C	POM121C-207	1.10*10-6	-1.55	5.48*10-5	-1.40	8.51*10-6	-1.29
DHRS7B	DHRS7B-207	1.83*10-6	-2.12	5.95*10-6	-2.31	1.21*10-4	-1.28
AC011899.2	AC011899.2-201	1.93*10-6	-1.58	2.25*10-4	-1.39	5.26*10-6	-1.32
EHMT2	EHMT2-202	4.19*10-6	-7.44	4.97*10-5	-1.09*10+1	6.28*10-5	-1.25
NOP14-AS1	NOP14-AS1-205	4.28*10-6	-1.59	4.95*10-5	-1.62	7.52*10-5	-1.21

ARAP3	ARAP3-202	4.39*10-6	-1.44	1.02*10-6	-3.26	1.74*10-3	1.48
STK10	STK10-213	4.84*10-6	-1.70	2.68*10-5	-1.75	1.53*10-4	-1.23
CDAN1	CDAN1-201	5.44*10-6	-1.83	3.82*10-5	-1.70	1.34*10-4	-1.36
ASPSCR1	ASPSCR1-202	5.97*10-6	-1.62	8.01*10-5	-1.65	8.09*10-5	-1.22
PSMA4	PSMA4-218	6.79*10-6	-2.17	9.40*10-3	-1.56	6.59*10-7	-1.69
CLCN7	CLCN7-209	8.90*10-6	-9.74	7.10*10-5	-7.56	5.06*10-5	-2.27
BRPF3	BRPF3-202	9.50*10-6	-1.72	7.89*10-5	-1.77	1.64*10-4	-1.24
HELLPAR	HELLPAR-201	1.03*10-5	-3.00	2.39*10-5	-2.91	4.70*10-4	-1.53
HMGN2	HMGN2-210	1.06*10-5	-1.91	1.12*10-4	-1.88	1.42*10-4	-1.33
NASP	NASP-210	1.32*10-5	-1.78	2.27*10-4	-1.62	1.04*10-4	-1.36
DOCK2	DOCK2-202	1.51*10-5	-2.00	7.81*10-5	-2.01	3.31*10-4	-1.33
DVL1	DVL1-202	1.69*10-5	-1.82	3.20*10-3	-1.56	1.07*10-5	-1.42
GATAD2A	GATAD2A-203	1.91*10-5	-2.39	3.61*10-5	-2.65	7.20*10-4	-1.31
TTC17	TTC17-207	2.34*10-5	-1.98	4.30*10-3	-1.46	1.28*10-5	-1.61
AC004492.1	AC004492.1-201	2.71*10-5	-1.79	6.86*10-4	-1.53	1.11*10-4	-1.41
SIAE	SIAE-205	2.81*10-5	-2.13	2.47*10-3	-1.78	9.10*10-5	-1.53
RNASEK	RNASEK-202	3.62*10-5	-2.10	6.69*10-5	-2.15	1.18*10-3	-1.33
HSPBP1	HSPBP1-202	3.65*10-5	-2.08	3.93*10-3	-1.88	2.97*10-5	-1.45
AC114490.3	AC114490.3-201	3.98*10-5	-2.13	9.01*10-4	-1.89	9.29*10-5	-1.47
PMS2CL	PMS2CL-204	4.54*10-5	-1.97	1.26*10-2	-1.34	1.07*10-5	-1.68
TBCE	TBCE-281	6.20*10-5	-1.91	4.90*10-3	-1.38	1.64*10-4	-1.60
TALDO1	TALDO1-209	6.42*10-5	-1.78	1.39*10-3	-1.59	2.06*10-4	-1.37
ANKHD1	ANKHD1-207	6.70*10-5	-1.82	2.79*10-4	-1.75	9.90*10-4	-1.33
STAMBP	STAMBP-202	7.08*10-5	-2.33	2.45*10-3	-2.49	1.41*10-4	-1.33
LRRC45	LRRC45-201	7.52*10-5	-2.15	4.18*10-3	-1.85	6.36*10-5	-1.51
NSUN6	NSUN6-201	7.55*10-5	-1.99	1.38*10-4	-2.24	1.81*10-3	-1.23
SVIL	SVIL-215	9.07*10-5	-2.33	6.67*10-3	-2.51	7.26*10-5	-1.33
RRP15	RRP15-201	9.50*10-5	-2.23	9.20*10-4	-2.20	3.58*10-4	-1.39
GUSBP11	GUSBP11-206	9.94*10-5	-2.37	1.72*10-3	-2.29	1.11*10-3	-1.44
EIF2D	EIF2D-210	1.02*10-4	-1.83	8.05*10-4	-1.81	7.24*10-4	-1.30

GDI2	GDI2-207	1.19*10-4	-1.83	4.29*10-2	-1.21	1.01*10-5	-1.65
TCF7L2	TCF7L2-216	1.27*10-4	-1.44	2.15*10-4	-1.25	2.95*10-3	-1.28
INSL6	INSL6-203	1.36*10-4	-2.03	4.69*10-3	-1.65	1.90*10-4	-1.53
LARS1	LARS1-205	1.72*10-4	-2.01	2.79*10-3	-1.80	4.93*10-4	-1.43
ACSS1	ACSS1-204	1.73*10-4	-2.23	4.02*10-3	-1.91	1.02*10-3	-1.53
ARAP3	ARAP3-204	1.78*10-4	-1.28	1.20*10-4	-2.12	7.51*10-3	1.22
ZC3HAV1	ZC3HAV1-204	2.14*10-4	-1.71	8.90*10-3	-1.59	1.97*10-4	-1.32
TYSND1	TYSND1-201	2.37*10-4	-2.51	6.74*10-3	-2.31	3.20*10-4	-1.52
PTBP3	PTBP3-203	2.63*10-4	-3.03	4.48*10-4	-3.60	3.70*10-3	-1.32
NDUFB10	NDUFB10-202	3.16*10-4	-2.32	1.98*10-3	-2.31	4.39*10-3	-1.40
UBE3A	UBE3A-227	4.50*10-4	-2.41	8.78*10-3	-2.19	5.53*10-4	-1.51
PARL	PARL-202	4.86*10-4	-2.24	1.92*10-3	-2.11	2.53*10-3	-1.44
CYB561D1	CYB561D1-201	5.04*10-4	-2.68	6.90*10-4	-2.79	8.03*10-3	-1.41
DMAC2	DMAC2-207	5.96*10-4	-1.69	1.74*10-3	-1.66	1.15*10-2	-1.27
MTND2P28	MTND2P28-201	6.84*10-4	3.73	2.92*10-3	3.84	7.86*10-3	2.35
SETD5	SETD5-214	6.95*10-4	-3.03	3.63*10-3	-2.78	2.37*10-3	-1.60
GSK3A	GSK3A-202	7.07*10-4	-2.26	1.25*10-3	-2.47	1.78*10-2	-1.30
RPL32P3	RPL32P3-206	7.09*10-4	-1.64	5.45*10-3	-1.27	5.24*10-3	-1.45
FMR1	FMR1-217	7.20*10-4	-2.38	4.70*10-4	-2.53	1.48*10-2	-1.35
ZNF708	ZNF708-202	7.20*10-4	-2.25	1.19*10-2	-2.11	2.69*10-3	-1.45
ANKRD50	ANKRD50-202	8.04*10-4	-1.95	1.10*10-2	-1.80	3.25*10-3	-1.40
SAP18	SAP18-207	9.48*10-4	-5.76	5.75*10-3	-5.51	3.58*10-3	-1.77
GVINP1	GVINP1-202	1.17*10-3	-2.33	1.85*10-3	-2.17	7.86*10-3	-1.47
RPN2	RPN2-202	1.24*10-3	-1.83	3.05*10-3	-1.50	6.67*10-3	-1.46
UNC13D	UNC13D-210	1.31*10-3	-2.32	1.14*10-3	-2.54	1.86*10-2	-1.31
AL603756.1	AL603756.1-201	1.33*10-3	-1.82	3.87*10-3	-1.97	6.70*10-3	-1.22
SLC39A7	SLC39A7-202	1.43*10-3	-2.14	8.56*10-3	-2.03	9.54*10-3	-1.41
PCID2	PCID2-215	1.48*10-3	-1.51	3.62*10-2	-1.26	9.03*10-4	-1.33
BTN3A2	BTN3A2-201	1.48*10-3	-2.86	1.68*10-2	-1.92	3.91*10-3	-1.95
ATP2B1	ATP2B1-210	1.52*10-3	-2.28	2.81*10-2	-2.05	2.81*10-3	-1.50

PPP1CB	PPP1CB-202	1.57*10-3	-2.13	2.16*10-3	-2.39	1.51*10-2	-1.26
NR2C2	NR2C2-210	1.66*10-3	-1.64	2.10*10-2	-1.42	1.46*10-3	-1.35
ZNF160	ZNF160-214	1.95*10-3	-2.54	1.35*10-3	-2.51	2.78*10-2	-1.45
EIF4G3	EIF4G3-201	1.98*10-3	-2.12	5.81*10-3	-2.03	7.46*10-3	-1.40
MLX	MLX-206	2.07*10-3	-2.25	3.47*10-3	-1.80	1.46*10-2	-1.61
ZZEF1	ZZEF1-210	2.18*10-3	-1.95	1.78*10-2	-1.84	7.61*10-3	-1.37
KHSRP	KHSRP-213	2.18*10-3	-3.22	1.51*10-2	-2.02	4.23*10-3	-2.13
RHBDD2	RHBDD2-202	2.28*10-3	-2.45	4.75*10-3	-2.81	9.73*10-3	-1.29
HP1BP3	HP1BP3-203	2.57*10-3	-1.89	1.11*10-2	-1.79	3.98*10-3	-1.35
TMEM94	TMEM94-201	2.59*10-3	-3.39	7.41*10-3	-2.81	6.83*10-3	-1.77
TNK2	TNK2-216	2.68*10-3	2.19	3.30*10-2	2.04	6.46*10-3	1.64
CXorf38	CXorf38-203	3.07*10-3	-1.72	9.97*10-3	-1.71	1.96*10-2	-1.27
MMS19	MMS19-218	3.34*10-3	-2.81	5.58*10-3	-2.84	1.96*10-2	-1.46
PLCL1	PLCL1-201	3.36*10-3	-1.76	1.91*10-2	-1.80	1.38*10-2	-1.26
DNHD1	DNHD1-214	3.56*10-3	-1.91	1.20*10-2	-1.63	8.48*10-3	-1.45
ANAPC5	ANAPC5-218	3.59*10-3	-2.55	1.81*10-2	-2.09	5.49*10-3	-1.65
NRIP1	NRIP1-202	3.62*10-3	-6.33	2.00*10-2	-6.47	1.50*10-2	-1.69
CLIP1	CLIP1-217	3.65*10-3	-2.49	3.27*10-2	-1.77	2.95*10-3	-1.79
RUBCN	RUBCN-202	3.67*10-3	-1.82	1.65*10-2	-1.68	1.08*10-2	-1.36
KDM6A	KDM6A-211	3.96*10-3	-5.76	2.63*10-2	-5.07	1.41*10-2	-1.90
SPATA5	SPATA5-202	3.98*10-3	-3.03	3.12*10-3	-3.93	3.49*10-2	-1.22
EHMT2	EHMT2-203	4.34*10-3	-1.54	5.16*10-3	-1.34	2.84*10-2	-1.32
EXTL3	EXTL3-214	4.67*10-3	-1.89	2.37*10-2	-2.06	1.94*10-2	-1.24
KIAA0753	KIAA0753-201	4.78*10-3	-1.82	3.11*10-2	-1.56	3.75*10-3	-1.42
POLD4	POLD4-209	5.09*10-3	-1.81	2.54*10-2	-1.31	1.02*10-2	-1.57
DDX3X	DDX3X-244	5.18*10-3	-2.83	3.42*10-3	-2.82	3.65*10-2	-1.48
RNPS1	RNPS1-221	5.18*10-3	-1.89	3.77*10-2	-1.81	1.37*10-2	-1.34
NBPF11	NBPF11-203	5.50*10-3	-3.35	1.06*10-2	-2.54	1.36*10-2	-1.88
ERAP2	ERAP2-202	6.08*10-3	-2.74	1.39*10-2	-2.58	4.10*10-2	-1.53
MCM7	MCM7-201	6.13*10-3	-2.27	1.97*10-2	-2.50	1.52*10-2	-1.30

AC018628.2	AC018628.2-201	6.35*10-3	-1.51	1.25*10-2	-1.48	2.33*10-2	-1.22
USP3	USP3-204	6.60*10-3	-1.58	4.58*10-2	-1.22	6.51*10-3	-1.42
PICALM	PICALM-206	7.18*10-3	-1.72	4.10*10-2	-1.59	2.06*10-2	-1.33
RAB33B	RAB33B-201	8.51*10-3	-1.99	4.99*10-2	-1.86	9.04*10-3	-1.39
MYO1G	MYO1G-205	9.60*10-3	-1.59	2.87*10-2	-1.32	1.95*10-2	-1.36
ZFAT	ZFAT-201	9.68*10-3	-1.91	2.40*10-2	-1.60	2.73*10-2	-1.46
TTPAL	TTPAL-202	1.01*10-2	-4.10	4.86*10-2	-3.86	2.55*10-2	-1.69
TNFAIP3	TNFAIP3-208	1.14*10-2	-1.69	3.37*10-2	-1.43	1.77*10-2	-1.38
FBXL5	FBXL5-211	1.31*10-2	1.61	4.11*10-2	1.55	4.38*10-2	1.33
GIGYF2	GIGYF2-201	1.36*10-2	-1.64	2.35*10-2	-1.61	4.61*10-2	-1.26
RNPS1	RNPS1-220	1.62*10-2	-1.90	4.31*10-2	-1.62	2.46*10-2	-1.44
C5orf58	C5orf58-205	1.83*10-2	-1.61	3.33*10-2	-1.56	3.53*10-2	-1.26

Note 1: The linear model contained the variables "grade of stenosis", "statin use" and the interaction term "grade of stenosis*statin use".

Note 2: Genes were included in the list if they met the criteria for at least one of the comparisons: $|FC| > 1.2$ and $p<0.05$.

Table VIII: List of 19 transcripts common to Table VI and Table VII

Gene symbol	Transcript ID
CC2D1B	CC2D1B-201
CD55	CD55-208
GATAD2A	GATAD2A-203
HELLPAR	HELLPAR-201
KIAA0753	KIAA0753-201
LRRC41	LRRC41-203
MLX	MLX-206
MTERF1	MTERF1-201
NAGK	NAGK-201
NOP14-AS1	NOP14-AS1-205
NSUN6	NSUN6-201
PPP1CB	PPP1CB-202
PTBP3	PTBP3-203
RHBDD2	RHBDD2-202
STK10	STK10-213
TMED3	TMED3-202
TNFAIP3	TNFAIP3-208
TYSND1	TYSND1-201
ZNF696	ZNF696-201