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Improved risk prediction of all-cause dementia, Alzheimer's disease, and vascular dementia by biomarkers of oxidative stress and inflammation

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Contents

List of figures

List of tables

List of abbreviations

8-iso-$PGF_{2\alpha}$	8-iso-prostaglandin $F_{2\alpha}$
α1-AT	A1-antichymotrypsin
AD	Alzheimer's disease
ANU-ADRI	Australian National University Alzheimer's Disease Risk Index
APOE	Apolipoprotein E
AUC	Area under the curve
Aβ	Amyloid beta
Aβ-	Amyloid beta negative
Aβ+	Amyloid beta positive
BDSI	Brief Dementia Screening Indicator
BMI	Body mass index
CAD	Coronary artery disease
CAIDE	Cardiovascular Risk Factors, Aging and Dementia
Casp-8	Caspase 8
CD244	Natural killer cell receptor 2B4
CHD	Coronary heart disease
CI	Confidence interval
CNS	Central nervous system
CO	Carbon monoxide
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CVD	Cardiovascular disease
CX3CL1	Fractalkine
CX3CR1	C-X3-C motif chemokine receptor 1
CXCL5	C-X-C motif chemokine 5
DRS	Dementia Risk Score
e.g.	Exempli gratia
ELISA kits	Enzyme-linked immunosorbent assay kits
EN-RAGE	S100-A12 protein
ESTHER study	Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung [German]
FDA	Food and Drug Administration
FDR	False discovery rate
FINDRISC	Finnish Diabetes Risk Score
FRS	Framingham cardiovascular Risk Score
GC	Gas-chromatography
GCV	Geometric coefficients of variation
GM	Geometric mean
GPs	General practitioners
GSD	Geometric standard deviation
HGF	Hepatocyte growth factor
HIC	High-income countries
HIF-1α-LCN2-VEGFA	hypoxia-inducible factor 1α-Lipocalin2-VEGFA
HPLC	High-performance liquid-chromatography
HR	Hazard ratio

i.e.	Id est
IL-10RB	Interleukin 10 Receptor Subunit Beta
IL-1β	Interleukin-1β
IL-6	Interleukin-6
IP-10	Interferon-γ-inducible protein 10
IQR	Interquartile range
LAP	Latency-associated peptide
LASSO	Least Absolute Shrinkage and Selection Operator
LC-MS/MS	Liquid chromatography tandem mass-spectrometry
LMIC	Low-income and middle-income countries
LOD	Limit of detection
mCAIDE	Modified CAIDE score
MCI	Mild cognitive impairment
MCI-	Cognitively normal individuals
MCI+	Patients with mild cognitive impairment
MCMC method	Markov chain Monte Carlo method
MCP-1	Monocyte protein-1
MOOSE	Meta-analysis of obsrvational studies in Epidemiology
mPPT	Mini Physical Performance Testing
MRI	Magnetic resonance imaging
n.a.	Not available
n.s.	Not significant
NFT	Neurofibrillary tangles
NO_2	Nitrogen dioxide
NOS	Newcastle-Ottawa scale
NPX	Normalized Protein eXpression
OND	Other neurological diseases
OR	Odds ratio
OS	Oxidative stress
PCA	Principal Component Analysis
PCR	Polymerase chain reaction
PDD	Parkinson's disease dementia
PEA	Proximity Extension Assay technology
PGHSs	Prostaglandin-endoperoxide synthases
PM2.5	Particulate matter
PS-1	Presenilin 1
qPCR	Quantitative polymerase chain reaction
Redox	Reduction-oxidation
ROS	Reactive oxygen species
SAS	Statistical Analysis System
SD	Standard deviation
SE	Standard error
SIRT2	SIR2-like protein 2
SMD	Standardised mean difference
SNP	TaqMan single-nucleotide polymorphism
TGF-β1	Transforming growth factor β-1
TNFα	Tumor necrosis factor α
TRAIL	Tumor Necrosis Factor Related Apoptosis Inducing Ligand

TβR1	Transforming growth factor-ß receptor type I
uPA	Urokinase-type plasminogen activator
VD	Vascular dementia
VEGF	Vascular endothelial growth factor
VEGF-A	Vascular endothelial growth factor A
VEGFR-1	Vascular endothelial growth factor receptor 1
Vs	versus
WHO	World Health Organization

1. Introduction

Dementia is a multifaceted syndrome mainly characterized by a decline in cognitive function and affecting an individual's daily living (World Health Organization 2019). The most common cause of dementia is Alzheimer's disease (AD), accounting for 60-80% of all dementia cases, followed by vascular dementia (VD) as the second most common form of dementia, accounting for 15-20% of all cases (O'Brien and Thomas 2015; Rizzi et al. 2014). Other common forms are dementia with Lewy Bodies and frontotemporal dementia (Gale et al. 2018). However, a mixed type of dementia is also likely to occur, especially in old age (>85 years). Joint pathologies are, for example, oxidative stress (OS), neuroinflammation, hypoxia, mitochondria bioenergetics, neurodegeneration, and blood-brain barrier permeability (Raz et al. 2016).

This dissertation will focus on all-cause dementia, AD, and VD as well as the mechanisms of OS and neuroinflammation. Hallmarks of AD pathology are the deposition of amyloid β (Aβ) peptides in the form of plaques and the formation of neurofibrillary tangles (NFTs) based on hyperphosphorylated tau (Lane et al. 2018). On the other hand, VD is characterized by a deteriorated cerebrovascular blood flow leading to cognitive decline. The deteriorated blood flow can be caused by various underlying cerebrovascular pathologies like micro bleedings, microinfarcts or arteriolosclerosis (Iadecola 2013).

1.1 Epidemiology of dementia

Dementia is a major challenge for global public health and social care systems. In 2015, 9.9 million incident dementia cases were registered worldwide, equivalent to one new case every three seconds. Overall, 46.8 million people were estimated to live with dementia in 2015 worldwide. As the number of dementia cases increases with rising life expectancy, it has been estimated that this number will double every 20 years and grow to 75 million cases in 2030. (Prince et al. 2015)

The global prevalence of dementia in people older than 60 was estimated to be 5.2% in 2015 (Prince et al. 2015). However, the prevalence varies between countries, especially between high-income countries (HIC) and low-income or middle-income countries (LMIC), being higher

in the latter two. For example, the prevalence of dementia in Central Europe was estimated to be 4.65% among individuals aged 60 or older in 2015, whereas it was at 7.15% in Southeast Asia or 8.34% in Latin America. Likewise, the projected increase in dementia cases is expected to have the greatest impact on LMIC since life expectancy rises and risk factor load is higher (Livingston et al. 2020). According to estimates from the World Alzheimer's Report 2015, 63% of all dementia cases will be attributable to people living in LMIC countries by 2030 (Prince et al. 2015).

Interestingly, the prevalence of dementia rapidly increases in older age groups. For instance, the prevalence of dementia among those 65 and older is 8.6% in Germany (Deutsche Alzheimer Gesellschaft e.V. 2020). In the age group of 65 to 69 years, the prevalence is still low with 1.3%. However, it increases to 8,1% in the age group 75-79 and 21.8% in 85 to 89 year-olds. In the oldest age group, including individuals aged 90 or older, the prevalence of dementia is already at 40.9%. Moreover, the prevalence of dementia is generally higher among women than men (Erol et al. 2015).

Costs related to the disease were at US$ 818 billion in 2015 and are expected to rise to US$2 trillion by 2030. Those estimates account for direct medical costs, direct social care costs, as well as costs of informal care (unpaid). Due to higher per-person costs in HIC than LMIC, most costs (87%) are caused in HIC, although dementia prevalence is lower.

Therefore, research on preventing or delaying the onset of dementia is one of the major challenges globally (Siva 2021).

1.2 Risk factors for dementia

Several risk factors condition the development of dementia. These include non-modifiable risk factors like age, sex, genetic factors, ethnicity and family history, with age being the greatest risk factor for dementia (World Health Organization 2019). However, a large number of modifiable risk factors for dementia have been identified. In the most recent report of the Lancet Commission, 12 lifestyle-related risk factors for dementia were identified and estimated to potentially prevent or delay 40% of all dementia cases (Livingston et al. 2020). This percentage might be even higher in LMIC. Even if a diagnosis has already been made, risk factors can be addressed to slow the progression of dementia (Livingston et al. 2020).

Modifiable risk factors of dementia occur in all stages of life and could reduce the prevalence of dementia to varying degrees. Already in **early life (< 45 years)**, less education is considered a risk factor for dementia, accounting for 7% of reducible prevalent dementia cases. This is because higher education provides a higher cognitive reserve which preserves longer in old age (Livingston et al. 2020). However, also in later life, cognitive training was shown to be beneficial and reduce the risk of cognitive impairment (Krell-Roesch et al. 2019).

During **mid-life (45-65 years)**, several potentially modifiable risk factors account for a total reduction of 15% in the prevalence of dementia. Hearing loss is the largest modifiable risk factor for dementia in mid-life, accounting for a decrease of 8% if wholly eliminated (Livingston et al. 2020). Lower cognitive stimulation and less social interaction resulting from hearing loss might promote cognitive decline and development of dementia in the following. However, hearing aids are a proven means to regain social interaction and reduce the risk of developing dementia (Amieva et al. 2018). Furthermore, excessive alcohol consumption (1%) is a risk factor known for a long time and is also related to early-onset dementia. It is assumed that weekly consumption of more than 21 units of alcohol (corresponding to two bottles of wine) poses an increased risk for the disease (Koch et al. 2019; Livingston et al. 2020; Piumatti et al. 2018). Traumatic brain injuries (3%), hypertension (2%), and obesity (1%) represent additional modifiable risk factors for dementia.

Smoking is the most preventable risk factor for dementia in **late-life (>65 years)**, accounting for 5% of the potentially reducible risk (Livingston et al. 2020). A recent longitudinal study showed that even in later life, smoking cessation can still reduce the risk for developing dementia distinctly (Choi et al. 2018; Livingston et al. 2020). Depression and social isolation both account for a 4% reduction in dementia prevalence if eliminated. In both cases, reverse causation cannot be ruled out, but social contact is now considered a protective factor for dementia (Livingston et al. 2020). Air pollution, which accounts for 2% of reducible dementia risk, was recently added to the list of dementia risk factors (Livingston et al. 2020). Pollutants like nitrogen dioxide (NO_2), carbon monoxide (CO) and fine ambient particulate matter (PM2.5) originating, for example, from traffic were shown to be associated with dementia (Delgado-Saborit et al. 2021; Peters et al. 2019). However, the underlying mechanisms are not fully clear yet. Finally, physical inactivity and diabetes are among the modifiable risk factors for dementia in later life, accounting for 2% and 1% of reducible dementia prevalence if eliminated (Livingston et al. 2020).

1.3 Oxidative stress and inflammation as key mechanisms in dementia pathogenesis

Apart from the discussed risk factors, OS and inflammation are well known to be key mechanisms involved in dementia pathogenesis (Raz et al. 2016).

OS is a common component of many diseases and is mainly driven by reactive oxygen species (ROS). ROS are created during reduction-oxidation (redox) reactions and are a permanent feature of metabolic processes like apoptosis, immune responses, protein folding and cell differentiation (Pizzino et al. 2017; Sies and Jones 2020). But also exogeneous factors like nutrition, exercise, drugs, air pollution or UV light can lead to redox reactions and ROS production (Sies et al. 2017). In the cell, levels of ROS are highly regulated and kept at a minimum (Pizzino et al. 2017). However, if ROS are excessively produced, oxidant production exceeds antioxidant defenses, leading to a disruption of redox signalling and controls. This condition is defined as OS in which the excessively produced ROS damage proteins, lipids and nucleic acids (Sies et al. 2017).

Due to high oxygen consumption, high energy production, and an impaired antioxidant defense mechanism, neurons are particularly prone to ROS (Wojsiat et al. 2018). Consequently, ROS induced damage of proteins and lipids in the brain cause neurodegeneration and cell death (Luca et al. 2015; Mao 2013; Wojsiat et al. 2018). Growing evidence has already shown that OS plays an important role in the development of dementia, especially AD and VD (Wojsiat et al. 2018). However, it has been observed that OS is not only associated with dementia but also with its risk factors, including hypertension, diabetes, hypercholesterolemia, obesity, depression, smoking, and low physical activity (Burke and FitzGerald 2003; Dias et al. 2014; Fernandez-Sanchez et al. 2011; Laufs et al. 2005; Lindqvist et al. 2017; Maritim et al. 2003; Montezano et al. 2015; Sies et al. 2017). Therefore, OS could be a mediator between risk factors of dementia and the disease itself.

Inflammation is an established defense mechanism of the body towards infection, toxins, or injury and a common feature of aging (Kinney et al. 2018). The latter condition is often described as “inflammaging” and constitutes chronic low-grade inflammation in older adults arising from cellular debris, cellular senescence and immunosenescence (Franceschi and

Campisi 2014; Walker 2018). This process poses a risk of morbidity and mortality among older adults (Franceschi and Campisi 2014).

However, several types of inflammation are defined in the literature, contributing directly or indirectly to dementia pathogenesis. Systemic inflammation, for example, is defined to occur outside the central nervous system (CNS), whereas neuroinflammation is defined as inflammatory processes within the CNS (Calsolaro and Edison 2016; Walker et al. 2019a). Persistent systemic inflammation causes the release of pro- and anti-inflammatory products, which can cross the blood-brain barrier and lead to neuroinflammation. In neuroinflammation, microglia and astrocytes are activated and transition into their reactive phenotypes M1 and A1, respectively. Subsequently, various pro-inflammatory products, including several cytokines, ROS or nitric oxides, are released. First, this is beneficial, e.g. for Aβ clearance (Lim et al. 2015; Walker et al. 2019a). However, if inflammation becomes chronic, the released pro-inflammatory products can also cause neurodegeneration. This cascade might promote the progression of cognitive decline and lead to dementia development in later life (Kinney et al. 2018; Lim et al. 2015; Walker 2018; Walker et al. 2019a).

1.4 Biomarkers of oxidative stress and inflammation

The definition of “biomarker” is as follows: “A defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or biological responses to an exposure or intervention, including therapeutic interventions.” (FDA NIH Biomarker Working Group 2016). In dementia research, biomarkers have the potential to be used for early diagnosis, as they can be measured already at an early stage of the disease (Calsolaro and Edison 2016; McGeer et al. 2016). Furthermore, identified new biomarkers could deepen our understanding of the pathogenetic processes leading to dementia and might represent novel drug targets (Khoury and Ghossoub 2019; Shen et al. 2018). The current challenge of biomarker research in dementia in general and distinct forms is to find reliable diagnostic and predictive biomarkers easily accessible in fluids like blood (Simrén et al. 2020; Zetterberg and Burnham 2019).

There are several biomarkers commonly used to assess OS and inflammation levels. F_2-isoprostanes, for example, are an established family of OS biomarkers discovered over 30 years ago (Milne et al. 2015). They are the gold standard for OS biomarkers (Ahmed et al. 2020). Also, the European Food Safety Authority (EFSA) recommended using F_2-isoprostanes as biomarkers for oxidative damage to lipids if measured with chromatographic techniques coupled with mass spectrometry (Efsa Panel on Dietetic Products et al. 2018). F_2-isoprostanes have preferable properties such as stability in biological fluids like blood, urine, and cerebrospinal fluid (CSF) (Czerska et al. 2016; Milne et al. 2005). They are produced in membranes by free ROS induced lipid peroxidation of polyunsaturated fatty acids like arachidonic acid. Depending on the degree of chemical reduction, different families of isoprostanes can be formed. F_2-isoprostanes, for example, originate from the pathway of complete chemical reduction and encompass 64 stereoisomers. Of those, 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) is the most abundantly produced F_2-isoprostane, followed by 5-F_{2t}-Isoprostane. (Ahmed et al. 2020; Menzel et al. 2021)

The formation of isoprostanes is a constant feature of various diseases. Levels of 8-iso-$PGF_{2\alpha}$ and other F_2-isoprostanes have previously been shown to be increased in plasma and/or urine samples of patients with diabetes, obesity, hypercholesterolemia, asthma, cardiovascular disease, stroke, or cancer (Gao et al. 2018; Kaufman et al. 2007; Lin et al. 2015; Samitas et al. 2009; Wang et al. 2006; Zhang 2013). Moreover, F_2-isoprostanes were often associated with neurodegenerative diseases like AD, multiple sclerosis, Huntington's disease and Creutzfeldt-Jakob disease (Miller et al. 2014). However, contradictory results on the association between F_2-isoprostanes or 8-iso-$PGF_{2\alpha}$ and AD have been published, and most studies utilized a cross-sectional study design in which reverse causation cannot be excluded (Trares et al. 2020). Thus, the predictive value of 8-iso-$PGF_{2\alpha}$ levels remains unclear in the context of dementia.

The multifactorial process of inflammation is accompanied by the release of various pro- and anti-inflammatory mediators like cytokines or chemokines and the subsequent activation of particular signalling cascades (Liu et al. 2017; Walker et al. 2019a). Especially when chronic inflammation becomes apparent, these inflammatory mediators can be measured and related to diseases (Liu et al. 2017). Several studies have found increased levels of pro-inflammatory cytokines and proteins like interleukin-6 (IL-6), interleukin-1β (IL-1β), C-reactive protein (CRP), or α1-antichymotrypsin (α1-AT) to be associated with the onset of all-cause dementia (Darweesh et al. 2018; Lai et al. 2017). Further studies also revealed that CRP, IL-1ß, IL-2, IL-4,

IL-6, IL-8, IL-10, IL-12, IL-18, monocyte protein-1 (MCP-1), MCP-3, interferon-γ-inducible protein 10 (IP-10), and tumour necrosis factor α (TNF-α) are associated with the incidence of AD (Park et al. 2020; Su et al. 2019). However, longitudinal studies on the association between biomarkers of the inflammation-related proteome and dementia are scarce (Walker 2018).

1.5 Dementia risk prediction

With the accelerated approval of *Aduhelm* as the first effective treatment against AD by the U.S. Food and Drug Administration (FDA), there is hope that the early stages of AD might be reversible. Although the efficacy, safety, and clinical application of the drug are still controversially discussed (Alexander et al. 2021; Mahase 2021), it can be considered a first step towards an effective dementia treatment. *Aduhelm* and future, improved drugs are likely to be most effective in early AD treatment. Thus, it is vital to perform dementia risk assessments and make diagnoses early (Goerdten et al. 2019; Hou et al. 2019). By this, intervention in dementia therapy, risk factor management or disease monitoring can be provided to prevent or delay the onset of dementia (Hou et al. 2019).

The scientific literature on dementia risk prediction increased rapidly since new risk factors and biomarkers were identified during the last years. However, sample sizes and follow-up durations varied extremely, and external validation is often lacking (Hou et al. 2019). Also, the underlying study populations are highly different. Risk prediction models combining demographic, cognition, physical and health risk factors are often best suited and versatile (Stephan et al. 2010; Tang et al. 2015). The Cardiovascular Risk Factors, Aging and Dementia (CAIDE) model, which is based on data from a Finnish, population-based study, is such a risk model (Kivipelto et al. 2006). Including several risk factors of dementia, the authors could predict the risk of developing dementia with an area under the curve (AUC) of 0.769 (95% confidence interval (CI): 0.709-0.829). A second model containing additionally Apolipoprotein E (*APOE*) ε4 performed slightly better (AUC [95% CI]: 0.776 [0.717 – 0.836]). The CAIDE model was internally and externally validated in many cohorts, including high-income countries and various ethnicities (Exalto et al. 2014; Fayosse et al. 2020; Licher et al. 2018; Torres et al. 2020; Virta et al. 2013). However, the performance of the model was attenuated when applied to low-income countries as well as late-life cohorts (Anstey et al. 2014; Stephan et al. 2020).

Recent systematic reviews on dementia risk prediction models recommended to reuse or enhance existing prediction models instead of creating new ones (Hou et al. 2019), to incorporate costs and applicability in the conceptualization of the prediction models (Hou et al. 2019; Tang et al. 2015; Tang et al. 2017), to validate models externally (Graille et al. 2020; Tang et al. 2015), and to perform analyses stratified for confounding factors as well as to test the interaction between variables (Tang et al. 2015; Tang et al. 2017). Furthermore, the use of biomarkers in dementia risk prediction models might help to accomplish the goal of identifying individuals at risk early (Ritchie and Muniz-Terrera 2019).

1.6 Aim of the dissertation

The overall aim of this thesis was to create a dementia risk prediction model by combining already known risk factors like age or physical inactivity with newly identified biomarkers from the field of OS and inflammation. By this, subjects who are likely to develop dementia should be identified to make diagnoses early. This goal was pursued by four aims.

A) To assess the association of urinary 8-iso-$PGF_{2\alpha}$ levels with all-cause dementia, AD, and VD in a large prospective cohort study with a 14-year follow-up (ESTHER study).

B) To summarize the existing literature from observational studies on the association between F_2-isoprostanes and AD in a systematic review with meta-analysis.

C) To assess the association of inflammation-related blood-based biomarkers from the Olink Target 96 inflammation panel with all-cause dementia, AD, and VD in a large prospective cohort study with a 17-year follow-up (ESTHER study).

D) To use the already existing, well-validated CAIDE model for dementia risk prediction in a large prospective cohort study with a 17-year follow-up (ESTHER study) and to improve it by including biomarkers of OS and inflammation.

2. Material and Methods

2.1 Associations of urinary 8-iso-prostaglandin $F_{2\alpha}$ levels with all-cause dementia, Alzheimer's disease and vascular dementia incidence

The first aim of the dissertation was pursued in a prospective cohort study on the associations of the OS biomarker 8-iso-prostaglandin $F_{2\alpha}$ with all-cause dementia, AD and VD incidence based on the 14-year follow-up data of the ESTHER study (Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung [German]).

2.1.1 Study population

The ESTHER study is a prospective cohort study established in Saarland, a German federal state. 9,940 study participants between the age of 50 and 75 years were recruited during a general health checkup from 2000 to 2002. Besides an age of 50-75 years, the inclusion criteria for the ESTHER study were physical and mental ability to participate in the study as well as knowledge of the German language. Participants were followed up 2, 5, 8, 11 and 14 years after baseline. Details have been described elsewhere (Löw et al. 2004). The distribution of sociodemographic baseline characteristics and common prevalent chronic diseases was similar to the distribution in the respective age categories in the German National Health Survey, which is a representative sample of the German population (Löw et al. 2004). The study was approved by the ethics committees of the Heidelberg University and the state medical board of Saarland, Germany.

2.1.2 Dementia ascertainment

Dementia information was collected at the 14-year-follow-up via questionnaires sent to the study participants' general practitioners (GPs). Details have been published elsewhere (Perna et al. 2019). In brief, the dementia ascertainment included sending standardized questionnaires to the GPs of all study participants, including those who dropped out during

follow-up due to ill health or had died. The GPs were asked several dementia-related questions, including whether they were aware of a dementia diagnosis for their patients. If so, they were asked to provide all available medical records of neurologists, psychiatrists, memory or other specialized providers that documented the diagnosis of dementia. If the GP provided a mixed dementia diagnosis, available medical records were screened for an underlying AD or VD background and considered as AD, VD, or both. The current guidelines in Germany for AD diagnosis follow the National Institute on Aging and the Alzheimer's Association (McKhann et al. 2011).

2.1.3 Measurement of 8-iso-prostaglandin $F_{2\alpha}$ levels

Urinary 8-iso-$PGF_{2\alpha}$ levels were measured from spot urine samples collected during the health check-up at baseline. Almost all urine samples were collected in the morning (98%), and there was no rule for a time distance to the last urination. Urine samples were shipped to the study center and were stored at -80°C for 14-16 years until 8-iso-$PGF_{2\alpha}$ levels were measured in summer/autumn 2016. Urinary levels of F_2-isoprostanes are generally considered stable in frozen samples, but long-term storage studies are still lacking in the literature. The 8iso1 enzyme-linked immunosorbent assay (ELISA) kits were purchased from Detroit R&D (Detroit, Michigan, USA) to determine 8-iso-$PGF_{2\alpha}$ levels in urine samples, which were not purified by high-performance liquid-chromatography (HPLC) before analysis. The assay was performed according to the manufacturer's protocol as described previously (Gao et al. 2018). In brief, this assay is based on the competition between 8-iso-$PGF_{2\alpha}$ in the sample and an 8-iso-$PGF_{2\alpha}$-horseradish peroxidase conjugate for a limited number of 8-iso-$PGF_{2\alpha}$-specific rabbit antiserum binding sites. According to the manufacturer, measurement of authentic 8-iso-$PGF_{2\alpha}$ and a panel of eicosanoids structurally similar to 8-iso-$PGF_{2\alpha}$ showed a specificity of this assay for 8-iso-$PGF_{2\alpha}$ of 100% with cross-reactivity to other compounds < 0.1%. I am not aware of a manufacturer-independent study, which checked these claims. Usually, results from ELISAs are not comparable with those from more precise gas-chromatography (GC) or liquid chromatography-tandem mass-spectrometry (LC-MS/MS) because they produce higher absolute values due to cross-reactivity (Klawitter et al. 2011). Generally, ELISA and LC-MS/MS results correlate better when measured in urine than in plasma samples, but still no correlation coefficients > 0.610 should be expected (Klawitter et al. 2011).

To correct for variability in dilution of 8-iso-$PGF_{2\alpha}$ molecules in the urine samples, they were standardized by urinary creatinine levels. Thus, 8-iso-$PGF_{2\alpha}$ levels are expressed in nmol/mmol creatinine. The creatinine concentration was determined by the kinetic Jaffe method (Hermida et al. 2014).

2.1.4 Covariate assessment

Information on age, sex, education, smoking status, alcohol consumption, physical activity, body mass index (BMI), and life-time history of depression were obtained from a standardized self-administered questionnaire. The history of coronary heart disease (CHD) and diabetes mellitus were obtained from physician diagnoses. Furthermore, anti-diabetic drugs reported by the GP were used to complement diabetes mellitus diagnoses. Participants were considered to have cardiovascular disease (CVD) based upon CHD diagnoses from GPs or self-reported history of myocardial infarction, stroke, pulmonary embolism, or revascularization of coronary arteries. The *APOE* genotypes were measured using TaqMan SNP genotyping assays with genotypes analyzed in an endpoint allelic discrimination read using a PRISM 7000 Sequence detection system (Applied Biosystems) (Nabers et al. 2018). Total cholesterol was measured from serum samples by an enzymatic colorimetric test with the Synchron LX multicalibrator system (Beckman Coulter, Galway, Ireland). Serum CRP levels were determined by immunoturbidimetry with the wrCRP antibody (Bayer, Leverkusen, Germany) on the ADVIA 2400.

2.1.5 In- and exclusion criteria

Participation in the ESTHER study (baseline age range 50-75 years) was the only inclusion criterion. Exclusion criteria were unavailability of information or uncertainty about a dementia diagnosis during follow-up and unavailability of an 8-iso-$PGF_{2\alpha}$ measurement. Dementia information could not be collected for participants who withdrew consent to contact their GP (n = 1,121) or whose GPs withdrew consent to be contacted (n = 304) during follow-up (see Flow-chart in **Figure 1**). Furthermore, dementia information was not available if GPs could not be contacted, e.g. due to closure of practice, retirement or death (n = 930), or due to other reasons like address changes (n = 105). In total, the dementia questionnaire was repeatedly sent to the GPs of n = 7,480 study participants. Information was received from the GPs of

n = 6,422 study participants (response rate: 85.9%). Participants were excluded if GPs did not have information about whether dementia was diagnosed (n = 288) or if dementia diagnosis was suspected (n = 108), which resulted in suitable dementia information for n = 6,026 study participants. A few of these study participants (n = 173) did not donate a urine sample, or the 8-iso-$PGF_{2\alpha}$ biomarker could not be measured. Therefore, in total, n = 5,853 study participants could be included in the present analysis.

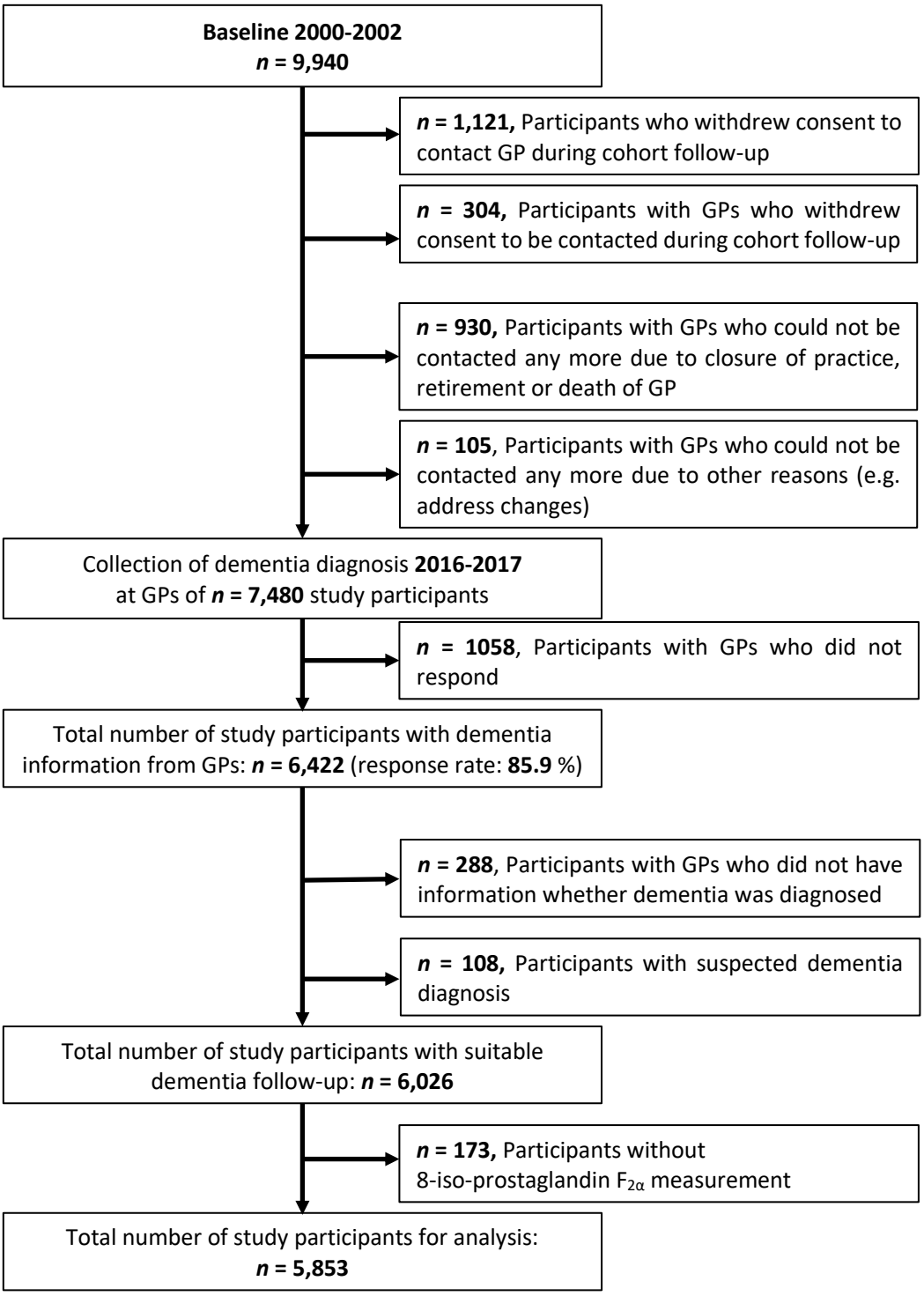

Figure 1. Flowchart of dementia ascertainment during the 14-year follow-up of the ESTHER study and selection of study population for this research project

Baseline characteristics of the included n = 5,853 and excluded n = 4,087 ESTHER study participants were reasonably comparable, supporting the absence of selection bias, although many factors showed statistically significant differences given the large sample size (**Supplementary Table 1**).

2.1.6 Statistical analyses

The associations of baseline characteristics with levels of 8-iso-$PGF_{2\alpha}$ in the top tertile (> 0.242 nmol/mmol creatinine) were determined by a multivariate logistic regression model. A Cox proportional hazards regression model was used to identify baseline characteristics statistically significantly associated with all-cause dementia, AD and VD incidence. Age and sex were pre-selected covariates adjusted for in the main Cox proportional hazards regression model, used to determine hazard ratios (HR) and 95% confidence intervals (95% CI) for the associations of 8-iso-$PGF_{2\alpha}$ levels with all-cause dementia, AD, and VD incidences. Baseline characteristics that were statistically significantly ($p<0.05$) associated with both 8-iso-$PGF_{2\alpha}$ levels and all-cause dementia were considered to be potential confounders and adjusted for in the main Cox model in addition to age and sex. In a sensitivity analysis, I adjusted for all baseline characteristics shown in Table 1 (see chapter 3.1). In a further sensitivity analysis, I considered the competing risk of death by estimating cause-specific hazards and Fine-Gray subdistribution hazards (Tullio et al. 2019). As all results of the main analysis were confirmed in competing risk models, these results are not shown.

To assess the dose-response relationship with total dementia incidence, 8-iso-$PGF_{2\alpha}$ levels were first modelled with restricted cubic splines (Desquilbet and Mariotti 2010). As this analysis suggested a non-linear relationship, fractional polynomials were used to discover the best fitting first-order term for 8-iso-$PGF_{2\alpha}$ levels (Royston et al. 1999). The natural logarithm had significantly better model fit than the linear term ($p = 0.002$), and therefore logarithmized isoprostane levels were used in all analyses in addition to 8-iso-$PGF_{2\alpha}$ tertiles. Analyses for dementia endpoints were carried out for the total population and stratified by sex and age. Potential interactions of logarithmized 8-iso-$PGF_{2\alpha}$ levels with the baseline characteristics selected for the main model were explored by adding interaction terms to the main model. In a further sensitivity analysis, patients diagnosed with dementia in the first 7 years of follow-

up were excluded to check for potential reverse causality. All analyses described in this study were carried out for all-cause dementia incidence because of the low case numbers for dementia subtypes.

To my knowledge, missing values of covariates were missing at random. The highest proportion of missing values for a covariate was 9.5% (*APOE* polymorphism). Therefore, multiple imputations could be applied to impute missing values for all study participants. Five data sets were imputed with the Markov chain Monte Carlo (MCMC) method separately by sex with the SAS procedure PROC MI. The variables for the imputation model were those shown in Table 1 (see chapter 3.1). All analyses were performed in the five imputed data sets, and results were combined by the SAS procedure PROC MIANALYZE.

Statistical tests were two-sided using an alpha level of 0.05. All statistical analyses were conducted with the Statistical Analysis System (SAS, version 9.4, Cary, North Carolina, USA).

2.2 Association of F_2-isoprostane levels with Alzheimer's disease in observational studies: a systematic review and meta-analysis

To achieve the second aim of this dissertation, a systematic review and meta-analysis on the association of F_2-isoprostane levels with AD in observational studies were performed. The protocol of this systematic review was registered at PROSPERO (no. CRD42020197315). Moreover, the systematic review was conducted according to the standards of reporting the meta-analysis of observational studies in epidemiology (MOOSE) statement (Stroup et al. 2000). The corresponding checklist is provided in **Supplemental Table 2**.

2.2.1 Search strategy and data extraction

Two medical databases, PubMed and Web of Science, were searched for relevant studies. The detailed search strings can be found in **Supplemental Tables 3 and 4**, respectively. The search query for both databases combined synonyms of the specific biomarker 8-iso-$PGF_{2\alpha}$ and the whole family of F_2-isoprostanes with different terms for the most common forms of dementia. No restrictions on language or publication period were made.

Publications gathered with the developed search string were maintained in the reference management software EndNote (Clarivate Analytics, Philadelphia, PA, version EndNote X9.2). Duplicates were first removed automatically and additionally by hand afterwards. Next, the titles and abstracts of the remaining articles were reviewed. Articles were excluded if the type of publication was not eligible for this review (e.g. reviews, comments, letters, or commentaries) or if they were irrelevant to the review topic. In the full-text review, articles were excluded if AD was not the outcome or combined with other outcomes if AD patients were not compared to a healthy control group, if the presented data was not plausible, or if no eligible data for the meta-analyses were presented. In the latter case, the corresponding authors of the respective articles were contacted and asked to provide the required data. Finally, cross-referencing of all included articles was performed to find further studies the search strings might have missed.

The full-text selection and data extraction were performed independently by two reviewers (myself and Li-Ju Chen). Disagreements between individual judgements were resolved by discussion or consultation of a third researcher (PD Dr Ben Schöttker).

2.2.2 Assessment of study quality

The Newcastle-Ottawa scale (NOS) was used to assess the risk of selection bias as well as confounding by indication and the adequacy of outcome assessment (Wells et al. 2019). This scale ranges from 0 to 9, with fewer points indicating a higher risk of bias.

2.2.3 Statistical analyses

Biomarker levels were assessed with various measurement techniques and units. Thus, standardised mean differences (SMDs) were used to pool the extracted data in random-effects models. In particular, Hedge's g was used as a measure of effect size as it also accounts for small sample sizes (Hedges 1981).

If the mean and SD of biomarker levels were not provided, the presented summary measure was extracted and transformed into mean and SD according to formulas or references provided in the Cochrane Handbook.

- If the mean and standard error (SE) of biomarker levels were provided, the SD was computed by $SD = SE \times \sqrt{N}$.
- If the median and range or interquartile range were provided, data was transformed according to Wan et al. 2014 (Higgins et al. 2021; Wan et al. 2014).
- If the geometric mean (GM) and the geometric coefficients of variation (GCV) were provided, the GCV was first transformed into the geometric standard deviation (GSD) (Kirkwood 1979). GM and GSD were then transformed into mean and SD according to Higgins et al. 2008 (Higgins et al. 2008).
- If data was reported in subgroups (e.g. *APOE* ε4 carrier and *APOE* ε4 non-carrier), the data were summarised for cases and controls according to the Cochrane Handbook (Higgins et al. 2021).

In some of the included longitudinal studies, the preferred effect measure to report the results was the HR, which I used for the meta-analyses in these cases.

The meta-analyses were first performed for subgroups of studies by study design (cross-sectional or longitudinal), biomarker (8-iso-$PGF_{2\alpha}$ or F_2-isoprostane family), and sample types (urine, blood, CSF, frontal lobe tissue). In addition, a meta-regression of all included cross-sectional studies was carried out to investigate potential causes of between-study heterogeneity and to judge whether it is appropriate to pool all cross-sectional studies in one meta-analysis. No such meta-regression was performed for longitudinal studies because too few studies were available. If multiple results of the same study population were published, the measurement with the highest Hedge's g was chosen. In a sensitivity analysis, the lowest Hedge's g was included in the meta-analysis.

Heterogeneity between the studies was examined with the I^2 statistic and τ^2. The risk of publication bias was assessed statistically using Egger's test of the intercept (one-tailored) and graphically by visual inspection of funnel plots (Rothstein et al. 2005). If publication bias was detected, the trim and fill method was used to estimate a pooled effect estimate with a random-effects model, which includes potentially unpublished studies (Duval and Tweedie 2000).

The software Comprehensive Meta-Analysis 2.0 (Biostat, Englewood, NJ) was used for all statistical analyses and to create funnel plots. Forest plots were created using the R package "forestplot" (R, version 3.6.3; forestplot package version 1.10.1) (Gordon and Lumley 2020; R Core Team 2020).

2.3 Association of the inflammation-related proteome with dementia development at older age

For the third aim of this dissertation, analyses on the association of the inflammation-related proteome with all-cause dementia, AD, and VD were conducted in a case-cohort study.

2.3.1 Study population

This study was based on the 14- and the meanwhile conducted 17-year follow up data of the ESTHER study. The ESTHER study was described in chapter 2.1.1. Data was analyzed in a case-cohort study design.

2.3.2 Dementia ascertainment

Acquisition of dementia data was described in chapter 2.1.2. Procedures remained the same for the 17-year follow-up. Due to the use of the 14- and 17-year follow up of the ESTHER study in this project, the median follow-up time was 16.3 years (interquartile-range: 13.5-17.0 years), and the maximum was 19.4 years due to the 2-year period of baseline recruitment.

2.3.3 Olink biomarker measurements

Inflammation-related, blood-based proteins were measured from serum samples collected during the health checkup at baseline (2000-2002). Blood samples were sent to the study centre and stored at -80°C until biomarker measurements took place in March 2018, December 2018, and September 2020 (referred to as time points t1, t2 and t3 in the following). At the time of the measurements, 10-25 µl of serum was extracted from different aliquots

that had been thawed twice and sent with dry ice to the laboratories, which analyzed the samples with the Olink Target 96 Inflammation panel, Olink Proteomics, Uppsala, Sweden. At t1 and t2, samples were analyzed in the laboratory of Olink Proteomics, Uppsala Science Park, SE-75183 Uppsala, Sweden. At t3, the measurements were performed in the Research Unit Protein Science, German Research Center for Environmental Health, Helmholtz Center Munich, Heidemannstraße 1, 80939 München, Germany.

The Olink panels are based on a Proximity Extension Assay technology (PEA) (Assarsson et al. 2014; Lundberg et al. 2011). Details on the reliability and stability of the technology are described elsewhere (Olink Proteomics AB 2016). In brief, oligonucleotide labelled antibody probe pairs are allowed to bind to their respective target proteins in the samples. Only if two antibodies are in close proximity, a polymerase chain reaction (PCR) reporter sequence is formed by DNA polymerization. This sequence is detected and quantified using high throughput real-time quantitative PCR (qPCR) (Fluidigm® Biomark™ HD system). The Olink Target 96 Inflammation panel allows the measurement of 92 biomarkers per sample. A list of all biomarkers of this panel is displayed in **Supplemental Table 5**.

At t1, t2 and t3, 22, 15 and 5 plates were used, respectively. To avoid batch effects, cases and controls were randomly distributed across plates and adjusted according to included interpolate controls. The average intra-assay coefficient of variance among all 92 measured biomarkers was 7%, 4% and 3% at t1, t2 and t3, respectively. The average inter-assay coefficient of variance was 12%, 10% and 10% at t1, t2 and t3, respectively. Furthermore, the quality of each serum sample was assessed by Olink technology (Olink Proteomics AB 2016). All samples were measured successfully, and the number of quality control warnings was below 4% in all three timepoints. Of the 1,435 randomly selected controls and 393 incident dementia cases, 46 serum samples of participants were excluded due to a quality control warning by Olink.

Protein levels are reported as Normalized Protein eXpression (NPX) values, a relative quantification unit logarithmically related to protein concentration. The number of samples with values below the lower limit of detection (LOD) varied strongly by biomarker and is shown in **Supplemental Table 5**. In total, 20 biomarkers with > 25 % of the values below LOD were excluded from all analyses (grey shaded biomarkers in Supplemental Table 5). Thereby, 72 out of the 92 biomarkers were considered evaluable markers. Biomarker values below the LOD were replaced by LOD/√2. The normalization of raw data was conducted with the R (R Core

Team, 2020, version 3.6.3) package “OlinkAnalyze”, developed and maintained by the Olink Proteomics Data Science Team (Olink Proteomics Data Science Team 2018). For this procedure, bridging samples were used to normalize data from three different measurement time points.

2.3.4 Covariate assessment

Assessment of sex, age, education, BMI, physical activity, lifetime history of depression, CVD, diabetes, and *APOE* genotypes were described in chapter 2.1.4.

2.3.5 In- and exclusion criteria

The selection of study participants from the ESTHER cohort for this case-cohort analysis is shown in **Figure 2**. ESTHER participants were eligible for selection as cases or random controls. Participants were excluded if dementia incidence status could not be ascertained by GP questionnaires (n = 3,583) or blood samples were not available (n = 73). Thus, information from 6,284 participants was available for analyses. Olink inflammation panel measurements were performed in a case-cohort design among 1,435 randomly selected study participants and all incident dementia cases of the rest of the cohort (n = 393). To check if the random selection was successful, I compared the baseline characteristics of selected and non-selected controls (**Supplemental Table 6**). After excluding participants with quality control warnings, 389 incident dementia cases and 1,393 randomly selected participants were available. As the random controls included 115 incident dementia cases, the study population comprised 504 participants with incident dementia and 1,278 randomly selected controls.

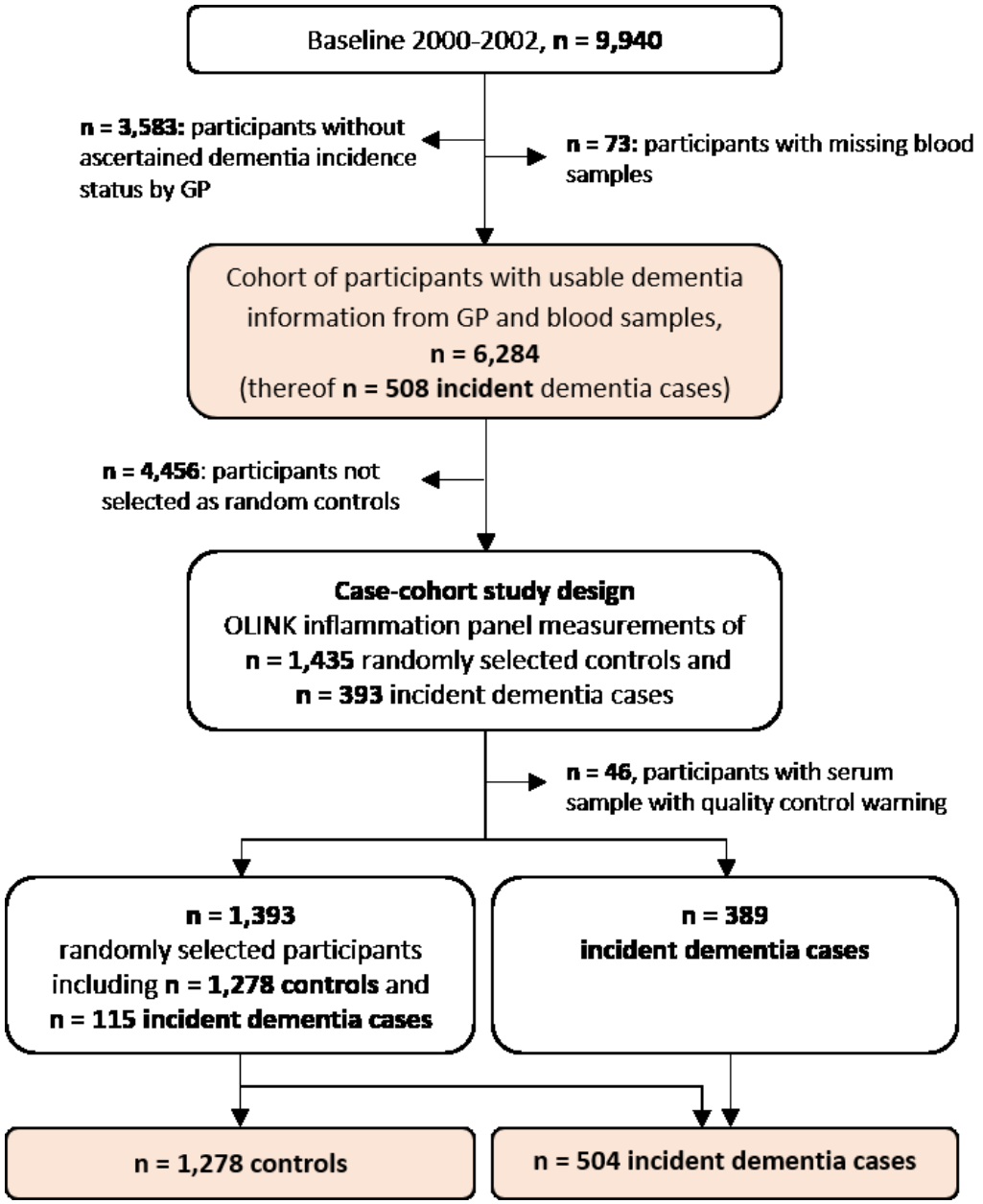

Figure 2. Flowchart of dementia ascertainment during the 14- and 17-year follow-up of the ESTHER study and selection of the study population for this research project.

Abbreviations: GP, general practitioner.

2.3.6 Statistical analyses

First, to describe factors associated with dementia risk, categorized baseline characteristics of all-cause dementia cases and controls were compared using the χ^2-test. Second, odds ratios (ORs) were estimated with a multivariate logistic regression model, including all baseline characteristics.

In a univariate, descriptive analysis, the median and IQR of all inflammation-related protein levels of all-cause dementia, AD, and VD cases were separately compared with those of controls, using the Wilcoxon Rank Sum test. Additionally, in a multivariate approach, the ORs per one SD increase of each inflammation-related protein were assessed separately with each outcome (all-cause dementia, AD, and VD incidence) in logistic regression models adjusted for potential confounders. In models for AD incidence, study participants with other (e.g. VD) or unknown dementia forms were excluded. The same was applied for the outcome of Vd incidence by excluding AD and other non-vascular dementia cases. The models were adjusted for age, sex, education, physical activity, BMI, CVD, diabetes, depression, and *APOE* genotype. All variables were used as categorical variables, as described in Table 5, except age, which was modelled continuously. The covariates were selected because they were statistically significantly associated with all-cause dementia, AD, or VD in the previous analysis with the ESTHER study participants, described in chapters 2.1, 3.1, and 4.1. Statistical test results were corrected for multiple testing by the Benjamini and Hochberg method for all tests carried out for one outcome (Benjamini and Hochberg 1995). A false discovery rate (FDR) < 0.05 was applied as the threshold for statistical significance.

I further aimed to identify those inflammation-related proteins whose association with a dementia outcome was independent of other inflammatory biomarkers. Therefore, all biomarkers, which were significantly associated with a dementia endpoint after FDR correction, were tested for the independence of the association by forward elimination. In detail, only biomarkers having the strongest, independent, positive association with the outcome entered the regression model with the threshold for statistical significance of $p<0.05$. The identified independent biomarkers were used for the naming of biomarker clusters. All other biomarkers of the Olink inflammation panel, which were highly correlated (Spearman's correlation coefficient $r > 0.5$) (Spearman 1904) with an independent biomarker, were put in its cluster. One biomarker might be in more than one cluster. I favoured this statistical approach over a principal component analysis (PCA) because it has a higher transparency, is easier to reproduce by others, its results are easier to interpret, and the associations of the biomarkers with the dementia outcomes are being acknowledged in the decision about the number of clusters.

The associations of the independent biomarkers with dementia endpoints were further analyzed in subgroup analyses based on age, sex, obesity, diabetes, history of CVD, and *APOE* ε4 polymorphism. Apart from this, interaction terms were tested. In addition, the dose-response relationships between the independent biomarkers and dementia endpoints were assessed with restricted cubic spline curves (Desquilbet and Mariotti 2010).

Several sensitivity analyses were performed. To check for potential reverse causality, the associations between the independent biomarkers and dementia endpoints were analyzed stratified by time of diagnosis (in the first ten years of follow-up vs later years). Competing risk of death was examined by excluding subjects without dementia diagnosis who died before their 80th birthday, the average life expectancy of the cohort's population. Fractional polynomials with first-order terms were used to determine each biomarker's best fitting function with the outcomes (Royston and Sauerbrei 2005). Since the linear function was the best fitting one for almost all biomarkers (68 of 72), all were modelled linearly. Finally, to examine the impact of persons with a potential acute infection on the overall results, subjects with CRP levels > 20 mg/L were excluded.

To my knowledge, missing values of covariates were missing at random. The highest proportion of missing values was found for *APOE* polymorphism (7.5%). Thus, multiple imputation was used to impute missing values. Variables shown in Table 5 were used for the imputation model. Twenty data sets were imputed with the MCMC method separately by sex with the SAS procedure PROC MI. All analyses were performed based on those 20 datasets with the SAS procedure PROC MIANALYZE.

Statistical tests were two-sided using an alpha level of 0.05. All statistical analyses were conducted with the Statistical Analysis System (SAS, version 9.4, Cary, North Carolina, USA).

2.4 Improved risk prediction of all-cause dementia, Alzheimer's disease, and vascular dementia by biomarkers of oxidative stress and inflammation

To achieve the fourth aim of this dissertation, an already existing, well-validated dementia risk prediction model was applied to a large prospective cohort study with 17 years of follow-up and extended by biomarkers of OS and inflammation.

2.4.1 Study population

This study was based on the 14- and 17-year follow up data of the ESTHER study. The ESTHER study was described in chapter 2.1.1. Data from the case-cohort study described in chapters 2.3, 3.3, and 4.3 was used.

2.4.2 Dementia ascertainment and case-cohort design sample

The collection of dementia information was described in chapter 2.1.2. In addition to the 14-year follow-up, data from the 17-year follow-up was used (median (interquartile range) follow-up time: 16.3 years (13.5-17.0 years)) (see chapter 2.3.2 for details).

Overall, information on whether dementia got diagnosed during 17 years of follow-up or not could be ascertained for n = 6,357 study participants (64% of the original cohort). A flowchart of the study population is shown in **Figure 3.**

After excluding subjects with missing blood samples (n = 73) from participants with ascertained dementia information, 6,284 participants were eligible to be drawn for the case-cohort sample and measurements of the Olink Target 96 inflammation panel. This sample primarily consisted of 1,435 randomly selected participants and 393 dementia cases. However, due to quality control warnings during the biomarker measurements, 46 participants were additionally excluded. Participants were further excluded in case of missing data for at least one of the aforementioned CAIDE model variables (n = 145). For the last exclusion step, I compared the data of included and excluded participants with respect to age, sex, and education, and no indication of selection bias was detected (**Supplemental Table 7**). Because the randomly selected controls entailed some incident dementia cases, the final sample included a total of 440 dementia cases and 1,197 controls. The predictive ability of 8-iso-$PGF_{2\alpha}$ was assessed in a sample including 432 cases and 1,609 controls because 8-iso-$PGF_{2\alpha}$ levels were missing for n = 28 of the included participants.

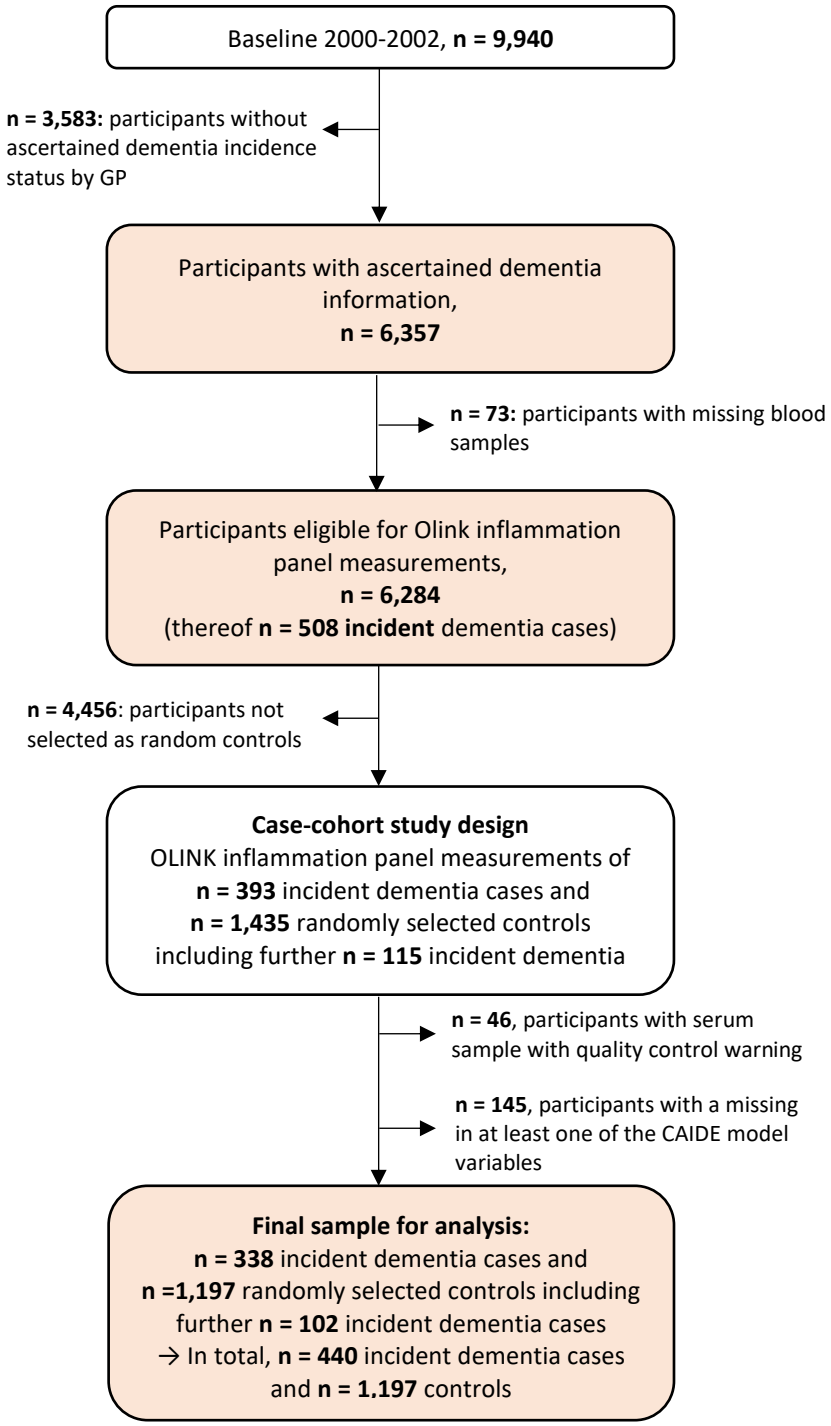

Figure 3. Flowchart of dementia ascertainment during the 14- and 17-year follow-up of the ESTHER study and study participant selection.
Abbreviations: GP, general practitioner

2.4.3 Origin, assessment, and modifications of the CAIDE model variables

The CAIDE model originates from the CAIDE study, a population-based cohort study from Finland assessing cardiovascular risk factors, aging, and dementia (Kivipelto et al. 2001). For the development of the CAIDE model, 1,409 participants aged between 39 and 64 years of the original CAIDE study were included (Kivipelto et al. 2006). Of those, 61 developed dementia during 20 years of follow-up. CAIDE model 1 consists of the variables age, education, sex, systolic blood pressure, body-mass index (BMI), total cholesterol, and physical activity, while CAIDE model 2 additionally includes *APOE* ε4 status.

Assessment of the CAIDE model variables age, sex, education, BMI, and physical activity of participants in the ESTHER study was described in chapter 2.1.4. *APOE* genotypes were determined by TaqMan single-nucleotide polymorphism (SNP) genotyping assays (Applied Biosystems, California, USA). Endpoint allelic discrimination reads were used to analyze genotypes with the Bio-RAD CFX Connect System (Bio-Rad Laboratories, CA, USA). Missing *APOE* information (n = 70) was imputed based on quality-controlled genetic data. For details, see Stocker et al. 2020 (Stocker et al. 2020).

All variables used in the CAIDE model were available but needed to be newly calibrated because the ESTHER cohort has a different age range, school education history and physical activity assessment than the CAIDE study. Fractional polynomials were utilized to determine the best fitting function of the continuous variables in the prediction of all-cause dementia, AD, and VD (Royston and Sauerbrei 2005) (data not shown). Because the linear function was the best fitting function for age, systolic blood pressure, BMI, and total cholesterol, they were kept as continuous variables. Education, physical activity, and *APOE* genotypes were dichotomized by summarizing categories with almost similar ORs for the association with all-cause dementia (data not shown).

2.4.4 Measurement of oxidative stress and inflammation-related biomarkers

Levels of inflammation-related proteins were measured in baseline serum samples using the Olink Target 96 inflammation panel (Olink Proteomics, Uppsala, Sweden). Details were

described in chapter 2.3.3. In addition, a list of all biomarkers is depicted in **Supplemental Table 5**.

Measurements of the biomarker 8-iso-$PGF_{2\alpha}$ were performed in urinary baseline samples of ESTHER study participants as described in chapter 2.1.3.

2.4.5 Statistical analyses

The associations of the CAIDE model variables with the outcomes of all-cause dementia, AD and VD were determined by a multivariate logistic regression model adjusted for age, education, sex, systolic blood pressure, BMI, total cholesterol, physical activity and *APOE* ε4 status.

The discriminative performance of all variables, including the CAIDE model variables and the biomarkers, was calculated using least absolute shrinkage and selection operator (LASSO) logistic regression models. LASSO is a form of linear regression that uses shrinkage to exclude variables that are not useful for the prediction (Tibshirani 1996). This makes the final equation simpler and easier to interpret. The CAIDE model variables were defined as not being penalized by the LASSO regression and thus forced into the model. In a sensitivity analysis, all variables were penalized. The parameter λ was determined by ten-fold cross-validation. The AUCs and 95% CIs were estimated using 10,000 bootstrap samples for the CAIDE model and CAIDE model + inflammatory biomarkers for all-cause dementia, AD, and VD as the outcome, respectively. While the CAIDE model only included the CAIDE model variables, the CAIDE model + inflammatory biomarkers additionally included those of the 72 inflammation-related biomarkers selected by the LASSO regression. Moreover, I distinguished between CAIDE model 1 and 2, with only the latter including *APOE* ε4 carrier status among the unpenalized CAIDE model variables. To determine if the differences between the CAIDE model and the CAIDE model + inflammatory biomarkers models were statistically significant, bootstrap intervals for the differences in AUCs were computed.

Besides calculations for the total sample, the models' discrimination performance was also evaluated in subgroups for mid-life (55-64 years) and late-life (65-75 years) for all three dementia outcomes and CAIDE model 1 and CAIDE model 2.

In an additional analysis, the predictive ability of the OS biomarker 8-iso-$PGF_{2\alpha}$ was assessed, adding the biomarker to the unpenalized CAIDE model variables.

The Statistical Analysis System (SAS, version 9.4, Cary, North Carolina, USA) was used for multivariate logistic regression. Statistical tests were two-sided using an alpha level of 0.05. LASSO regression was performed using the R package "*glmnet"* (R, version 3.6.3; glmnet package version 4.1-2) (Friedman et al. 2010). For AUC computation and bootstrapping, the R package *ModelGood* (R, version 3.6.3; ModelGood package version 1.0.9) was used (Gerds 2014).

3. Results

3.1 Associations of urinary 8-iso-prostaglandin $F_{2\alpha}$ levels with all-cause dementia, Alzheimer's disease and vascular dementia incidence

Table 1 shows the baseline characteristics of the study population and their associations with increased 8-iso-$PGF_{2\alpha}$ levels. Approximately two-thirds of the participants were between 50 and 64 years old, while one-third of the participants were aged 65-75. Slightly more females (55%) than males (45%) were included in the sample. The median [interquartile range (IQR)] 8-iso-$PGF_{2\alpha}$ level was 0.20 [0.15–0.27] nmol/mmol creatinine. The median (IQR) 8-iso-$PGF_{2\alpha}$ level was statistically significantly higher ($p = 0.02$, Wilcoxon rank-sum test) among study participants that developed all-cause dementia during follow-up (0.25 [0.16-0.28] nmol/mmol creatinine) than among those who did not (0.20 [0.15–0.27] nmol/mmol creatinine).

Among the baseline characteristics, current smoking, high alcohol consumption, obesity (BMI>30 kg/m²), diabetes, increased CRP levels, and the *APOE* ε4/ε4 genotype were positively associated ($p<0.05$) with increased levels of 8-iso-$PGF_{2\alpha}$. Moreover, 8-iso-$PGF_{2\alpha}$ levels were statistically significantly lower in males, study participants with longer school education, and individuals with higher physical activity.

Table 1. Baseline characteristics of included study participants (n = 5,853) and their associations with 8-iso-prostaglandin $F_{2\alpha}$ levels in the top tertile (> 0.242 nmol/mmol creatinine)

Baseline characteristics	*n* (%)	8-iso-$PGF_{2\alpha}$ levels (nmol/mmol creatinine)	Association with 8-iso-$PGF_{2\alpha}$ levels > 0.242 nmol/mmol creatinine	
		Median (IQR)	Odds ratio (95%CI)*	p-value
Age (years)				
50-64	3740 (63.9)	0.20 (0.16-0.27)	1.00 (Ref.)	Ref
65-69	1309 (22.4)	0.20 (0.15-0.26)	0.97 (0.88-1.07)	0.606
70-75	804 (13.7)	0.204(0.16-0.27)	0.98 (0.88-1.10)	0.741
Sex				
Female	3200 (54.7)	0.21 (0.16-0.28)	1.00 (Ref.)	Ref
Male	2653 (45.3)	0.19 (0.15-0.25)	**0.60 (0.53-0.69)**	**<0.001**
Education (years)				
< 9	4236 (74.1)	0.21 (0.16-0.27)	1.00 (Ref.)	Ref
9-11	819 (14.3)	0.19 (0.15-0.26)	**0.84 (0.71-0.99)**	**0.048**
≥ 12	661 (11.6)	0.19 (0.14-0.25)	0.85 (0.70-1.03)	0.089
Smoking status				
Never smoker	2909 (50.8)	0.20 (0.15-0.26)	1.00 (Ref.)	Ref
Former smoker	1939 (33.9)	0.19 (0.15-0.26)	1.13 (0.98-1.29)	0.101
Current smoker	874 (15.3)	0.26 (0.19-0.35)	**3.12 (2.65-3.68)**	**<0.001**
Alcohol consumption†				
None	1611 (30.3)	0.21 (0.16-0.27)	1.00 (Ref.)	Ref
Low or moderate	3333 (62.6)	0.20 (0.15-0.26)	**1.17 (1.01-1.35)**	**0.032**
High	381 (7.2)	0.22 (0.16-0.30)	**1.50 (1.17-1.93)**	**0.002**
Physical activity‡				
Inactive	1133 (19.4)	0.22 (0.16-0.29)	1.00 (Ref.)	Ref
Low	2645 (45.3)	0.20 (0.15-0.27)	**0.83 (0.71-0.96)**	**0.015**
Medium or high	2061 (35.3)	0.19 (0.150-0.26)	**0.76 (0.64-0.90)**	**0.001**
BMI (kg/m²)				
< 25	1632 (27.9)	0.20 (0.15-0.27)	1.00 (Ref.)	Ref
25-<30	2738 (46.9)	0.20 (0.15-0.26)	0.94 (0.87-1.02)	0.136
≥30	1473 (25.2)	0.21 (0.160-0.28)	**1.15 (1.05-1.26)**	**0.003**
CVD§				
No	4709 (80.5)	0.20 (0.16-0.27)	1.00 (Ref.)	Ref
Yes	1143 (19.5)	0.20 (0.15-0.27)	1.01 (0.87-1.18)	0.865
Diabetes				
No	4951 (85.8)	0.20 (0.15-0.27)	1.00 (Ref.)	Ref
Yes	821 (14.2)	0.22 (0.16-0.29)	**1.25 (1.06-1.48)**	**0.007**
Life-time history of depression				
No	4997 (85.5)	0.20 (0.16-0.27)	1.00 (Ref.)	Ref
Yes, without current pharmacotherapy	660 (11.3)	0.20 (0.15-0.27)	0.90 (0.75-1.07)	0.235
Yes, with current pharmacotherapy	187 (3.2)	0.20 (0.16-0.27)	1.00 (0.73-1.38)	0.977

Baseline characteristics	*n* (%)	8-iso-PGF$_{2\alpha}$ levels (nmol/mmol creatinine)	Association with 8-iso-PGF$_{2\alpha}$ levels > 0.242 nmol/mmol creatinine	
		Median (IQR)	Odds ratio (95%CI)*	p-value
Total cholesterol levels (mg/dl)				
< 200	1913 (32.7)	0.20 (0.15-0.27)	1.00 (Ref.)	Ref
200-<240	1983 (33.9)	0.20 (0.16-0.27)	0.98 (0.90-1.06)	0.529
≥240	1957 (33.4)	0.21 (0.16-0.27)	1.07 (0.99-1.16)	0.101
CRP levels (mg/L)				
< 1	1563 (26.7)	0.19 (0.15-0.25)	1.00 (Ref.)	Ref
1-<3	2202 (37.6)	0.20 (0.15-0.27)	1.07 (0.99-1.16)	0.093
≥3	2088 (35.7)	0.21 (0.16-0.28)	**1.09 (1.00-1.19)**	**0.041**
***APOE* genotypes**				
ε4 non-carrier	3913 (66.8)	0.20 (0.15-0.27)	1.00 (Ref.)	Ref
ε2/ε4	194 (3.7)	0.21 (0.15-0.27)	0.91 (0.64-1.28)	0.570
ε3/ε4	1109 (20.9)	0.20 (0.16-0.27)	1.04 (0.90-1.21)	0.578
ε4/ε4	83 (1.6)	0.22 (0.15-0.29)	**1.60 (1.02-2.50)**	**0.041**

Abbreviations: 8-iso-PGF$_{2\alpha}$, 8-iso-prostaglandin F$_{2\alpha}$; *APOE*, apolipoprotein E; BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; IQR, interquartile range; NSAIDs, nonsteroidal anti-inflammatory drugs.

NOTE: Numbers printed in bold are statistically significant ($p < 0.05$).

* Multivariate logistic regression model including all variables shown in this table.

† Definition of low or moderate alcohol consumption: women 0-19.99 grams ethanol/day (g/d) or men 0-39.99 g/d; Definition of high alcohol consumption: women ≥ 20-39.99g/d or men ≥ 40g/d.

‡ "Inactive" was defined by < 1 h of vigorous or < 1 h light physical activity per week. "Medium or high" was defined by ≥ 2 h of vigorous and ≥ 2 h of light physical activity/week. All other amounts of physical activity were grouped into the category "Low".

§ CVD was defined as coronary artery disease or a self-reported history of myocardial infarction, stroke, pulmonary embolism, or revascularization of coronary arteries.

Among all included n = 5,853 study participants, 365 cases of all-cause dementia were diagnosed during a median follow-up of 13.7 years. Thereof, 109 study participants were diagnosed with AD and 127 with VD. Increasing age, male sex, low school education, physical inactivity, an increased BMI, diabetes, medically treated depression, and the *APOE ε3/ε4*, as well as ε4/ε4 genotype, were statistically significantly associated with an increased all-cause dementia incidence (**Supplemental Table 8**). From this list of baseline characteristics, education, physical activity, BMI, diabetes, and the *APOE ε4* polymorphism were selected for the main model in addition to age and sex because they were statistically significantly associated with both 8-iso-PGF$_{2\alpha}$ levels (**Table 1**) and dementia and therefore could be confounders.

Table 2 shows the associations for 8-iso-$PGF_{2\alpha}$ levels with all-cause dementia, AD, and VD. Continuously modelled, logarithmized 8-iso-$PGF_{2\alpha}$ levels were statistically significantly associated with all-cause dementia incidence (HR [95% CI] per 1 standard deviation (SD): 1.47 [1.19-1.82]) and AD incidence (HR [95% CI] per 1 SD: 1.55 [1.05-2.29]), whereas the association with VD was not statistically significant although the HR point estimate was increased (HR [95% CI] per 1 SD: 1.20 [0.83-1.73]). When 8-iso-$PGF_{2\alpha}$ levels were modelled in tertiles, which reduces the statistical power, only the effect estimate for all-cause dementia remained statistically significant when comparing top to bottom tertile (HR [95% CI]: 1.45 [1.12-1.88]). In sensitivity analyses adjusting for all assessed baseline characteristics, effect estimates were very similar, and associations found to be statistically significant in the main model remained statistically significant (**Supplemental Table 9**). In a further sensitivity analysis excluding patients diagnosed with dementia in the first 7 years of follow-up, the HR point estimates for all-cause dementia and AD were somewhat attenuated, but the association of logarithmized 8-iso-$PGF_{2\alpha}$ levels and all-cause dementia incidence remained statistically significant (**Supplemental Table 10**).

Table 2. Associations of 8-iso-prostaglandin $F_{2\alpha}$ levels with all-cause and common subtype dementia incidences

8-iso-prostaglandin $F_{2\alpha}$ [nmol/mmol creatinine]	n_{total}	All-cause dementia		Alzheimer's disease		Vascular dementia	
		n_{cases}	HR (95%CI)*	n_{cases}	HR (95%CI)*	n_{cases}	HR (95%CI)*
Per 1 SD †	5853	365	**1.47 (1.19-1.82)**	109	**1.55 (1.05-2.29)**	127	1.20 (0.83-1.73)
Tertile 1 (≤0.169)	1951	105	1.00 (ref.)	33	1.00 (ref.)	37	1.00 (ref.)
Tertile 2 (>0.169-0.242)	1952	123	1.16 (0.89-1.51)	27	0.80 (0.48-1.34)	48	1.30 (0.85-2.01)
Tertile 3 (>0.242)	1950	137	**1.45 (1.12-1.88)**	49	1.54 (0.98-2.41)	42	1.28 (0.82-2.00)

Abbreviations: CI, confidence interval; HR, hazard ratio; SD, standard deviation.

NOTE: Numbers printed in bold are statistically significant ($p < 0.05$).

* The model was adjusted for age (continuously), sex, education, physical activity, BMI (categorical), diabetes, and *APOE* **ε**4 polymorphism. The HR per 1 SD was obtained in analysis with logarithmized 8-iso-prostaglandin $F_{2\alpha}$ levels.

† 1 SD of 8-iso-prostaglandin $F_{2\alpha}$ levels = 0.278 nmol/mmol creatinine.

The dose-response curve showed a steady increasing dementia risk with increasing 8-iso-$PGF_{2\alpha}$ levels until the 75th percentile (0.268 nmol/mmol creatinine) and plateaued thereafter (**Figure 4**), which is typical for logarithmic relationships. The interaction term of logarithmized

8-iso-$PGF_{2\alpha}$ levels and the *APOE* ε4/ε4 genotype was statistically significantly associated with all-cause dementia incidence on the p<0.05 significance level (ß = 1.95, p = 0.02) but not after correction for multiple testing (Bonferroni-corrected threshold for statistical significance: p<0.007). **Table 3** shows the additive risks of the *APOE* ε4/ε4 genotype and increased 8-iso-$PGF_{2\alpha}$ levels (defined by top tertile; >0.242 nmol/mmol creatinine) for dementia development**.** If both risk factors were present, the dementia risk was almost 9-fold increased (HR [95% CI]: 8.63 [4.55-16.39]). In contrast, if only increased 8-iso-$PGF_{2\alpha}$ levels were present, the dementia risk was 1.3-fold increased (HR [95% CI]: 1.30 [1.04-1.61]) and if only the *APOE* ε4/ε4 genotype was present, the dementia risk was approximately 2-fold increased (HR [95% CI]: 2.10 [0.93-4.75]).

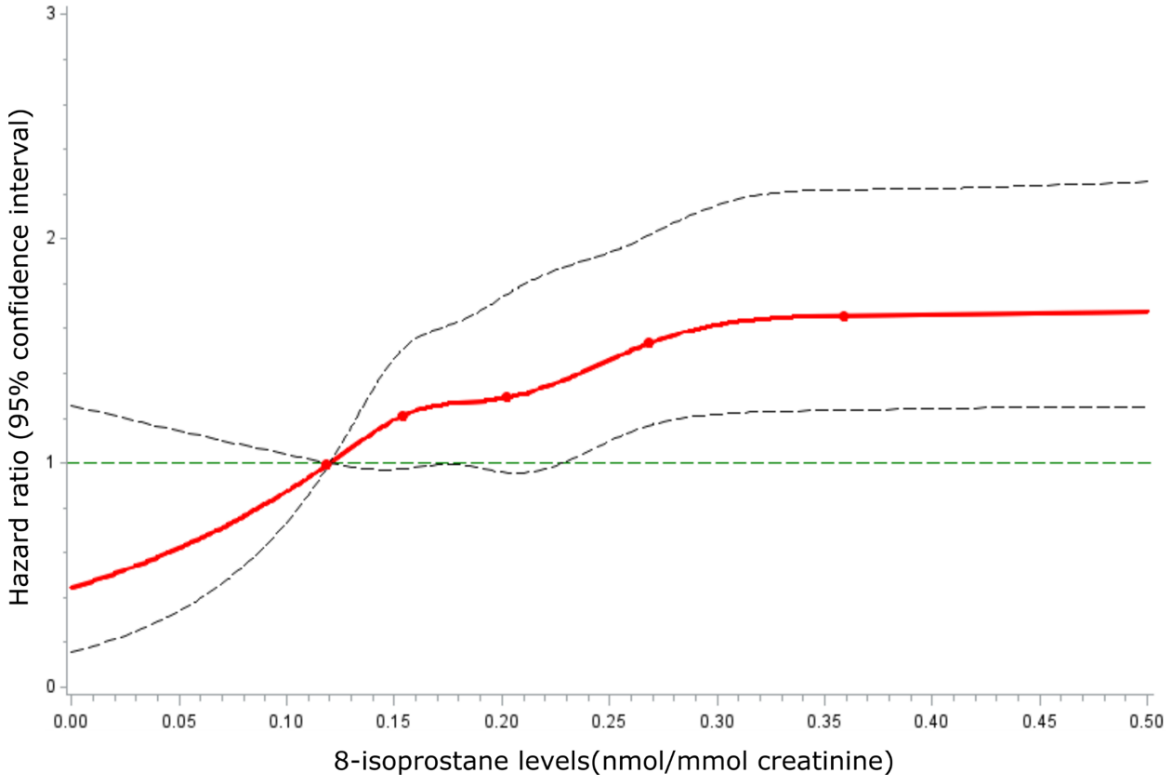

Figure 4. Dose-response curve for the association of 8-iso-prostaglandin $F_{2\alpha}$ levels with all-cause dementia incidence

Solid red line: estimation; dashed curved lines: 95% confidence interval limits; dashed green line: reference line (hazard ratio = 1); dots: knots (10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentile).

Note: The model was adjusted for age (continuously), sex, education, physical activity, BMI (categorical), diabetes, and *APOE* ε4 polymorphism.

Table 3. Interaction of *APOE* ε4/ε4 genotype and 8-iso-prostaglandin $F_{2\alpha}$ levels for all-cause dementia incidence

***APOE* ε4/ε4**	**8-iso-$PGF_{2\alpha}$ > 0.242 nmol/mmol creatinine**	**All-cause dementia**		
		n_{total}	n_{cases}	**HR (95%CI)***
No	No	3853	222	Ref
No	Yes	1916	127	**1.30 (1.04-1.61)**
Yes	No	50	6	2.10 (0.93-4.75)
Yes	Yes	34	10	**8.63 (4.55-16.39)**

Abbreviations: 8-iso-$PGF_{2\alpha}$, 8-iso-prostaglandin $F_{2\alpha}$; CI, confidence interval; hazard ratio.

NOTE: Numbers printed in bold are statistically significant ($p < 0.05$). The *P* value for the interaction term of *APOE* ε4/ε4 genotype and 8-iso-prostaglandin $F_{2\alpha}$ levels (continuous) was $p = 0.0002$.

* The model was adjusted for age (continuously), sex, education, physical activity, BMI (categorical), and diabetes.

Last, I show results for all-cause dementia stratified by age and sex groups in **Supplemental Tables 11 and 12**, respectively. The association of logarithmized 8-iso-$PGF_{2\alpha}$ levels and dementia incidence was much weaker in younger (age 50-64 years) than in older (age 65-75 years) study participants and only statistically significant in the older age groups. In contrast, the association was comparably strong and statistically significant in both men and women.

3.2 Association of F_2-isoprostane levels with Alzheimer's disease in observational studies: a systematic review and meta-analysis

3.2.1 Study selection

The process of the study selection is depicted in **Figure 5**. In total, 158 individual studies were reviewed, and of those, 39 were considered in the full-text screening. In addition, via cross-referencing, six more were considered to include. A list of studies excluded in the full-text selection and the respective criteria can be found in **Supplemental Table 13**. Since the study of Praticò et al. 1998 used different study populations for measurements in CSF and frontal lobe tissue samples, both results were included (Praticò et al. 1998). Thus, 28 publications were finally included in this systematic review, which comprised data from 29 studies.

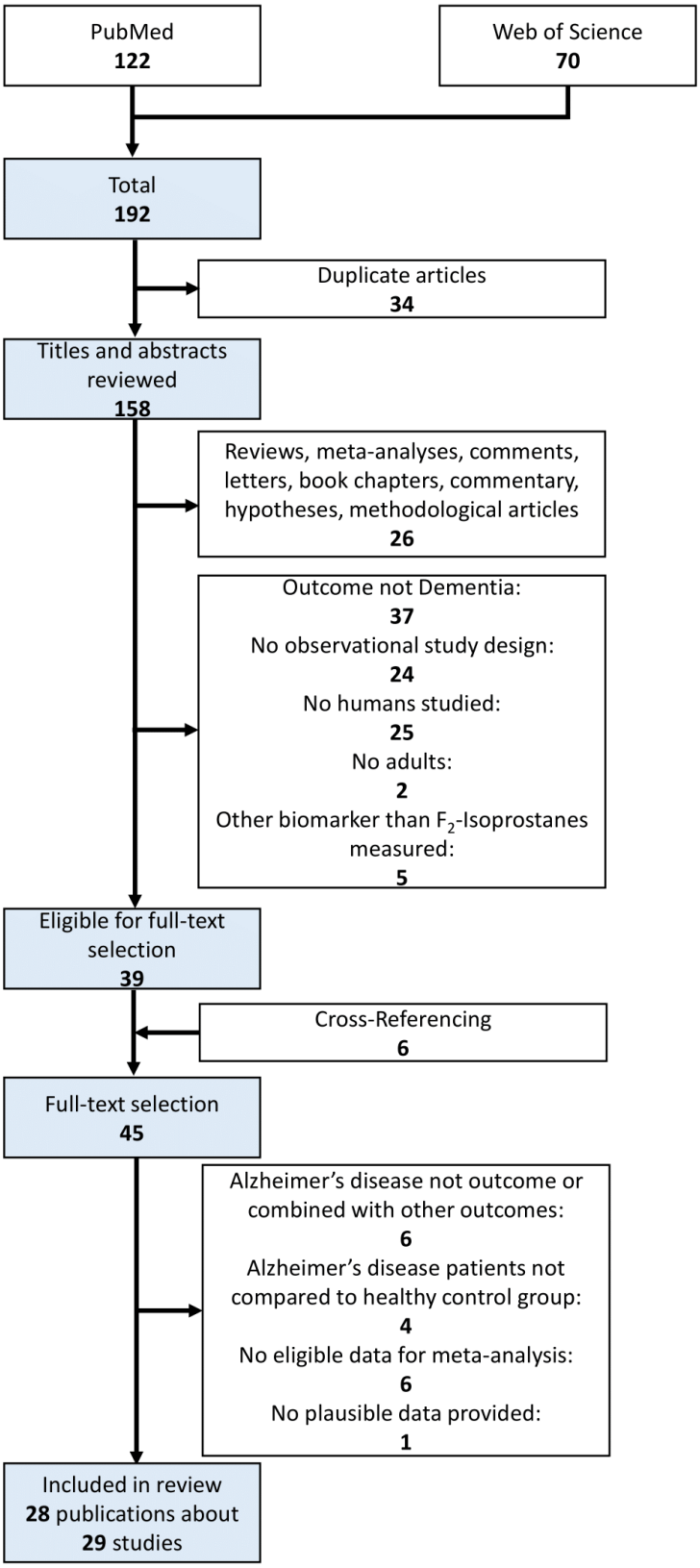

Figure 5. Flow chart describing literature search

3.2.2 Description of included studies

General information about the included studies can be found in **Supplemental Table 14**. Of the 29 studies, 25 had a cross-sectional study design, and only four were longitudinal. Since 1998, studies reporting in this research field have been continuously published. Most studies were conducted in the United States (n = 15), 12 were carried out in Europe, and two studies originated from Asia. Ten studies measured biomarker levels in urinary samples, ten in blood samples, nine in CSF samples, and four in tissue samples of the frontal lobe (some studies used multiple sample types). Measurements performed in urine and blood samples varied between using an immunological or analytical measurement technique, while all measurements in CSF or frontal lobe tissue samples were performed by analytical techniques. All studies examined participants older than 60 years. The number of AD cases of the 29 studies ranged from n = 4 to n = 160, but only four studies had more than 50 AD cases.

3.2.3 Risk of bias and confounding assessment

The results of the NOS scale can be found in **Supplemental Table 15**. The evaluation revealed a moderate risk of bias for most studies (scores of 4, 5, or 6 points). Four studies achieved 7 points and thus were regarded to have a low risk of bias (Ciabattoni et al. 2007; Montine et al. 2001; Peuchant et al. 2008). However, all these studies did not adjust for potential confounders. Sundelöf et al. and Trares et al. were the only ones adjusting their results for confounders (see **Supplemental Table 17** for details) and scored a maximum of 9 points on the NOS scale (Sundelöf et al. 2009; Trares et al. 2020).

3.2.4 Meta-analyses on cross-sectional studies

3.2.4.1 Biomarker and sample type-specific meta-analyses for cross-sectional studies

The studies were grouped according to biomarker (general F_2-isoprostanes or specific 8-iso-$PGF_{2\alpha}$ measurements) and sample type (urine, blood, CSF or frontal lobe tissue sample). Studies were available for all eight groups. However, only seven meta-analyses were performed because only one study measured 8-iso-$PGF_{2\alpha}$ levels in frontal lobe tissue samples

(Figure 6). The detailed results of studies reporting on general or specific F_2-isoprostanes are shown in **Supplemental Table 16,** and those reporting on the specific biomarker 8-iso-$PGF_{2\alpha}$ are shown in **Supplemental Table 17.**

Pooling the data gained from urinary samples, neither the general F_2-isoprostanes nor the specific 8-iso-$PGF_{2\alpha}$ measurements showed a statistically significant effect (Hedge's g [95% CI]: 0.60 [-0.01-1.21]; and 0.68 [-0.05-1.41]), respectively **(Figures 6A and 6B)**. Meta-analyses performed on studies conducted with blood samples yielded no significant differences for F_2-isoprostanes, but for 8-iso-$PGF_{2\alpha}$ levels (Hedge's g [95% CI]: 0.91 [-0.14-1.96]; and 0.68 [0.05-1.32], respectively) **(Figure 6C and 6D)**. With regards to CSF samples, a statistically significant difference between AD patients and controls was found for F_2-isoprostane levels (Hedge's g [95% CI]: 1.48 [0.97-1.98]; **Figure 6E)** but not for the specific biomarker 8-iso-$PGF_{2\alpha}$ (Hedge's g [95% CI]: 0.40 [-0.06-0.86]); **Figure 6F).** In total, four out of five studies, which used frontal lobe tissue samples, obtained statistically significant results, including the single study of Praticò et al. 1998 on 8-iso-$PGF_{2\alpha}$ with a Hedge's g [95% CI] of 1.93 [0.98-2.88] **(Figure 6H)**. The meta-analysis of the four studies with F_2-isoprostanes obtained a statistically significant pooled effect estimate as well (Hedge's g [95% CI]: 1.98 [0.77-3.20]); **(Figure 6G)**.

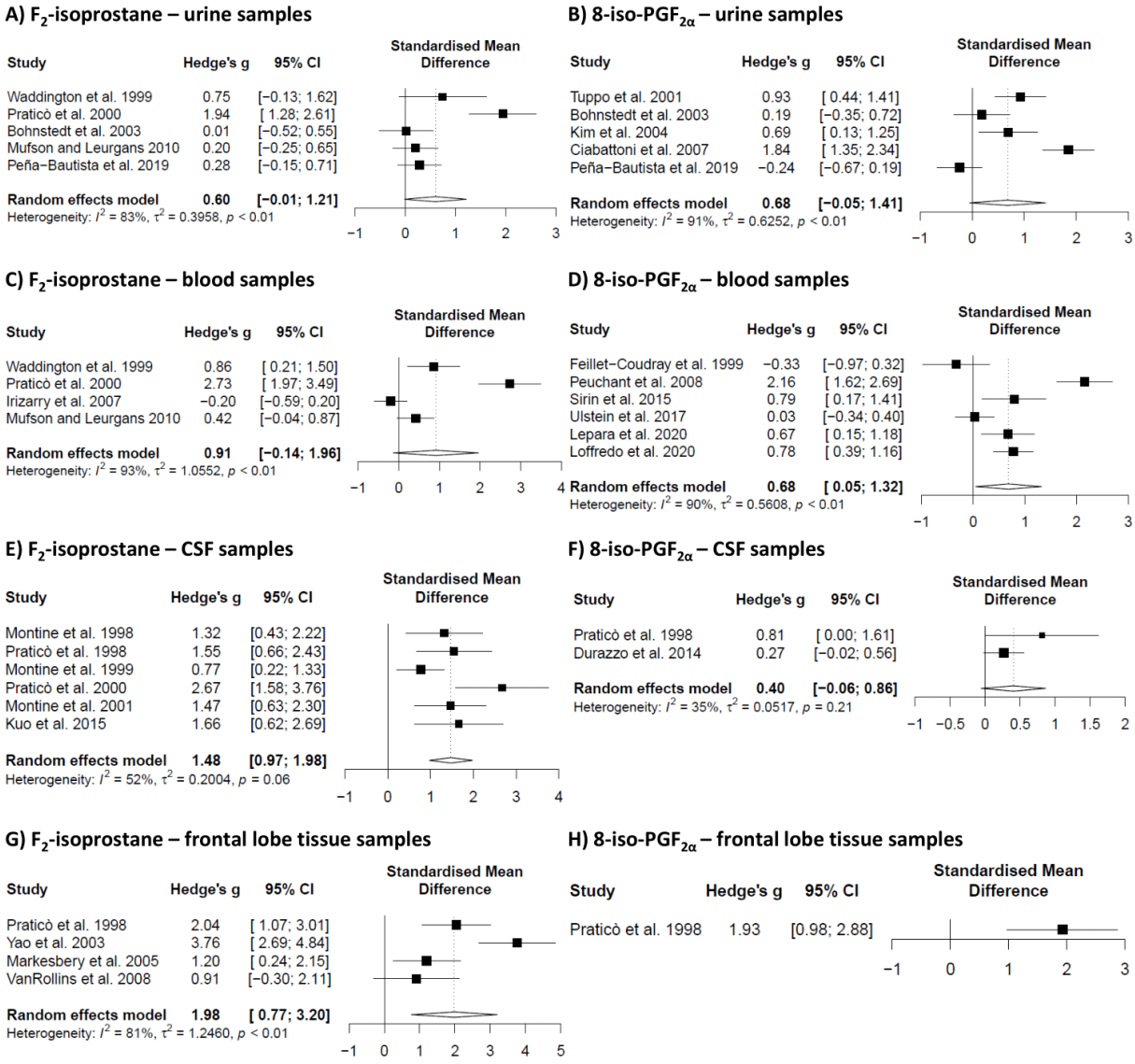

Figure 6. Meta-analyses assessing the association of F_2-isoprostane (left column) and 8-iso-prostaglandin $F_{2\alpha}$ levels (right column) with Alzheimer's disease in urine (A,B), blood (C,D), CSF (E,F) and tissue samples of the frontal lobe (G,H) in cross-sectional case control studies

3.2.4.2 Meta-regression

A meta-regression with all 25 cross-sectional studies was carried out to investigate sources of heterogeneity. If studies reported multiple results, either on biomarkers or sample type, only those with the highest Hedge's g were used for the meta-regression (Bohnstedt et al. 2003; Mufson and Leurgans 2010; Peña-Bautista et al. 2019; Praticò et al. 2000; Praticò et al. 1998; Waddington et al. 1999). Neither sample type, year of publication, biomarker, measurement technique, sample purification, AD diagnosis criteria, nor study quality had a statistically significant influence on the pooled effect of a meta-analysis of all cross-sectional studies **(Table 4)**.

Table 4. Results of meta-regression of included cross-sectional studies

Factor	β coefficient[a]	p-value
Sample type		
Urine	-1.17	0.3261
Blood	-1.11	0.2239
CSF	Ref.	Ref.
Tissue	0.41	0.6387
Year of publication	-0.03	0.4761
Biomarker		
F_2-isoprostanes	Ref.	Ref.
8-iso-prostaglandin $F_{2\alpha}$	-0.30	0.6874
Measurement technique		
Immunological method	Ref.	Ref.
Analytical method	-0.63	0.4413
Sample purification		
Not specified	-1.18	0.2040
Total F_2-isoprostanes	Ref.	Ref.
Free F_2-isoprostanes	-0.37	0.7462
Alzheimer's disease diagnosis criteria		
Not specified	Ref.	Ref.
NINCDS-ADRDA criteria	0.18	0.7673
DSM-IV criteria	0.34	0.7235
Other criteria	-0.14	0.8905
Study quality measured by Newcastle Ottawa Scale	0.27	0.4070

Abbreviations: CSF, cerebrospinal fluid; NINCDS-ADRDA, National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer's Disease and Related Disorders Association; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders Fourth Edition;

Notes: Numbers printed in bold are statistically significant ($p<0.05$).

[a]Linear regression model including all variables shown in this table.

3.2.4.3 Meta-analysis of all cross-sectional studies

Thus, as no relevant causes of heterogeneity were identified, it was judged to be appropriate also to present a pooled analysis of all cross-sectional studies **(Figure 7)**. The meta-analysis of the 25 studies showed significantly increased F_2-isoprostane/8-iso-$PGF_{2\alpha}$ levels among AD cases compared to healthy controls (Hedge's g [95% CI]: 1.00 [0.69-1.32]). Overall, 17 out of the 25 studies included in this analysis reported statistically significant differences between AD cases and controls. In the sensitivity analysis, using the lowest Hedge's g from studies with multiple results for the same population, the pooled effect estimate of the meta-analysis was attenuated but remained statistically significant (Hedge's g [95% CI]: 0.90 [0.58-1.22]) **(Figure 8)**. If multiple results of the same study population were published, the measurement with the lowest Hedge's g was chosen in this sensitivity analysis.

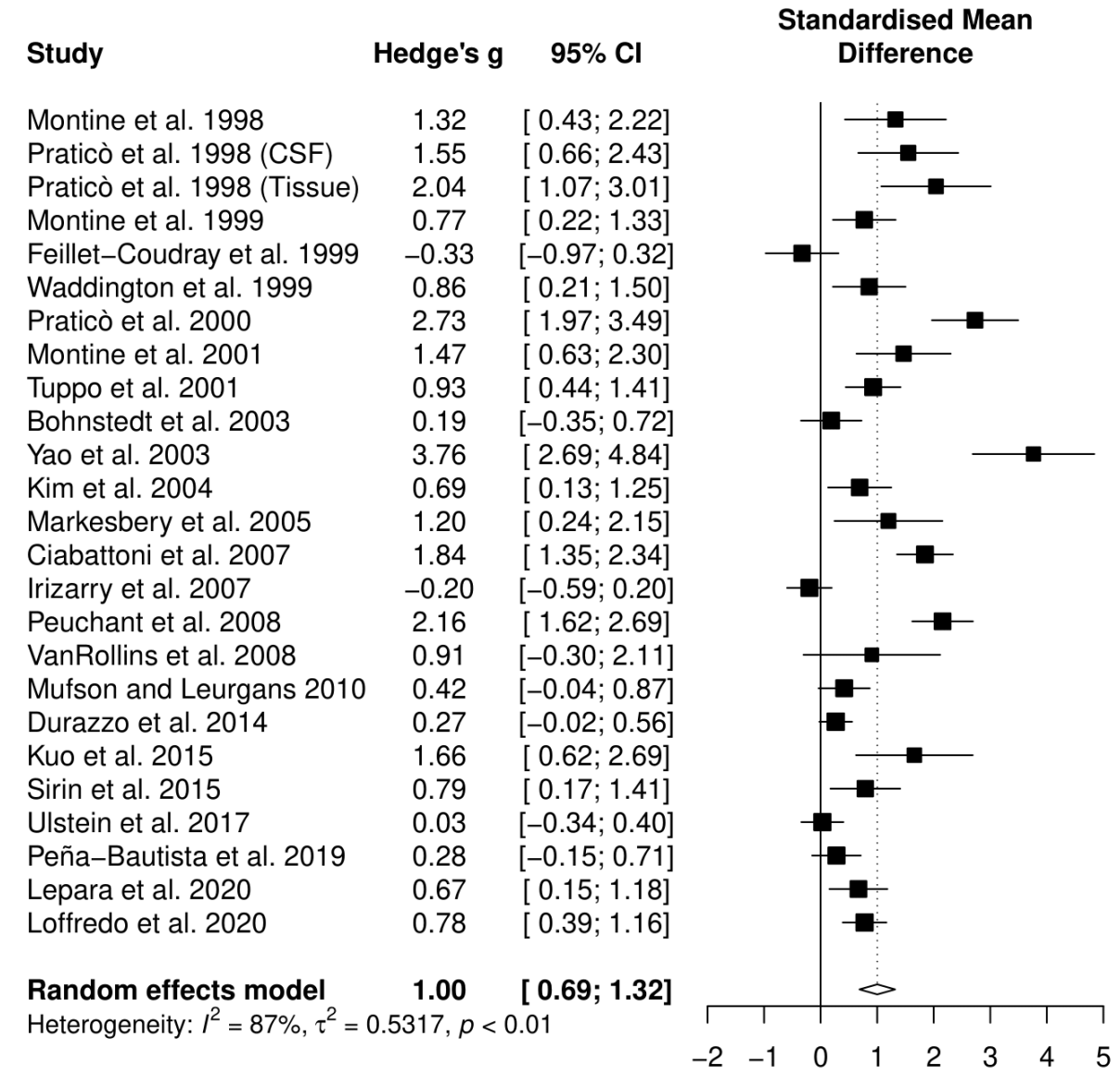

Figure 7. Forest plot of studies assessing the association between F_2-isoprostane levels and Alzheimer's disease incidence in different sample types in cross-sectional studies.
Results were pooled by random-effects meta-analysis.

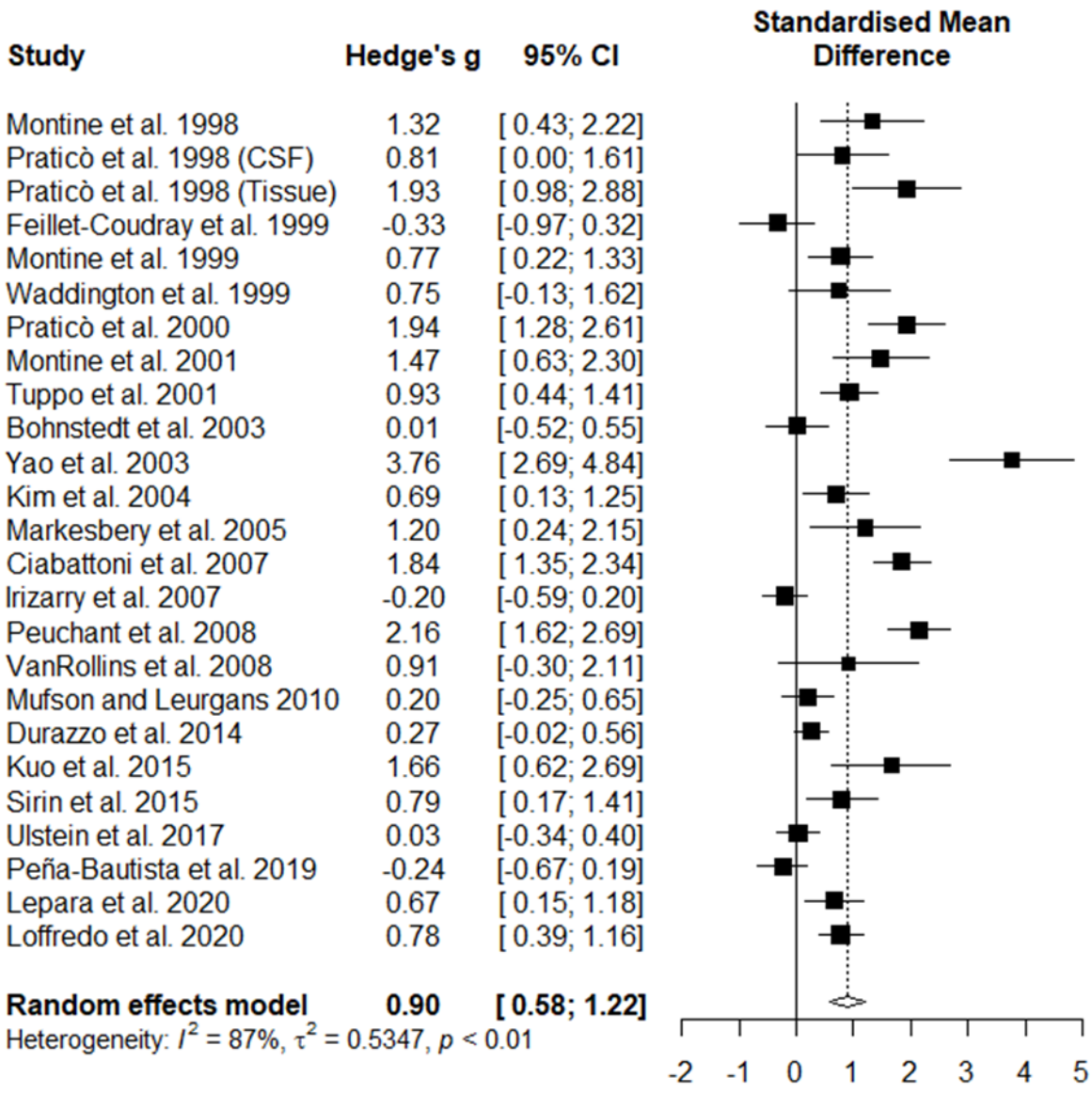

Figure 8. Sensitivity analysis for forest plot of studies assessing the association between F_2-isoprostane levels and Alzheimer's disease incidence in different sample types in cross-sectional studies.

3.2.5 Meta-analyses on longitudinal studies

Two studies examined F_2-isoprostane levels in longitudinal studies (Kester et al. 2012; Li et al. 2014). In both cases, CSF samples were used to determine the biomarker concentration. Contrary to the analysis on cross-sectional studies, the meta-analysis of these two longitudinal studies resulted in a non-significant difference between AD patients and controls (Hedge's g [95% CI]: -0.10 [-0.50-0.29]) **(Figure 9A)**. However, the samples sizes of the two studies were small. Kester and colleagues included 68 AD patients and 24 healthy controls, while Li and

colleagues included 7 AD patients and 135 healthy controls. Furthermore, the mean follow-up time was rather short, with about two years in the study of Kester and about four years in the study of Li.

Urinary 8-iso-$PGF_{2\alpha}$ levels were also examined by two longitudinal studies. The pooling of the two studies showed inconclusive results (HR [95% CI]: 1.14 [0.62-2.08] **(Figure 9B)**). Sundelöf and colleagues conducted their study in 2009 with a median follow-up of 5.1 years (Sundelöf et al. 2009). The second study was my own study from 2020 (Trares et al. 2020), which I updated for this systematic review by including three more years of follow-up (now: 17 years). I applied the same model as in my previous publication (described in chapter 2.1) but used a dichotomous 8-iso-$PGF_{2\alpha}$ level variable (</≥ median) this time to perform the same statistical methods as reported in the study of Sundelöf and colleagues. In contrast to their study, my study observed a statistically significant association of 8-iso-$PGF_{2\alpha}$ levels and AD incidence (HR [95% CI]: 1.44 [1.05-1.95]). The sample size in my study (n = 160 AD cases, n = 5,666 controls) was substantially higher compared to Sundelöf et al. (n = 47 AD cases, n = 681 controls) and all other individual studies included in this systematic review.

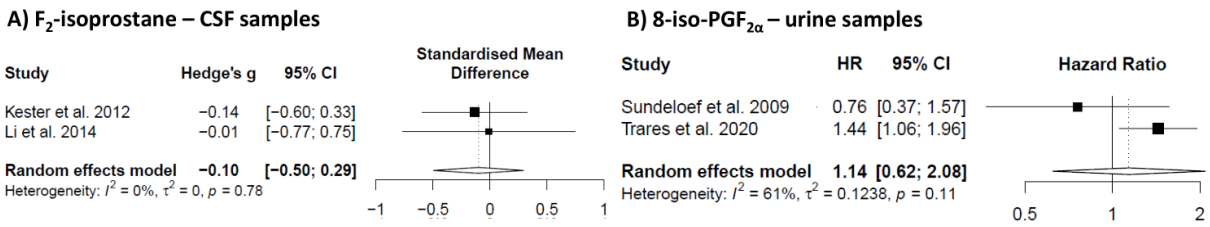

Figure 9. Forest plot of studies assessing the association between F_2-isoprostane levels and Alzheimer's disease incidence in different sample types in cross-sectional studies.
Results were pooled by random-effects meta-analysis.

3.2.6 Heterogeneity and publication bias

The heterogeneity was substantial (I^2 > 50%) in all meta-analyses except for cross-sectional studies on 8-iso-$PGF_{2\alpha}$ measured in CSF samples and longitudinal studies on F_2-isoprostane levels measured in CSF samples.

Publication bias was only observed in the meta-analysis of all 25 cross-sectional studies (Egger's test for symmetry of funnel plots: t = 3.61, df = 23, p = 0.0007). The funnel plot

indicated that small studies reporting insignificant results might not have been published **(Figure 10A)**. I used Duval and Tweedie's trim and fill method to impute the results of ten potentially not published studies to obtain a symmetric funnel plot **(Figure 10B)**. The random-effects summary estimate, including the potentially unpublished studies, was attenuated compared to the main result but still showed a statistically significant association of F_2-isoprostane levels with AD (Hedge's g [95% CI]: 0.42 [0.07-0.77]).

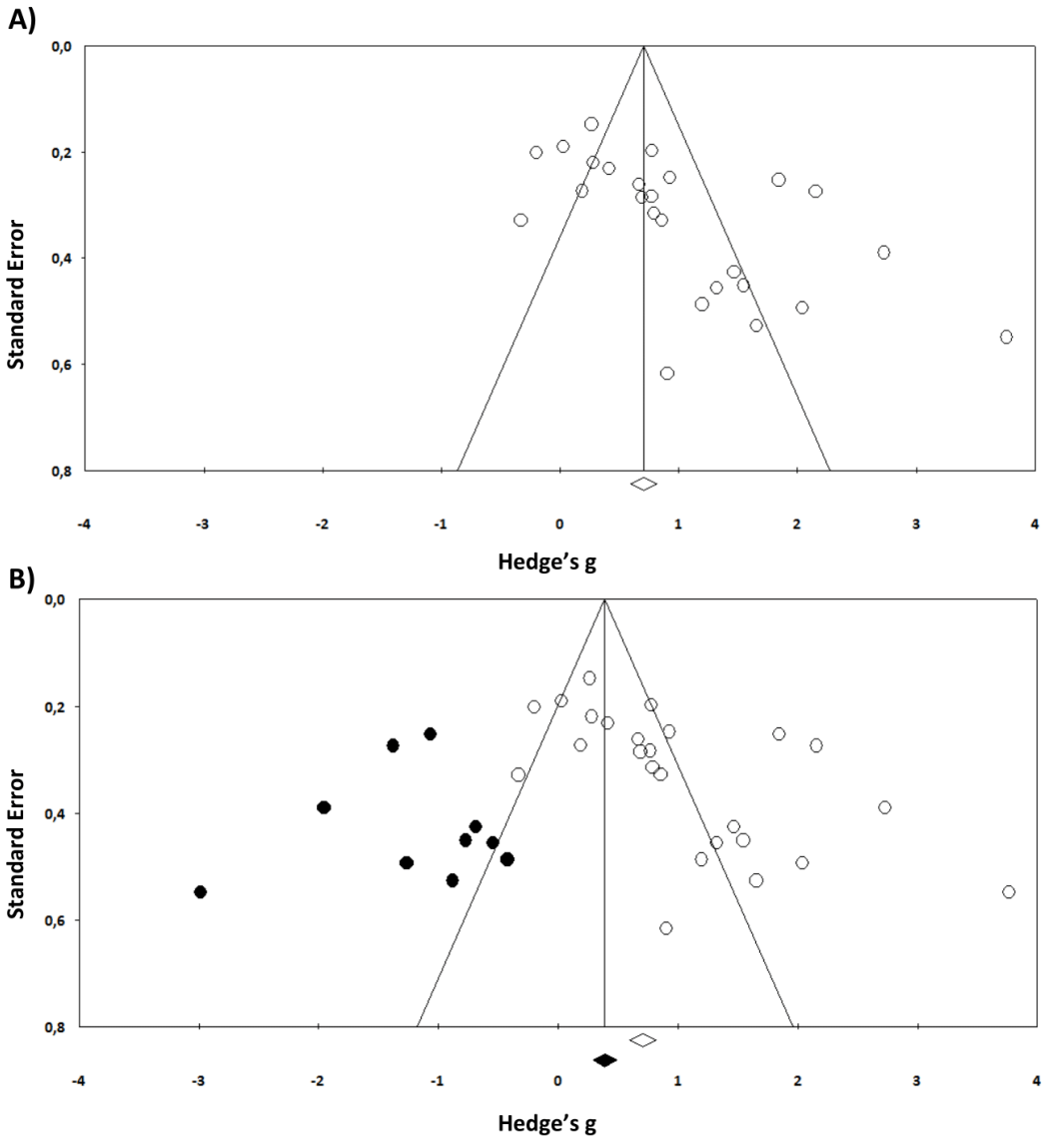

Figure 10. Funnel Plots

A) Funnel plot for the main meta-analysis of all 25 cross-sectional studies assessing publication bias with Egger's test for symmetry of funnel plots (t = 3.61, df = 23, p = 0.0007)29. Random effects model estimate: Hedge's g (95%CI): 1.00 (0.69-1.32). **B)** Funnel plot including imputed studies by Duval and Tweedie's trim and fill method30 indicated in black. Random effects model estimate: Hedge's g (95%CI): 0.42 (0.07-0.77).

3.3 Association of the inflammation-related proteome with dementia development at older age

Table 5 shows the baseline characteristics of 504 cases with incident dementia from any cause and 1,278 controls. The χ^2 test revealed significant differences between cases and controls in terms of age, physical activity, CVD, diabetes, lifetime history of depression, and *APOE* genotype. In the multivariate logistic regression analysis, CVD and diabetes lost statistical significance, but a trend towards an increased dementia risk could still be seen in the OR point estimates. Age, sex, lifetime history of depression with current pharmacotherapy and having at least one ε4 allele of the *APOE* gene remained significantly positively associated with all-cause dementia incidence. In contrast, physical activity remained significantly inversely associated.

Table 5. Baseline characteristics of included study participants (n = 1,782)

Baseline characteristics	*n* (%)	All-cause dementia cases (n = 504)	Controls (n = 1278)	χ^2 test p-value	Multivariate Odds Ratio (95%CI) ‡
Age (years)				**< 0.0001**	
50-64	956 (53.65)	154 (30.56)	802 (62.75)		1.00 Ref.
65-69	458 (25.70)	157 (31.15)	301 (23.55)		**2.57 (1.96-3.37)**
70-75	368 (20.65)	193 (38.29)	175 (13.69)		**5.37 (4.03-7.15)**
Sex				0.2486	
Female	965 (54.15)	262 (51.98)	703 (55.01)		1.00 Ref.
Male	817 (45.85)	242 (48.02)	575 (44.99)		**1.28 (1.01-1.63)**
Education (years)				0.0868	
< 9	1344 (77.42)	391 (80.79)	953 (76.12)		1.00 Ref.
9-11	216 (12.44)	48 (9.92)	168 (13.42)		0.84 (0.58-1.23)
≥ 12	176 (10.14)	45 (9.30)	131 (10.46)		0.93 (0.62-1.38)
Physical activity*				**< 0.0001**	
Inactive	383 (21.54)	150 (29.82)	233 (18.27)		1.00 Ref.
Low	814 (45.78)	220 (43.74)	594 (46.59)		**0.65 (0.49-0.86)**
Medium or high	581 (32.68)	133 (26.44)	448 (35.14)		**0.60 (0.44-0.83)**
BMI (kg/m²)				0.5708	
< 25	478 (26.91)	144 (28.63)	334 (26.24)		1.00 Ref.
25-<30	832 (46.85)	228 (45.33)	604 (47.45)		0.85 (0.65-1.12)
≥30	466 (26.24)	131 (26.04)	335 (26.32)		0.85 (0.62-1.17)
CVD†				**< 0.0001**	
No	1373 (77.05)	350 (69.44)	1023 (80.05)		1.00 Ref.
Yes	409 (22.95)	154 (30.56)	255 (19.95)		1.20 (0.92-1.56)
Diabetes				**0.0001**	
No	1469 (83.61)	386 (78.14)	1083 (85.75)		1.00 Ref.
Yes	288 (16.39)	108 (21.86)	180 (14.25)		1.29 (0.96-1.74)

Baseline characteristics	*n* (%)	All-cause dementia cases (n = 504)	Controls (n = 1278)	χ^2 test p-value	Multivariate Odds Ratio (95%CI) ‡
Life-time history of depression				**0.0225**	
No	1527 (85.69)	427 (84.72)	1100 (86.07)		1.00 Ref.
Yes, without current pharmacotherapy	184 (10.33)	47 (9.33)	137 (10.72)		1.01 (0.69-1.49)
Yes, with current pharmacotherapy	71 (3.98)	30 (5.95)	41 (3.21)		**2.26 (1.33-3.85)**
APOE genotypes				**< 0.0001**	
ε2/ε2	18 (1.09)	1 (0.22)	17 (1.42)		0.25 (0.04-1.47)
ε2/ε3	238 (14.43)	57 (12.58)	181 (15.13)		1.06 (0.75-1.52)
ε2/ε4	55 (3.34)	22 (4.86)	33 (2.76)		**2.77 (1.52-5.06)**
ε3/ε3	929 (56.34)	218 (48.12)	711 (59.45)		1.00 Ref.
ε3/ε4	379 (22.98)	135 (29.80)	244 (20.40)		**1.79 (1.35-2.37)**
ε4/ε4	30 (1.82)	20 (4.42)	10 (0.84)		**7.15 (3.18-16.08)**

Abbreviations: CI, confidence interval; BMI, body mass index; CVD, cardiovascular disease; *APOE*, apolipoprotein E;

NOTE: Numbers printed in bold are statistically significant (p < 0.05).

* "Inactive" was defined by < 1 h of vigorous or < 1 h light physical activity per week. "Medium or high" was defined by ≥ 2 h of vigorous and ≥ 2 h of light physical activity/week. All other amounts of physical activity were grouped into the category "Low".

† CVD was defined as coronary artery disease or a self-reported history of myocardial infarction, stroke, pulmonary embolism, or revascularization of coronary arteries.

‡ Results of multivariate logistic regression model including all variables shown in this table (imputed dataset).

Among the included 504 all-cause dementia cases, 163 and 195 participants developed AD and VD, respectively. The medians of all inflammation-related protein levels of all-cause dementia, AD, and VD cases were separately compared with those of controls (**Supplemental Tables 18-20**). In this univariate analysis, n = 60, n = 51 and n = 52 biomarker levels of the Olink inflammation panel were significantly increased in all-cause dementia, AD, and VD cases, respectively (FDR < 0.05).

Tables 6-8 show the multivariate logistic regression model results for those 58, 22, and 33 biomarkers, significantly associated with all-cause dementia, AD, and VD incidence, respectively, after FDR correction. **Supplemental Tables 21-23** show the non-significant ones. The associations' strengths were comparable and ranged for the various biomarker-outcome associations from OR point estimates of 1.12 to 1.51 per 1 SD increase. The forward selection

revealed that only two (CX3CL1 and EN-RAGE), two (EN-RAGE and LAP TGF-beta-1), and one (VEGF-A) inflammation-related proteins were independently, positively associated with all-cause dementia, AD, and VD, respectively. The reason for the low number of independent inflammation biomarkers was mainly due to high inter-correlation. Overall, 18, 26, 16, and 28 biomarkers of the Olink inflammation panel had a Spearman's r > 0.5 with CX3CL1, EN-RAGE, LAP TGF-beta 1, and VEGF-A, respectively (**Supplemental Tables 24-27**).

Table 6. Associations of significantly associated Olink Biomarker levels with all-cause dementia incidence.

Olink Biomarker	Value of 1 SD	All-cause dementia (n=504 cases)		
		OR (95% CI) per 1 SD*	p-value per 1 SD	FDR corrected p-value†
ADA	0.635	1.17 (1.05-1.32)	**0.0055**	**0.0098**
AXIN1	1.140	1.14 (1.02-1.28)	**0.0257**	**0.0352**
CASP-8	1.364	1.15 (1.02-1.29)	**0.0194**	**0.0279**
CCL3	1.508	1.14 (1.02-1.27)	**0.0259**	**0.0352**
CCL4	1.099	1.19 (1.06-1.33)	**0.0032**	**0.0066**
CCL11	0.697	1.29 (1.14-1.46)	**0.0001**	**0.0003**
CCL19	1.199	1.17 (1.05-1.32)	**0.0063**	**0.0108**
CCL20	1.540	1.18 (1.05-1.32)	**0.0041**	**0.0082**
CCL23	0.732	1.29 (1.14-1.46)	**<0.0001**	**0.0003**
CCL25	0.763	1.17 (1.03-1.32)	**0.0122**	**0.0187**
CCL28	0.548	1.27 (1.13-1.43)	**<0.0001**	**0.0003**
CD5	0.523	1.26 (1.12-1.42)	**0.0002**	**0.0007**
CD6	0.757	1.19 (1.05-1.34)	**0.0048**	**0.0093**
CD40	0.734	1.20 (1.07-1.36)	**0.0022**	**0.0050**
CD244	0.587	1.38 (1.22-1.57)	**<0.0001**	**0.0003**
CDCP1	0.894	1.20 (1.06-1.36)	**0.0030**	**0.0064**
CSF-1	0.425	1.24 (1.09-1.41)	**0.0013**	**0.0032**
CST5	0.698	1.17 (1.04-1.32)	**0.0112**	**0.0179**
CX3CL1	0.669	1.41 (1.24-1.60)	**<0.0001**	**0.0003**
CXCL1	0.901	1.17 (1.05-1.32)	**0.0065**	**0.0109**
CXCL5	0.957	1.33 (1.17-1.51)	**<0.0001**	**0.0003**
CXCL6	0.848	1.28 (1.14-1.44)	**0.0001**	**0.0003**
CXCL9	0.953	1.19 (1.05-1.34)	**0.0052**	**0.0096**
CXCL10	0.953	1.16 (1.03-1.30)	**0.0138**	**0.0207**
CXCL11	1.051	1.18 (1.05-1.33)	**0.0051**	**0.0096**
DNER	0.488	1.36 (1.20-1.55)	**<0.0001**	**0.0003**
EN-RAGE	1.307	1.41 (1.25-1.60)	**<0.0001**	**0.0003**
FGF-19	1.089	1.21 (1.08-1.35)	**0.0013**	**0.0032**
Flt3L	0.629	1.21 (1.07-1.36)	**0.0018**	**0.0042**
GDNF	0.506	1.16 (1.03-1.30)	**0.0149**	**0.0219**
HGF	0.719	1.34 (1.18-1.52)	**<0.0001**	**0.0003**
IL-7	0.798	1.14 (1.02-1.28)	**0.0246**	**0.0347**
IL-10	0.863	1.20 (1.07-1.35)	**0.0023**	**0.0050**
IL-18	0.763	1.33 (1.17-1.50)	**<0.0001**	**0.0003**

Olink Biomarker	Value of 1 SD	All-cause dementia (n=504 cases)		
		OR (95% CI) per 1 SD*	p-value per 1 SD	FDR corrected p-value†
IL-10RA	0.788	1.12 (1.01-1.25)	**0.0362**	**0.0461**
IL-10RB	0.533	1.29 (1.14-1.46)	**0.0001**	**0.0003**
IL-15RA	0.359	1.22 (1.09-1.38)	**0.0009**	**0.0026**
IL-18R1	0.602	1.27 (1.13-1.44)	**0.0001**	**0.0003**
LAP TGF-beta-1	0.574	1.37 (1.21-1.55)	**<0.0001**	**0.0003**
LIF-R	0.503	1.37 (1.21-1.56)	**<0.0001**	**0.0003**
MCP-2	0.769	1.18 (1.05-1.33)	**0.0069**	**0.0113**
MCP-4	0.927	1.23 (1.08-1.39)	**0.0015**	**0.0036**
MMP-10	0.761	1.22 (1.08-1.37)	**0.0010**	**0.0028**
NT-3	0.544	1.24 (1.10-1.39)	**0.0003**	**0.0009**
OPG	0.609	1.39 (1.22-1.58)	**<0.0001**	**0.0003**
PD-L1	0.600	1.30 (1.15-1.47)	**<0.0001**	**0.0003**
SCF	0.624	1.15 (1.01-1.29)	**0.0281**	**0.0375**
SIRT2	1.157	1.13 (1.01-1.27)	**0.0371**	**0.0461**
ST1A1	1.304	1.13 (1.01-1.27)	**0.0365**	**0.0461**
STAMBP	0.833	1.18 (1.05-1.32)	**0.0056**	**0.0098**
TGF-alpha	0.829	1.21 (1.08-1.37)	**0.0011**	**0.0029**
TNFRSF9	0.636	1.23 (1.09-1.39)	**0.0006**	**0.0018**
TNFSF14	1.064	1.16 (1.03-1.30)	**0.0119**	**0.0186**
TRAIL	0.513	1.31 (1.16-1.49)	**<0.0001**	**0.0003**
TRANCE	0.753	1.14 (1.01-1.28)	**0.0344**	**0.0450**
TWEAK	0.647	1.35 (1.19-1.53)	**<0.0001**	**0.0003**
VEGF-A	0.794	1.40 (1.24-1.59)	**<0.0001**	**0.0003**
uPA	0.604	1.36 (1.20-1.54)	**<0.0001**	**0.0003**

Abbreviations: SD, standard deviation; CI, confidence interval; FDR, false discovery rate; For biomarker abbreviations, see Supplemental Table 5.

Notes: Numbers printed in bold are statistically significant ($p < 0.05$). Grey shade: Independently associated with the outcome.

For associations of not significantly associated biomarkers, see Supplemental Table 21.

* Multivariate logistic regression model adjusted for age (continuously), sex, education, physical activity, BMI (categorical), CVD, diabetes, depression, *APOE* genotype.

† P-values corrected for multiple testing by the Benjamini and Hochberg method.

Table 7. Associations of significantly associated Olink Biomarker levels with Alzheimer's disease incidence

Olink Biomarker	Value of 1 SD	Alzheimer's disease (n = 163 cases)		
		OR (95% CI) per 1 SD*	p-value per 1 SD	FDR corrected p-value†
CASP-8	1.364	1.31 (1.10-1.57)	**0.0025**	**0.0156**
CCL23	0.732	1.43 (1.17-1.75)	**0.0004**	**0.0086**
CCL28	0.548	1.36 (1.14-1.61)	**0.0005**	**0.0086**
CD6	0.757	1.30 (1.08-1.58)	**0.0067**	**0.0254**
CD244	0.587	1.39 (1.14-1.70)	**0.0010**	**0.0120**
CX3CL1	0.669	1.35 (1.10-1.65)	**0.0034**	**0.0175**
CXCL5	0.957	1.37 (1.12-1.68)	**0.0023**	**0.0156**

Olink Biomarker	Value of 1 SD	Alzheimer's disease (n = 163 cases)		
		OR (95% CI) per 1 SD*	p-value per 1 SD	FDR corrected p-value†
CXCL6	0.848	1.34 (1.11-1.62)	**0.0026**	**0.0156**
DNER	0.488	1.37 (1.12-1.68)	**0.0025**	**0.0156**
EN-RAGE	1.307	1.51 (1.25-1.83)	**<0.0001**	**0.0036**
HGF	0.719	1.36 (1.12-1.66)	**0.0017**	**0.0153**
IL-10RB	0.533	1.33 (1.08-1.63)	**0.0066**	**0.0254**
LAP TGF-beta-1	0.574	1.46 (1.21-1.76)	**0.0001**	**0.0036**
LIF-R	0.503	1.31 (1.08-1.60)	**0.0062**	**0.0254**
PD-L1	0.600	1.31 (1.09-1.57)	**0.0034**	**0.0175**
ST1A1	1.304	1.30 (1.08-1.57)	**0.0062**	**0.0254**
STAMBP	0.833	1.26 (1.06-1.51)	**0.0108**	**0.0370**
TGF-alpha	0.829	1.26 (1.05-1.52)	**0.0140**	**0.0458**
TRAIL	0.513	1.30 (1.06-1.59)	**0.0104**	**0.0370**
TWEAK	0.647	1.38 (1.13-1.69)	**0.0016**	**0.0153**
VEGF-A	0.794	1.32 (1.09-1.60)	**0.0042**	**0.0202**
uPA	0.604	1.40 (1.16-1.71)	**0.0006**	**0.0086**

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Abbreviations: SD, standard deviation; CI, confidence interval; FDR, false discovery rate; For biomarker abbreviations, see Supplemental Table 5.

Notes: Numbers printed in bold are statistically significant ($p < 0.05$). Grey shade: Independently associated with the outcome. Only Alzheimer's disease cases were included in this analysis.

For associations of not significantly associated biomarkers, see Supplemental Table 22.

* Multivariate logistic regression model adjusted for age (continuously), sex, education, physical activity, BMI (categorical), CVD, diabetes, depression, *APOE* genotype.

† P-values corrected for multiple testing by the Benjamini and Hochberg method.

Table 8. Associations of significantly associated Olink Biomarker levels with vascular dementia incidence.

Olink Biomarker	Value of 1 SD	Vascular dementia (n = 195 cases)		
		OR (95% CI) per 1 SD*	p-value per 1 SD	FDR corrected p-value†
CCL11	0.697	1.30 (1.09-1.56)	**0.0042**	**0.0137**
CCL23	0.732	1.24 (1.04-1.48)	**0.0148**	**0.0347**
CD5	0.523	1.32 (1.11-1.56)	**0.0016**	**0.0091**
CD244	0.587	1.39 (1.16-1.66)	**0.0004**	**0.0072**
CDCP1	0.894	1.25 (1.05-1.48)	**0.0122**	**0.0313**
CX3CL1	0.669	1.35 (1.13-1.61)	**0.0011**	**0.0072**
CXCL1	0.901	1.22 (1.04-1.43)	**0.0151**	**0.0347**
CXCL5	0.957	1.39 (1.16-1.67)	**0.0004**	**0.0072**
CXCL6	0.848	1.32 (1.11-1.57)	**0.0018**	**0.0091**
CXCL9	0.953	1.25 (1.06-1.47)	**0.0089**	**0.0256**
CXCL10	0.953	1.28 (1.09-1.50)	**0.0029**	**0.0116**
DNER	0.488	1.37 (1.13-1.65)	**0.0010**	**0.0072**
EN-RAGE	1.307	1.41 (1.18-1.68)	**0.0001**	**0.0036**
Flt3L	0.629	1.22 (1.03-1.45)	**0.0226**	**0.0493**

HGF	0.719	1.32 (1.11-1.58)	**0.0019**	**0.0091**
IL-7	0.798	1.24 (1.05-1.47)	**0.0107**	**0.0296**
IL-10	0.863	1.27 (1.09-1.48)	**0.0017**	**0.0091**
IL-18	0.763	1.36 (1.14-1.63)	**0.0006**	**0.0072**
IL-18R1	0.602	1.30 (1.09-1.55)	**0.0034**	**0.0122**
LAP TGF-beta-1	0.574	1.33 (1.12-1.57)	**0.0011**	**0.0072**
LIF-R	0.503	1.36 (1.14-1.63)	**0.0007**	**0.0072**
MCP-2	0.769	1.24 (1.04-1.47)	**0.0154**	**0.0347**
MCP-4	0.927	1.26 (1.05-1.51)	**0.0114**	**0.0304**
MMP-10	0.761	1.30 (1.10-1.54)	**0.0022**	**0.0099**
NT-3	0.544	1.26 (1.08-1.46)	**0.0025**	**0.0106**
OPG	0.609	1.38 (1.15-1.67)	**0.0007**	**0.0072**
PD-L1	0.600	1.26 (1.07-1.49)	**0.0056**	**0.0175**
TGF-alpha	0.829	1.23 (1.05-1.46)	**0.0126**	**0.0313**
TNFRSF9	0.636	1.26 (1.06-1.49)	**0.0073**	**0.0219**
TRAIL	0.513	1.32 (1.09-1.59)	**0.0037**	**0.0127**
TWEAK	0.647	1.36 (1.13-1.63)	**0.0010**	**0.0072**
VEGF-A	0.794	1.43 (1.20-1.70)	**0.0001**	**0.0036**
uPA	0.604	1.30 (1.09-1.55)	**0.0034**	**0.0122**

Abbreviations: SD, standard deviation; CI, confidence interval; FDR, false discovery rate; For biomarker abbreviations, see Supplemental Table 5.

Notes: Numbers printed in bold are statistically significant ($p < 0.05$). Grey shad: Independently associated with the outcome. Only vascular dementia cases were included in this analysis.

For associations of not significantly associated biomarkers, see Supplemental Table 23.

* Multivariate logistic regression model adjusted for age (continuously), sex, education, physical activity, BMI (categorical), CVD, diabetes, depression, *APOE* genotype.

† P-values corrected for multiple testing by the Benjamini and Hochberg method.

When the two independent biomarkers for all-cause dementia were added simultaneously to the logistic regression models, the OR point estimates per 1 SD increase were attenuated but remained statistically significant (CX3CL1, OR [95% CI]: 1.29 [1.13-1.47], p = 0.0002; EN-RAGE, OR [95% CI]: 1.31 [1.15-1.49], p<0.0001). This was also the case for the two independent biomarkers for AD (EN-RAGE, OR [95% CI]: 1.37 [1.10-1.68], p = 0.0048; LAP TGF-beta-1, OR [95% CI]: 1.28 [1.04-1.58], p = 0.0187). For VD, only one independent biomarker was included (VEGF-A, OR [95% CI]: 1.43 [1.20-1.70], p<0.0001). The dose-response curves of these five biomarker-dementia outcome associations are shown in **Figure 11**. The risk of VD seems to start to increase only at higher VEGF-A levels (> 60th percentile). The other four biomarker-dementia associations show a more or less linear risk increase over the whole biomarker level distribution.

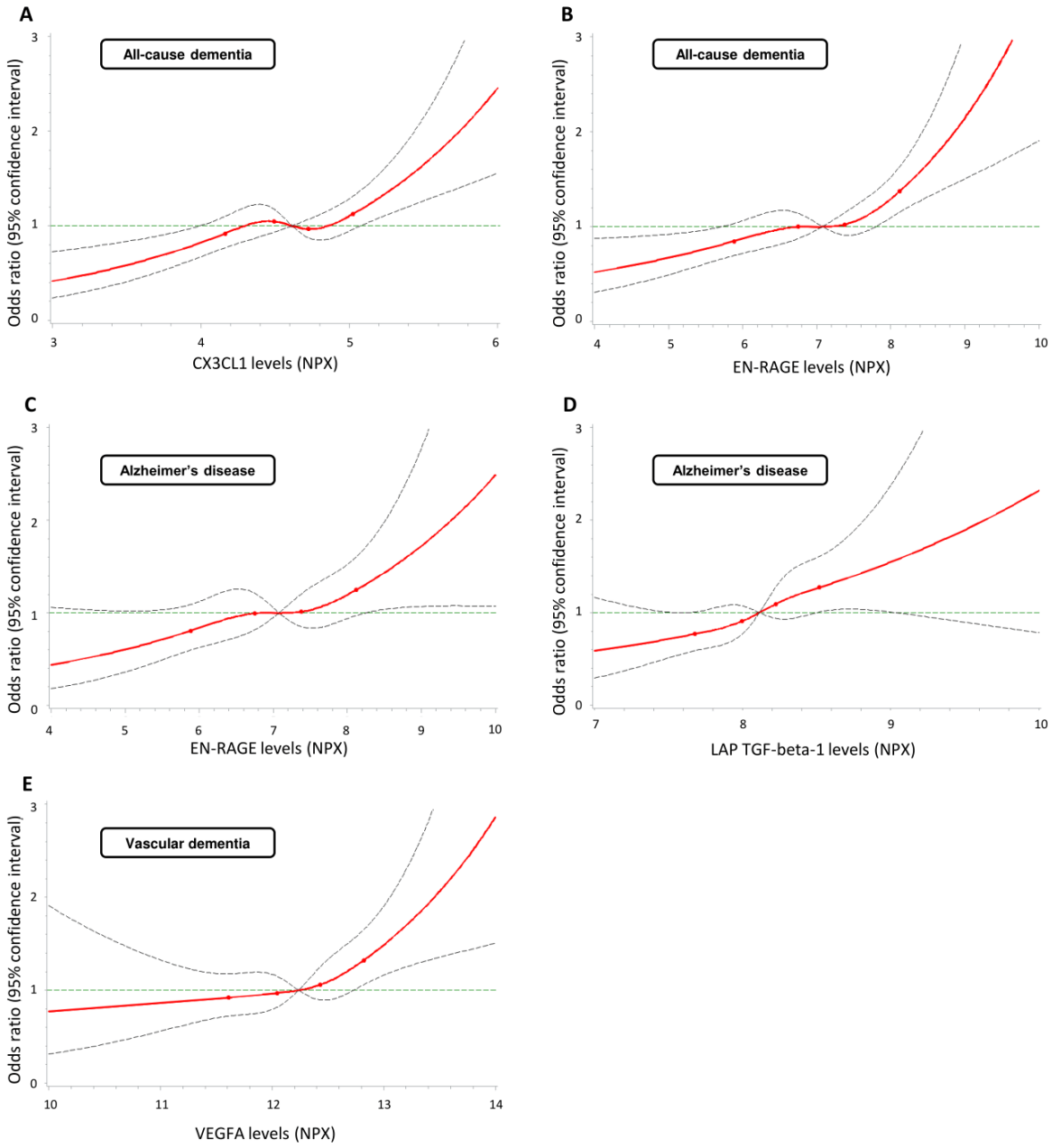

Figure 11. Association of all-cause dementia with (A) CX3CL1 and (B) EN-RAGE, Alzheimer's disease with (C) EN-RAGE and (D) LAP TGF-beta-1, and vascular dementia with (E) VEGFA in a spline regression model adjusted for age (continuously), sex, education, physical activity, BMI (categorical), cardiovascular disease, diabetes, depression, *APOE* genotype.

Solid red lines: estimation; dashed curved lines (black): 95% confidence interval limits; dashed horizontal line (green): reference line (hazard ratio = 1); dots: knots (20th, 40th, 60th, and 80th percentile).
Abbreviations: NPX, Normalized Protein eXpression

The results for these five selected biomarker-dementia endpoint associations are shown stratified for age, sex, obesity, diabetes, CVD, *APOE* ε4 in **Supplemental Tables 28-32**. Generally, results were similar in subgroups defined by the first four factors. For *APOE* ε4, there was a consistent pattern towards stronger associations of inflammation biomarkers

among *APOE* ε4 negative subjects. In line with this observation, the only statistically significant interaction found was between *APOE* ε4 polymorphism and the biomarker EN-RAGE for all-cause dementia (p = 0.024, **Supplemental Table 29**).

The results of the sensitivity analyses are also shown in **Supplemental Tables 28-32**. When stratified by time of diagnosis, all selected biomarkers had a stronger association with dementia diagnoses occurring in the first ten years of follow-up. However, significant associations were also observed for diagnoses in later years of follow-up. Besides, excluding subjects who died before their 80th birthday or had a sign of acute infection (CRP level >20mg/L) did not alter the results to any relevant extent.

3.4 Improved risk prediction of all-cause dementia, Alzheimer's disease, and vascular dementia by biomarkers of oxidative stress and inflammation

Table 9 shows the CAIDE model variables of all included study participants separately for all-cause dementia (n = 440), AD (n = 147), and VD (n = 167) cases, as well as healthy controls (n = 1197). The mean age of the all-cause dementia cases (mean (±SD): 66.9 (5.2) years) was five years higher than that of the controls (61.9 (6.4) years). Furthermore, more subjects among controls had a higher school education than the basic education of 9 years (23.1%) than among the all-cause dementia cases (18.9%). More men were among cases (55.6%) than controls (44.4%). Mean values for systolic blood pressure, BMI, and total cholesterol levels were comparable between all-cause dementia cases and controls. In addition, all-cause dementia cases included a higher proportion of participants being physically inactive (28.4% compared to 17.8%) and a distinctly increased number of *APOE* ε4 carriers than controls (40.9% compared to 23.8%). In a multivariate logistic regression model, only age, total cholesterol (inversely), physical activity (inversely) and *APOE* genotype were statistically significantly associated with all-cause dementia (**Supplemental Table 33**). In the model for AD (**Supplemental Table 34**), total cholesterol was additionally not significant, and in the model for VD (**Supplemental Table 35**), total cholesterol and physical activity were not statistically significant. Age and *APOE* genotype were statistically significantly associated with all dementia outcomes.

Table 9. CAIDE model variables of included participants (n = 1,637)

CAIDE model variables	Controls (n=1,197)	Cases		
		All-cause dementia (n=440)	Alzheimer's disease (n=147)	Vascular dementia (n=167)
Age (years), mean (SD)	61.9 (6.4)	66.9 (5.2)	66.7 (5.1)	67.1 (4.9)
Mid-life (50-64 years), n (%)	751 (62.7)	137 (31.1)	48 (32.7)	50 (29.9)
Late-life (65-75 years), n (%)	446 (37.3)	303 (68.9)	99 (67.3)	117 (70.1)
Education (years), mean (SD)				
≤ 9	920 (76.9)	357 (81.1)	122 (83.0)	135 (80.8)
> 9	277 (23.1)	83 (18.9)	25 (17.0)	32 (19.2)
Sex, n (%)				
Female	665 (55.6)	224 (50.9)	82 (55.8)	83 (49.7)
Male	532 (44.4)	216 (49.1)	65 (44.2)	84 (50.3)
SBP (mmHg), mean (SD)	139.4 (19.6)	142.61 (18.7)	142.22 (19.0)	142.87 (19.1)
BMI (kg/m^2), mean (SD)	27.86 (4.4)	27.51 (3.9)	27.13 (3.8)	27.57 (3.9)
Total cholesterol (mmol/L), mean (SD)	5.86 (1.2)	5.70 (1.3)	5.75 (1.3)	5.72 (1.3)
Physical activity [a], n (%)				
Inactive	213 (17.8)	125 (28.4)	47 (32.0)	43 (25.8)
Active	984 (82.2)	315 (71.6)	100 (68.0)	124 (74.3)
***APOE* genotypes, n (%)**				
ε4 non-carrier	912 (76.2)	260 (59.1)	73 (49.67)	106 (63.5)
ε4 carrier	285 (23.8)	180 (40.9)	74 (50.3)	61 (36.5)

Abbreviations: *APOE*, apolipoprotein E; SBP, systolic blood pressure; BMI, body mass index.

[a]"Inactive" was defined by <1 hour of vigorous or <1 hour of light physical activity per week. All other amounts of physical activity were grouped into the category "Active."

3.4.1 Predictive ability of inflammation-related biomarkers

Table 10 shows the discriminative performances of various prediction models for all-cause dementia, AD, and VD. In the total cohort, all CAIDE models had a high discriminative performance with an AUC > 0.71 and improved by 0.019-0.037 AUC increments when inflammatory biomarkers selected by the LASSO logistic regression were added to the model. The AUC differences for both CAIDE model 1 and 2 were statistically significant for all-cause dementia but neither for AD nor VD. The inflammation-related biomarkers selected by LASSO regression are shown in **Table 11**. In total, 16, 11, and 26 inflammatory biomarkers were added to the CAIDE model 1 for all-cause dementia, AD, and VD, respectively. The selected biomarkers differed between the outcomes but were similar for CAIDE model 1 and 2 for each outcome. The β-coefficients of all variables needed to calculate risk scores for the CAIDE + inflammatory biomarkers models for all-cause dementia, AD and VD can be found in **Supplemental Tables 36-38**, respectively.

Table 10. Discrimination performance of models

		n_{total}	n_{cases}	CAIDE Model 1[a]		CAIDE Model 2[b]	
				AUC (95% CI)	Δ AUC (95% CI)[c]	AUC (95% CI)	Δ AUC (95% CI)[c]
Total cohort	**All-cause dementia**						
	CAIDE Model	1637	440	0.725 (0.689 – 0.759)	-	0.751 (0.716 – 0.784)	-
	CAIDE Model + inflam. biomarkers[d]			0.756 (0.723 – 0.789)	**0.032 (0.007 – 0.053)**	0.776 (0.743 – 0.809)	**0.025 (0.001 – 0.045)**
	Alzheimer's disease						
	CAIDE Model	1344	147	0.713 (0.656 – 0.767)		0.767 (0.714 – 0.815)	-
	CAIDE Model + inflam. biomarkers[d]			0.750 (0.694 – 0.804)	0.037 (-0.012 – 0.078)	0.791 (0.738 – 0.842)	0.024 (-0.017 – 0.057)
	Vascular dementia						
	CAIDE Model	1364	167	0.716 (0.665 – 0.766)	-	0.725 (0.673 – 0.776)	-
	CAIDE Model + inflam. biomarkers[d]			0.737 (0.685 – 0.786)	0.021 (-0.029 – 0.061)	0.744 (0.692 – 0.794)	0.019 (-0.029 – 0.059)
Mid-Life (50-64 years)	**All-cause dementia**						
	CAIDE Model	888	137	0.685 (0.624 – 0.744)	-	0.721 (0.660 – 0.778)	-
	CAIDE Model + inflam. biomarkers[e]			0.739 (0.681 – 0.795)	0.054 (-0.007 – 0.107)	0.758 (0.701 – 0.813)	0.037 (-0.017 – 0.086)
	Alzheimer's disease						
	CAIDE Model	799	48	0.677 (0.576 – 0.774)	-	0.755 (0.659 – 0.843)	-
	CAIDE Model + inflam. biomarkers[e]			0.730 (0.631 – 0.824)	0.053 (-0.030 – 0.118)	0.785 (0.694 – 0.870)	0.030 (-0.034 – 0.079)
	Vascular dementia						
	CAIDE Model	801	50	0.664 (0.566 – 0.752)	-	0.665 (0.566 – 0.758)	-
	CAIDE Model + inflam. biomarkers[e]			0.778 (0.687 – 0.859)	**0.114 (0.020 – 0.198)**	0.778 (0.689 – 0.858)	**0.113 (0.021 – 0.196)**
Late-life (65-75 years)	**All-cause dementia**						
	CAIDE Model	749	303	0.608 (0.553 – 0.662)	-	0.651 (0.598 – 0.703)	-
	CAIDE Model + inflam. biomarkers[f]			0.631 (0.576 – 0.684)	0.023 (-0.012 – 0.049)	0.663 (0.611 – 0.714)	0.012 (-0.012 – 0.034)
	Alzheimer's disease						
	CAIDE Model	545	99	0.582 (0.495 – 0.665)	-	0.658 (0.574 – 0.736)	-
	CAIDE Model + inflam. biomarkers[f]			0.663 (0.578 – 0.744)	0.081 (0.000 – 0.157)	0.699 (0.619 – 0.773)	0.041 (-0.026 – 0.095)
	Vascular dementia						
	CAIDE Model	563	117	0.577 (0.495 – 0.655)	-	0.607 (0.525 – 0.686)	-
	CAIDE Model + inflam. biomarkers[f]			-[g]	-	0.617 (0.537 – 0.695)	0.010 (-0.046 – 0.052)

Abbreviations: inflam., inflammatory; AUC, area under the curve; CI, confidence interval.

[a]The CAIDE model 1 includes age, education, sex, systolic blood pressure, body-mass index, total cholesterol and physical activity.

[b]The CAIDE model 2 includes the variables of CAIDE model 1 and *APOE* ε4 status.

[c]The 95% CI is the bootstrap interval for the differences in AUCs.

[d]The inflammatory biomarkers selected by the LASSO regression are shown in Table 11.
[e]The inflammatory biomarkers selected by the LASSO regression for all-cause dementia, Alzheimer's disease and vascular dementia are shown in Suppl. Tables 39, 40 and 41, respectively.
[f]The inflammatory biomarkers selected by the LASSO regression for all-cause dementia, Alzheimer's disease and vascular dementia are shown in Suppl. Tables 42, 43 and 44, respectively.
[g]None of the inflammation-related biomarkers was selected by the LASSO regression. Thus only the AUC of the CAIDE model was estimated.

Table 11. Inflammatory biomarkers improving dementia prediction models in the total cohort (n = 1,637) selected by LASSO regression.

Inflammatory biomarkers	Improvement of the CAIDE models' predictive ability for dementia outcomes		
	All-cause dementia	Alzheimer's disease	Vascular dementia
Beta-NGF	Model 1 + 2	Model 1 + 2	Model 1 + 2
CASP-8	-	-	Model 1 + 2
CCL19	-	Model 1 + 2	Model 1 + 2
CCL20	Model 1	-	-
CCL28	-	Model 1 + 2	-
CD244	Model 1 + 2	-	Model 1 + 2
CD5	-	-	Model 1 + 2
CDCP1	-	-	Model 1 + 2
CST5	-	-	Model 1 + 2
CX3CL1	Model 1 + 2	-	-
CXCL5	Model 1 + 2	Model 1	Model 1 + 2
CXCL6	-	-	Model 1 + 2
CXCL9	-	-	Model 1 + 2
EN-RAGE	Model 1 + 2	Model 1 + 2	Model 1 + 2
FGF-19	-	Model 1	-
FGF-23	Model 1 + 2	Model 1 + 2	Model 1 + 2
IL-10	-	-	Model 1 + 2
IL-12B	Model 2	-	-
IL-18	Model 2	-	Model 1 + 2
IL-7	-	Model 1	-
LAP TGF-beta-1	Model 1 + 2	Model 1 + 2	Model 1 + 2
LIFR	Model 1 + 2	-	-
MCP-3	-	Model 1 + 2	-
MMP-1	-	-	Model 1
MMP-10	-	-	Model 1 + 2
NT3	-	-	Model 1 + 2
OPG	Model 1	-	Model 1 + 2
OSM	Model 1 + 2	-	Model 1 + 2
SCF	Model 1 + 2	-	Model 1 + 2
SIRT2	-	-	Model 1 + 2
SLAMF1	Model 1 + 2	Model 1 + 2	Model 1 + 2
ST1A1	-	Model 2	-
STAMBP	-	-	Model 1 + 2
TGF-alpha	Model 1	-	-
TNFB	Model 1 + 2	-	Model 1 + 2
VEGF-A	Model 1 + 2	-	Model 1 + 2

Abbreviations: For inflammatory biomarker abbreviations, see Supplemental Table 5.

NOTE: Model 1 and Model 2 refer to CAIDE Model 1 and CAIDE Model 2, respectively.

When *APOE* genotypes were additionally included in CAIDE model 2, the prediction improved more for AD and all-cause dementia than VD. Overall, the highest discriminative performance of all models was achieved for AD when both *APOE* genotypes and inflammatory biomarkers were included in the model (AUC [95% CI]: 0.791 [0.738-0.842]).

In a further step, I split the cohort into a mid-life (55-64 years) and late-life (65-75 years) sub-sample. A clear difference between dementia prediction became apparent (**Table 10**). While the AUCs for the various models for all-cause dementia, AD and VD varied between 0.664 and 0.785 in the mid-life sample, the AUCs in the late-life sample were always lower and varied between 0.577 and 0.699. However, inflammatory biomarkers selected by the LASSO regression consistently led to improvements of the AUCs of the models in both the mid-life and late-life subsample (not statistically significant in most models). The inflammatory biomarkers selected by the LASSO regression and the β-coefficients for their associations with all-cause dementia, AD and VD, as well as the other CAIDE variables needed to calculate the risk prediction models, are shown in **Supplemental Tables 39-41** for mid-life and **Supplemental Tables 42-44** for the late-life sample, respectively. Comparable to the total cohort, the highest AUCs were achieved for AD when the inflammatory biomarkers and *APOE* were included in the model (AUC [95% CI]: 0.785 [0.694-0.870] and 0.699 [0.619-0.773] for the mid-life and late-life sample, respectively). However, the largest increase in the AUC, which was also the only statistical significant one in this age-specific sub-group analysis, was observed for VD in the mid-life sample (by 0.114 and 0.113 increments in CAIDE model 1 and 2, respectively). In the late-life sample, none of the inflammatory biomarkers was selected by LASSO regression for a potentially improved VD prediction with CAIDE model 1, and the four selected biomarkers for CAIDE model 2 only led to a minor improvement in the AUC of 0.010 increments.

In a sensitivity analysis, I penalized not only the OLINK inflammation biomarkers but also the variables of the CAIDE model 1 in the LASSO regression. This analysis was exemplarily conducted for CAIDE model 1 and the outcome of all-cause dementia. Interestingly, all CAIDE model variables except education and systolic blood pressure were selected, and the same list of inflammatory biomarkers with only two additions was selected (CDCP1 and IL-12B). In addition, the AUC of this sensitivity analysis (0.757 [0.723-0.790]) was almost identical to the one from the main analysis (0.756 [0.723-0.789]).

3.4.2 Predictive ability of 8-iso-$PGF_{2\alpha}$

Adding 8-iso-$PGF_{2\alpha}$ to the CAIDE model, did not improve the predictive ability of the model (**Figure 12**) (AUC CAIDE model 1 [95% CI]: 0.727 [0.692-0.762]); AUC CAIDE model 1 + 8-iso-$PGF_{2\alpha}$: 0.726 [0.691-0.761]). The curves for the two models overlap so strongly that it appears if only one curve is shown in the figure.

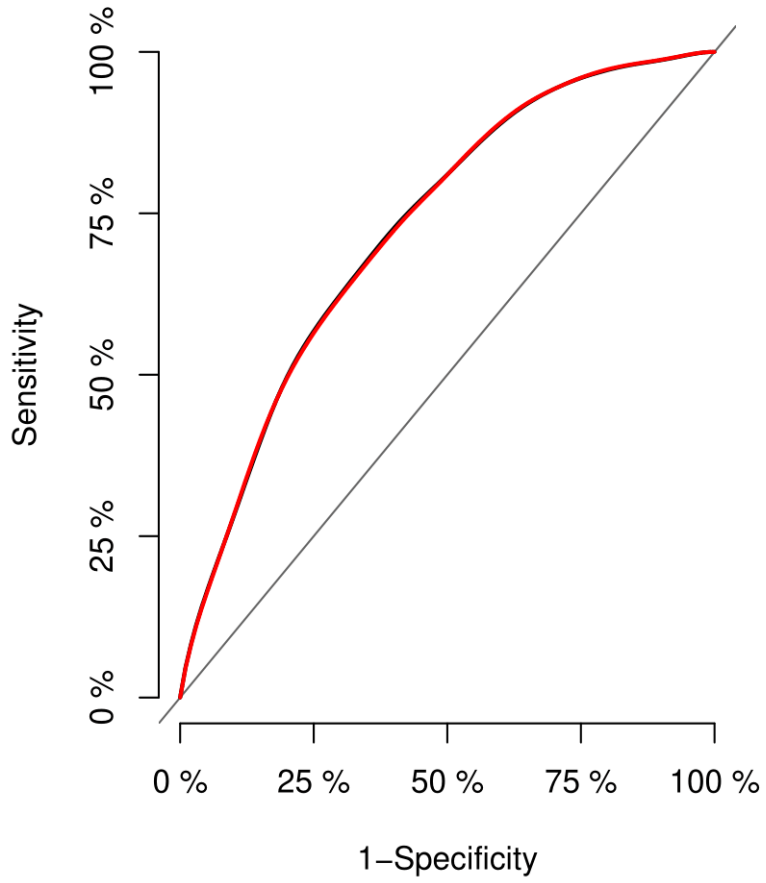

Figure 12. Receiver-operating characteristic (ROC) curves of the CAIDE model and the CAIDE model + 8-iso-$PGF_{2\alpha}$ predicting the risk for all-cause dementia.
The AUC for the CAIDE model (black curve) was 0.727 (95% CI: 0.692-0.762) and the AUC for the CAIDE model + 8-iso-$PGF_{2\alpha}$ (red curve) was 0.726 (0.691-0.761).

4. Discussion

4.1 Associations of urinary 8-iso-prostaglandin $F_{2\alpha}$ levels with all-cause dementia, Alzheimer's disease and vascular dementia incidence

When pursuing the first aim of this dissertation, logarithmized 8-iso-$PGF_{2\alpha}$ levels were found to be significantly associated with all-cause dementia. Furthermore, an interaction test revealed that the simultaneous presence of the *APOE* ε4/ε4 genotype and increased 8-iso-$PGF_{2\alpha}$ levels substantially increased the risk of dementia. Regarding dementia sub-types, logarithmized 8-iso-$PGF_{2\alpha}$ levels were statistically significantly associated with AD but not with VD. However, case numbers were limited for VD, and future larger studies may also establish an association of 8-iso-$PGF_{2\alpha}$ levels with VD incidence. This could be expected because of the important role of OS in atherosclerosis (Kattoor et al. 2017).

4.1.1 Comparison with previous studies

To my knowledge, only one previous prospective study has assessed the association of 8-iso-$PGF_{2\alpha}$ with dementia. Sundelöf et al. measured urinary 8-iso-$PGF_{2\alpha}$ levels in a cohort of 679 men, all aged 77 years (Sundelöf et al. 2009). During a median follow-up of 5.1 years, 80 all-cause dementia and 47 AD cases were identified. However, neither a statistically significant association between a comparison of 8-iso-$PGF_{2\alpha}$ above and below the median (0.18 nmol/mmol creatinine) with all-cause dementia (HR [95% CI]: 0.99 [0.61-1.61]) nor with AD incidence (HR [95% CI]: 0.76 [0.37-1.57]) was observed. The inclusion of only males and the higher mean age most likely does not explain the divergent findings because I also observed strong effect sizes in men and the age group 70-75 years, which is close to the 77-years-old study population of Sundelöf et al. The most likely reason for the difference to the results of my study is the lower case number for all-cause dementia (80 vs 365) and AD (47 vs 109). A low validity of the 8-iso-$PGF_{2\alpha}$ radioimmunoassay used by Sundelöf et al. is also a possible explanation.

Results from five other studies with a cross-sectional study design are available for the association of urinary levels of F_2-isoprostanes and AD and all observed increased levels in AD

patients (three with statistically significant findings for 8-iso-$PGF_{2\alpha}$ (Ciabattoni et al. 2007; Guan et al. 2012; Tuppo et al. 2001) and two with non-significant findings for 8-iso-$PGF_{2\alpha}$ levels but statistically significant results for other F_2-isoprostane molecules (Kim et al. 2004; Peña-Bautista et al. 2019)). In addition, Montine et al. observed increased F_2-isoprostane levels in *post-mortem* collected cerebrospinal fluid of 11 AD patients compared to 11 control patients (Montine et al. 1998). This finding was confirmed by further *post-mortem* studies for AD and other neurodegenerative diseases (Miller et al. 2014). However, the cross-sectional studies may not be comparable to my study because dementia could have influenced the 8-iso-$PGF_{2\alpha}$ levels (reverse causality). To the best of my knowledge, cross-sectional studies on the associations between urinary 8-iso-$PGF_{2\alpha}$ and all-cause dementia, VD, or other dementia subtypes are currently not available.

4.1.2 Interpretation of the findings

The association of a biomarker of lipid peroxidation with all-cause dementia incidence and especially AD incidence observed in this study could be potentially explained by oxidative damages to neural cells. F_2-isoprostanes are produced by ROS induced peroxidation of arachidonic acid. In the brain, neurons are prone to ROS and oxidative damage because of high oxygen consumption, high energy production, and an impaired antioxidant defence mechanism (Wojsiat et al. 2018). If redox homeostasis fails and ROS are excessively generated, F_2-isoprostanes can be formed. Oxidative damage then becomes apparent through alteration of integrity, fluidity, and permeability of neuronal membranes (Miller et al. 2014).

However, OS is related to other dementia risk factors and could also be a mediator. The potential pathways to dementia involving lipid oxidation are summarized in **Figure 13**. Low physical activity and diabetes, which have been recognized as risk factors for dementia also by other studies (Luck et al. 2014; Wium-Andersen et al. 2019), were associated with dementia incidence and 8-iso-$PGF_{2\alpha}$ levels in my study. Moreover, it is well known that low physical activity promotes type 2 diabetes (Smith et al. 2016) and that OS is also a risk factor for type 2 diabetes (Houstis et al. 2006). Therefore, there is a cluster of three important risk factors for dementia that influence each other. In addition, 8-iso-$PGF_{2\alpha}$ levels were statistically significantly increased in subjects with the *APOE* ε4/ε4 genotype. However, physical activity,

diabetes, the *APOE* ε4/ε4 genotype, and 8-iso-$PGF_{2\alpha}$ levels were also independently associated with all-cause dementia incidence in my study. HR point estimates for the associations of physical activity, diabetes, and the *APOE* ε4/ε4 genotype with dementia outcomes changed only slightly when the model was additionally adjusted for 8-iso-$PGF_{2\alpha}$ levels (**Supplemental Table 45**). These results suggest that physical activity, diabetes, the *APOE* ε4/ε4 genotype, and increased 8-iso-$PGF_{2\alpha}$ levels are independent risk factors for all-cause dementia, although they are interrelated.

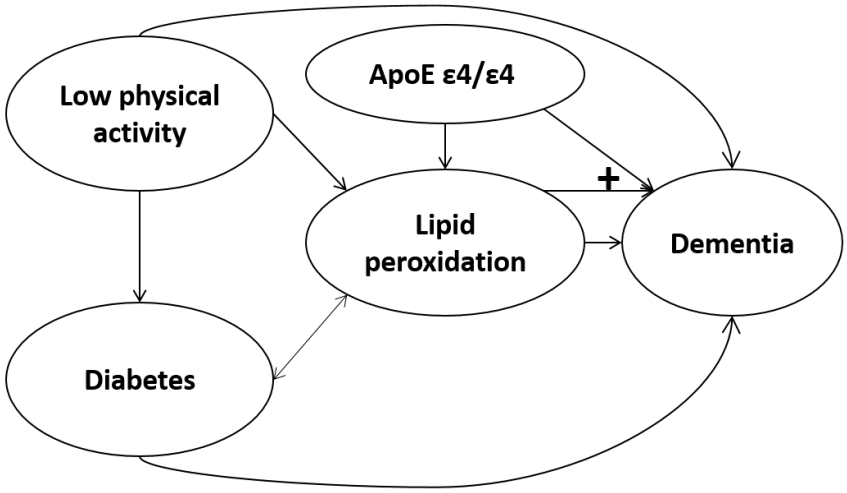

Figure 13. Potential pathways involving a contribution of lipid peroxidation to dementia development
The arrows symbolize associations. Double-headed arrows represent bidirectional associations. The plus sign indicates an interaction between two risk factors.

Interestingly, an interaction between the *APOE* ε4/ε4 genotype and increased 8-iso-$PGF_{2\alpha}$ levels was observed for all-cause dementia incidence. A possible mechanism for the interaction could be that individuals with the *APOE* ε4/ε4 genotype are more susceptible to the neurotoxic effects of OS and amyloid pathology (McCaffrey 2019). Normally, in the unimpaired lipid metabolism, apoE transfers toxic peroxidized lipids to astrocytes, preventing neuronal degradation (Fernandez et al. 2019). However, during the state of OS, microglia are activated and induce A1-reactive astrocytes. Instead of promoting neuronal survival, these cause neurodegeneration (Liddelow et al. 2017).

4.1.3 Strengths and limitations

The strengths of this study include the prospective cohort design, a representative sample of an older adult population (study participants with and without dementia information had similar baseline characteristics, **Supplemental Table 1**), the large sample size (n = 5,835), and a long follow-up period (14 years).

A general limitation is the observational study design. Although results were controlled for important potential confounders, residual confounding cannot be excluded. Another limitation is that the latency period from the onset of AD pathogenesis until a clinical dementia diagnosis can be even longer than 10 years (Ritchie et al. 2016). Hence, the long follow-up period of 14 years cannot totally exclude reverse causality. However, the observation of a statistically significant association of logarithmized 8-iso-$PGF_{2\alpha}$ levels and all-cause dementia even after exclusion of events in the first 7 years refute a strong impact of reverse causality on the main results.

The dementia diagnoses collected in the ESTHER study reflect the community-based clinic setting. No screening for dementia was performed at baseline, and no specific diagnostic procedures were used for the study. Therefore, the specific dementia subtype was often not determined with certainty, which may explain the low proportion of diagnosed AD among the all-cause dementia cases. However, other studies showed that most dementia patients have a mixed dementia type anyway (Boyle et al. 2018; Robinson et al. 2018). Another limitation is that 8-iso-$PGF_{2\alpha}$ levels were only measured once at baseline (not in duplicates or triplicates at baseline and not repeatedly measured during follow-up) and with an ELISA (instead of more precise GC- or LC-MS/MS methods). These limitations were necessary to enable measuring the large number of samples in this cohort study in a cost-efficient manner. Potentially resulting imprecision of the measurements can be expected to be non-differential with respect to dementia outcomes and might have led to some underestimation of associations. Finally, a limitation of my study is that it can only be generalized to Caucasian populations aged 50-75 years.

4.2 Association of F_2-isoprostane levels with Alzheimer's disease in observational studies: a systematic review and meta-analysis

The performed systematic review and meta-analysis revealed that F_2-isoprostane levels were significantly increased in AD patients compared to healthy controls in cross-sectional case-control studies when all studies with different measurement methods and sample types (CSF, blood, urine, or frontal lobe tissue) were pooled. These increased levels were also visible in separate meta-analyses by sample type for studies using CSF, blood, and frontal lobe tissue samples. Only four longitudinal studies were available, using either urine or CSF samples. Both meta-analyses showed no significant association of F_2-isoprostane or 8-iso-$PGF_{2\alpha}$ levels with AD incidence.

4.2.1 Previous systematic review

To the best of my knowledge, this is the first systematic review with meta-analyses and a focus on the associations of F_2-isoprostanes and 8-iso-$PGF_{2\alpha}$ with AD. Only one previous review, performed by van 't Erve and colleagues in 2017, included AD as one of 50 human health outcomes in a systematic review with meta-analyses (van 't Erve et al. 2017). In a fixed-effects model, the authors found slightly increased 8-iso-$PGF_{2\alpha}$ levels in AD patients compared to controls (Hedge's g [95% CI]: 0.5 [0.3-0.7]). However, only six studies were covered by the literature search and included in the analysis (Bohnstedt et al. 2003; Kim et al. 2004; Montine et al. 1999; Montine et al. 2001; Tuppo et al. 2001; Ulstein and Bøhmer 2017).

4.2.2 Interpretation of results

The revealed increase of F_2-isoprostane levels in AD patients indicates that OS is increased during the course of AD. Nevertheless, most studies did not adjust their results for potential confounders, previously shown to be associated with 8-iso-$PGF_{2\alpha}$ levels, like age, sex, education, current smoking, alcohol consumption, physical activity, body mass index, diabetes, and *APOE* ε4 polymorphism. In addition, publication bias was detected for the meta-analysis of all cross-sectional studies. Thus, results have to be interpreted with caution. In

addition, the causality of the association is uncertain because evidence from the few well-conducted, longitudinal studies was conflicting.

For a detailed discussion of the possible role of OS in the aetiology of AD, I would like to refer to chapter 4.1.2. In brief, F_2-isoprostanes are produced during the state of OS, when ROS induce lipid peroxidation of arachidonic acid and can cause neurodegeneration of neuronal membranes. OS is a multifactorial process and can be caused by many factors including, obesity/high-caloric diet, smoking, low physical activity, high alcohol consumption, hypertension, or hypercholesterolemia (Anusruti et al. 2020a; Anusruti et al. 2020b; Dias et al. 2014; Lindqvist et al. 2017; Montezano et al. 2015; Peskind et al. 2014; Schöttker et al. 2015; Schöttker et al. 2021).

4.2.3 Methodological differences between studies

To investigate the high heterogeneity between the analysed studies, I investigated several methodological differences prominently discussed in the literature in a meta-regression. The following factors will be discussed: sample purification (are total or free F_2-isoprostanes measured), the group of F_2-isoprostanes (total F_2-isoprostanes or specific 8-iso-$PGF_{2\alpha}$), the measurement technique (immunological or analytical technique), sample types for F_2-isoprostane measurement (i.e. urine, blood, CSF, frontal lobe tissue), sample storage, and nomenclature of isoprostanes.

F_2-isoprostanes can be either measured as free or total F_2-isoprostanes. The latter comprises the quantification of free and esterified molecules. Depending on the sample type and research question, different forms can be suitable to be analyzed. For example, F_2-isoprostanes are overrepresented in their free form in urinary samples (Nikolaidis et al. 2011; van 't Erve et al. 2017). In contrast, in tissue samples, F_2-isoprostanes mainly occur in esterified form. While urinary measurements of free F_2-isoprostanes are rather suited for detecting systemic changes, total or rather esterified isoprostanes might better represent oxidative damage to specific organs like the brain (Nikolaidis et al. 2011). To date, the majority of studies used free F_2-isoprostane levels for quantification (van 't Erve et al. 2017). However, this is not the case in all studies, thus making it difficult to compare them. Moreover, authors often did not report clearly if free or total F_2-isoprostanes were measured. For example, in this

systematic review, only 12 of the included 29 studies stated which part of F_2-isoprostanes was measured. In their systematic review, van ’t Erve and colleagues showed that total and free 8-iso-$PGF_{2\alpha}$ levels varied in different conditions, although no statistically significant difference was detected. In the meta-regression, studies, which measured free F_2-isoprostanes levels, tended to show smaller differences between AD cases and controls than those measuring total F_2-isoprostanes. However, the difference was not statistically significant (ß = -0.37, p = 0.7462).

In this systematic review, I analysed specific 8-iso-$PGF_{2\alpha}$ measurements separately from other F_2-isoprostanes. Comparing the results sample type-wise, I did not see any differences in urine or blood samples. In addition, the results of the meta-regression also showed no significant impact by this classification. However, not enough studies measuring the specific biomarker 8-iso-$PGF_{2\alpha}$ in CSF and frontal lobe tissue samples of AD patients were available.

Moreover, there is a debate about whether F_2-isoprostanes need to be quantified by an analytical method (chromatography coupled with mass spectrometry) or whether immunological methods (e.g. enzyme-linked immunosorbent assay (ELISA) kits) are likewise appropriate. While immunological methods are better suited for large sample sizes because they are cost and time-efficient, analytical techniques are supposed to have higher sensitivity and specificity in biomarker quantification (Ahmed et al. 2020). However, due to cross-reactivity, ELISA kits are assumed to generate much higher results (Efsa Panel on Dietetic Products et al. 2018; Menzel et al. 2021). This was confirmed by several studies reporting that immunological and analytical quantification methods yielded significantly different results (Graille et al. 2020; Klawitter et al. 2011; Menzel et al. 2021). In contrast, sensitivity analysis results in the systematic review of van’ t Erve and colleagues (van 't Erve et al. 2017) and the meta-regression in this systematic review did not reveal any significant impact on the pooled results by different measurement techniques.

Finally, F_2-isoprostanes can be measured in various sample types. In this systematic review, I performed meta-analyses for measurements obtained from urine, blood, CSF, and frontal lobe tissue. CSF and frontal lobe tissue samples seem best suited for F_2-isoprostane quantification because they showed the lowest levels of between-study heterogeneity. Surprisingly, measurements in urine samples were not as consistent, although this sample type usually presents the highest F_2-isoprostane concentrations, and F_2-isoprostanes are supposed to be

stable in urine (Cracowski et al. 2000; Menzel et al. 2021; van 't Erve et al. 2017). However, urinary F_2-isoprostane concentrations can differ depending on sample collection method (e.g., spot urine samples or 12- or 24h urine collection) and level of physical activity (Martinez-Moral and Kannan 2019). A 24h urine collection is preferred and also recommended by the EFSA as it is more representative for long-term F_2-isoprostane exposure (Efsa Panel on Dietetic Products et al. 2018; Menzel et al. 2021). F_2-isoprostane measurements in blood samples are considered the least suited, and heterogeneity was particularly high using this sample type (Efsa Panel on Dietetic Products et al. 2018). Nevertheless, the meta-analysis about studies measuring 8-iso-$PGF_{2\alpha}$ levels in AD cases compared to controls in this sample type was statistically significant. Although the meta-regression suggested overall weaker effect estimates in studies using urine or blood samples compared to studies using CSF or frontal lobe tissue, the differences did not have a significant impact on the overall pooled effect estimate. Furthermore, for accurate measurement results, samples should be quickly frozen after extraction and not thawed until analysis to prevent ex-vivo oxidation and repeated thawing, and freezing should be avoided, especially in plasma and tissue samples containing a high amount of arachidonic acid (Nikolaidis et al. 2011) (Menzel et al. 2021). However, the pre-analytical sample handling is rarely reported in publications and should be more transparent in future studies.

In addition to the methodological differences discussed above, the year of publication, the AD diagnosis criteria, and the general study quality also had no significant effect on the meta-analyses result. Thus, the high heterogeneity between the studies remains unresolved and may be related to differences between study populations.

Finally, it should be noted that several nomenclatures are used for isoprostanes (Cracowski et al. 2000), which hampers the search for the relevant literature. Besides the first nomenclature introduced by Morrow et al. when F_2-isoprostanes were first discovered, Taber and Rokach proposed two alternative nomenclatures quite early in isoprostane research (Morrow et al. 1990; Rokach et al. 1997; Taber et al. 1997). In contrast to the first nomenclature, Taber and Rockach differentiate between various isomeric structures (Cracowski et al. 2000). However, all three nomenclatures are still used concurrently, which might be misleading, especially for non-experts to the field, and thus hampers the search for relevant literature.

4.2.4 Population characteristics with potential impact on the results

Age and AD disease stage may have a great impact on F_2-isoprostanes levels (Kester et al. 2012; Peskind et al. 2014) and thus could be factors contributing to the between-study heterogeneity. Although most studies included in this systematic review examined patients above the age of 65 and only results of properly diagnosed AD patients were included, the age and stage of the disease could still deviate significantly. In the first part of this dissertation (see chapters 2.1, 3.1, and 4.1), I observed that sex, education, current smoking, alcohol consumption, physical activity, body mass index, diabetes, and the *APOE* ε4 polymorphism were statistically significantly associated with 8-iso-$PGF_{2\alpha}$ levels (chapters 2.1, 3.1, and 4.1). In addition, the study of Duits and colleagues observed a significantly higher annual increase in F_2-isoprostane levels of *APOE* ε4 carriers compared to non-carriers (Duits et al. 2013). The possible biological mechanism for this relation concerning the vulnerability of *APOE* ε4/ε4 carriers to the neurotoxic effects of OS was discussed in detail in chapter 4.1.2. Thus, it is essential that studies in this field properly adjust for the above mentioned potential confounders, which is a major limitation in this research field (see 4.2.5 below).

4.2.5 Strengths and limitations

This systematic review comprises an extensive review of the published literature on F_2-isoprostanes and their association with AD. Due to an SMD approach and data transformation, most of the available studies could be included. Effect sizes were cautiously estimated by random-effects meta-analyses, and the risk of bias of both individual studies and the meta-analyses was assessed by appropriate methods. Another strength of this systematic review is meta-analyses conducted distinctly by study design, measured biomarkers and sample type. Last but not least, a meta-regression was carried out, showing that pooling of all cross-sectional studies was appropriate despite methodological differences.

Based on the available literature, this article was limited to associations between F_2-isoprostane levels and AD. Associations with other types of dementia could not be examined due to an insufficient number of publications. Another inherent limitation was that meta-analyses on cross-sectional studies were based on unadjusted analyses, possibly leading to residual confounding. The low NOS scores for study quality of most of the cross-sectional

studies also reflected this circumstance. In addition, a significant risk for publication bias was found in the analysis of the included 25 cross-sectional studies. However, the random effects summary estimate remained statistically significant after imputing ten smaller, potentially unpublished, insignificant studies. Despite all efforts to assure accurate meta-analyses, the quality of results is conditioned by the quality of the actual published studies. In addition, most studies had a cross-sectional study design, and thus, reverse causation cannot be excluded. The few longitudinal studies, which also had good NOS scores for study quality, yielded conflicting results, which I could not explain based on the available information from the publications.

4.2.6 Future recommendations

More longitudinal studies with long-term follow-up (> 10 years) should be conducted to address the research question, whether F_2-isoprostane levels are causally related to AD development. Since OS is a multifactorial process, these future studies should be adjusted for potential confounders, previously shown to be associated with 8-iso-$PGF_{2\alpha}$ levels, like age, sex, education, current smoking, alcohol consumption, physical activity, body mass index, diabetes, and *APOE* ε4 polymorphism (Trares et al. 2020). Mass spectrometry-based methods are recommended for biomarker measurements because they have a higher sensitivity (Efsa Panel on Dietetic Products et al. 2018; Milne et al. 2005). In addition, future studies are urged to adhere to standard measurement protocols for this particular group of biomarkers. For example, Holder and colleagues developed such a measurement protocol for 8-iso-$PGF_{2\alpha}$ levels in urine samples, which is suitable for large sample sizes although using LC-MS/MS (Holder et al. 2020).

Besides ROS induced lipid peroxidation of arachidonic acid, 8-iso-$PGF_{2\alpha}$ can also be formed enzymatically by prostaglandin-endoperoxide synthases (PGHSs) (Klein et al. 1997; Praticò and FitzGerald 1996; Praticò et al. 1995). Among the F_2-isoprostanes, this alternative formation is unique to 8-iso-$PGF_{2\alpha}$ and occurs independently of the non-enzymatic pathway in the context of inflammation (Praticò et al. 1995). However, this might falsify the actual concentration of 8-iso-$PGF_{2\alpha}$ evoked by OS and might have previously been misinterpreted. Therefore, contrary to the current practice, it is advisable to measure total or rather esterified instead of free

levels of 8-iso-$PGF_{2\alpha}$ as these are less affected by the enzymatic formation of 8-iso-$PGF_{2\alpha}$ formation (Ahmed et al. 2020; Menzel et al. 2021). In addition, a recommended approach to elude this problem when measuring free 8-iso levels is to calculate the ratio of 8-iso-$PGF_{2\alpha}$/$PGF_{2\alpha}$ instead (van 't Erve et al. 2015). By this, the contribution of enzymatic formation and lipid peroxidation can be distinguished, and OS related 8-iso-$PGF_{2\alpha}$ levels can be clearly determined. However, some concerns about applying this ratio in urinary samples have been raised (Ahmed et al. 2020).

In addition to F_2-isoprostanes, there are many other OS biomarker classes derived from arachidonic acid as well as other PUFAs (Ahmed et al. 2020; Miller et al. 2014). Isofurans, for example, also originate from lipid peroxidation of arachidonic acid and are formed in a competitive reaction to isoprostanes under high oxygen tension (Cuyamendous et al. 2016). Moreover, lipid peroxidation of the PUFAs eicosapentaenoic acid and docosahexaenoic acid, on the other hand, results in F_3-isoprostanes and F_4-neuroprostanes, respectively (Ahmed et al. 2020). F_4-isoprostanes and isofurans have previously been shown to be elevated in patients diagnosed with AD or other neurological diseases (García-Blanco et al. 2018; Miller et al. 2014; Peña-Bautista et al. 2019). Thus, instead of only focusing on one specific biomarker like 8-iso-$PGF_{2\alpha}$ or the family of F_2-isoprostanes, it might be advisable to take further biomarkers into account as well. This might additionally improve our understanding of the link between OS and AD. In two previous studies, combined models of OS biomarkers were already used to differentiate successfully between different AD stages and controls (García-Blanco et al. 2018; Peña-Bautista et al. 2019).

4.3 Association of the inflammation-related proteome with dementia development at older age

To my knowledge, the study conducted to achieve the third aim of this dissertation contains the first prospective cohort study to analyze a whole panel of inflammation-related, blood-based biomarkers for all-cause dementia, AD, and VD incidence. I identified a high number of statistically significantly associated proteins with at least one of the outcomes, even after FDR correction. However, only a few biomarkers were strongly and independently associated with dementia outcomes because of a high inter-correlation between the biomarkers. The

identified independent biomarkers include CX3CL1 (associated with all-cause dementia), EN-RAGE (associated with all-cause dementia and AD), LAP TGF-beta-1 (associated with AD), and VEGF-A (associated with VD). Each of these biomarkers is only one marker of an inflammatory protein cluster, in which the majority of biomarkers are associated with dementia.

4.3.1 Previous studies examining a set of inflammatory biomarkers

A few previous studies, mostly with a cross-sectional study design, investigated the association between single inflammatory biomarkers and all-cause dementia or AD (Darweesh et al. 2018; Lai et al. 2017; Park et al. 2020; Su et al. 2019). To my knowledge, only two previous studies examined a whole panel of inflammatory biomarkers for dementia as the outcome in a cross-sectional design. In the BioFINDER study, Whelan and colleagues (Whelan et al. 2019) measured 270 proteins with the Olink immunoassay in CSF and plasma of 161 AD patients, 75 amyloid beta positive (Aβ+) patients with mild cognitive impairment (MCI+), and 415 amyloid beta negative (Aβ-) cognitively normal individuals (MCI-). Interestingly, approximately half of the CSF proteins correlated at least modestly with their analogues in plasma, indicating that findings in plasma samples partially reflected the situation in CSF. Compared to Aβ-/MCI- individuals, CSF levels of 32 proteins and plasma levels of 33 proteins were statistically significantly associated with AD (False discovery corrected p-value < 0.05). The comparison of Aβ+/MCI+ patients with Aβ-/MCI- individuals, were replicated in an independent cohort. Thereby, 10 CSF and six plasma markers could be replicated. However, a replication analysis in an independent sample was not performed for AD. Six of the identified 33 proteins for AD in plasma samples corresponded with my findings in serum samples and can now be considered replicated by my study (Casp-8, CXCL5, CXCL6, ST1A1, TRAIL, uPA). Gaetani and colleagues measured biomarker levels of the Olink inflammation panel in CSF samples of 34 AD-MCI cases and 25 controls having other neurological diseases (OND) (Gaetani et al. 2021). In univariate analyses, 11 of 46 analyzed biomarkers were found to have the highest discriminatory ability between AD-MCI and OND. Four of those biomarkers (SIRT2, HGF, MMP-10, CXCL5) were also selected as discriminatory factors during penalized logistic regression (LASSO regression).

However, the studies of Whelan and Gaetani were cross-sectional, and evidence from longitudinal studies on this field is still sparse (Walker 2018). The recently published

longitudinal study of Walker et al. (Walker et al. 2019b) reported statistically significant associations between inflammatory biomarkers measured in midlife (CRP and a composite score of fibrinogen, white blood cell count, von Willebrand factor, and factor VIII) and cognitive decline over 20 years in a population-based cohort study with 12,336 participants. My longitudinal results with a broad panel of inflammatory proteins and the endpoints all-cause dementia, AD and VD complement and expand these findings.

4.3.2 Independently associated biomarkers

CX3CL1

The biomarker CX3CL1, which is also commonly known as Fractalkine in humans, was independently associated with all-cause dementia and among the list of statistically significant biomarkers for AD and VD in my study. This biomarker is a chemokine binding to its receptor C-X3-C motif chemokine receptor 1 (CX3CR1) in a one-to-one relationship. While CX3CL1 is usually expressed in neurons, CX3CR1 is expressed on microglia. In the case of neuroinflammation, CX3CL1 regulates microglial activation by reducing the release of pro-inflammatory products (Finneran and Nash 2019). Whether the effect of CX3CL1 is neuroprotective or neurotoxic in diseases like dementia is still controversially discussed in the literature. The current opinion is that this depends on the disease state, the affected CNS area, and the local concentration of the CX3CL1/CX3CR1 complex (Finneran and Nash 2019; Pawelec et al. 2020).

Nevertheless, due to the regulatory function in inflammation, this biomarker is a promising therapeutic target. A recent Polish study reported on the predictive ability of CX3CL1 as a biomarker in the early development of MCI and AD (Kulczyńska-Przybik et al. 2020). In this study, significantly higher CSF and blood levels of CX3CL1 were found in MCI and AD patients compared to cognitively healthy controls. I now confirm these results with longitudinal data, including 17 years of follow-up.

EN-RAGE

EN-RAGE was independently associated with all-cause dementia and AD. In addition, EN-RAGE was significantly associated with VD. EN-RAGE is also often referenced as S100-A12. The S100-

protein family has already been shown multiple times to be related to AD (Cristóvão and Gomes 2019). However, the S100-A12 protein (EN-RAGE) is the least studied S100 protein in the context of AD and dementia (Cristóvão and Gomes 2019). In the only available study, Shepherd and colleagues (Shepherd et al. 2006) revealed associations of EN-RAGE with senile plaques, reactive glia, and neurons in brain samples of sporadic and familial (PS-1) AD cases in a cross-sectional study. My study is the first longitudinal cohort study reporting on this association.

EN-RAGE is a calcium-, zinc-, and copper-binding protein. In previous studies, it was shown to be associated with diseases like heart failure (He et al. 2015) and coronary artery disease (CAD) in diabetes patients (Zhao et al. 2013). Recently, Feng and colleagues (Feng et al. 2018) reported significantly elevated EN-RAGE concentrations in patients with traumatic brain injury compared to controls. In this study, EN-RAGE showed great potential as a marker for ongoing inflammatory processes in the brain. RAGE, the receptor EN-RAGE binds to, is additionally known to be involved in inflammatory processes related to ageing and neurodegeneration (Derk et al. 2018; Teissier and Boulanger 2019).

LAP TGF-beta-1

LAP TGF-beta-1 is an anti-inflammatory cytokine that was independently associated with AD in my study (OR [95% CI]: 1.46 [1.21-1.76]). Additionally, it was significantly associated with all-cause dementia and VD. This biomarker consists of two components, latency-associated peptide (LAP) and transforming growth factor beta-1 (TGF-beta-1), which are non-covalently linked to each other in the intracellular environment. Thereby, LAP keeps TGF-beta-1 biologically inactive (Khalil 1999). When activated, TGF-beta-1 binds to its receptor transforming growth factor-ß receptor type I (TßR-1), protecting neurons against Aβ deposits and apoptosis (Estrada et al. 2018; Fang et al. 2018). However, controversial findings have been reported on concentrations of this biomarker in AD (Diniz et al. 2019). The current theory is that the level of TGF-beta-1 in the body might depend on disease progression (Diniz et al. 2019). According to this theory, the reported elevated levels of TGF-beta-1 in my study might show an early response to commencing neurodegenerative processes in AD. A recent study additionally reported on the specificity of TGF-beta-1 for AD and VD compared to Parkinson's disease dementia (PDD) (Khedr et al. 2020).

VEGF-A

In this cohort, VEGF-A was independently associated with VD (OR [95% CI]: 1.43 [1.20-1.70]) and also significantly associated with all-cause dementia and AD. VEGF-A belongs to the vascular endothelial growth factor (VEGF) family and induces endothelial cell growth, cell migration, and permeabilization of blood vessels. Like other members of this family, VEGF-A induces the receptors VEGF receptor 1 and 2 (VEGFR-1 and VEGFR-2) (Shibuya 2012). In VD, VEGF-A is reported to be involved in microvessel loss and blood-brain barrier breakdown (Kim et al. 2017). It was shown in the same study in mice that VEGF-A is involved in the hypoxia-inducible factor 1α-Lipocalin2-VEGFA (HIF-1α-LCN2-VEGFA) axis. Other groups have also shown an involvement of VEGF-A in increasing blood-brain barrier permeability (Chapouly et al. 2015; Jiang et al. 2014). Hence, blocking VEGF-A signalling might be a promising therapeutic target (Shaik et al. 2020).

4.3.3 Inflammatory proteins prominently discussed in dementia research and biomarker clusters

Interestingly, the frequently discussed inflammatory biomarker IL-6 was not significantly associated with any dementia outcome in my study but highly correlated with EN-RAGE and VEGF-A (Darweesh et al. 2018; Lai et al. 2017; Su et al. 2019). However, apart from the inflammatory biomarkers discussed above, many others were statistically significantly associated with dementia outcomes as well but highly correlated with the highlighted proteins. IL-10, for example, is currently discussed by others as a risk factor for AD (Park et al. 2020). In this study, IL-10 was also statistically significantly associated with all-cause dementia and VD even after correction for multiple testing. In addition, a subunit of the IL-10 receptor (IL-10RB) was significantly associated with all-cause dementia and AD. Both IL-10 and IL-10RB were highly correlated with VEGF-A and IL-10RB, additionally with LAP TGF-beta-1 and CX3CL1. Due to the high correlation of these biomarkers, it is not possible to decide with the used study design which of the biomarkers are the most clinically relevant ones and are causally associated with the outcome. Basic research is needed to elucidate this open question and the role of the identified biomarkers in the aetiology of dementia. The underlying mechanisms are likely to be complex because it is known that the multifactorial process of inflammation comes along with increases in the levels of many inflammatory proteins.

Therefore, it might be necessary to look into inflammatory protein networks/clusters rather than focusing on single proteins in future studies. For example, for AD, two such protein clusters were identified in this study. The EN-RAGE and the LAP TGF-beta-1 cluster consist of respectively nine inflammatory proteins significantly associated with AD (**Supplemental Tables 25 and 26**). The overlap of the two clusters is only three proteins (HGF, CD244 and uPA).

4.3.4 Role of *APOE* ε4 polymorphism

APOE ε4 negative subjects had stronger associations between inflammation biomarkers and dementia outcomes than *APOE* ε4 positive individuals. Interestingly, the interaction of *APOE* ε4 and EN-RAGE for all-cause dementia was statistically significant. One explanation could be that the absolute AD risk of *APOE* ε4 carriers is so pronounced that the additional presence or absence of a weaker risk factor, such as inflammation, may not have much impact. In contrast, the potential impact of inflammation on the total dementia risk of *APOE* ε4 non-carriers is relatively high compared to other dementia risk factors.

4.3.5 Strengths and limitations

The strengths of this study comprise a large sample size, the representative sample of an older adult population, a long follow-up period (17 years), and the prospective cohort design. Moreover, the diversity of inflammatory biomarkers (72 biomarkers analyzed) and the high sensitivity and specificity of Olink's proximity extension assays (Assarsson et al. 2014; Lundberg et al. 2011) used for the biomarker measurements can be assigned to the study's strengths.

The observational study design is one of the limitations of this study. Although analyses were controlled for confounders, residual confounding cannot be entirely excluded. Apart from this, the latency between the onset and the clinical diagnosis of dementia can be longer than the follow-up time of 17 years (Ritchie et al. 2016). However, results were still statistically significant after excluding events in the first ten years of follow-up. Thus, it can be assumed that there is no strong indication of reverse causality in the study results.

In the ESTHER study, dementia information is collected via GPs. After a referral to various neurologists, psychiatrists, memory clinics, or other specialized providers in the study region, diagnoses were obtained from the medical records of specialists. This process reflects the community-based clinical setting in Germany. Therefore, dementia diagnostics were performed heterogeneously, and dementia subtypes were often not assessed. This may be one reason why the ratio of AD to VD diagnoses is not as high in my study as in other studies with homogenous subtype diagnostics based on biomarkers of AD pathology among all study participants.

Due to cost issues, biomarker measurements were only performed for baseline blood samples and could not be repeated in follow-ups. Furthermore, findings could not be replicated in another independent study, which should be aimed at future research.

The biomarkers TNF and IFN-gamma had to be excluded since the proportion of values below LOD was > 25% in the total study sample. After Olink improved the inflammation panel in 2019, better results could be achieved for these biomarkers. However, the improved panel was only used in a fraction of the study sample (n = 440). When analyzing the data only in this subsample, TNF and IFN-gamma showed significant associations for all three analyzed dementia outcomes.

Lastly, it has to be stated that my study results refer to an almost exclusively Caucasian population with blood samples taken between the ages of 50 and 75 years and may not be generalized to other types of populations.

4.4 Improved risk prediction of all-cause dementia, Alzheimer's disease, and vascular dementia by biomarkers of oxidative stress and inflammation

In the prospective cohort study performed to pursue the fourth aim of this dissertation, I aimed to improve the CAIDE model by including the serum levels of inflammation-related proteins. Adding the biomarkers to different models for all-cause dementia, AD, and VD led to a substantial improvement in the discriminative ability of the models, but due to lower case numbers for AD and VD, only the AUC improvement for all-cause dementia was statistically significant. In addition, a better dementia prediction in mid-life than late-life became

apparent, which was further improved by the inflammation-related biomarkers. In addition, all models improved when *APOE* ε4 carrier status was included.

4.4.1 Previous studies

The CAIDE score showed good external validity in four cohorts without any adjustments to the model (Exalto et al. 2014; Fayosse et al. 2020; Licher et al. 2018; Virta et al. 2013). All of them reported a similar discriminative performance of the score. Besides, the CAIDE risk score was evaluated as a tool for dementia risk prediction in different ethnicities and showed good predictive ability in subgroups for Asians and dark-skinned people (Exalto et al. 2014). However, the prognosis was poor in cohorts of Hispanic/Latino Americans and Japanese American men (Chosy et al. 2019; Torres et al. 2020). Furthermore, Stephan and colleagues recently showed that the CAIDE score has poor predictive ability in low- and middle-income countries ($0.52 \leq c \leq 0.63$) (Stephan et al. 2020). Finally, it was demonstrated that the CAIDE score is not applicable to late-life cohorts (Anstey et al. 2014). Thus, despite its unquestionable advantages, improvements of the CAIDE score are needed.

To my knowledge, three modifications of the CAIDE score are available: Tolea and colleagues designed a modified version of the CAIDE score (mCAIDE) to simplify the application of the model in a community-based setting (Tolea et al. 2021). Therefore, laboratory measurements of cholesterol levels were replaced by self-reported information about high cholesterol levels (yes or no). In addition, physical activity assessment was replaced by the mini Physical Performance Testing (mPPT). The mCAIDE score was first applied to a cohort of 230 community-dwelling older adults in which it slightly improved the discrimination between cognitively impaired and unimpaired individuals (AUC mCAIDE: 0.78 [0.71-0.85], AUC CAIDE: 0.71 [0.61-0.80]). Afterwards, the score was additionally validated in an independent clinical cohort of 219 participants and demonstrated to discriminate well between different stages of dementia.

Exalto and colleagues aimed to improve the CAIDE score by including diabetes mellitus, depressed mood, head trauma, central obesity, lung function, and smoking as additional mid-life risk factors (Exalto et al. 2014). However, the added variables did not improve its predictive abilities.

Harrison and colleagues tested if adding a composite score of two biomarkers of inflammation (IL-6 and CRP) and one of OS (homocysteine) to the CAIDE score would improve the predictive ability of cognitive decline for study participants of two cohorts aged 85 years or older (Harrison et al. 2017). Adding the biomarkers to the CAIDE score increased the HR for comparison of a high- and low-risk group from 1.14 (95% CI: 0.64-2.03, p = 0.65) to 1.96 (1.27-3.42, p = 0.02) in the first cohort and from 1.64 (1.04-2.58, p = 0.03) to 1.89 (1.18-3.02, p = 0.08) in the second cohort. However, AUC differences were not reported.

4.4.2 Interpretation of findings

Compared to the original CAIDE model, the predictive ability in this study was lower but still good (AUCs of 0.769 and 0.776 for CAIDE model 1 and 2 in the original study compared to 0.725 and 0.751, respectively, for all-cause dementia in this study). Nonetheless, the discriminative ability increased in all prediction models when the inflammation-related biomarkers were added. This increase was statistically significant in the main analysis for all-cause dementia in the total cohort. Inflammation is considered to have a crucial role in dementia pathogenesis (Kinney et al. 2018; Raz et al. 2016). During a continuous inflammatory response outside of the central nervous system (systemic inflammation), pro- and anti-inflammatory proteins are released and permeate through the blood-brain barrier. Thereby, the transition of microglia and astrocytes into their active phenotypes is triggered (Walker et al. 2019a). Pro-inflammatory products are then released and are first beneficial as part of a defence mechanism. However, if inflammation becomes chronic, the permanent release of pro-inflammatory cytokines can cause neuronal damage and neurodegeneration, possibly leading to cognitive decline and neurodegenerative processes (Kinney et al. 2018; Lim et al. 2015; Walker et al. 2019a). Thus, increased inflammatory protein levels might be predictive for dementia in later life.

Notably, CX3CL1 and EN-RAGE, LAP TGF-beta-1 and EN-RAGE, and VEGF-A were among the biomarkers chosen by the LASSO regression for all-cause dementia, AD, and VD, respectively. In the previous analysis with the case-cohort sample from the ESTHER study, I showed that these biomarkers are independently associated with the respective dementia outcomes and discussed the potential mechanisms (chapters 2.3, 3.3, and 4.3).

The OS biomarker 8-iso-$PGF_{2\alpha}$ did not improve the model's discriminative ability for all-cause dementia. This can be explained by the biomarker being already associated with many CAIDE model variables like sex, education, BMI, or physical activity, as shown in chapters 2.1, 3.1 and 4.1.

A clear difference in the predictive ability of the CAIDE model between mid-life and late-life with a better predictive ability for mid-life was also shown in this study. Similarly, a poor performance of the CAIDE model in late-life samples was observed in a previous study by Anstey et al. (Anstey et al. 2014). Furthermore, Fayosse and colleagues showed that CAIDE was only significantly associated with dementia at a mean age of 55 years but not at 60 or 65 years when examining participants separately (Fayosse et al. 2020). Other risk prediction models reviewed in this study (Framingham cardiovascular Risk Score (FRS) and Finnish Diabetes Risk Score (FINDRISC)) also performed poorly in the split age categories. Finally, Licher et al. demonstrated that age nearly has a similar predictive ability to four prominent models (CAIDE, Brief Dementia Screening Indicator (BDSI), Australian National University Alzheimer's Disease Risk Index (ANU-ADRI), and Dementia Risk Score (DRS)) (Licher et al. 2018). This indicates that age is the most determining factor in dementia risk prediction, and some other factors in the CAIDE model might be dispensable. In the sensitivity analysis penalizing all CAIDE model variables, education and systolic blood pressure were not selected by the LASSO regression, implying that they did not contribute to the predictive ability of the CAIDE model in this study.

Due to the outstanding importance of age in dementia prediction, cohorts with a large age range should be analyzed separately for mid-life and late-life samples. Especially in the case of CAIDE, it seems advisable to apply it only to mid-life samples. Interestingly, in this study, inflammation-related biomarkers improved the predictive abilities of the CAIDE model for all-cause dementia prediction more in the mid-life than the late-life sample. A possible explanation is that chronic low-grade inflammation is a symptom of advancing age (also known as "inflammaging") (Zuo et al. 2019). In late-life, inflammatory biomarker levels might also be increased in individuals, not at risk of dementia and differentiate less well subjects at higher and lower risk for dementia.

Finally, *APOE* ε4 polymorphism is a well-known risk factor of dementia, especially for AD, and is a constant component of most dementia risk prediction models (Tang et al. 2015). Also, in

this study, *APOE* ε4 carrier status was one of the strongest predictors. Nevertheless, even the CAIDE model 2, including *APOE* ε4, was further improved by adding inflammation-related biomarkers. While the *APOE* ε4 genotype is known to be involved in nearly every pathology leading to AD, inflammation might link these processes (Kloske and Wilcock 2020). However, the interplay between *APOE* and inflammation is not fully understood yet.

4.4.3 Application of the created prediction models

Another essential feature of dementia risk prediction models is their applicability in the clinical and community-based setting (Hou et al. 2019). Identifying individuals at risk of developing dementia makes early intervention and prevention possible. For example, a recent report of the Lancet Commission demonstrated that 40% of dementia cases could be prevented or delayed by modifying 12 risk factors of daily living (Livingston et al. 2020). In addition, diagnoses could be made early, before symptoms occur. In this study, I developed dementia risk prediction models, which can be easily transferred to the community-based and clinical setting. The model with the best predictive abilities for all-cause dementia in the total cohort with an AUC of 0.776 was CAIDE model 2 (including *APOE* ε4 genotype) extended by 16 inflammation-related proteins. Thus, in clinical practice, only a brief physical examination and interview with the patient and a blood sample sent to a laboratory offering the needed analyses would be required to obtain all necessary information to estimate the dementia risk with this prediction model. Although cerebral magnetic resonance imaging (MRI) measures would provide higher accuracy, their use in prediction models is disadvantageous because of their extensive costs (Hou et al. 2019; Stephan et al. 2010; Tang et al. 2015).

4.4.4 Strengths and limitations

This study is characterized by the prospective cohort design, a long follow-up period of 17 years, its large sample size and the representativeness for the German health care setting. In addition, appropriate measures were taken to prevent overfitting of the developed models by applying LASSO logistic regression and bootstrapping (Ranstam and Cook 2018; Tibshirani 1996).

Although this study has a long follow-up time, reverse causality cannot be completely excluded. The latency period between dementia onset and clinical diagnosis of the disease might be longer than 10 years (Ritchie et al. 2016). Thus, the observed predictive ability of the inflammation-related biomarkers could result to some extent from early dementia detection.

In the ESTHER study, dementia diagnoses are being collected in a community-based setting. Thus, diagnoses were collected from medical records, and subtypes often have not or have not been accurately assessed. This might also explain the comparatively low proportion of AD cases. However, the most important outcome for dementia risk assessment in the community setting is all-cause dementia. Moreover, due to a different age structure, education system, and physical activity assessment in the ESTHER study compared to the CAIDE study, the CAIDE variables needed to be adopted. This hampers a direct comparison to the results of the CAIDE score. Furthermore, the created prediction models were not validated externally, which should be addressed in future studies. Finally, the results of this study originate from a study population that comprises mainly Caucasians aged 50 to 75 years. Hence, the results might not be generalized to other populations.

4.5 Conclusion

The overall aim of this dissertation was to identify biomarkers from the fields of oxidative stress and inflammation and combine them with already known dementia risk factors in a comprehensive dementia risk prediction model. By this, individuals at risk of dementia should be identified early.

The associations between the oxidative stress biomarker 8-iso-$PGF_{2\alpha}$ and all-cause dementia, AD, and VD were analyzed for the first aim of this dissertation. 8-iso-$PGF_{2\alpha}$ levels were statistically significantly associated with all-cause dementia and AD incidence. However, due to the relatively low case numbers for VD in my study, the non-significant finding for this outcome should not be interpreted as the absence of an association. Larger studies are needed for dementia sub-types that are less frequent than AD. Furthermore, future studies should corroborate the observed interaction of the *APOE* ε4/ε4 genotype and 8-iso-$PGF_{2\alpha}$ levels, which was not statistically significant after correction for multiple testing but

biologically plausible. In addition, the role of lipid peroxidation in dementia development should be explored by further basic research studies.

To achieve the second aim, a systematic review and meta-analysis of 29 observational studies on the association of F_2-isoprostane levels and AD were performed. A statistically significant association was observed in the meta-analysis of 25 cross-sectional studies but not in meta-analyses of four longitudinal studies. More longitudinal studies adjusting for potential confounders, previously shown to be associated with 8-iso-$PGF_{2\alpha}$ levels, like age, sex, education, current smoking, alcohol consumption, physical activity, body mass index, diabetes, and *APOE* ε4 polymorphism, are needed to clarify whether OS plays a causal role in AD development.

The association of inflammation-related blood-based biomarkers from the Olink inflammation panel with all-cause dementia, AD, and VD was assessed for the third aim. 58 out of 72 tested proteins were significantly associated with all-cause dementia incidence even after correction for multiple testing. In addition, several inflammatory proteins were further associated with AD and VD. The biomarkers CX3CL1, EN-RAGE, LAP TGF-beta-1, and VEGF-A had strong and independent associations with dementia outcomes and may have great potential as drug targets, early diagnostic markers, and components of dementia prediction scores. However, due to the observed high inter-correlation of inflammatory biomarkers, it should be noted that not only single biomarkers but also clusters of increased inflammatory protein levels may play a role in dementia pathogenesis or risk prediction. The complex interrelationships in these clusters are not yet understood and require further research.

Finally, the CAIDE model was applied to the ESTHER study. Adding 16 inflammation-related blood-based biomarkers to the CAIDE model, including the *APOE* ε4 genotype, significantly improves the model's discriminative ability for all-cause dementia. The factors needed for this improved CAIDE model are easy to obtain in clinical routine, indicating a potential utility for dementia risk screening. The model's predictive abilities improvements were of similar magnitude for AD and VD but not statistically significant due to lower case numbers for dementia subtypes. The discriminative ability of all models was higher in the mid-life compared to the late-life subsample. However, it is more important to have suitable dementia risk assessment tools in mid-life because targeting dementia risk factors in mid-life has a greater potential to prevent or delay the onset of dementia than in late-life. Therefore, future

studies are warranted to externally validate this improved CAIDE model, including inflammatory serum proteins in mid-life populations with an extended dementia follow-up (ideally more than 15 years).

In conclusion, future longitudinal studies should further examine the association between the oxidative stress biomarker 8-iso-$PGF_{2\alpha}$ and dementia. Although the biomarker did not have a predictive value, it might be helpful for a better understanding of dementia pathogenesis. Furthermore, the investigation of inflammatory biomarker clusters could shed more light on the complex interrelationships of inflammatory metabolites in dementia pathogenesis. However, more importantly, inflammatory biomarkers significantly increased the discriminative ability of dementia risk prediction models. The improved CAIDE model has great potential to be applied in the clinical setting and should be externally validated in future studies.

5. Summary

5.1 English summary

Dementia is a major challenge for global public health and social care systems. With increasing life expectancy and constantly rising numbers of dementia cases, preventing or delaying the onset of dementia are of tremendous importance. Therefore, it is indispensable to identify individuals at risk of dementia early. This dissertation aimed to combine established dementia risk factors with newly identified biomarkers from the field of oxidative stress and inflammation in a dementia risk prediction model.

First, to assess the associations between oxidative stress and dementia, the gold-standard biomarker 8-iso-prostaglandin $F_{2\alpha}$ was utilized and measured in urinary samples from 5,853 participants aged between 50 and 75 years of the German population-based ESTHER study. Over 14 years of follow-up, 365 all-cause dementia cases were diagnosed, including 127 vascular dementia and 109 Alzheimer's disease cases. Participants in the top compared to the bottom 8-iso-prostaglandin $F_{2\alpha}$ tertile had a 45% increased risk of all-cause dementia incidence. Furthermore, continuously modelled, logarithmized 8-iso-prostaglandin $F_{2\alpha}$ levels were statistically significantly associated with Alzheimer's disease incidence. Moreover, an interaction between high 8-iso-prostaglandin $F_{2\alpha}$ levels and the *APOE* ε4/ε4 genotype was detected. Participants with both risk factors had an almost 9-fold increased risk of dementia.

Next, I put my own study results in context with the pre-existing literature and conducted a systematic review. In a random-effects model meta-analysis of 25 cross-sectional studies, F_2-isoprostane levels were statistically significantly associated with Alzheimer's disease (Hedge's g [95% confidence interval]: 1.00 [0.69-1.32]). In addition, when studies were grouped by biomarker and sample specimen, F_2-isoprostanes and 8-iso-prostaglandin $F_{2\alpha}$ levels were statistically significantly elevated in tissue samples of the frontal lobe of Alzheimer's disease patients. Moreover, F_2-isoprostane levels in cerebrospinal fluid and 8-iso-prostaglandin $F_{2\alpha}$ levels in blood samples of Alzheimer's disease patients were significantly increased. The 4 longitudinal studies did not yield significant associations in meta-analyses raising doubts about the causality of the association found in the cross-sectional studies. In addition, none of the cross-sectional studies was adjusted for potential confounders. Thus, the quality of evidence in this field is poor and further adjusted longitudinal studies are required to reinforce results.

After examining an oxidative stress biomarker in dementia, the Olink Target 96 Inflammation panel was used in the ESTHER study to evaluate whether inflammation-related biomarkers are associated with incident all-cause dementia, Alzheimer's disease, and vascular dementia cases ascertained until the 17-year follow-up. In a case-cohort study design, biomarker levels were measured in serum samples of 504 all-cause dementia cases (including 163 Alzheimer's disease and 195 vascular dementia cases) and 1,278 controls. After correction for multiple testing, 58 out of 72 tested (80.6%) biomarkers were statistically significantly associated with all-cause dementia, 22 with Alzheimer's disease, and 33 with vascular dementia incidence. Four biomarker clusters were identified. Among those, the strongest representatives, CX3CL1, EN-RAGE, LAP TGF-beta-1 and VEGF-A, were significantly associated with dementia endpoints independently from other inflammation-related proteins. In addition, all named associations were stronger among *APOE* ε4 negative subjects.

Finally, to create the intended dementia risk prediction model, the established and well-validated Cardiovascular Risk Factors, Aging and Dementia (CAIDE) model was applied to the ESTHER case-cohort sample described above. I aimed to improve it by adding the investigated biomarkers using LASSO regression. Different models for all-cause dementia, Alzheimer's disease, and vascular dementia were created. The oxidative stress biomarker 8-iso-prostaglandin $F_{2\alpha}$ did not improve dementia risk prediction. However, adding 16 biomarkers from the Olink Target 96 Inflammation to the CAIDE model version, including *APOE* ε4 significantly improved the predictive ability for all-cause dementia (area under the curve (AUC) increase by 0.032 increments) and resulted in an AUC of 0.776. The AUC increase of prediction models for Alzheimer's disease and vascular dementia was of similar magnitude but not statistically significant due to lower case numbers. The CAIDE model generally performed better in mid-life (50-64 years) than in late-life (65-75 years) subsamples. All AUC increases by inflammation-related biomarkers were larger in the mid-life subsample.

Overall, this work showed that oxidative stress is involved in dementia pathogenesis, but the representative biomarker 8-iso-prostaglandin $F_{2\alpha}$ does not improve dementia risk prediction models. In contrast, the majority of the studied inflammation-related biomarkers were associated with all-cause dementia and additionally significantly improved the predictive ability of the established CAIDE model. Especially the predictive abilities of these blood-based biomarkers in mid-life are of clinical relevance to guide early preventive measures against dementia.

5.2 Deutsche Zusammenfassung

Demenz stellt weltweit eine große Herausforderung für das öffentliche Gesundheitswesen und die Sozialfürsorgesysteme dar. Angesichts der steigenden Lebenserwartung und der ständig wachsenden Zahl von Demenzfällen ist es von enormer Bedeutung, den Beginn einer Demenzerkrankung zu verhindern oder zu verzögern. Daher ist es unerlässlich, Personen mit einem Demenzrisiko frühzeitig zu identifizieren. Ziel dieser Dissertation war es, etablierte Demenz-Risikofaktoren mit neu identifizierten Biomarkern für oxidativen Stresses und Entzündung in einem Demenz-Risikovorhersagemodell zu kombinieren.

Um den Zusammenhang zwischen oxidativem Stress und Demenz zu bewerten, wurde zunächst der Goldstandard-Biomarker 8-iso-Prostaglandin $F_{2\alpha}$ verwendet und in Urinproben von 5.853 Teilnehmern der deutschen bevölkerungsbasierten ESTHER-Studie im Alter zwischen 50 und 75 Jahren gemessen. Während der 14-jährigen Nachbeobachtungszeit wurden 365 Fälle von Demenz diagnostiziert, darunter 127 Fälle von vaskulärer Demenz und 109 Fälle von Alzheimer-Krankheit. Teilnehmer aus dem obersten im Vergleich zum untersten 8-iso-Prostaglandin $F_{2\alpha}$-Terzil hatten ein um 45% erhöhtes Risiko für das Auftreten von allgemeiner Demenz. Darüber hinaus waren die kontinuierlich modellierten, logarithmierten 8-iso-prostaglandin $F_{2\alpha}$-Werte statistisch signifikant mit dem Auftreten von Alzheimer-Erkrankungen verbunden. Außerdem wurde eine Wechselwirkung zwischen hohen 8-iso-Prostaglandin $F_{2\alpha}$-Spiegeln und dem *APOE* ε4/ε4-Genotyp festgestellt: Teilnehmer, die beide Risikofaktoren aufwiesen, hatten ein fast 9-fach erhöhtes Risiko, an Demenz zu erkranken. Als Nächstes habe ich meine eigenen Studienergebnisse in den Kontext der bereits vorhandenen Literatur gestellt und eine systematische Übersichtsarbeit durchgeführt. In einer Meta-Analyse mit Random-Effects-Modell, die 25 Querschnittsstudien einschloss, wurde ein statistisch signifikanter Zusammenhang zwischen dem F_2-Isoprostan-Spiegel und der Alzheimer-Krankheit festgestellt (Hedge's g [95% Konfidenzintervall]: 1,00 [0,69-1,32]). Wenn die Studien nach Biomarker und Probenmaterial gruppiert wurden, waren die Werte von F_2-Isoprostanen und 8-iso-Prostaglandin $F_{2\alpha}$ in Gewebeproben des Frontallappens von Alzheimer-Patienten statistisch signifikant erhöht. Darüber hinaus waren die F_2-Isoprostan-Werte in der Cerebrospinalflüssigkeit und die 8-iso-Prostaglandin $F_{2\alpha}$-Werte in Blutproben von Alzheimer-Patienten signifikant erhöht. Über 4 Längsschnittstudien ergaben sich in den Metaanalysen keine signifikanten Zusammenhänge, was Zweifel aufkommen lässt, ob der in den Querschnittsstudien gefundene Zusammenhang kausal ist. Darüber hinaus wurde keine der Querschnittsstudien für potenzielle Störfaktoren adjustiert. Daher ist die Qualität der Nachweise in diesem Bereich mangelhaft und es sind weitere adjustierte Längsschnittstudien

erforderlich, um die Ergebnisse zu bestärken. Nach der Untersuchung eines Biomarkers für oxidativen Stress bei Demenz wurde das Olink Target 96 Inflammation Panel in der ESTHER-Studie verwendet, um zu untersuchen, ob entzündungsbezogene Biomarker mit neu auftretenden Fällen von allgemeiner Demenz, Alzheimer-Krankheit und vaskulärer Demenz assoziiert sind, die in der 17-jährigen Nachbeobachtungszeit festgestellt wurden. Im Rahmen einer Fall-Kohorten-Studie wurden die Biomarkerwerte in Serumproben von 504 Demenzfällen (darunter 163 Fälle von Alzheimer und 195 Fälle von vaskulärer Demenz) und 1.278 Kontrollen gemessen. Nach Korrektur für multiples Testen waren 58 von 72 getesteten Biomarkern (80,6 %) statistisch signifikant mit allgemeiner Demenz, 22 mit Alzheimer-Krankheit und 33 mit vaskulärer Demenz assoziiert. Es wurden vier Biomarker-Cluster identifiziert. Unter diesen waren die stärksten Vertreter, CX3CL1, EN-RAGE, LAP TGF-beta-1 und VEGF-A, unabhängig von anderen entzündungsbezogenen Proteinen signifikant mit Demenz-Endpunkten assoziiert. Darüber hinaus waren alle genannten Assoziationen bei *APOE*-ε4-negativen Probanden stärker ausgeprägt. Um schließlich das geplante Demenzrisikomodell zu erstellen, wurde das etablierte und gut validierte Modell für kardiovaskuläre Risikofaktoren, Alterung und Demenz (CAIDE) auf die oben beschriebene ESTHER-Fall-Kohorten Stichprobe angewendet. Ich habe versucht, das Modell durch Hinzufügen der untersuchten Biomarker mittels LASSO-Regression zu verbessern. Es wurden verschiedene Modelle für allgemeine Demenz, Alzheimer-Krankheit und vaskuläre Demenz erstellt. Der Biomarker für oxidativen Stress, 8-iso-Prostaglandin $F_{2\alpha}$, verbesserte die Vorhersage des Demenzrisikos nicht. Das Hinzufügen von 16 Biomarkern aus dem Olink Target 96 Inflammation zur CAIDE-Modellversion, einschließlich *APOE* ε4, verbesserte jedoch die Vorhersagefähigkeit für allgemeine Demenz signifikant (Anstieg der Fläche unter der Kurve (AUC) um 0,032 Schritte) und führte zu einer AUC von 0,776. Die AUC-Erhöhung der Vorhersagemodelle für die Alzheimer-Krankheit und die vaskuläre Demenz war ähnlich hoch, aber aufgrund der geringeren Fallzahlen statistisch nicht signifikant. Das CAIDE-Modell schnitt im mittleren Lebensalter (50-64 Jahre) im Allgemeinen besser ab als in den Unterstichproben im späten Lebensalter (65-75 Jahre). Alle AUC-Erhöhungen durch entzündungsbezogene Biomarker waren in der Teilstichprobe des mittleren Lebensalters größer.

Insgesamt zeigte diese Arbeit, dass oxidativer Stress an der Pathogenese der Demenz beteiligt ist, der repräsentative Biomarker 8-iso-Prostaglandin $F_{2\alpha}$ jedoch die Modelle zur Vorhersage des Demenzrisikos nicht verbessert. Im Gegensatz dazu waren die meisten der untersuchten entzündungsbezogenen Biomarker mit allgemeiner Demenz assoziiert und verbesserten zusätzlich die Vorhersagefähigkeit des etablierten CAIDE-Modells erheblich. Insbesondere die Vorhersagefähigkeiten dieser blutbasierten Biomarker im mittleren Lebensalter sind von klinischer Relevanz, um frühzeitige Präventionsmaßnahmen gegen Demenz zu ergreifen.

6. References

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7. Own publications and contributions

This dissertation was conducted based on data from the ESTHER study led by Prof. Dr Hermann Brenner. I organized and coordinated additional biomarker measurements with the Olink Target 96 inflammation panel (Olink Proteomics, Uppsala Sweden) for the study and performed the data normalization for the newly gained data. The analysis and evaluation of the ESTHER study data were conducted by myself and is the central result of this dissertation. The data analysis of the first project was accomplished together with Xin Gào, which is reflected by a shared first authorship on the paper (1st publication in the list below).

The full-text selection and data extraction for the systematic review were performed independently by two researchers, which were my co-doctoral fellow Li-Ju Chen and me. The design of the search strategy, the preparation and analysis of the data, and the interpretation of results for the systematic review were carried out by me.

All results of the present work were or will be published in peer-reviewed journals within the scope of the following 4 articles:

1. Trares, K., Gào, X., Perna, L., Rujescu, D., Stocker, H., Möllers, T., Beyreuther, K., Brenner, H. and Schöttker, B. (2020). **Associations of urinary 8-iso-prostaglandin F(2α) levels with all-cause dementia, Alzheimer's disease, and vascular dementia incidence: results from a prospective cohort study**. Alzheimers Dement *16 (5)*, 804-813, doi: 10.1002/alz.12081.

2. Trares, K., Chen, L.-J. and Schöttker, B. (2022). **Association of F2-isoprostane levels with Alzheimer's disease in observational studies: A systematic review and meta-analysis**. Ageing Research Reviews *74*, 101552, [epub ahead of print], doi: https://doi.org/10.1016/j.arr.2021.101552.

3. Trares, K., Bhardwaj, M., Perna, L., Stocker, H., Petrera, A., Hauck, S. M., Beyreuther, K., Brenner, H. and Schöttker, B. (2021). **Association of the inflammation-related proteome with dementia development at older age: results from a large, prospective, population-based cohort study**. medRxiv, 2021.2006.2015.21258913, doi: 10.1101/2021.06.15.21258913. *[Under review at Neurology]*

4. Trares, K., Wiesenfarth, M., Stocker, H., Perna, L., Beyreuther, K., Brenner, H. and Schöttker, B. (202X). **Improved risk prediction of all-cause dementia, Alzheimer's disease, and vascular dementia by inflammation-related proteins**. *[In preperation]*

Chapters 2.1, 3.1, 4.1, 4.5, and parts of chapter 1 of this dissertation originate from **publication 1**. My own contributions to this publication include data analysis, interpretation of results and writing of the manuscript, which was revised by my supervisor PD Dr Ben Schöttker. Xin Gào prepared the datasets and contributed to the data analysis.

The dissertation chapters 2.2, 3.2, 4.2, 4.5, and parts of chapter 1 are based on **publication 2**. My own contributions to this publication include the organization and coordination of the biomarker measurements, the normalization of the biomarker data, preparing the data sets, planning and conducting data analyses, interpretation of results and writing of the manuscript. My supervisor PD Dr Ben Schöttker revised the manuscript.

Publication 3 provides the content for chapters 2.3, 3.3, 4.3, 4.5 and parts of chapter 1, 2.1, 3.1, and 4.1 of this dissertation. My own contributions to this publication include the design of the search strategy, the preparation and analysis of the data, the interpretation of results, and the writing of the manuscript, which was revised by my supervisor PD Dr Ben Schöttker. The selection of the relevant literature and the data extraction were performed independently by two researchers, which were my co-doctoral fellow Li-Ju Chen and me.

Chapters 2.4, 3.4, 4.4, 4.5, and parts of chapter 1 of this dissertation are based on **publication 4**. My own contributions to this publication include the planning of the data analysis, preparing the data sets, the implementation of data analysis, interpretation of the results and writing the manuscript. The manuscript is in preparation and was revised by my supervisor PD Dr Ben Schöttker.

Further own and co-authored publications:

5. Chen, L.-J., Trares, K., Laetsch, D. C., Nguyen, T. N. M., Brenner, H. and Schöttker, B. (2020). **Systematic Review and Meta-Analysis on the Associations of Polypharmacy and Potentially Inappropriate Medication With Adverse Outcomes in Older Cancer Patients**. The Journals of Gerontology: Series A, doi: 10.1093/gerona/glaa128.

6. Mai Nguyen, T. N., Chen, L.-J., Trares, K., Stocker, H., Holleczek, B., Beyreuther, K., Brenner, H. and Schöttker, B. (2021). **Long-term low-dose acetylsalicylic use shows protective potential for the development of both vascular dementia and Alzheimer's disease in patients with coronary heart disease but not in other individuals from the general population: results from two large cohort studies**. medRxiv, 2021.2009.2020.21263830, doi: 10.1101/2021.09.20.21263830.

7. Perna, L., Trares, K., Perneczky, R., Tato, M., Stocker, H., Möllers, T., Holleczek, B., Schöttker, B. and Brenner, H. (2021). **Risk of Late-Onset Depression and Cognitive Decline: Results From Inflammatory Proteome Analyses in a Prospective Population-Based Cohort Study**. The American Journal of Geriatric Psychiatry, doi: https://doi.org/10.1016/j.jagp.2021.12.001.

8. Ponce-de-Leon, M., Linseisen, J., Peters, A., Linkohr, B., Heier, M., Grallert, H., Schöttker, B., Trares, K., Bhardwaj, M., Gào, X., Brenner, H., Kamiński, K. A., Paniczko, M., Kowalska, I., Baumeister, S. E. and Meisinger, C. (2021). **Novel associations between inflammation-related proteins and adiposity: A targeted proteomics approach across four population-based studies**. Translational Research, doi: 10.1016/j.trsl.2021.11.004.

9. Salem, A. A., Trares, K., Kohl, M., Jansen, E., Brenner, H. and Schöttker, B. (2022). **Long-term effects of smoking on serum concentrations of oxidative stress biomarkers: Results of a large, population-based cohort study**. Environmental Research *204*, 111923, doi: https://doi.org/10.1016/j.envres.2021.111923.

10. Schmiedek, F., Kroehne, U., Goldhammer, F., Prindle, J. J., Lindenberger, U., Kliner-Köonig, J., Grabe, H. J., Riedel-Heller, S. G., Pabst, A., Streit, F., Zillich, L., Kleineidam, L., Wagner, M., Rietschel, M., Rujescu, D., Schmidt, B., NAKO Investigators and Berger, K. (2021). **General cognitive ability assessment in the German National Cohort (NAKO) – The block-adaptive number series task**. The World Journal of Biological Psychiatry. *[Accepted for publication]*

11. Schönstein, A., Trares, K. and Wahl, H.-W. (202X). **Subjective Views of Aging and Objective Aging Biomarkers: An Emerging Research Area's Achievements and Questions**. In: Subjective Views of Aging: Theory, Research, and Practice, eds. Shrira, A., Palgi, Y. and Diehl, M., Springer. *[Accepted for publication]*

Oral presentations at scientific conferences and video journals:

15. Jahrestagung der Deutschen Gesellschaft für Epidemiologie

29th September, 2020 (online)

Oral presentation: „Associations of urinary 8-isoprostane levels with all-cause dementia, Alzheimer's disease and vascular dementia incidence: results from a prospective cohort study"

Alzheimer's Association International Conference (AAIC) 2021

26th-30th July, 2021, Denver, USA and online

Oral presentation: "Inflammation biomarkers for future development of all-cause dementia, Alzheimer's disease, and vascular dementia"

The Video Journal of Dementia

Video interviews in the scope of the AAIC 2021

"Inflammatory biomarkers for the prediction of dementia onset":

https://vjdementia.com/video/gwpqskbo8eg-inflammatory-biomarkers-for-the-prediction-of-dementia-onset/

"The role of inflammation in dementia":

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"The roles of inflammatory biomarkers in the pathogenesis of dementia":

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Supplement

Supplemental Table 1. Comparison of baseline characteristics of included n = 5,853 and excluded n = 4,087 study participants of the ESTHER cohort study

Baseline characteristics	*Excluded study participants*	*Included study participants*	P *
	n (%)	*n* (%)	
Age (years)			**< 0.001**
50-64	2364 (57.8)	3740 (63.9)	
65-69	966 (23.6)	1309 (22.3)	
70-75	757 (18.5)	804 (13.7)	
Sex			0.507
Female	2262 (55.4)	3200 (54.7)	
Male	1825 (44.7)	2653 (45.3)	
Education (years)			0.230
< 9	2999 (75.5)	4236 (74.1)	
9-11	553 (13.9)	819 (14.3)	
≥ 12	420 (10.6)	661 (11.6)	
Smoking status			**< 0.001**
Never smoker	1923 (48.8)	2909 (50.8)	
Former smoker	1246 (31.6)	1939 (33.9)	
Current smoker	775 (19.7)	874 (15.3)	
Alcohol consumption†			**< 0.001**
Abstainer	1303 (35.7)	1611 (30.3)	
Low or moderate	2108 (57.8)	3333 (62.6)	
High	239 (6.6)	381 (7.2)	
Physical activity‡			**< 0.001**
Inactive	986 (24.2)	1133 (19.4)	
Low	1881 (46.2)	2645 (45.3)	
Medium or high	1204 (29.6)	2061 (35.3)	
BMI (kg/m²)			0.428
< 25	1092 (26.8)	1632 (27.9)	
25-<30	1937 (47.5)	2738 (46.9)	
≥30	1052 (25.8)	1473 (25.2)	
CVD §			0.156
No	3239 (79.3)	4709 (80.5)	
Yes	845 (20.7)	1143 (19.5)	
Diabetes			**0.008**
No	3279 (83.8)	4951 (85.8)	
Yes	634 (16.2)	821 (14.2)	
Life-time history of depression			0.535
No	3498 (86.1)	4997 (85.5)	
Yes, without current pharmacotherapy	451 (11.1)	660 (11.3)	
Yes, with current pharmacotherapy	115 (2.8)	187 (3.2)	
Total cholesterol levels (mg/dl)			**0.001**
< 200	1472 (36.0)	1913 (32.7)	
200-<240	1270 (31.1)	1983 (33.9)	
≥240	1345 (32.9)	1957 (33.4)	

Baseline characteristics	*Excluded study participants*	*Included study participants*	P *
	n (%)	*n* (%)	
CRP levels (mg/L)			**0.016**
< 1	1100 (26.9)	1563 (26.7)	
1-<3	1431 (35.0)	2202 (37.6)	
≥3	1556 (38.1)	2088 (35.7)	
***APOE* genotypes**			0.328
ε4 non-carrier	2590 (73.9)	3913 (73.8)	
ε2/ε4	118 (3.4)	194 (3.7)	
ε3/ε4	724 (20.7)	1109 (20.9)	
ε4/ε4	72 (2.1)	83 (1.6)	

Abbreviations: *APOE*, apolipoprotein E; BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; IQR, interquartile range; NSAIDs, nonsteroidal anti-inflammatory drugs.

NOTE: Bold print: Statistically significant difference.

* Result of a χ^2 test

† Definition of low or moderate alcohol consumption: women 0-19.99 grams ethanol/day (g/d) or men 0-39.99 g/d; Definition of high alcohol consumption: women ≥ 20-39.99g/d or men ≥ 40g/d.

‡ "Inactive" was defined by < 1 h of vigorous or < 1 h light physical activity per week. "Medium or high" was defined by ≥ 2 h of vigorous and ≥ 2 h of light physical activity/week. All other amounts of physical activity were grouped into the category "Low".

§ CVD was defined as coronary artery disease or a self-reported history of myocardial infarction, stroke, pulmonary embolism, or revascularization of coronary arteries.

Supplemental Table 2. MOOSE checklist for systematic review

Reporting of Background	
Problem definition	Chapter 1.1-1.4
Hypothesis statement	Chapter 1.3, Chapter 1.4
Description of study outcome(s)	Chapter 1.1-1.4
Type of exposure or intervention used	Not applicable
Type of study designs used	Chapter 2.2
Study population	Chapter 1.6, Chapter 3.2.1
Reporting of Search Strategy	
Qualifications of searchers (e.g. librarians and investigators)	Chapter 2.2.1
Search strategy, including time period included in the synthesis and keywords	Chapter 2.2.1
Effort to include all available studies, including contact with authors	Chapter 2.2.1
Databases and registries searched	Chapter 2.2.1
Search software used, name and version, including special features used (e.g. explosion)	Chapter 2.2.1
Use of hand searching (e.g. reference lists of obtained articles)	Chapter 2.2.1
List of citations located and those excluded, including justification	Suppelemental Table 13
Method of addressing articles published in languages other than English	Not applicable
Method of handling abstracts and unpublished studies	Not applicable
Description of any contact with authors	Chapter 2.2.1
Reporting of Methods	
Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Chapter 2.2.1, Chapter 2.2.2
Rationale for the selection and coding of data (e.g. sound clinical principles or convenience)	Chapter 2.2
Documentation of how data were classified and coded (e.g. multiple raters, blinding, and interrater reliability)	Chapter 2.2.3
Assessment of confounding (e.g. comparability of cases and controls in studies where appropriate)	Chapter 2.2.2
Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	Chapter 2.2
Assessment of heterogeneity	Chapter 2.2.3
Description of statistical methods (e.g. complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-	Chapter 2.2.3

response models, or cumulative meta-analysis) in sufficient detail to be replicated	
Provision of appropriate tables and graphics	Table 4, Figures 5-10, Supplemental Tables 2-4 and 13-17
Reporting of Results	
Graphic summarizing individual study estimates and overall estimate	Figures 5-10
Table giving descriptive information for each study included	Supplemental Tables 14, 16-17
Results of sensitivity testing (e.g. subgroup analysis)	Chapter 3.2.4.3, Figure 8
Indication of statistical uncertainty of findings	Chapter 3.2.3 – Chapter 3.2.6, Chapter 4.3
Reporting of Discussion	
Quantitative assessment of bias (e.g. publication bias)	Chapter 2.2.2, Chapter 2.2.3, Chapter 3.2.6, Figure 10, Chapter 4.2.2, Chapter 4.2.5
Justification for exclusion (e.g. exclusion of non-English-language citations)	Chapter 2.1.1, Supplemental Table 13
Assessment of quality of included studies	Chapter 2.2.2, Chapter 3.2.4.2, Chapter 4.2.3, Chapter 4.2.5
Reporting of Conclusions	
Consideration of alternative explanations for observed results	Chapter 4.2.2, Chapter 4.2.3, Chapter 4.2.4, Chapter 4.2.5
Generalization of the conclusions (e.g. appropriate for the data presented and within the domain of the literature review)	Chapter 4.2.5, Chapter 4.2.6, Chapter 4.5 paragraph 3
Guidelines for future research	Chapter 4.2.6
Disclosure of funding source	Not applicable

Supplemental Table 3. Literature search of systematic review on PubMed Search (13.07.2021)

Search No.	Search terms	Hits
1	8-isoprostane [tw] OR 8-isoprostane [tiab] OR 8-iso Prostaglandin F2alpha [tw] OR 8-iso Prostaglandin F2alpha [tiab] OR 8-Isoprostaglandin F2alpha [tw] OR 8-Isoprostaglandin F2alpha [tiab] OR 8-IsoP [tw] OR 8-IsoP [tiab] OR 8-iso-PGF2alpha-III [tw] OR 8-iso-PGF2alpha-III [tiab] OR 8-iso-PGF2alpha [tw] OR 8-iso-PGF2alpha [tiab] OR 8-iso-15-S-Prostaglandin F2alpha [tw] OR 8-iso-15-S-Prostaglandin F2alpha [tiab] OR 15-F2t-IsoP [tw] OR 15-F2t-IsoP [tiab] OR 15-F2t-Isoprostane [tw] OR 15-F2t-Isoprostane [tiab] OR 15-F2t-Iso prostaglandin [tw] OR 15-F2t-Iso prostaglandin [tiab] OR Isoprostaglandin F2alpha type III [tw] OR Isoprostaglandin F2alpha type III [tiab] OR 8-epi-PGF2alpha [tw] OR 8-epi-PGF2alpha [tiab] OR 8-Epiprostaglandin F2alpha [tw] OR 8-Epiprostaglandin F2alpha [tiab] OR 8-Epi-prostaglandin F2alpha [tw] OR 8-Epi-prostaglandin F2alpha [tiab] OR 9,11,15-Trihydroxy-prosta-5,13-dien-1-oic acid [tw] OR 9,11,15-Trihydroxy-prosta-5,13-dien-1-oic acid [tiab] OR F2-isoprostane*[tw] OR F2-isoprostane*[tiab] OR “F2-Isoprostanes"[Mesh] OR iPF2alpha-I [tw] OR iPF2alpha-I [tiab] OR iPF2alpha-II [tw] OR iPF2alpha-II [tiab] OR iPF2alpha-III [tw] OR iPF2alpha-III [tiab] OR iPF2alpha-IV [tw] OR iPF2alpha-IV [tiab] OR iPF2alpha-V [tw] OR iPF2alpha-V [tiab] OR iPF2alpha-VI [tw] OR iPF2alpha-VI [tiab]	4,465
2	“Dementia” [Mesh] OR Dementia [tw] OR Dementia [tiab] OR “Alzheimer Disease” [Mesh] OR Alzheimer Disease [tw] OR Alzheimer Disease [tiab] OR Alzheimer’s Disease [tw] OR Alzheimer’s Disease [tiab] OR	277,779

	Alzheimer* [tw] OR Alzheimer* [tiab] OR “Dementia, Vascular” [Mesh] OR Vascular dementia [tw] OR Vascular dementia [tiab] OR “Dementia, Multi-Infarct” [Mesh] OR Multi infarct dementia [tw] OR Multi infarct dementia [tiab] OR Multi-infarct dementia [tw] OR Multi-infarct dementia [tiab] OR Multiinfarct dementia [tw] OR Multiinfarct dementia [tiab] OR “Frontotemporal Dementia” [Mesh] OR Frontotemporal dementia [tw] OR Frontotemporal dementia [tiab] OR Fronto-temporal dementia [tw] OR Fronto-temporal dementia [tiab] OR Lewy body dementia [tw] OR Lewy body dementia [tiab] OR Lewy-body dementia [tw] OR Lewy-body dementia [tiab] OR “Lewy Body Disease” [Mesh] OR Lewy Body Disease [tw] OR Lewy Body Disease [tiab]	
3	1 AND 2	122

Supplemental Table 4. Literature search of systematic review on Web of Science Search (13.07.2021)

Search No.	Search terms	Hits
1	TS = (“8 isoprostane” OR “8-isoprostane” OR “8-iso Prostaglandin F2alpha” OR “8-Isoprostaglandin F2alpha” OR “8-IsoP” OR “8-iso-PGF2alpha-III” OR “8-iso-PGF2alpha” OR “8-iso-15-S-Prostaglandin F2alpha” OR “15-F2t-IsoP” OR “15-F2t-Isoprostane” OR “15-F2t-Iso prostaglandin” OR “Isoprostaglandin F2alpha type III” OR “8-epi-PGF2alpha” OR “8-Epiprostaglandin F2alpha” OR “8-Epi-prostaglandin F2alpha” OR “9,11,15-Trihydroxy-prosta-5,13-dien-1-oic acid” OR “F2-isoprostane*” OR “iPF2alpha-I” OR “iPF2alpha-II” OR “iPF2alpha-III” OR “iPF2alpha-IV” OR “iPF2alpha-V” OR	2,547

	"iPF2alpha-VI")	
2	TI = ("8 isoprostane" OR "8-isoprostane" OR "8-iso Prostaglandin F2alpha" OR "8-Isoprostaglandin F2alpha" OR "8-IsoP" OR "8-iso-PGF2alpha-III" OR "8-iso-PGF2alpha" OR "8-iso-15-S-Prostaglandin F2alpha" OR "15-F2t-IsoP" OR "15-F2t-Isoprostane" OR "15-F2t-Iso prostaglandin" OR "Isoprostaglandin F2alpha type III" OR "8-epi-PGF2alpha" OR "8-Epiprostaglandin F2alpha" OR "8-Epi-prostaglandin F2alpha" OR "9,11,15-Trihydroxy-prosta-5,13-dien-1-oic acid" OR "F2-isoprostane*" OR "iPF2alpha-I" OR "iPF2alpha-II" OR "iPF2alpha-III" OR "iPF2alpha-IV" OR "iPF2alpha-V" OR "iPF2alpha-VI")	385
3	AB = ("8 isoprostane" OR "8-isoprostane" OR "8-iso Prostaglandin F2alpha" OR "8-Isoprostaglandin F2alpha" OR "8-IsoP" OR "8-iso-PGF2alpha-III" OR "8-iso-PGF2alpha" OR "8-iso-15-S-Prostaglandin F2alpha" OR "15-F2t-IsoP"2 OR "15-F2t-Isoprostane" OR "15-F2t-Iso prostaglandin" OR "Isoprostaglandin F2alpha type III" OR "8-epi-PGF2alpha" OR "8-Epiprostaglandin F2alpha" OR "8-Epi-prostaglandin F2alpha" OR "9,11,15-Trihydroxy-prosta-5,13-dien-1-oic acid" OR "F2-isoprostane*" OR "iPF2alpha-I" OR "iPF2alpha-II" OR "iPF2alpha-III" OR "iPF2alpha-IV" OR "iPF2alpha-V" OR "iPF2alpha-VI")	1,968
4	TS = ("Dementia" OR "Alzheimer Disease" OR "Alzheimer's Disease" OR Alzheimer* OR "Vascular dementia" OR "Multi infarct dementia" OR "Multi-infarct dementia" OR "Multiinfarct dementia" OR "Frontotemporal dementia" OR "Fronto-temporal dementia" OR "Lewy body dementia" OR	331,269

	"Lewy-body dementia" OR "Lewy Body Disease")	
5	TI = ("Dementia" OR "Alzheimer Disease" OR "Alzheimer's Disease" OR Alzheimer* OR "Vascular dementia" OR "Multi infarct dementia" OR "Multi-infarct dementia" OR "Multiinfarct dementia" OR "Frontotemporal dementia" OR "Fronto-temporal dementia" OR "Lewy body dementia" OR "Lewy-body dementia" OR "Lewy Body Disease")	148,862
6	AB = ("Dementia" OR "Alzheimer Disease" OR "Alzheimer's Disease" OR Alzheimer* OR "Vascular dementia" OR "Multi infarct dementia" OR "Multi-infarct dementia" OR "Multiinfarct dementia" OR "Frontotemporal dementia" OR "Fronto-temporal dementia" OR "Lewy body dementia" OR "Lewy-body dementia" OR "Lewy Body Disease")	183,610
7	(1 OR 2 OR 3) AND (4 OR 5 OR 6)	70

Supplemental Table 5. List of biomarkers measured with Olink Proseek® Multiplex Inflammation I^{96x96} kits.

Abbreviation	Biomarker name	Values < LOD
4E-BP1	Eukaryotic translation initiation factor 4E-binding protein 1	0%
ADA	Adenosine Deaminase	0%
ARTN	Artemin	76%
AXIN1	Axin-1	5%
Beta-NGF	Beta-nerve growth factor	12%
CASP-8	Caspase-8	0%
CCL11	Eotaxin	0%
CCL19	C-C motif chemokine 19	0%
CCL20	C-C motif chemokine 20	0%
CCL23	C-C motif chemokine 23	0%
CCL25	C-C motif chemokine 25	0%
CCL28	C-C motif chemokine 28	2%
CCL3	C-C motif chemokine 3	0%
CCL4	C-C motif chemokine 4	0%
CD244	Natural killer cell receptor 2B4	0%
CD40	CD40L receptor	0%
CD5	T-cell surface glycoprotein CD5	0%
CD6	T cell surface glycoprotein CD6 isoform	0%
CD8A	T-cell surface glycoprotein CD8 alpha chain	0%
CDCP1	CUB domain-containing protein 1	0%
CSF-1	Macrophage colony-stimulating factor 1	0%
CST5	Cystatin D	3%
CX3CL1	Fractalkine	0%
CXCL1	C-X-C motif chemokine 1	0%
CXCL10	C-X-C motif chemokine 10	0%
CXCL11	C-X-C motif chemokine 11	0%
CXCL5	C-X-C motif chemokine 5	0%
CXCL6	C-X-C motif chemokine 6	0%
CXCL9	C-X-C motif chemokine 9	0%
DNER	Delta and Notch-like epidermal growth factor-related receptor	0%
EN-RAGE	Protein S100-A12	0%
FGF-19	Fibroblast growth factor 19	0%
FGF-21	Fibroblast growth factor 21	0%
FGF-23	Fibroblast growth factor 23	25%
FGF-5	Fibroblast growth factor 5	40%
Flt3L	Fms-related tyrosine kinase 3 ligand	0%
GDNF	Glial cell line-derived neurotrophic factor	25%
HGF	Hepatocyte growth factor	0%
IFN_gamma	Interferon gamma	81%
IL1_alpha	Interleukin-1 alpha	85%
IL-10	Interleukin-10	3%
IL-10RA	Interleukin-10 receptor subunit alpha	22%
IL-10RB	Interleukin-10 receptor subunit beta	0%
IL-12B	Interleukin-12 subunit beta	0%
IL13	Interleukin-13	86%

Abbreviation	Biomarker name	Values < LOD
IL-15RA	Interleukin-15 receptor subunit alpha	20%
IL-17A	Interleukin-17A	28%
IL-17C	Interleukin-17C	32%
IL-18	Interleukin-18	0%
IL-18R1	Interleukin-18 receptor 1	0%
IL2	Interleukin-2	98%
IL20	Interleukin-20	78%
IL-20RA	Interleukin-20 receptor subunit alpha	47%
IL22-RA1	Interleukin-22 receptor subunit alpha-1	83%
IL24	Interleukin-24	90%
IL2RB	Interleukin-2 receptor subunit beta	66%
IL33	Interleukin-33	97%
IL4	Interleukin-4	85%
IL-5	Interleukin-5	62%
IL-6	Interleukin-6	1%
IL-7	Interleukin-7	0%
IL-8	Interleukin-8	0%
LAP TGF-beta-1	Latency-associated peptide transforming growth factor beta-1	0%
LIF	Leukemia inhibitory factor	65%
LIFR	Leukemia inhibitory factor receptor	0%
MCP-1	Monocyte chemotactic protein 1	0%
MCP-2	Monocyte chemotactic protein 2	0%
MCP-3	Monocyte chemotactic protein 3	5%
MCP-4	Monocyte chemotactic protein 4	0%
MMP-1	Matrix metalloproteinase-1	0%
MMP-10	Matrix metalloproteinase-10	0%
NRTN	Neurturin	72%
NT-3	Neurotrophin-3	13%
OPG	Osteoprotegerin	0%
OSM	Oncostatin-M	0%
PD-L1	Programmed cell death 1 ligand 1	0%
SCF	Stem cell factor	0%
SIRT2	SIR2-like protein 2	7%
SLAMF1	Signaling lymphocytic activation molecule	9%
ST1A1	Sulfotransferase 1A1	4%
STAMBP	STAM-binding protein	0%
TGF-alpha	Transforming growth factor alpha	0%
TNF	Tumor necrosis factor	70%
TNFB	TNF-beta	0%
TNFRSF9	Tumor necrosis factor receptor superfamily member 9	0%
TNFSF14	Tumor necrosis factor ligand superfamily member 14	0%
TRAIL	TNF-related apoptosis-inducing ligand	0%
TRANCE	TNF-related activation-induced cytokine	0%
TSLP	Thymic stromal lymphopoietin	87%
TWEAK	Tumor necrosis factor (Ligand) superfamily, member 12	0%
uPA	Urokinase-type plasminogen activator	0%
VEGF-A	Vascular endothelial growth factor-A	0%

Note: Grey shade indicates biomarkers with 25% or more of the values below the lower limit of detection (LOD)

Supplemental Table 6. Baseline characteristics of selected (n = 1,278) and non-selected (n = 4,456) controls

Baseline characteristics	Non-selected controls (n = 4,456)	Selected controls (n = 1,278)
Age (years)		
50-64	3028 (67.9)	802 (62.8)
65-69	941 (21.1)	301 (23.6)
70-75	489 (11.0)	175 (13.7)
Sex		
Female	2426 (54.4)	703 (55.0)
Male	2032 (45.6)	575 (45.0))
Education (years)		
< 9	3138 (71.8)	953 (76.1)
9-11	673 (15.4)	168 (13.4)
≥ 12	549 (12.6)	131 (10.5)
Physical activity*		
Inactive	820 (18.4)	233 (18.3)
Low	2001 (45.0)	594 (46.6)
Medium or high	1626 (36.6)	448 (35.1)
BMI (kg/m²)		
< 25	1251 (28.1)	334 (26.2)
25-<30	2107 (47.3)	604 (47.5)
≥30	1095 (24.6)	335 (26.3)
CVD†		
No	3649 (81.9)	1023 (80.1)
Yes	808 (18.1)	255 (19.9)
Diabetes		
No	3793 (86.2)	1083 (85.8)
Yes	607 (13.8)	180 (14.3)
Life-time history of depression		
No	3785 (85.1)	1100 (86.1)
Yes, without current pharmacotherapy	533 (12.0)	137 (10.7)
Yes, with current pharmacotherapy	129 (2.9)	41 (3.2)
***APOE* genotypes**		
ε2/ε2	24 (0.6)	17 (1.4)
ε2/ε3	592 (14.8)	181 (15.1)
ε2/ε4	145 (3.6)	33 (2.8)
ε3/ε3	2371 (59.3)	711 (59.5)
ε3/ε4	812 (20.3)	244 (20.4)
ε4/ε4	52 (1.3)	10 (0.8)

Abbreviations: CI, confidence interval; BMI, body mass index; CVD, cardiovascular disease; *APOE*, apolipoprotein E;

NOTE: Numbers printed in bold are statistically significant ($p < 0.05$).

* “Inactive” was defined by < 1 h of vigorous or < 1 h light physical activity per week. “Medium or high” was defined by ≥ 2 h of vigorous and ≥ 2 h of light physical activity/week. All other amounts of physical activity were grouped into the category “Low”.

† CVD was defined as coronary artery disease or a self-reported history of myocardial infarction, stroke, pulmonary embolism, or revascularization of coronary arteries.

‡ Results of multivariate logistic regression model including all variables shown in this table (imputed dataset).

Supplemental Table 7. Comparison of age, education, and sex of included and excluded study participants of the ESTHER study

CAIDE model variables	Excluded study participants (n=145)	Included study participants (n=1637)
Age (years), mean (SD)	64.4 (6.8)	63.2 (6.5)
Education (years), n (%)		
< 9	67 (67.7)	1277 (78.0)
≥ 9	32 (32.3)	360 (22.0)
Sex, n (%)		
Female	76 (52.4)	889 (54.3)
Male	69 (47.6)	748 (45.7)

Note: values are either presented as Mean (±SD) or in categories (n (%)).

Supplemental Table 8. Associations of baseline characteristics with incidence of all-cause dementia and common dementia subtypes

Baseline characteristics	n_{total}	All-cause dementia		Alzheimer's disease		Vascular dementia	
		n_{cases}	HR (95% CI) *	n_{cases}	HR (95% CI) *	n_{cases}	HR (95% CI) *
Age (per year)	5853	365	**1.20 (1.17-1.23)**	109	**1.19 (1.14-1.23)**	127	**1.19 (1.15-1.24)**
Sex							
Female	3200	188	1.00 (Ref.)	62	1.00 (Ref.)	67	1.00 (Ref.)
Male	2653	177	**1.48 (1.15-1.89)**	47	1.23 (0.78-1.94)	60	1.34 (0.88-2.04)
Education (years)							
< 9	4236	290	1.00 (Ref.)	88	1.00 (Ref.)	100	1.00 (Ref.)
9-11	819	27	**0.58 (0.39-0.87)**	9	0.62 (0.31-1.24)	10	0.62 (0.32-1.20)
≥ 12	661	30	**0.67 (0.45-1.00)**	9	0.70 (0.34-1.44)	10	0.71 (0.37-1.40)
Smoking status							
Never smoker	2909	196	1.00 (Ref.)	60	1.00 (Ref.)	67	1.00 (Ref.)
Former smoker	1939	113	0.83 (0.64-1.08)	30	0.78 (0.48-1.28)	39	0.90 (0.58-1.40)
Current smoker	874	43	1.22 (0.86-1.73)	15	1.43 (0.79-2.58)	18	1.49 (0.86-2.59)
Alcohol consumption							
None	1611	115	1.00 (Ref.)	36	1.00 (Ref.)	38	1.00 (Ref.)
Low or moderate	3333	166	0.82 (0.64-1.04)	48	0.78 (0.49-1.25)	65	0.91 (0.60-1.40)
High	381	20	0.99 (0.62-1.58)	7	1.16 (0.52-2.60)	3	0.40 (0.12-1.34)
Physical activity							
Inactive	1133	118	1.00 (Ref.)	40	1.00 (Ref.)	38	1.00 (Ref.)
Low	2645	155	**0.69 (0.54-0.88)**	40	**0.53 (0.33-0.83)**	59	0.81 (0.53-1.24)
Medium or high	2061	91	**0.62 (0.46-0.83)**	29	**0.59 (0.35-0.99)**	30	0.66 (0.40-1.11)
BMI (kg/m^2**)**							
< 25	1632	108	1.00 (Ref.)	38	1.00 (Ref.)	33	1.00 (Ref.)
25-<30	2738	165	**0.73 (0.57-0.94)**	45	**0.62 (0.40-0.98)**	63	0.88 (0.57-1.37)
≥30	1473	91	0.77 (0.57-1.04)	25	0.64 (0.37-1.11)	31	0.79 (0.47-1.33)
CVD							
No	4709	246	1.00 (Ref.)	82	1.00 (Ref.)	80	1.00 (Ref.)
Yes	1143	119	1.21 (0.96-1.53)	27	0.83 (0.53-1.32)	47	**1.56 (1.07-2.28)**
Diabetes							
No	4951	272	1.00 (Ref.)	82	1.00 (Ref.)	93	1.00 (Ref.)
Yes	821	87	**1.57 (1.21-2.02)**	26	**1.68 (1.04-2.70**)	32	**1.64 (1.07-2.52)**
Life-time history of depression							
No	4997	313	1.00 (Ref.)	93	1.00 (Ref.)	108	1.00 (Ref.)
Yes, without current pharmacotherapy	660	32	0.86 (0.59-1.24)	11	0.96 (0.50-1.81)	11	0.90 (0.48-1.68)
Yes, with current pharmacotherapy	187	20	**1.99 (1.25-3.15)**	5	1.65 (0.66-4.10)	8	**2.25 (1.08-4.70)**
Total cholesterol levels (mg/dl)							
< 200	1913	128	1.00 (Ref.)	36	1.00 (Ref.)	44	1.00 (Ref.)
200-<240	1983	121	0.82 (0.64-1.06)	35	0.77 (0.48-1.24)	43	0.90 (0.58-1.38)
≥240	1957	116	0.86 (0.66-1.11)	38	0.87 (0.54-1.40)	40	0.91 (0.58-1.42)
CRP levels (mg/L)							
< 1	1563	103	1.00 (Ref.)	35	1.00 (Ref.)	32	1.00 (Ref.)
1-<3	2202	139	0.97 (0.74-1.27)	41	0.87 (0.54-1.41)	44	0.95 (0.59-1.53)
≥3	2088	123	0.92 (0.69-1.23)	33	0.78 (0.46-1.32)	51	1.15 (0.71-1.86)
***APOE* genotypes**							
ε4 non-carrier	3913	205	1.00 (Ref.)	53	1.00 (Ref.)	70	1.00 (Ref.)
ε2/ε4	194	12	1.31 (0.73-2.38)	2	0.98 (0.28-3.44)	7	**2.22 (1.02-4.81)**
ε3/ε4	1109	91	**1.64 (1.28-2.08)**	36	**2.43 (1.58-3.72)**	32	**1.68 (1.08-2.61)**
ε4/ε4	83	16	**4.27 (2.46-7.39)**	7	**7.19 (3.15-16.38)**	1	1.06 (0.13-8.38)

Abbreviations: CI, confidence interval; BMI, body-mass index, CVD, cardiovascular disease; HR, hazard ratio.
NOTE: Numbers printed in bold are statistically significant ($p < 0.05$).
* Multivariate Cox proportional hazards regression model with all variables shown in this table.

Supplemental Table 9. Associations of 8-iso-prostaglandin $F_{2\alpha}$ levels with all-cause and common subtype dementia incidences in a model adjusting for all assessed covariates

8-iso-prostaglandin $F_{2\alpha}$ levels [nmol/mmol creatinine]	n_{total}	All-cause dementia		Alzheimer's disease		Vascular dementia	
		n_{cases}	HR (95%CI)*	n_{cases}	HR (95%CI)*	n_{cases}	HR (95%CI)*
Per 1 SD †	5853	365	**1.48 (1.19-1.84)**	109	**1.54 (1.03-2.30)**	127	1.15 (0.79-1.68)
Tertile 1 (≤0.169)	1951	105	1.00 (ref.)	33	1.00 (ref.)	37	1.00 (ref.)
Tertile 2 (>0.169-0.242)	1952	123	1.17 (0.90-1.52)	27	0.79 (0.47-1.32)	48	1.28 (0.83-1.97)
Tertile 3 (>0.242)	1950	137	**1.45 (1.11-1.88)**	49	1.53 (0.97-2.42)	42	1.21 (0.77-1.90)

Abbreviations: CI, confidence interval; HR, hazard ratio; SD, standard deviation.

NOTE: Numbers printed in bold are statistically significant ($p < 0.05$).

* The model was adjusted for age (continuously), sex, education, smoking status, alcohol consumption, physical activity, BMI (categorical), cardiovascular disease, diabetes, life-time history of depression, total cholesterol levels (continuously), CRP levels (continuously, logarithmized) and *APOE* ε4 polymorphism. The HR per 1 SD was obtained in analysis with logarithmized 8-iso-prostaglandin $F_{2\alpha}$ levels.

† 1 SD of 8-iso-prostaglandin $F_{2\alpha}$ levels = 0.278 nmol/mmol creatinine.

Supplemental Table 10. Association of 8-iso-prostaglandin F2α levels with all-cause and common subtype dementia incidences in a sensitivity analysis excluding dementia cases in the first 7 years of follow-up

8-iso-prostaglandin $F_{2\alpha}$ levels [nmol/mmol creatinine]	n_{total}	All-cause dementia		Alzheimer's disease		Vascular dementia	
		n_{cases}	HR (95%CI)*	n_{cases}	HR (95%CI)*	n_{cases}	HR (95%CI)*
Per 1 SD †	5768	280	**1.42 (1.11-1.82)**	79	1.38 (0.86-2.21)	102	1.24 (0.81-1.87)
Tertile 1 (≤0.169)	1935	89	1.00 (ref.)	28	1.00 (ref.)	30	1.00 (ref.)
Tertile 2 (>0.169-0.242)	1919	90	1.00 (0.75-1.35)	19	0.65 (0.36-1.17)	39	1.34 (0.83-2.16)
Tertile 3 (>0.242)	1914	101	1.29 (0.97-1.73)	32	1.20 (0.71-2.01)	33	1.29 (0.78-2.13)

Abbreviations: CI, confidence interval; HR, hazard ratio; SD, standard deviation.

NOTE: Numbers printed in bold are statistically significant ($p < 0.05$).

* The model was adjusted for age (continuously), sex, education, physical activity, BMI (categorical), diabetes, and *APOE* ε4 polymorphism. The HR per 1 SD was obtained in analysis with logarithmized 8-iso-prostaglandin $F_{2\alpha}$ levels.

† 1 SD of 8-iso-prostaglandin $F_{2\alpha}$ levels = 0.278 nmol/mmol creatinine.

Supplemental Table 11. Association of 8-iso-prostaglandin $F_{2\alpha}$ levels with all-cause dementia incidence stratified by age

8-iso-prostaglandin $F_{2\alpha}$ levels [nmol/mmol creatinine]	Age 50-64 years			Age 65-69 years			Age 70-75 years		
	n_{total}	n_{cases}	HR (95%CI)*	n_{total}	n_{cases}	HR (95%CI)*	n_{total}	n_{cases}	HR (95%CI)*
Per 1 SD †	3740	96	1.18 (0.77-1.81)	1309	107	**1.65 (1.09-2.51)**	804	162	**1.52 (1.11-2.08)**
Tertile 1 (≤0.169)	1209	31	1.00 (ref.)	485	32	1.00 (ref.)	257	42	1.00 (ref.)
Tertile 2 (>0.169-0.242)	1249	30	0.96 (0.58-1.59)	412	36	1.27 (0.78-2.05)	291	57	1.30 (0.87-1.95)
Tertile 3 (>0.242)	1282	35	1.18 (0.72-1.93)	412	39	1.46 (0.90-2.35)	256	63	**1.63 (1.10-2.43)**

Abbreviations: CI, confidence interval; HR, hazard ratio; SD, standard deviation.

NOTE: Numbers printed in bold are statistically significant ($p < 0.05$).

* The model was adjusted for age (continuously), sex, education, physical activity, BMI (categorical), diabetes, and *APOE* **ε**4 polymorphism. The HR per 1 SD was obtained in analysis with logarithmized 8-iso-prostaglandin $F_{2\alpha}$ levels.

† 1 SD of 8-iso-prostaglandin $F_{2\alpha}$ levels = 0.278 nmol/mmol creatinine.

Supplemental Table 12. Associations of 8-iso-prostaglandin $F_{2\alpha}$ levels with all-cause dementia incidence stratified by sex

8-iso-prostaglandin $F_{2\alpha}$ levels [nmol/mmol creatinine]	Men			Women		
	n_{total}	n_{cases}	HR (95%CI)*	n_{total}	n_{cases}	HR (95%CI)*
Per 1 SD †	2653	177	**1.47 (1.09-1.97)**	3200	188	**1.42 (1.05-1.94)**
Tertile 1 (≤0.169)	1010	55	1.00 (ref.)	941	50	1.00 (ref.)
Tertile 2 (>0.169-0.242)	892	67	1.38 (0.96-1.97)	1060	56	0.97 (0.66-1.42)
Tertile 3 (>0.242)	751	55	**1.48 (1.01-2.16)**	1199	82	1.36 (0.95-1.94)

Abbreviations: CI, confidence interval; HR, hazard ratio; SD, standard deviation.

NOTE: Numbers printed in bold are statistically significant ($p < 0.05$).

* The model was adjusted for age (continuously), education, physical activity, BMI (categorical), diabetes, and *APOE* **ε**4 polymorphism. The HR per 1 SD was obtained in analysis with logarithmized 8-iso-prostaglandin $F_{2\alpha}$ levels.

† 1 SD of 8-iso-prostaglandin $F_{2\alpha}$ levels = 0.278 nmol/mmol creatinine.

Supplemental Table 13. Excluded studies and reasons

Reason	Study
Alzheimer's disease not outcome or combined with other outcomes	1. Downey LA, Simpson T, Timmer J, et al. Impaired verbal episodic memory in healthy older adults is marked by increased F(2)-Isoprostanes. *Prostaglandins, leukotrienes, and essential fatty acids.* 2018;129:32-37. 2. Connolly J, Siderowf A, Clark CM, Mu D, Pratico D. F2 isoprostane levels in plasma and urine do not support increased lipid peroxidation in cognitively impaired Parkinson disease patients. *Cognitive and behavioral neurology : official journal of the Society for Behavioral and Cognitive Neurology.* 2008;21(2):83-86. 3. Dietrich M, Hu Y, Block G, et al. Associations between apolipoprotein E genotype and circulating F2-isoprostane levels in humans. *Lipids.* 2005;40(4):329-334. 4. Montine TJ, Peskind ER, Quinn JF, Wilson AM, Montine KS, Galasko D. Increased cerebrospinal fluid F2-isoprostanes are associated with aging and latent Alzheimer's disease as identified by biomarkers. *Neuromolecular medicine.* 2011;13(1):37-43. 5. Peskind ER, Li G, Shofer JB, et al. Influence of lifestyle modifications on age-related free radical injury to brain. *JAMA neurology.* 2014;71(9):1150-1154. 6. Pratico D. Biomarkers of Alzheimer's disease: F2-isoprostanes. *Neurobiology of aging.* 2004;25:S5-S5.
Alzheimer's disease patients not compared to healthy control group	7. Hatanaka H, Hanyu H, Fukasawa R, Sato T, Shimizu S, Sakurai H. Peripheral oxidative stress markers in diabetes-related dementia. *Geriatrics & gerontology international.* 2016;16(12):1312-1318. 8. Quinn JF, Bussiere JR, Hammond RS, et al. Chronic dietary alpha-lipoic acid reduces deficits in hippocampal memory of aged Tg2576 mice. *Neurobiology of aging.* 2007;28(2):213-225. 9. Swardfager W, Yu D, Scola G, et al. Peripheral lipid oxidative stress markers are related to vascular risk factors and subcortical small vessel disease. *Neurobiology of aging.* 2017;59:91-97. 10. Bhatia HS, Baron J, Hagl S, Eckert GP, Fiebich BL. Rice bran derivatives alleviate microglia activation: possible involvement of MAPK pathway. *Journal of neuroinflammation.* 2016;13(1):148.
No eligible data for meta-analysis	11. Montine TJ, Quinn JF, Milatovic D, et al. Peripheral F2-isoprostanes and F4-neuroprostanes are not increased in Alzheimer's disease. *Annals of neurology.* 2002;52(2):175-179.[a] 12. Sonnen JA, Larson EB, Brickell K, et al. Different patterns of cerebral injury in dementia with or without diabetes. *Archives of neurology.* 2009;66(3):315-322. 13. Casadesus G, Smith MA, Basu S, et al. Increased isoprostane and prostaglandin are prominent in neurons in Alzheimer disease. *Molecular neurodegeneration.* 2007;2:2.

	14. Fessel JP, Hulette C, Powell S, Roberts LJ, 2nd, Zhang J. Isofurans, but not F2-isoprostanes, are increased in the substantia nigra of patients with Parkinson's disease and with dementia with Lewy body disease. *Journal of neurochemistry.* 2003;85(3):645-650.[b] 15. Duits FH, Kester MI, Scheffer PG, et al. Increase in cerebrospinal fluid F2-isoprostanes is related to cognitive decline in APOE ε4 carriers. *Journal of Alzheimer's disease : JAD.* 2013;36(3):563-570.[a] 16. Forman MS, Mufson EJ, Leurgans S, et al. Cortical biochemistry in MCI and Alzheimer disease: lack of correlation with clinical diagnosis. *Neurology.* 2007;68(10):757-763.[b]
No plausible data provided	17. Guan JZ, Guan WP, Maeda T, Makino N. Effect of vitamin E administration on the elevated oxygen stress and the telomeric and subtelomeric status in Alzheimer's disease. *Gerontology.* 2012;58(1):62-69.[c]

[a]Possibly overlapping study population in another included publication.

[b]Authors could not be reached anymore to provide eligible data for meta-analysis.

[c]Clear outlier in the meta-analysis with extremely increased levels of 8-iso-$PGF_{2\alpha}$ in AD patients compared to controls. I assume that the authors reported the standard error instead of the standard deviation, as the ratio between the standard deviation and the mean is much lower for cases and controls than in other studies.

Supplemental Table 14. General information of included studies

First Author, year	Study design	Country	Sample type	Analytical technique	Type of isopros-tanes	Study population						Follow-up time (years)
						Sample size		Female (%)		Age (years) (± SD)		
						Cases	Controls	Cases	Controls	Cases	Controls	
Cross-sectional												
Waddington et al. 1999	cross-sectional	USA	urine	GC-MS	free	9	11	n.a.	n.a.	55-95[b,c]		n.a.
Praticò et al. 2000	cross-sectional	USA	urine	GC-MS	total	25	25	92.0	76.0	76.0 (1.4)	74.5 (2.0)	n.a.
Tuppo et al. 2001	cross-sectional	USA	urine	EIA kit	n.a.	38	33	76.3	81.8	80.2 (6.2)[a]	78.0 (7.0)[a]	n.a.
Bohnstedt et al. 2003	cross-sectional	Sweden	urine	LC-MS	n.a.	23	30	52.2	60.0	75.5 (10.0)	63.1 (10.0)	n.a.
Kim et al. 2004	cross-sectional	South Korea	urine	GC-MS	n.a.	34	20	n.a.	n.a.	75.4 (8.7)	71.3 (6.9)	n.a.
Ciabattoni et al. 2007	cross-sectional	Italy	urine	RIA	n.a.	44	44	56.8	61.4	73.0 (8.0)	75.0 (7.0)	n.a.
Mufson and Leurgans 2010	cross-sectional	USA	urine	GC-MS	n.a.	21	167	67.0	74.0	86.3 (7.0)	79.8 (6.3)	n.a.
Peña-Bautista et al. 2019	cross-sectional	Spain	urine	UPLC-MS	n.a.	70	29	40.0	62.1	70.5 (68.0-74.0)[c]	66.0 (62.0-72.0)[c]	n.a.
Feillet-Coudray et al. 1999	cross-sectional	France	blood (plasma)	EIA Kit	total	25	14	72.0	57.1	75.0 (1.0)	76.0 (1.0)	n.a.
Waddington et al. 1999	cross-sectional	USA	blood (plasma)	GC-MS	free	19	20	n.a.	n.a.	76.0 (2.3)	77.0 (2.7)	n.a.
Praticò et al. 2000	cross-sectional	USA	blood (plasma)	GC-MS	n.a.	25	25	92.0	76.0	76.0 (1.4)	74.5 (2.0)	n.a.
Irizarry et al. 2007	cross-sectional	USA	blood (plasma)	GC-MS	n.a.	49	48	51.0	52.0	75.7 (7.2)	71.4 (9.4)	n.a.

First Author, year	Study design	Country	Sample type	Analytical technique	Type of isopros-tanes	Study population						Follow-up time (years)
						Sample size		Female (%)		Age (years) (± SD)		
						Cases	Controls	Cases	Controls	Cases	Controls	
Peuchant et al. 2008	cross-sectional	France	blood (plasma)	ELISA Kit	n.a.	25	76	49.0	48.0	74.8 (10.9)	75.2 (4.8)	n.a.
Mufson and Leurgans 2010	cross-sectional	USA	blood (plasma)	GC-MS	n.a.	21	167	67.0	74.0	86.3 (7.0)	79.8 (6.3)	n.a.
Sirin et al. 2015	cross-sectional	Turkey	blood (plasma)	EIA Kit	Total	20	22	40.0	40.9	79.1 (8.9)	71.6 (6.7)	n.a.
Ulstein et al. 2017	cross-sectional	Norway	blood (n.a.)	LC-MS/MS	n.a.	48	63	52.1	60.3	71.0 (8.2)	72.7 (6.3)	n.a.
Lepara et al. 2020	cross-sectional	Bosnia and Herzegovina	blood (serum)	EIA Kit	n.a.	30	30	100.0	100.0	80.76 (1.1)[a]	80.13 (1.1)[a]	n.a.
Loffredo et al. 2020	cross-sectional	Italy	blood (sreum)	colorimetric assay kit	n.a.	47	64	48.9	43.8	75.0 (8.0)	72.0 (8.0)	n.a.
Montine et al. 1998	cross-sectional	USA	CSF[e]	GC-NICI-MS	free	11	11	63.6	72.7	78.4 (1.6)	82.2 (1.8)	n.a.
Praticò et al. 1998	cross-sectional	USA	CSF[e]	GC-MS	free	15	10	n.a.	n.a.	n.a.	n.a.	n.a.
Montine et al. 1999	cross-sectional	USA	CSF	GC-NICI-MS	total	27	25	54.6	47.6	67.2 (1.6)	57.6 (2.7)	n.a.
Praticò et al. 2000	cross-sectional	USA	CSF	GC-MS	n.a.	14	10	78.6	70.0	74.0 (1.3)	74.0 (2.0)	n.a.
Montine et al. 2001	cross-sectional	USA	CSF	GC-NICI-MS	free	19	10	28.6	40.0	65.3 (2.7)	66.4 (2.9)	n.a.
Durazzo et al. 2014	cross-sectional	USA	CSF	HPLC-MS	n.a.	101 (59;42)[d]	83 (60;23)[d]	53.0; 29.0[d]	60.0; 35.0[d]	74.2 (8.0); 75.0 (7.3)[d]	76.2 (5.5); 75.6 (5.3)[d]	n.a.
Kuo et al. 2015	cross-sectional	Taiwan	CSF	GC-NICI-MS	free	9	9	44.4	33.3	77.0 (64.0-79.0)[c]	69 (65.5-74.5)[c]	n.a.

First Author, year	Study design	Country	Sample type	Analytical technique	Type of isopros-tanes	Study population						Follow-up time (years)
						Sample size		Female (%)		Age (years) (± SD)		
						Cases	Controls	Cases	Controls	Cases	Controls	
Praticò et al. 1998	cross-sectional	USA	tissue[f]	GC-MS	free	19	8	47.4	37.5	79.0 (9.2)	76.0 (13.6)	n.a.
Yao et al. 2003	cross-sectional	USA	tissue[f]	GC-MS	Total	23	14	52.2	42.9	75.7 (6.0)	76.0 (5.0)	n.a.
Markesbery et al. 2005	cross-sectional	USA	tissue[f]	GC-NICI-MS	n.a.	7	13	71.4	53.9	87.6 (3.8)	84.6 (6.9)	n.a.
VanRollins et al. 2008	cross-sectional	USA	tissue[f]	GC-NICI-MS	free	4	6	n.a.	n.a.	84.0 (9.0)	87.0 (5.0)	n.a.
Longitudinal												
Sundelöf et al. 2009	longitudinal	Sweden	urine	RIA	free	47	681	0.0	0.0	77.5 (0.8)[b]		5.1 (0.2-7.9)[g]
Trares et al. 2020 (updated with the 17-year-FUP data for this review)[h]	longitudinal	Germany	urine	ELISA kit	free	160	5666	58.8	55.1	66.8 (5.2)	61.23 (6.5)	16.39 (13.70-17.08)[i]
Kester et al. 2012	longitudinal	The Netherlands	CSF	LC-MS/MS	n.a.	68	24	46.0	29.0	65.0 (7.0)	64.0 (10.0)	AD: 1.9 (1.0); CN: 2.5 1.7)[j,k]
Li et al. 2014	longitudinal	USA	CSF	GC-MS-SIM	n.a.	7	135	71.0	61.0	77.1 (5.9)	65.6 (11.2)	4.4 (2.3)[j]

Abbreviations: SD, standard deviation; CSF, cerebrospinal fluid; LC-MS, liquid chromatography-mass spectrometry; GC-MS, gas chromatography-mass spectrometry; GC-NICI-MS, gas chromatography - negative chemical ionization - mass spectrometry; LC-MS, liquid chromatography-tandem mass spectrometry; RIA, radioimmunoassay; EIA kit, enzyme immunoassay kit; ELISA, Enzyme Linked Immuno Sorbent Assay Kit; GC-MS-SIM, gas chromatography-mass spectrometry with selective ion monitoring; IQR, interquartile range; FUP, follow-up.

[a]Standard Error of the Mean (SEM) instead of SD provided.

[b]Data of the whole study population.

[c]Range instead of SD provided.

[d]Data divided into non-smoking AD cases/controls and smoking AD cases/controls
[e]Extracted *post mortem*.
[f]Extracted *post mortem* from the frontal lobe.
[g]Data provided as median and range.
[h]Initial data from Trares et al. 2020: Number of cases=109, Number of controls=5488, %Female cases=56.9, %Female controls=54.9, Age cases=67.3 (4.8), Age controls=61.34 (6.4), Follow-up time=13.7 (IQR: 13.31-14.10)
[i]Data provided as median and IQR
[j]Data provided as mean and SD.
[k]Data reported separately for cases (AD) and controls (CN).

Supplemental Table 15. Risk of Bias Evaluation with the Newcastle Ottawa Scale for case-control studies

First Author, year	Selection				Comparability*	Exposure			Study Quality Score (maximum: 9 points)
	Is the case definition adequate	Representativeness of the cases	Selection of controls	Definition of controls	Comparability of cases and controls on the basis of the design or analysis	Ascertainment of exposure	Same method of ascertainment for cases and controls	Non-Response rate	
(Montine et al. 1998)	A → 1P	B → 0P	C → 0P	A → 1P	A → 1P[a]	A → 1P	A → 1P	C → 0P	5P
(Praticò et al. 1998) (tissue)	A → 1P	B → 0P	C → 0P	A → 1P	C → 0P	A → 1P	A → 1P	C → 0P	4P
Praticò et al. 1998 (CSF)	A → 1P	B → 0P	C → 0P	A → 1P	C → 0P	A → 1P	A → 1P	C → 0P	4P
(Feillet-Coudray et al. 1999)	A → 1P	A → 1P	C → 0P	A → 1P	C → 0P	A → 1P	A → 1P	C → 0P	5P
Montine et al. 1999	A → 1P	B → 0P	B → 0P	A → 1P	C → 0P	A → 1P	A → 1P	C → 0P	4P
(Waddington et al. 1999)	A → 1P	A → 1P	A → 1P	B → 0P	A → 1P[a]	A → 1P	A → 1P	B → 0P	6P
(Praticò et al. 2000)	A → 1P	A → 1P	A → 1P	A → 1P	A → 1P[b]	A → 1P	A → 1P	C → 0P	7P
(Montine et al. 2001)	A → 1P	A → 1P	A → 1P	A → 1P	A → 1P[a]	A → 1P	A → 1P	C → 0P	7P
(Tuppo et al. 2001)	A → 1P	A → 1P	A → 1P	A → 1P	C → 0P	A → 1P	A → 1P	C → 0P	6P
(Bohnstedt et al. 2003)	A → 1P	A → 1P	A → 1P	A → 1P	C → 0P	A → 1P	A → 1P	C → 0P	6P
(Yao et al. 2003)	A → 1P	B → 0P	C → 0P	A → 1P	C → 0P	A → 1P	A → 1P	C → 0P	4P
(Kim et al. 2004)	A → 1P	A → 1P	C → 0P	A → 1P	C → 0P	A → 1P	A → 1P	C → 0P	5P
(Markesbery et al. 2005)	A → 1P	A → 1P	A → 1P	A → 1P	C → 0P	A → 1P	A → 1P	C → 0P	6P
(Ciabattoni et al. 2007)	A → 1P	A → 1P	A → 1P	A → 1P	A → 1P[b]	A → 1P	A → 1P	C → 0P	7P
(Irizarry et al. 2007)	A → 1P	A → 1P	A → 1P	A → 1P	C → 0P	A → 1P	A → 1P	C → 0P	6P

(Peuchant et al. 2008)	A → 1P	A → 1P	A → 1P	A → 1P	A → 1P[b]	A → 1P	A → 1P	B → 0P	7P
(VanRollins et al. 2008)	A → 1P	B → 0P	C → 0P	A → 1P	C → 0P	A → 1P	A → 1P	C → 0P	4P
(Sundelöf et al. 2009)	A → 1P	A → 1P	A → 1P	A → 1P	A,B → 2P[a,c]	A → 1P	A → 1P	A → 1P[d]	9P
(Mufson and Leurgans 2010)	A → 1P	A → 1P	A → 1P	A → 1P	C → 0P	A → 1P	A → 1P	C → 0P	6P
(Kester et al. 2012)	A → 1P	B → 0P	C → 0P	A → 1P	C → 0P	A → 1P	A → 1P	C → 0P	4P
(Durazzo et al. 2014)	A → 1P	A → 1P	A → 1P	A → 1P	C → 0P	A →1P	A → 1P	C → 0P	6P
(Li et al. 2014)	A → 1P	A → 1P	A → 1P	A → 1P	C → 0P	A → 1P	A → 1P	C → 0P	6P
(Kuo et al. 2015)	A → 1P	A → 1P	A → 1P	A → 1P	C → 0P	A → 1P	A → 1P	C → 0P	6P
(Sirin et al. 2015)	A → 1P	A → 1P	A → 1P	A → 1P	C→ 0P	A → 1P	A → 1P	C → 0P	6P
(Ulstein and Bøhmer 2017)	A → 1P	B → 0P	B → 0P	A → 1P	A → 1P[a]	A → 1P	A → 1P	C → 0P	5P
(Peña-Bautista et al. 2019)	A → 1P	A → 1P	A → 1P	A → 1P	C → 0P	A → 1P	A → 1P	C → 0P	6P
(Lepara et al. 2020)	A → 1P	A → 1P	A → 1P	A → 1P	C → 0P	A → 1P	A → 1P	C → 0P	6P
(Loffredo et al. 2020)	C → 0P	A → 1P	A → 1P	B → 0P	A → 1P[b]	A → 1P	A → 1P	C → 0P	5P
Trares et al. 2020	A → 1P	A → 1P	A → 1P	A → 1P	A,B → 2P[a,c]	A → 1P	A → 1P	A → 1P[d]	9P

Abbreviations: P, point.

*Counting of the comparability of cases and controls on the basis of the design or analysis: Zero points are allocated (indicated as option C) if cases and controls were not controlled based on the design or analysis.

[a]Age matched controls

[b]Age and sex matched controls

[c]Results adjusted for potential confounders

[d]Reported for the whole cohort

Supplemental Table 16. Results of studies measuring general or specific F_2-isoprostanes except 8-iso-$PGF_{2\alpha}$

First Author, year	Biomarker measured	Unit of biomarker levels	Form of result	Result		p-value (Test)	Adjusted covariates
				Cases	Controls		
cross-sectional							
Waddington et al. 1999	F_2-isoprostanes	pmol/mmol	Mean (± SEM)	295.00 (12.00)	238.00 (28.00)	n.s.[b]	None
Praticò et al. 2000	8,12-iso-$iPF_{2\alpha}$	ng/mg creatinine	Mean (± SE)	4.93 (0.42)	1.77 (0.17)	**<0.0001[a]**	None
Bohnstedt et al. 2003	8-iso-15(R)-Prostaglandin $F_{2\alpha}$ (iP15R)	pg/mg creatinine	Mean (± SD)	346.00 (271.00)	342.00 (259.00)	n.s. (n.a.)	None
Mufson and Leurgans 2010	iPα-VI	ng/mg creatinine	GM (± GCV)	3.90 (105%)	2.80 (126%)	0.246 (ANOVA)	None
Peña-Bautista et al. 2019	5-F_{2t}-IsoP	ng/mg creatinine	Median (IQR)	2.67 (1.68-5.07)	2.37 (1.76-3.37)	n.a.	None
Waddington et al. 1999	F_2-isoprostanes	pmol/L	Mean (± SEM)	1420.00 (136.00)	1009.00 (69.00)	**<0.0100[b]**	None
Praticò et al. 2000	8,12-iso-$iPF_{2\alpha}$	ng/ml	Mean (± SE)	0.68 (0.05)	0.18 (0.01)	**<0.0001[a]**	None
Irizarry et al. 2007	$iP_{2\alpha}$-IV	pg/ml	Mean (±SD)	1075.60 (651.20)	1196.10 (547.50)	n.s. (n.a.)	None
Mufson and Leurgans 2010	$iP_{2\alpha}$-VI	pg/ml	GM (±GCV)	138.00 (70%)	107.00 (73%)	**0.2500[c]**	None
Montine et al. 1998	F_2-Isoprostanes	pg/ml	Mean (±SEM)	72.00 (7.00)	46.00 (4.00)	**0.0100[d]**	None
Praticò et al. 1998	$iPF_{2\alpha}$-VI	pg/ml	Median (range)	102.00 (33.00-220.00)	38.00 (22.00-80.00)	**0.0090[e]**	None
Montine et al. 1999	F_2-Isoprostanes	pg/ml	Mean (±SEM)	31.00 (2.60)	22.90 (1.00)	**<0.0500[f]**	None
Praticò et al. 2000	8,12-iso-$iPF_{2\alpha}$-VI	pg/ml	Mean (± SE)	66.00 (4.6)	25.00 (3.30)	**<0.0001[a]**	None
Montine et al. 2001	F_2-Isoprostanes	pg/ml	Mean (± SD)	35.40 (7.8)	25.00 (4.56)	**<0.0200 (n.a.)**	None
Kuo et al. 2015	F_2-Isoprostanes	pg/ml	Median (range)	22.60 (13-29)	14.30 (12.70-18.10)	0.0570[a,g]	None
Markesbery et al. 2005	F_2-Isoprostanes	ng/g	Mean (±SEM)	3.50 (0.80)	1.80 (0.20)	**<0.0040[h]**	None
Praticò et al. 1998	$iPF_{2\alpha}$-VI	pg/g wet tissue	Median (range)	1100.00 (700.00-1880.00)	480.00 (320.00-650.00)	n.a.	None
VanRollins et al. 2008	F_2-Isoprostanes	ng/g wet gissue	Mean (± SEM)	2.50 (0.80)	1.50 (0.10)	n.s.[b]	None
Yao et al. 2003	8,12-iso-$iPF_{2\alpha}$-VI	pg/mg tissue	Mean (± SD)	355.00 (58.00)	159.00 (36.00)	**< 0.0010[a]**	None
longitudinal							
Kester et al. 2012	$iPF_{2\alpha}$-VI	pg/mL	Mean (±SD)	14.60 (4.30)[k]	15.20 (4.70)[l]	n.s.[i]	None
Li et al. 2014	F_2-Isoprostanes	pg/ml	Mean (±SD)	30.30 (8.90)	30.40 (9.60)	n.a.	None

Abbreviations: SE, Standard Error; SD, Standard Deviation; SEM, Standard Error of the Mean; GM, Geometric Mean; GCV, Geometric Coefficient of Variation; IQR, Interquartile Range; n.s., not significant

Note: Numbers printed in bold are statistically significant (p<0.05).

[a]Kurskal Wallis test.

[b]Student's T-test.

[c]ANOVA.

[d]Unpaired T-test.

[e]ANOVA with pairwise T-test.

[f]Kurskal Wallis test coupled with Dunn's post-test.

[g]Among three groups: control, mild cognitive impairment, mild AD.

[h]Kruskal Wallis test with post-hoc Wilcoxon's rank sum.

[i]ANOVA with post-hoc Bonferroni.

[k]Baseline data of 64 of 68 AD patients, values at follow-up: 18.1 (7.7) [mean (SD)].

[l] Baseline data of 21 of 24 controls, values at follow-up: 19.8 (9.8) [mean (SD)].

Supplemental Table 17. Results of studies measuring 8-iso-$PGF_{2\alpha}$ levels

First Author, year	Biomarker measured	Unit of biomarker levels	Form of result	Result		p-value (Test)	Adjusted covariates
				Cases	Controls		
cross-sectional							
Tuppo et al. 2001	8-iso-$PGF_{2\alpha}$ ($iPF_{2\alpha}$-III)	pg/mg creatinine	Mean (± SEM)	24.00 (2.70)	11.30 (1.50)	**<0.0001**[c]	None
Bohnstedt et al. 2003	8-iso Prostaglandin $F_{2\alpha}$ ($iPF_{2\alpha}$)	pg/mg creatinine	Mean (± SD)	241 (163)	216 (101)	n.s. (n.a.)	None
Kim et al. 2004	8-iso$PGF_{2\alpha}$	ng/mg creatinine	Mean (± SD)	6.09 (6.07)	2.50 (2.81)	n.s.[b]	None
Ciabattoni et al. 2007	8-iso-$PGF_{2\alpha}$	pg/mg creatinine	Median (IQR)	938.50 (665.50 - 1337.50)	304.00 (218.50 - 348.50)	**< 0.0001**[a]	None
Peña-Bautista et al. 2019	15-keto-15-F_{2t}-IsoP	ng/mg creatinine	Median (IQR)	0.84 (0.22-1.94)	1.33 (0.58-2)	n.a.	None
Feillet-Coudray et al. 1999	8-epi$PGF_{2\alpha}$	ng/L	Mean (± SEM)	15.50 (2.50)	20.00 (4.00)	n.s.[b,c]	None
Peuchant et al. 2008	8-epi-prostaglandin $F_{2\alpha}$	pg/ml	Mean (± SD)	30.65 (3.10)	24.61 (2.67)	**<0.0010**[d]	None
Sirin et al. 2015	8-iso-$PGF_{2\alpha}$	pg/ml	Mean (± SD)	3254.80 (2066.10)	2014.90 (809.10)	**0.0220**[e]	None
Ulstein et al. 2017	8-iso-$PGF_{2\alpha}$	pg/ml	Mean (± SD)	65.5 (51.1)	64.3 (26.8)	0.6900[f]	None
Lepara et al. 2020	8-iso-$PGF_{2\alpha}$	pg/ml	Median (IQR)	74.00 (0.00-212.50)	17.50 (0.00-29.25)	0.015[a,d]	None
Loffredo et al. 2020	8-iso-$PGF_{2\alpha}$	pmol/l	Mean (± SD)	221 (110)	152 (68)	**<0.0500**[a]	None
Praticò et al. 1998	$iPF_{2\alpha}$-III	pg/ml	Median (range)	49 (30-84)	41 (22-60)	0.1400[i]	None
Durazzo et al. 2014	8-iso-$PGF_{2\alpha}$ ($iPF_{2\alpha}$-III)	pg/ml	Mean (± SEM)	ns:2.01 (0.06); s:2.26 (0.05)[g]	ns: 1.91 (0.06); s: 2.19 (0.10)	0.0140[h]	None
Praticò et al. 1998	$iPF_{2\alpha}$-III	pg/g wet tissue	Median (range)	410 (240-880)	200 (81-260)	**0.0020**[k]	None
longitudinal							
Sundelöf et al. 2009	8-iso-$PGF_{2\alpha}$	mg/mmol	HR (95% CI)	0.76 (0.37-1.57)[l]		0.4600	[m]
Trares et al. 2020 (updated with 17-year FUP data for this review)	8-iso-$PGF_{2\alpha}$	nmol/mmol ceatinine	HR (95% CI)[o]	1.44 (1.05-1.95)[l]		**0.0237**	[n]

Abbreviations: SD, standard deviation; IQR, interquartile range, SEM, standard error of the mean; HR, Hazard ratio; CI, confidence interval; FUP, follow-up.

Note: Numbers printed in bold are statistically significant ($p<0.05$).

[a]Kurskal Wallis test.

[b]Student's T-test.

[c]Mann-Whitney U Rank Sum Test.

[d]Mann-Whitney U Test with Bonferroni correction.

[e]ANOVA paired with post hoc Bonferroni test.

[f]ANOVA.

[g]Data divided into non-smoking (ns) and smoking (s) subgroups.

[h]T-tests with Bonferroni correction resulted in: $p = 0.014$ (nsCN vs. sCN); $p<0.001$ (sAD vs. nsCN); $p = 0.005$ (sAD vs. nsAD).

[i]ANOVA with pairwise T-test.

[k]Pairwise comparison by two-tailed T-test.

[l]Results above the median with below/at the median as reference.

[m]Adjusted covariates: age (continuous), *APOE* genotype (binary e4 carriers and non-carriers), diabetes (binary), NSAID treatment (binary), aspirin treatment (binary), smoking status (binary), BMI (continuous), hypertension (binary), serum cholesterol (continuous), energy adjusted intake of dietary vitamin E (continuous), vitamin C (continuous), stroke (binary), educational levels (ordinal).

[n]Adjusted covariates: age (continuously), sex, education, physical activity, BMI (categorical), diabetes, *APOE* e4 polymorphism.

[o]Mean 8-iso-$PGF_{2\alpha}$ values were 0.245 nmol/mmol creatinine (SD: 0.234) for cases and 0.232 nmol/mmol creatinine (SD: 0.280) for controls; Hedge's g [95% CI]: 0.05 [-0.11-0.20].

Supplemental Table 18. Comparison of median biomarker levels between all-cause dementia cases (n=504) and controls (n=1,278)

Olink Biomarker	All-cause dementia cases (n=504) Median (IQR)	Controls (n=1,278) Median (IQR)	p-value[a]	FDR[b]
ADA	6.47 (6.09-6.82)	6.38 (6.02-6.73)	**0.0041**	**0.0029**
AXIN1	3.72 (2.61-4.35)	3.60 (2.49-4.26)	0.0662	0.0536
Beta-NGF	0.61 (0.60-0.67)	0.61 (0.43-0.77)	0.3080	0.2807
CASP-8	6.09 (5.15-7.09)	5.93 (5.02-6.85)	**0.0248**	**0.0197**
CCL3	7.25 (6.65-8.00)	7.03 (6.49-7.77)	**<0.0001**	**<0.0001**
CCL4	7.41 (6.87-8.10)	7.25 (6.71-7.87)	**0.0006**	**0.0004**
CCL11	8.67 (8.27-9.06)	8.54 (8.15-8.89)	**<0.0001**	**<0.0001**
CCL19	9.12 (8.49-9.84)	8.84 (8.31-9.54)	**<0.0001**	**<0.0001**
CCL20	6.74 (6.04-7.57)	6.44 (5.76-7.27)	**<0.0001**	**<0.0001**
CCL23	10.00 (9.62-10.41)	9.86 (9.46-10.21)	**<0.0001**	**<0.0001**
CCL25	6.61 (6.16-7.01)	6.39 (5.94-6.83)	**<0.0001**	**<0.0001**
CCL28	2.81 (2.53-3.15)	2.70 (2.42-3.00)	**<0.0001**	**<0.0001**
CD5	5.99 (5.66-6.27)	5.84 (5.54-6.14)	**<0.0001**	**<0.0001**
CD6	7.42 (6.99-7.83)	7.34 (6.83-7.73)	**0.0038**	**0.0026**
CD40	12.61 (12.12-13.01)	12.44 (11.98-12.89)	**0.0002**	**0.0001**
CD244	6.75 (6.44-7.07)	6.63 (6.34-6.89)	**<0.0001**	**<0.0001**
CD8A	9.47 (8.86-10.07)	9.33 (8.73-9.91)	**0.0016**	**0.0010**
CDCP1	3.97 (3.45-4.49)	3.64 (3.15-4.14)	**<0.0001**	**<0.0001**
CSF-1	11.01 (10.82-11.19)	10.93 (10.73-11.12)	**<0.0001**	**<0.0001**
CST5	7.30 (6.94-7.67)	7.15 (6.81-7.50)	**<0.0001**	**<0.0001**
CX3CL1	4.72 (4.46-5.06)	4.57 (4.20-4.88)	**<0.0001**	**<0.0001**
CXCL1	10.39 (9.94-10.88)	10.31 (9.84-10.74)	**0.0091**	**0.0066**
CXCL5	12.64 (12.00-13.20)	12.44 (11.75-13.03)	**0.0002**	**0.0001**
CXCL6	9.39 (8.94-9.89)	9.24 (8.73-9.70)	**<0.0001**	**<0.0001**
CXCL9	7.31 (6.87-7.89)	6.98 (6.49-7.54)	**<0.0001**	**<0.0001**
CXCL10	9.96 (9.46-10.54)	9.72 (9.26-10.24)	**<0.0001**	**<0.0001**
CXCL11	8.33 (7.63-8.92)	8.06 (7.46-8.69)	**<0.0001**	**<0.0001**
DNER	9.65 (9.40-9.86)	9.56 (9.32-9.77)	**<0.0001**	**<0.0001**
EN-RAGE	7.27 (6.46-8.18)	7.00 (6.01-7.77)	**<0.0001**	**<0.0001**
FGF-19	8.82 (8.04-9.49)	8.53 (7.82-9.24)	**<0.0001**	**<0.0001**
FGF-21	5.92 (5.05-6.70)	5.65 (4.82-6.51)	**0.0008**	**0.0005**
FGF-23	0.93 (0.84-1.09)	0.93 (0.64-1.15)	0.3844	0.3552
Flt3L	9.77 (9.45-10.13)	9.65 (9.33-9.98)	**<0.0001**	**<0.0001**
GDNF	1.44 (1.29-2.04)	1.43 (1.29-1.90)	**<0.0001**	**<0.0001**
HGF	9.93 (9.45-10.28)	9.73 (9.29-10.10)	**<0.0001**	**<0.0001**
IL-6	2.52 (1.98-3.43)	2.30 (1.71-3.17)	**<0.0001**	**<0.0001**
IL-7	3.16 (2.65-3.73)	3.09 (2.53-3.63)	**0.0169**	**0.0132**
IL-8	9.40 (7.74-11.96)	9.19 (7.56-11.68)	0.2557	0.2266
IL-10	3.49 (3.09-3.88)	3.36 (3.00-3.76)	**0.0002**	**0.0001**
IL-18	9.15 (8.68-9.54)	8.90 (8.48-9.37)	**<0.0001**	**<0.0001**
IL-10RA	0.71 (0.48-1.09)	0.66 (0.43-0.98)	**0.0079**	**0.0057**
IL-10RB	6.75 (6.50-7.01)	6.64 (6.34-6.87)	**<0.0001**	**<0.0001**

IL-15RA	1.51 (1.36-1.74)	1.38 (1.28-1.67)	**<0.0001**	**<0.0001**
IL-18R1	9.17 (8.84-9.55)	9.07 (8.68-9.40)	**<0.0001**	**<0.0001**
LAP TGF-beta-1	8.22 (7.89-8.55)	8.07 (7.73-8.39)	**<0.0001**	**<0.0001**
LIF-R	4.83 (4.59-5.09)	4.71 (4.48-4.92)	**<0.0001**	**<0.0001**
MCP-1	12.52 (12.05-13.22)	12.45 (12.00-12.98)	**0.0131**	**0.0098**
MCP-2	9.86 (9.34-10.24)	9.69 (9.22-10.09)	**0.0001**	**0.0001**
MCP-3	3.89 (2.63-6.38)	3.54 (2.46-6.13)	0.0955	0.0810
MCP-4	15.16 (14.69-15.65)	15.04 (14.51-15.49)	**0.0002**	**0.0001**
MMP-1	15.90 (15.32-16.37)	15.91 (15.18-16.43)	0.6484	0.6319
MMP-10	9.91 (9.48-10.30)	9.75 (9.31-10.17)	**<0.0001**	**<0.0001**
NT-3	1.37 (1.14-1.65)	1.31 (1.04-1.56)	**0.0006**	**0.0004**
OPG	10.48 (10.16-10.79)	10.26 (9.94-10.56)	**<0.0001**	**<0.0001**
OSM	7.85 (6.97-8.99)	7.87 (6.97-8.91)	0.8246	0.8246
PD-L1	6.26 (5.97-6.54)	6.10 (5.82-6.38)	**<0.0001**	**<0.0001**
SCF	9.69 (9.33-9.98)	9.59 (9.24-9.86)	**<0.0001**	**<0.0001**
SIRT2	4.51 (3.61-5.17)	4.38 (3.49-5.10)	0.0794	0.0653
SLAMF1	2.94 (2.66-3.34)	2.87 (2.47-3.21)	**0.0002**	**0.0001**
ST1A1	4.20 (2.84-4.92)	4.02 (2.87-4.81)	0.0955	0.0801
STAMBP	5.77 (5.09-6.21)	5.65 (5.05-6.11)	**0.0079**	**0.0057**
TGF-alpha	4.91 (4.42-5.41)	4.83 (4.30-5.30)	**0.0164**	**0.0126**
TNFB	5.35 (5.03-5.65)	5.35 (4.98-5.66)	0.6034	0.5805
TNFRSF9	7.61 (7.30-7.96)	7.44 (7.11-7.78)	**<0.0001**	**<0.0001**
TNFSF14	7.86 (6.97-8.46)	7.71 (6.94-8.33)	**0.0161**	**0.0122**
TRAIL	8.40 (8.14-8.66)	8.33 (8.05-8.57)	**<0.0001**	**<0.0001**
TRANCE	5.29 (4.85-5.77)	5.31 (4.80-5.75)	0.5578	0.5296
TWEAK	9.27 (8.93-9.53)	9.16 (8.87-9.43)	**<0.0001**	**<0.0001**
VEGF-A	12.43 (11.88-12.89)	12.17 (11.69-12.63)	**<0.0001**	**<0.0001**
4E-BP1	8.03 (7.23-8.75)	7.83 (6.90-8.60)	**0.0034**	**0.0023**
uPA	9.94 (9.68-10.27)	9.84 (9.53-10.11)	**<0.0001**	**<0.0001**

Abbreviations: IQR, interquartile range; FDR, false discovery rate; For biomarker abbreviations, see Supplemental Table 5

NOTE: Numbers printed in bold are statistically significant ($p < 0.05$).

[a]Results of a Wilcoxon rank-sum test.

[b]P-values corrected for multiple testing by Benjamini and Hochberg method.

Supplemental Table 19. Comparison of median biomarker levels between Alzheimer's disease cases (n=163) and controls (n=1,278)

Olink Biomarker	Alzheimer's disease (n=163) Median (IQR)	Controls (n=1,278) Median (IQR)	p-value[a]	FDR[b]
ADA	6.44 (6.06-6.76)	6.38 (6.02-6.73)	0.2526	0.3041
AXIN1	3.80 (2.89-4.36)	3.60 (2.49-4.26)	**0.0197**	**0.0331**
Beta-NGF	0.61 (0.56-0.74)	0.61 (0.43-0.77)	0.3371	0.3916
CASP-8	6.29 (5.31-7.44)	5.93 (5.02-6.85)	**0.0012**	**0.0037**
CCL3	7.17 (6.60-7.96)	7.03 (6.49-7.77)	0.0508	0.0704
CCL4	7.36 (6.89-8.12)	7.25 (6.71-7.87)	**0.0363**	0.0522
CCL11	8.67 (8.29-9.01)	8.54 (8.15-8.89)	**0.0007**	**0.0031**
CCL19	9.28 (8.60-9.82)	8.84 (8.31-9.54)	**0.0002**	**0.0010**
CCL20	6.81 (6.04-7.61)	6.44 (5.76-7.27)	**0.0018**	**0.0050**
CCL23	10.03 (9.63-10.41)	9.86 (9.46-10.21)	**<0.0001**	**0.0004**
CCL25	6.51 (6.14-6.92)	6.39 (5.94-6.83)	**0.0099**	**0.0190**
CCL28	2.83 (2.54-3.24)	2.70 (2.42-3.00)	**<0.0001**	**0.0004**
CD5	5.99 (5.70-6.25)	5.84 (5.54-6.14)	**0.0003**	**0.0013**
CD6	7.43 (7.05-7.86)	7.34 (6.83-7.73)	**0.0027**	**0.0069**
CD40	12.55 (12.18-13.03)	12.44 (11.98-12.89)	**0.0040**	**0.0093**
CD244	6.76 (6.45-7.10)	6.63 (6.34-6.89)	**0.0001**	**0.0009**
CD8A	9.45 (8.80-10.00)	9.33 (8.73-9.91)	0.1043	0.1350
CDCP1	3.94 (3.40-4.40)	3.64 (3.15-4.14)	**0.0001**	**0.0009**
CSF-1	11.02 (10.82-11.16)	10.93 (10.73-11.12)	**0.0029**	**0.0070**
CST5	7.25 (6.95-7.65)	7.15 (6.81-7.50)	**0.0009**	**0.0031**
CX3CL1	4.73 (4.46-5.03)	4.57 (4.20-4.88)	**<0.0001**	**0.0001**
CXCL1	10.38 (9.88-10.96)	10.31 (9.84-10.74)	0.0872	0.1148
CXCL5	12.63 (12.09-13.25)	12.44 (11.75-13.03)	**0.0008**	**0.0031**
CXCL6	9.39 (8.95-9.91)	9.24 (8.73-9.70)	**0.0045**	**0.0101**
CXCL9	7.36 (6.87-7.91)	6.98 (6.49-7.54)	**<0.0001**	**<0.0001**
CXCL10	9.90 (9.43-10.61)	9.72 (9.26-10.24)	**0.0012**	**0.0037**
CXCL11	8.39 (7.69-8.99)	8.06 (7.46-8.69)	**0.0008**	**0.0031**
DNER	9.67 (9.40-9.84)	9.56 (9.32-9.77)	**0.0021**	**0.0057**
EN-RAGE	7.33 (6.56-8.17)	7.00 (6.01-7.77)	**<0.0001**	**0.0005**
FGF-19	8.82 (8.09-9.61)	8.53 (7.82-9.24)	**0.0017**	**0.0050**
FGF-21	5.93 (5.00-6.70)	5.65 (4.82-6.51)	**0.0478**	0.0674
FGF-23	0.93 (0.79-1.04)	0.93 (0.64-1.15)	0.6932	0.7301
Flt3L	9.76 (9.42-10.11)	9.65 (9.33-9.98)	**0.0050**	**0.0107**
GDNF	1.44 (1.29-1.99)	1.43 (1.29-1.90)	**0.0253**	**0.0391**
HGF	9.89 (9.49-10.26)	9.73 (9.29-10.10)	**0.0006**	**0.0027**
IL-6	2.52 (1.90-3.60)	2.30 (1.71-3.17)	**0.0139**	**0.0249**
IL-7	3.05 (2.48-3.44)	3.09 (2.53-3.63)	0.4597	0.5114
IL-8	9.38 (7.64-12.41)	9.19 (7.56-11.68)	0.4592	0.5114
IL-10	3.48 (3.08-3.84)	3.36 (3.00-3.76)	**0.0331**	**0.0484**
IL-18	9.02 (8.64-9.45)	8.90 (8.48-9.37)	**0.0134**	**0.0247**
IL-10RA	0.73 (0.50-1.07)	0.66 (0.43-0.98)	**0.0322**	**0.0479**
IL-10RB	6.77 (6.48-6.93)	6.64 (6.34-6.87)	**0.0001**	**0.0008**

IL-15RA	1.46 (1.36-1.70)	1.38 (1.28-1.67)	**0.0244**	**0.0385**
IL-18R1	9.14 (8.80-9.50)	9.07 (8.68-9.40)	**0.0151**	**0.0265**
LAP TGF-beta-1	8.30 (7.91-8.61)	8.07 (7.73-8.39)	**<0.0001**	**0.0001**
LIF-R	4.81 (4.50-5.04)	4.71 (4.48-4.92)	**0.0008**	**0.0031**
MCP-1	12.49 (12.04-13.22)	12.45 (12.00-12.98)	0.1333	0.1699
MCP-2	9.82 (9.29-10.15)	9.69 (9.22-10.09)	0.0828	0.1109
MCP-3	3.57 (2.43-5.93)	3.54 (2.46-6.13)	0.9037	0.9271
MCP-4	15.15 (14.71-15.68)	15.04 (14.51-15.49)	**0.0173**	**0.0297**
MMP-1	15.89 (15.36-16.38)	15.91 (15.18-16.43)	0.5209	0.5561
MMP-10	9.90 (9.48-10.30)	9.75 (9.31-10.17)	**0.0029**	**0.0070**
NT-3	1.32 (1.13-1.59)	1.31 (1.04-1.56)	0.4101	0.4695
OPG	10.44 (10.15-10.75)	10.26 (9.94-10.56)	**<0.0001**	**<0.0001**
OSM	7.98 (6.94-9.06)	7.87 (6.97-8.91)	0.5066	0.5482
PD-L1	6.25 (5.98-6.54)	6.10 (5.82-6.38)	**<0.0001**	**0.0001**
SCF	9.72 (9.38-9.99)	9.59 (9.24-9.86)	**0.0002**	**0.0010**
SIRT2	4.52 (3.79-5.18)	4.38 (3.49-5.10)	**0.0301**	**0.0458**
SLAMF1	2.90 (2.51-3.31)	2.87 (2.47-3.21)	0.3032	0.3575
ST1A1	4.37 (3.30-5.04)	4.02 (2.87-4.81)	**0.0074**	**0.0151**
STAMBP	5.82 (5.22-6.21)	5.65 (5.05-6.11)	**0.0093**	**0.0184**
TGF-alpha	4.92 (4.48-5.33)	4.83 (4.30-5.30)	0.0555	0.0756
TNFB	5.39 (5.08-5.69)	5.35 (4.98-5.66)	0.2404	0.2967
TNFRSF9	7.60 (7.35-7.95)	7.44 (7.11-7.78)	**<0.0001**	**0.0003**
TNFSF14	7.90 (7.27-8.49)	7.71 (6.94-8.33)	**0.0102**	**0.0193**
TRAIL	8.43 (8.13-8.63)	8.33 (8.05-8.57)	**0.0054**	**0.0113**
TRANCE	5.32 (4.92-5.79)	5.31 (4.80-5.75)	0.2541	0.3041
TWEAK	9.30 (8.94-9.58)	9.16 (8.87-9.43)	**0.0025**	**0.0065**
VEGF-A	12.36 (11.87-12.76)	12.17 (11.69-12.63)	**0.0012**	**0.0037**
4E-BP1	8.07 (7.29-8.68)	7.83 (6.90-8.60)	**0.0219**	**0.0361**
uPA	9.97 (9.67-10.30)	9.84 (9.53-10.11)	**<0.0001**	**0.0004**

Abbreviations: IQR, interquartile range; FDR, false discovery rate; For biomarker abbreviations, see Supplemental Table 5

NOTE: Numbers printed in bold are statistically significant ($p < 0.05$).

[a]Results of a Wilcoxon rank-sum test. Study participants with other (e.g. VD) or unknown dementia forms were excluded.

[b]P-values corrected for multiple testing by Benjamini and Hochberg method.

Supplemental Table 20. Comparison of median biomarker levels between vascular dementia cases (n=195) and controls (n=1,278)

Olink Biomarker	Vascular dementia (n=195) Median (IQR)	Controls (n=1,278) Median (IQR)	p-value[a]	FDR[b]
ADA	6.45 (6.06-6.75)	6.38 (6.02-6.73)	0.2154	0.2701
AXIN1	3.70 (2.39-4.37)	3.60 (2.49-4.26)	0.5743	0.6312
Beta-NGF	0.61 (0.59-0.67)	0.61 (0.43-0.77)	0.4956	0.5758
CASP-8	6.04 (5.12-7.10)	5.93 (5.02-6.85)	0.2717	0.3302
CCL3	7.30 (6.76-8.11)	7.03 (6.49-7.77)	**0.0006**	**0.0015**
CCL4	7.53 (6.88-8.10)	7.25 (6.71-7.87)	**0.0025**	**0.0050**
CCL11	8.68 (8.27-9.06)	8.54 (8.15-8.89)	**<0.0001**	**0.0003**
CCL19	9.06 (8.44-9.73)	8.84 (8.31-9.54)	**0.0114**	**0.0166**
CCL20	6.79 (6.03-7.74)	6.44 (5.76-7.27)	**<0.0001**	**0.0002**
CCL23	9.98 (9.63-10.44)	9.86 (9.46-10.21)	**<0.0001**	**0.0004**
CCL25	6.63 (6.09-7.06)	6.39 (5.94-6.83)	**0.0001**	**0.0004**
CCL28	2.82 (2.55-3.07)	2.70 (2.42-3.00)	**0.0005**	**0.0014**
CD5	6.06 (5.71-6.31)	5.84 (5.54-6.14)	**<0.0001**	**<0.0001**
CD6	7.45 (6.97-7.78)	7.34 (6.83-7.73)	0.0529	0.0733
CD40	12.62 (12.12-13.03)	12.44 (11.98-12.89)	**0.0069**	**0.0106**
CD244	6.77 (6.44-7.09)	6.63 (6.34-6.89)	**<0.0001**	**0.0002**
CD8A	9.49 (8.95-10.11)	9.33 (8.73-9.91)	**0.0057**	**0.0091**
CDCP1	4.01 (3.47-4.61)	3.64 (3.15-4.14)	**<0.0001**	**<0.0001**
CSF-1	11.03 (10.85-11.19)	10.93 (10.73-11.12)	**<0.0001**	**0.0004**
CST5	7.28 (6.94-7.64)	7.15 (6.81-7.50)	**0.0008**	**0.0019**
CX3CL1	4.71 (4.42-5.05)	4.57 (4.20-4.88)	**<0.0001**	**<0.0001**
CXCL1	10.48 (10.07-10.89)	10.31 (9.84-10.74)	**0.0027**	**0.0051**
CXCL5	12.69 (12.04-13.21)	12.44 (11.75-13.03)	**0.0015**	**0.0032**
CXCL6	9.38 (8.99-9.91)	9.24 (8.73-9.70)	**0.0006**	**0.0015**
CXCL9	7.34 (6.89-7.98)	6.98 (6.49-7.54)	**<0.0001**	**<0.0001**
CXCL10	10.00 (9.50-10.73)	9.72 (9.26-10.24)	**<0.0001**	**<0.0001**
CXCL11	8.30 (7.68-8.89)	8.06 (7.46-8.69)	**0.0015**	**0.0032**
DNER	9.64 (9.39-9.87)	9.56 (9.32-9.77)	**0.0019**	**0.0040**
EN-RAGE	7.22 (6.48-8.18)	7.00 (6.01-7.77)	**0.0002**	**0.0005**
FGF-19	8.91 (8.02-9.48)	8.53 (7.82-9.24)	**0.002**	**0.0041**
FGF-21	5.94 (5.18-6.71)	5.65 (4.82-6.51)	**0.0028**	**0.0053**
FGF-23	0.93 (0.79-1.02)	0.93 (0.64-1.15)	0.8527	0.8749
Flt3L	9.78 (9.47-10.15)	9.65 (9.33-9.98)	**0.0002**	**0.0005**
GDNF	1.42 (1.29-2.06)	1.43 (1.29-1.90)	**0.0063**	**0.0100**
HGF	9.97 (9.50-10.26)	9.73 (9.29-10.10)	**<0.0001**	**<0.0001**
IL-6	2.57 (1.99-3.43)	2.30 (1.71-3.17)	**0.0012**	**0.0026**
IL-7	3.28 (2.75-3.78)	3.09 (2.53-3.63)	**0.0031**	**0.0056**
IL-8	9.42 (7.98-12.28)	9.19 (7.56-11.68)	0.1985	0.2529
IL-10	3.53 (3.11-3.97)	3.36 (3.00-3.76)	**0.0006**	**0.0015**
IL-18	9.16 (8.69-9.55)	8.90 (8.48-9.37)	**<0.0001**	**0.0002**
IL-10RA	0.71 (0.47-1.10)	0.66 (0.43-0.98)	0.0681	0.0928
IL-10RB	6.74 (6.48-7.01)	6.64 (6.34-6.87)	**<0.0001**	**0.0004**

IL-15RA	1.55 (1.36-1.73)	1.38 (1.28-1.67)	**<0.0001**	**0.0003**
IL-18R1	9.20 (8.87-9.56)	9.07 (8.68-9.40)	**<0.0001**	**0.0002**
LAP TGF-beta-1	8.20 (7.89-8.50)	8.07 (7.73-8.39)	**0.0001**	**0.0004**
LIF-R	4.83 (4.62-5.10)	4.71 (4.48-4.92)	**<0.0001**	**<0.0001**
MCP-1	12.54 (11.99-13.21)	12.45 (12.00-12.98)	0.0722	0.0967
MCP-2	9.92 (9.38-10.30)	9.69 (9.22-10.09)	**0.0003**	**0.0009**
MCP-3	3.85 (2.65-6.56)	3.54 (2.46-6.13)	0.1077	0.1419
MCP-4	15.15 (14.70-15.68)	15.04 (14.51-15.49)	**0.0049**	**0.0082**
MMP-1	15.91 (15.27-16.36)	15.91 (15.18-16.43)	0.9038	0.9154
MMP-10	9.95 (9.57-10.42)	9.75 (9.31-10.17)	**<0.0001**	**<0.0001**
NT-3	1.40 (1.13-1.65)	1.31 (1.04-1.56)	**0.0044**	**0.0075**
OPG	10.48 (10.15-10.79)	10.26 (9.94-10.56)	**<0.0001**	**<0.0001**
OSM	7.77 (7.09-9.10)	7.87 (6.97-8.91)	0.7958	0.8382
PD-L1	6.27 (5.95-6.58)	6.10 (5.82-6.38)	**<0.0001**	**<0.0001**
SCF	9.70 (9.32-9.99)	9.59 (9.24-9.86)	**0.0050**	**0.0082**
SIRT2	4.51 (3.45-5.08)	4.38 (3.49-5.10)	0.9709	0.9709
SLAMF1	2.93 (2.66-3.34)	2.87 (2.47-3.21)	**0.0096**	**0.0143**
ST1A1	4.13 (2.70-4.85)	4.02 (2.87-4.81)	0.5753	0.6312
STAMBP	5.71 (5.04-6.13)	5.65 (5.05-6.11)	0.6045	0.6541
TGF-alpha	4.95 (4.49-5.51)	4.83 (4.30-5.30)	**0.0192**	**0.0275**
TNFB	5.35 (5.01-5.62)	5.35 (4.98-5.66)	0.8368	0.8698
TNFRSF9	7.63 (7.29-8.01)	7.44 (7.11-7.78)	**<0.0001**	**<0.0001**
TNFSF14	7.89 (6.87-8.49)	7.71 (6.94-8.33)	0.0518	0.0731
TRAIL	8.38 (8.14-8.68)	8.33 (8.05-8.57)	**0.0030**	**0.0055**
TRANCE	5.29 (4.83-5.86)	5.31 (4.80-5.75)	0.3947	0.4725
TWEAK	9.25 (8.92-9.54)	9.16 (8.87-9.43)	**0.0033**	**0.0058**
VEGF-A	12.45 (11.87-12.95)	12.17 (11.69-12.63)	**<0.0001**	**<0.0001**
4E-BP1	8.00 (7.21-8.65)	7.83 (6.90-8.60)	0.2272	0.2805
uPA	9.94 (9.66-10.28)	9.84 (9.53-10.11)	**0.0003**	**0.0009**

Abbreviations: IQR, interquartile range; FDR, false discovery rate; For biomarker abbreviations, see Supplemental Table 5

NOTE: Numbers printed in bold are statistically significant ($p < 0.05$).

[a]Results of a Wilcoxon rank-sum test. Study participants with other (e.g. AD) or unknown dementia forms were excluded.

[b]P-values corrected for multiple testing by Benjamini and Hochberg method.

Supplemental Table 21. Non-significant associations of Olink Inflammation panel biomarker levels with <u>all-cause dementia</u> incidence (FDR ≥ 0.05)

Olink Biomarker	Value of 1 SD	All-cause dementia (n=504 cases)		
		OR (95% CI) per 1 SD[a]	p-value per 1 SD	FDR corrected p-value[b]
Beta-NGF	0.397	0.97 (0.86-1.11)	0.6887	0.6984
CD8A	0.944	1.13 (1.00-1.27)	**0.0492**	0.0581
FGF-21	1.315	1.11 (0.99-1.25)	0.0716	0.0818
FGF-23	0.449	0.99 (0.88-1.11)	0.8587	0.8587
IL-6	1.871	1.10 (0.98-1.23)	0.1092	0.1210
IL-8	2.570	1.09 (0.97-1.22)	0.1471	0.1605
IL-12B	0.795	1.10 (0.98-1.24)	0.1086	0.1210
MCP-1	1.001	1.12 (1.00-1.26)	**0.0469**	0.0563
MCP-3	2.846	1.05 (0.94-1.18)	0.3626	0.3784
MMP-1	0.981	1.04 (0.93-1.18)	0.4651	0.4784
OSM	1.501	1.08 (0.96-1.21)	0.2165	0.2292
SLAMF1	0.633	1.12 (1.00-1.27)	0.0506	0.0588
TNFB	0.592	1.08 (0.96-1.22)	0.1823	0.1959
4E-BP1	1.300	1.13 (1.00-1.27)	**0.0457**	0.0558

Abbreviations: SD, standard deviation; CI, confidence interval; FDR, false discovery rate; For biomarker abbreviations, see Supplemental Table 5.

[a] Multivariate logistic regression model adjusted for age (continuously), sex, education, physical activity, BMI (categorical), CVD, diabetes, depression, *APOE* genotype.

[b] P-values corrected for multiple testing by the Benjamini and Hochberg method.

Supplemental Table 22. Non-significant associations of Olink Inflammation panel biomarker levels with <u>Alzheimer's disease</u> incidence (FDR ≥ 0.05)

Olink Biomarker	Value of 1 SD	Alzheimer's disease (n=163 cases)		
		OR (95% CI) per 1 SD[a]	p-value per 1 SD	FDR corrected p-value[b]
ADA	0.635	1.07 (0.90-1.28)	0.4191	0.4572
AXIN1	1.140	1.24 (1.04-1.50)	**0.0196**	0.0564
Beta-NGF	0.397	0.98 (0.81-1.18)	0.8200	0.8315
CCL3	1.508	1.11 (0.94-1.31)	0.2045	0.2727
CCL4	1.099	1.16 (0.98-1.38)	0.0792	0.1326
CCL11	0.697	1.26 (1.03-1.53)	**0.0215**	0.0594
CCL19	1.199	1.24 (1.04-1.47)	**0.0162**	0.0501
CCL20	1.540	1.17 (1.00-1.38)	0.0520	0.0891
CCL25	0.763	1.08 (0.90-1.30)	0.4293	0.4613
CD5	0.523	1.24 (1.03-1.49)	**0.0253**	0.0594
CD40	0.734	1.24 (1.03-1.50)	**0.0259**	0.0594
CD8A	0.944	1.09 (0.91-1.32)	0.3392	0.3939
CDCP1	0.894	1.16 (0.96-1.40)	0.1324	0.1945
CSF-1	0.425	1.22 (1.00-1.50)	0.0512	0.0891
CST5	0.698	1.25 (1.03-1.51)	**0.0226**	0.0594
CXCL1	0.901	1.15 (0.97-1.37)	0.1021	0.1598
CXCL9	0.953	1.23 (1.02-1.48)	**0.0264**	0.0594
CXCL10	0.953	1.09 (0.91-1.31)	0.3288	0.3881
CXCL11	1.051	1.21 (1.01-1.45)	**0.0341**	0.0701
FGF-19	1.089	1.21 (1.02-1.45)	**0.0328**	0.0695
FGF-21	1.315	1.10 (0.91-1.32)	0.3247	0.3881

Olink Biomarker	Value of 1 SD	Alzheimer's disease (n=163 cases)		
		OR (95% CI) per 1 SD[a]	p-value per 1 SD	FDR corrected p-value[b]
FGF-23	0.449	0.87 (0.72-1.06)	0.1694	0.2301
Flt3L	0.629	1.18 (0.98-1.41)	0.0875	0.1400
GDNF	0.506	1.06 (0.88-1.28)	0.5480	0.5802
IL-6	1.871	1.10 (0.93-1.30)	0.2483	0.3136
IL-7	0.798	0.97 (0.81-1.16)	0.7445	0.7658
IL-8	2.570	1.07 (0.90-1.28)	0.4121	0.4565
IL-10	0.863	1.08 (0.91-1.29)	0.3790	0.4331
IL-18	0.763	1.22 (1.01-1.48)	**0.0393**	0.0765
IL-12B	0.795	1.15 (0.96-1.38)	0.1361	0.1960
IL-10RA	0.788	1.13 (0.96-1.33)	0.1555	0.2195
IL-15RA	0.359	1.12 (0.93-1.35)	0.2414	0.3104
IL-18R1	0.602	1.18 (0.98-1.42)	0.0862	0.1400
MCP-1	1.001	1.13 (0.95-1.34)	0.1663	0.2301
MCP-2	0.769	1.08 (0.90-1.31)	0.3892	0.4379
MCP-3	2.846	0.99 (0.84-1.18)	0.9418	0.9418
MCP-4	0.927	1.22 (1.00-1.49)	**0.0465**	0.0873
MMP-1	0.981	1.10 (0.92-1.32)	0.2830	0.3512
MMP-10	0.761	1.21 (1.01-1.45)	**0.0393**	0.0765
NT-3	0.544	1.10 (0.92-1.31)	0.2878	0.3512
OPG	0.609	1.28 (1.05-1.57)	**0.0167**	0.0501
OSM	1.501	1.12 (0.94-1.33)	0.2121	0.2777
SCF	0.624	1.26 (1.03-1.56)	**0.0277**	0.0604
SIRT2	1.157	1.20 (1.00-1.43)	0.0517	0.0891
SLAMF1	0.633	1.05 (0.87-1.27)	0.5886	0.6142
TNFB	0.592	1.17 (0.96-1.41)	0.1144	0.1716
TNFRSF9	0.636	1.24 (1.03-1.50)	**0.0250**	0.0594
TNFSF14	1.064	1.24 (1.03-1.49)	**0.0245**	0.0594
TRANCE	0.753	1.21 (1.00-1.45)	**0.0473**	0.0873
4E-BP1	1.300	1.16 (0.97-1.39)	0.1135	0.1716

Abbreviations: SD, standard deviation; CI, confidence interval; FDR, false discovery rate; For biomarker abbreviations, see Supplemental Table 5.

[a] Multivariate logistic regression model adjusted for age (continuously), sex, education, physical activity, BMI (categorical), CVD, diabetes, depression, *APOE* genotype. Study participants with other (e.g. VD) or unknown dementia forms were excluded.

[b] P-values corrected for multiple testing by the Benjamini and Hochberg method.

Supplemental Table 23. Non-significant associations of Olink Inflammation panel biomarker levels with vascular dementia incidence (FDR ≥ 0.05)

Olink Biomarker	Value of 1 SD	Vascular dementia (n=195 cases)		
		OR (95% CI) per 1 SD[a]	p-value per 1 SD	FDR corrected p-value[b]
ADA	0.635	1.08 (0.92-1.27)	0.3647	0.4376
AXIN1	1.140	1.06 (0.90-1.25)	0.4768	0.5364
Beta-NGF	0.397	0.98 (0.83-1.16)	0.8471	0.8839
CASP-8	1.364	1.08 (0.92-1.28)	0.3416	0.4198
CCL3	1.508	1.17 (1.00-1.36)	**0.0473**	0.0811
CCL4	1.099	1.19 (1.01-1.39)	**0.0329**	0.0623

Olink Biomarker	Value of 1 SD	Vascular dementia (n=195 cases)		
		OR (95% CI) per 1 SD[a]	p-value per 1 SD	FDR corrected p-value[b]
CCL19	1.199	1.08 (0.92-1.27)	0.3440	0.4198
CCL20	1.540	1.19 (1.02-1.39)	**0.0260**	0.0520
CCL25	0.763	1.18 (1.00-1.41)	0.0564	0.0864
CCL28	0.548	1.17 (1.00-1.38)	0.0503	0.0823
CD6	0.757	1.15 (0.97-1.37)	0.0966	0.1419
CD40	0.734	1.18 (1.00-1.40)	0.0555	0.0864
CD8A	0.944	1.14 (0.96-1.35)	0.1316	0.1858
CSF-1	0.425	1.24 (1.03-1.49)	**0.0240**	0.0508
CST5	0.698	1.08 (0.91-1.27)	0.3916	0.4622
CXCL11	1.051	1.21 (1.02-1.43)	**0.0250**	0.0514
FGF-19	1.089	1.18 (1.01-1.39)	**0.0404**	0.0727
FGF-21	1.315	1.14 (0.96-1.35)	0.1277	0.1839
FGF-23	0.449	0.96 (0.83-1.12)	0.6277	0.6745
GDNF	0.506	1.16 (0.99-1.35)	0.0655	0.0983
IL-6	1.871	1.11 (0.95-1.30)	0.1709	0.2322
IL-8	2.570	1.11 (0.94-1.30)	0.2125	0.2732
IL-12B	0.795	1.13 (0.96-1.34)	0.1397	0.1934
IL-10RA	0.788	1.16 (1.00-1.34)	0.0501	0.0823
IL-10RB	0.533	1.21 (1.01-1.44)	**0.0372**	0.0687
IL-15RA	0.359	1.20 (1.02-1.42)	**0.0278**	0.0541
MCP-1	1.001	1.11 (0.95-1.30)	0.2037	0.2667
MCP-3	2.846	1.06 (0.90-1.24)	0.5008	0.5547
MMP-1	0.981	1.00 (0.86-1.17)	0.9633	0.9769
OSM	1.501	1.07 (0.91-1.26)	0.4036	0.4687
SCF	0.624	1.12 (0.94-1.32)	0.1974	0.2632
SIRT2	1.157	1.00 (0.85-1.18)	0.9941	0.9941
SLAMF1	0.633	1.07 (0.91-1.26)	0.4175	0.4771
ST1A1	1.304	1.09 (0.92-1.29)	0.3037	0.3836
STAMBP	0.833	1.04 (0.88-1.22)	0.6686	0.7079
TNFB	0.592	1.05 (0.89-1.24)	0.5723	0.6243
TNFSF14	1.064	1.18 (1.00-1.39)	0.0546	0.0864
TRANCE	0.753	1.19 (1.00-1.40)	**0.0455**	0.0799
4E-BP1	1.300	1.00 (0.85-1.19)	0.9593	0.9769

Abbreviations: SD, standard deviation; CI, confidence interval; FDR, false discovery rate; For biomarker abbreviations, see Supplemental Table 5.

[a] Multivariate logistic regression model adjusted for age (continuously), sex, education, physical activity, BMI (categorical), CVD, diabetes, depression, *APOE* genotype. Study participants with other (e.g. AD) or unknown dementia forms were excluded.

[b] P-values corrected for multiple testing by the Benjamini and Hochberg method.

Supplemental Table 24. Olink Inflammation Panel Biomarkers in the CX3CL1 cluster

No.	Biomarker	Spearman's r with CX3CL1[a]
1	IL-10RB[b,c]	0.672
2	LIF-R[b,c,d]	0.661
3	OPG[b,d]	0.621
4	CCL23[b,c,d]	0.603
5	PD-L1[b,c,d]	0.602
6	TNFRSF9[b,d]	0.600
7	CSF-1[b]	0.589
8	IL-15RA[b]	0.568
9	TWEAK[b,c,d]	0.561
10	CCL25[b]	0.555
11	TRAIL[b,c,d]	0.550
12	Flt3L[b]	0.546
13	DNER[b,c,d]	0.537
14	uPA[b,c,d]	0.523
15	CD244[b,c,d]	0.522
16	CD5[b,d]	0.522
17	SCF[b]	0.508
18	IL-12B	0.506

[a]All biomarkers with r > 0.5 with CX3CL1 were selected for the cluster.

[b]Significantly associated with all-cause dementia.

[c]Significantly associated with Alzheimer's disease.

[d]Significantly associated with vascular dementia.

Note: For biomarker abbreviations, see Supplemental Table 5.

Supplemental Table 25. Olink Inflammation Panel Biomarkers in the EN-RAGE cluster

No.	Biomarker	Spearman's r with EN-RAGE[a]
1	TNFSF14[b]	0.817
2	OSM	0.799
3	CASP-8[b,c]	0.784
4	CD40[b]	0.753
5	STAMBP[b,c]	0.750
6	HGF[b,c,d]	0.735
7	SIRT2[b]	0.731
8	TGF-alpha[b,c,d]	0.715
9	IL-8	0.705
10	AXIN1[b]	0.675
11	CCL3[b]	0.664
12	uPA[b,c,d]	0.643
13	ADA[b]	0.632
14	CCL4[b]	0.620
15	CD244[b,c,d]	0.599

No.	Biomarker	Spearman's r with EN-RAGE[a]
1	TNFSF14[b]	0.817
16	VEGF-A[b,c,d]	0.593
17	MCP-3	0.591
18	CSF-1[b]	0.574
19	ST1A1[b,c]	0.561
20	CXCL11[b]	0.559
21	MCP-1	0.552
22	IL-6	0.542
23	CD6[b,c]	0.536
24	CD5[b,d]	0.535
25	IL-18[b,d]	0.517
26	CXCL1[b,d]	0.511

[a]All biomarkers with r > 0.5 with EN-RAGE were selected for the cluster.

[b]Significantly associated with all-cause dementia.

[c]Significantly associated with Alzheimer's disease.

[d]Significantly associated with vascular dementia.

Note: For biomarker abbreviations, see Supplemental Table 5.

Supplemental Table 26. Olink Inflammation Panel Biomarkers in the LAP TGF-beta-1 cluster

No.	Biomarker	Spearman's r with LAP TGF-beta-1[a]
1	HGF[b,c,d]	0.601
2	CD244[b,c,d]	0.577
3	TWEAK[b,c,d]	0.560
4	uPA[b,c,d]	0.557
5	OPG[b,d]	0.543
6	LIF-R[b,c,d]	0.540
7	CSF-1	0.539
8	IL-10RB[b,c]	0.539
9	PD-L1[b,c,d]	0.518
10	VEGF-A[b,d]	0.516
11	DNER[b,c,d]	0.513
12	CD5[b,d]	0.510
13	CCL11[b,d]	0.509
14	MCP-4[b,d]	0.504
15	CXCL6[b,c,d]	0.501
16	CD40[b]	0.500

[a]All biomarkers with r > 0.5 with LAP TGF-beta-1 were selected for the cluster.

[b]Significantly associated with all-cause dementia.

[c]Significantly associated with Alzheimer's disease.

[d]Significantly associated with vascular dementia.

Note: For biomarker abbreviations, see Supplemental Table 5.

Supplemental Table 27. Olink Inflammation Panel Biomarkers in the VEGF-A cluster

No.	Biomarker	Spearman's r with VEGF-A[a]
1	HGF[b,c,d]	0.680
2	CD40[b]	0.646
3	CSF-1	0.620
4	uPA[b,c,d]	0.608
5	EN-RAGE[b,c,d]	0.593
6	CD244[b,c,d]	0.584
7	MCP-1	0.563
8	OSM	0.561
9	CXCL1[b,d]	0.558
10	CCL3[b]	0.556
11	TGF-alpha[b,c,d]	0.554
12	TNFSF14[b]	0.551
13	IL-6	0.538
14	CCL4[b]	0.534
15	STAMBP[b,c]	0.531
16	PD-L1[b,c,d]	0.529
17	IL-10RB[b,c]	0.526
18	CD5[b,d]	0.522
19	TNFRSF9[b,d]	0.520
20	CXCL11[b]	0.519
21	IL-10[b,d]	0.518
22	OPG[b,d]	0.518
23	SIRT2[b]	0.518
24	LAP TGF-beta-1[b,c,d]	0.516
25	ADA[b]	0.514
26	TWEAK[b,c,d]	0.504
27	IL-18R1[b,d]	0.504
28	CCL11[b,d]	0.502

[a]All biomarkers with r > 0.5 with VEGF-A were selected for the cluster.

[b]Significantly associated with all-cause dementia.

[c]Significantly associated with Alzheimer's disease.

[d]Significantly associated with vascular dementia.

For biomarker abbreviations, see Supplemental Table 5.

Supplemental Table 28. Exploratory subgroup and sensitivity analyses for the association of CX3CL1 and all-cause dementia

Group	n_{total}	n_{cases}	OR (95% CI)[a]	p-value interaction
Total cohort	1782	504	1.41 (1.24-1.60)	
Stratified by age				0.2767
<68 years	1258	254	1.51 (1.29-1.78)	
≥68 years	524	250	1.49 (1.22-1.83)	
Stratified by sex				0.6705
Women	965	262	1.36 (1.14-1.61)	
Men	817	242	1.48 (1.23-1.78)	
Stratified by obesity				0.9649
BMI < 30 kg/m²	479	144	1.46 (1.14-1.87)	
BMI ≥ 30 kg/m²	1303	360	1.40 (1.21-1.62)	
Stratified by diabetes				0.0804
No	1489	394	1.47 (1.28-1.69)	
Yes	293	110	1.19 (0.87-1.64)	
Stratified by CVD				0.8759
No	1373	350	1.40 (1.21-1.63)	
Yes	409	154	1.45 (1.13-1.86)	
Stratified by *APOE* ε4				0.5879
Negative	1276	313	1.42 (1.22-1.66)	
Positive	506	193	1.34 (1.07-1.69)	
Stratified by time of diagnosis				NA
In first 10 years of FUP	1498	220	1.65 (1.36-2.00)	
In year 11-19 of FUP	1562	284	1.28 (1.10-1.48)	
Excluding subjects free of dementia who died prior to 80th birthday				NA
No	1782	504	1.41 (1.24-1.60)	
Yes	1601	504	1.47 (1.29-1.67)	
Excluding subjects with sign of acute infection (CRP level >20mg/L)				NA
No	1782	504	1.41 (1.24-1.60)	
Yes	1745	497	1.41 (1.24-1.60)	

Abbreviations: CX3CL1, Fractalkine; OR, odds ratio; CI, confidence interval; CVD, cardiovascular disease; *APOE*, apolipoprotein E; CRP, C-reactive protein;

NOTE: Numbers printed in bold are statistically significant ($p < 0.05$).

[a]Results of multivariate logistic regression model adjusted for age (continuously), sex, education, physical activity, BMI (categorical), CVD, diabetes, depression, *APOE* genotype.

Supplemental Table 29. Exploratory subgroup and sensitivity analyses for the association of EN-RAGE and all-cause dementia

Group	n_{total}	n_{cases}	OR (95% CI)[a]	p-value interaction
Total cohort	1782	504	1.41 (1.25-1.60)	
Stratified by age				0.3201
<68 years	1258	254	1.41 (1.21-1.64)	
≥68 years	524	250	1.34 (1.11-1.61)	
Stratified by sex				0.2769
Women	965	262	1.55 (1.30-1.85)	
Men	817	242	1.32 (1.11-1.56)	
Stratified by obesity				0.2911
BMI < 30 kg/m²	479	144	1.31 (1.04-1.66)	
BMI ≥ 30 kg/m²	1303	360	1.48 (1.28-1.71)	
Stratified by diabetes				0.3445
No	1489	394	1.45 (1.27-1.66)	
Yes	293	110	1.31 (0.99-1.74)	
Stratified by CVD				0.9128
No	1373	350	1.43 (1.24-1.66)	
Yes	409	154	1.37 (1.09-1.73)	
Stratified by *APOE* ε4				**0.0241**
Negative	1276	313	1.56 (1.34-1.81)	
Positive	506	193	1.16 (0.94-1.42)	
Stratified by time of diagnosis				NA
In first 10 years of FUP	1498	220	1.46 (1.22-1.73)	
In year 11-19 of FUP	1562	284	1.36 (1.18-1.57)	
Excluding subjects free of dementia who died prior to 80th birthday				NA
No	1782	504	1.41 (1.25-1.60)	
Yes	1601	504	1.49 (1.31-1.68)	
Excluding subjects with sign of acute infection (CRP level >20mg/L)				NA
No	1782	504	1.41 (1.25-1.60)	
Yes	1745	497	1.43 (1.27-1.62)	

Abbreviations: EN-RAGE, Protein S100-A12; OR, odds ratio; CI, confidence interval; CVD, cardiovascular disease; *APOE*, apolipoprotein E; CRP, C-reactive protein;

NOTE: Numbers printed in bold are statistically significant ($p < 0.05$).

[a] Results of multivariate logistic regression model adjusted for age (continuously), sex, education, physical activity, BMI (categorical), CVD, diabetes, depression, *APOE* genotype.

Supplemental Table 30. Exploratory subgroup and sensitivity analyses for the association of EN-RAGE and Alzheimer's disease

Group	n_{total}	n_{cases}	OR (95% CI)[a]	p-value interaction
Total cohort	1782	163	1.51 (1.25-1.83)	
Stratified by age				0.7589
<68 years	1258	88	1.43 (1.12-1.81)	
≥68 years	524	75	1.54 (1.12-2.11)	
Stratified by sex				0.6325
Women	965	95	1.46 (1.14-1.88)	
Men	817	68	1.58 (1.16-2.15)	
Stratified by obesity				0.9321
BMI < 30 kg/m²	479	53	1.53 (1.06-2.22)	
BMI ≥ 30 kg/m²	1303	110	1.50 (1.19-1.89)	
Stratified by diabetes				0.2142
No	1489	131	1.40 (1.14-1.72)	
Yes	293	32	2.01 (1.22-3.30)[b]	
Stratified by CVD				0.4966
No	1373	124	1.56 (1.25-1.95)	
Yes	409	39	1.31 (0.90-1.91)[b]	
Stratified by *APOE* ε4				0.0798
Negative	1276	87	1.78 (1.35-2.33)	
Positive	506	76	1.22 (0.93-1.61)	
Stratified by time of diagnosis				NA
In first 10 years of FUP	1699	80	1.79 (1.34-2.36)	
In year 11-19 of FUP	1702	83	1.31 (1.02-1.67)	
Excluding subjects free of dementia who died prior to 80th birthday				NA
No	1782	163	1.51 (1.25-1.83)	
Yes	1601	163	1.58 (1.30-1.92)	
Excluding subjects with sign of acute infection (CRP level >20mg/L)				NA
No	1782	163	1.51 (1.25-1.83)	
Yes	1745	160	1.51 (1.24-1.83)	

Abbreviations: EN-RAGE, Protein S100-A12; OR, odds ratio; CI, confidence interval; CVD, cardiovascular disease; *APOE*, apolipoprotein E; CRP, C-reactive protein;

NOTE: Numbers printed in bold are statistically significant ($p < 0.05$).

[a]Results of multivariate logistic regression model adjusted for age (continuously), sex, education, physical activity, BMI (categorical), CVD, diabetes, depression, *APOE* genotype. Study participants with other (e.g. VD) or unknown dementia forms were excluded.

[b]Results of multivariate logistic regression model adjusted for age (continuously), sex, education, physical activity, BMI (categorical), CVD, diabetes. Study participants with other (e.g. VD) or unknown dementia forms were excluded.

Supplemental Table 31. Exploratory subgroup and sensitivity analyses for the association of LAP TGF-beta-1 and Alzheimer's disease

Group	n_{total}	n_{cases}	OR (95% CI)[a]	p-value interaction
Total cohort	1782	163	1.46 (1.21-1.76)	
Stratified by age				0.6941
<68 years	1258	88	1.41 (1.12-1.78)	
≥68 years	524	75	1.49 (1.09-2.03)	
Stratified by sex				0.9685
Women	965	95	1.51 (1.17-1.94)	
Men	817	68	1.47 (1.09-1.97)	
Stratified by obesity				0.3398
BMI < 30 kg/m²	479	53	1.30 (0.88-1.93)	
BMI ≥ 30 kg/m²	1303	110	1.53 (1.23-1.91)	
Stratified by diabetes				0.7049
No	1489	131	1.48 (1.20-1.84)	
Yes	293	32	1.64 (1.05-2.54)[b]	
Stratified by CVD				0.3792
No	1373	124	1.55 (1.25-1.93)	
Yes	409	39	1.28 (0.88-1.85)[b]	
Stratified by *APOE* ε4				0.3987
Negative	1276	87	1.59 (1.21-2.07)	
Positive	506	76	1.33 (1.02-1.74)	
Stratified by time of diagnosis				NA
In first 10 years of FUP	1699	80	1.47 (1.13-1.91)	
In year 11-19 of FUP	1702	83	1.46 (1.15-1.85)	
Excluding subjects free of dementia who died prior to 80th birthday				NA
No	1782	163	1.46 (1.21-1.76)	
Yes	1601	163	1.56 (1.28-1.90)	
Excluding subjects with sign of acute infection (CRP level >20mg/L)				NA
No	1782	163	1.46 (1.21-1.76)	
Yes	1745	160	1.46 (1.21-1.77)	

Abbreviations: LAP TGF-beta-1, Latency-associated peptide transforming growth factor beta-1; OR, odds ratio; CI, confidence interval; CVD, cardiovascular disease; *APOE*, apolipoprotein E; CRP, C-reactive protein;

NOTE: Numbers printed in bold are statistically significant ($p < 0.05$).

[a]Results of multivariate logistic regression model adjusted for age (continuously), sex, education, physical activity, BMI (categorical), CVD, diabetes, depression, *APOE* genotype. Study participants with other (e.g. VD) or unknown dementia forms were excluded.

[b]Results of multivariate logistic regression model adjusted for age (continuously), sex, education, physical activity, BMI (categorical), CVD, diabetes. Study participants with other (e.g. VD) or unknown dementia forms were excluded.

Supplemental Table 32. Exploratory subgroup and sensitivity analyses for the association of VEGF-A and vascular dementia

Group	n_{total}	n_{cases}	OR (95% CI)[a]	p-value interaction
Total cohort	1782	195	1.43 (1.20-1.70)	
Stratified by age				0.6534
<68 years	524	98	1.24 (1.00-1.54)	
≥68 years	1258	97	1.75 (1.31-2.34)	
Stratified by sex				0.7408
Women	965	100	1.41 (1.10-1.79)	
Men	817	95	1.45 (1.13-1.86)	
Stratified by obesity				0.5126
BMI < 30 kg/m²	479	49	1.34 (0.95-1.90)	
BMI ≥ 30 kg/m²	1303	146	1.48 (1.21-1.81)	
Stratified by diabetes				0.7305
No	1489	147	1.44 (1.19-1.74)	
Yes	293	48	1.40 (0.91-2.16)	
Stratified by CVD				0.1381
No	1373	124	1.34 (1.08-1.65)	
Yes	409	71	1.74 (1.24-2.44)	
Stratified by *APOE* ε4				0.5134
Negative	1276	127	1.47 (1.19-1.82)	
Positive	506	68	1.32 (0.97-1.81)	
Stratified by time of diagnosis				NA
In first 10 years of FUP	1666	79	1.75 (1.33-2.32)	
In year 11-19 of FUP	1703	116	1.29 (1.04-1.59)	
Excluding subjects free of dementia who died prior to 80th birthday				NA
No	1782	195	1.43 (1.20-1.70)	
Yes	1601	195	1.52 (1.27-1.82)	
Excluding subjects with sign of acute infection (CRP level >20mg/L)				NA
No	1782	195	1.43 (1.20-1.70)	
Yes	1745	192	1.46 (1.22-1.74)	

Abbreviations: VEGF-A, Vascular endothelial growth factor-A; OR, odds ratio; CI, confidence interval; CVD, cardiovascular disease; *APOE*, apolipoprotein E; CRP, C-reactive protein;

NOTE: Numbers printed in bold are statistically significant ($p < 0.05$).

[a]Results of multivariate logistic regression model adjusted for age (continuously), sex, education, physical activity, BMI (categorical), CVD, diabetes, depression, *APOE* genotype. Study participants with other (e.g. AD) or unknown dementia forms were excluded.

Supplemental Table 33. Associations of CAIDE model variables with <u>all-cause dementia</u>.

CAIDE model variables	CAIDE model 1[a]		CAIDE model 2[b]	
	Multivariate Odds Ratio (95%CI)[a]	χ^2 test p-value	Multivariate Odds Ratio (95%CI)[b]	χ^2 test p-value
Age (years), per 1 year	**1.15 (1.12-1.17)**	**<0.0001**	**1.15 (1.13-1.18)**	**<0.0001**
Education (years)				
< 9	1.00 Ref.		1.00 Ref.	
≥ 9	0.92 (0.68-1.24)	0.5870	0.91 (0.67-1.23)	0.5255
Sex				
Female	1.00 Ref.		1.00 Ref.	
Male	1.24 (0.97-1.59)	0.0842	1.28 (1.00-1.64)	0.0516
Systolic blood pressure (mm Hg), per 1 mmHg	1.00 (0.996-1.01)	0.5364	1.00 (0.995-1.01)	0.5666
Body-mass index (kg/m²), per 1 kg/m²	0.97 (0.95-1.00)	0.0768	0.98 (0.95-1.01)	0.1190
Total cholesterol (mmol/L), per 1 mmol/L	**0.90 (0.82-0.99)**	**0.0308**	**0.88 (0.80-0.97)**	**0.0118**
Physical activity[c]				
Inactive	1.00 Ref.		1.00 Ref.	
Active	**0.64 (0.48-0.85)**	**0.0022**	**0.66 (0.49-0.88)**	**0.0044**
***APOE* genotypes**				
ε4 non-carrier	-	-	1.00 Ref.	
ε4 carrier	-	-	**2.45 (1.90-3.16)**	**<0.0001**

Note: Numbers printed in bold are statistically significant.

Abbreviations: CI, Confidence Interval; *APOE*, apolipoprotein E.

[a]The CAIDE model 1 includes age, education, sex, systolic blood pressure, body-mass index, total cholesterol and physical activity.

[b]The CAIDE model 2 includes the variables of CAIDE model 1 and *APOE* ε4 status.

[c]"Inactive" was defined by <1 hour of vigorous or <1 hour light physical activity per week. All other amounts of physical activity were grouped into the category "Active."

Supplemental Table 34. Associations of CAIDE model variables with Alzheimer's disease.

CAIDE model variables	CAIDE model 1[a]		CAIDE model 2[b]	
	Multivariate Odds Ratio (95%CI)[a]	χ^2 test p-value	Multivariate Odds Ratio (95%CI)[b]	χ^2 test p-value
Age (years), per 1 year	**1.14 (1.10-1.17)**	**<0.0001**	**1.14 (1.10-1.18)**	**<0.0001**
Education (years)				
≤ 9	1.00 Ref.		1.00 Ref.	
> 9	0.85 (0.53-1.37)	0.4975	0.85 (0.52-1.39)	0.5167
Sex				
Female	1.00 Ref.		1.00 Ref.	
Male	1.01 (0.69-1.47)	0.9575	1.08 (0.74-1.59)	0.6921
SBP (mmHg), per 1 mmHg	1.00 (0.99-1.01)	0.5551	1.00 (0.99-1.01)	0.5633
BMI (kg/m^2), per 1 kg/m²	**0.95 (0.91-0.99)**	**0.0216**	**0.95 (0.91-0.99)**	**0.0341**
Total cholesterol (mmol/L), per 1 mmol/L	0.91 (0.79-1.06)	0.2176	0.90 (0.77-1.05)	0.1692
Physical activity[c]				
Inactive	1.00 Ref.		1.00 Ref.	
Active	**0.55 (0.37-0.83)**	**0.0040**	**0.56 (0.37-0.85)**	**0.0070**
***APOE* genotypes**				
ε4 non-carrier	-	-	1.00 Ref.	
ε4 carrier	-	-	**3.48 (2.40-5.04)**	**<0.0001**

Note: Numbers printed in bold are statistically significant.

Abbreviations: CI, Confidence Interval; APOE, apolipoprotein E; SBP, systolic blood pressure; BMI, body mass index.

[a]The CAIDE model 1 includes age, education, sex, systolic blood pressure, body-mass index, total cholesterol and physical activity.

[b]The CAIDE model 2 includes the variables of CAIDE model 1 and *APOE* ε4 status.

[c]"Inactive" was defined by <1 hour of vigorous or <1 hour light physical activity per week. All other amounts of physical activity were grouped into the category "Active."

Supplemental Table 35. Associations of CAIDE model variables with vascular dementia.

CAIDE model variables	CAIDE model 1[a]		CAIDE model 2[b]	
	Multivariate Odds Ratio (95%CI)[a]	χ^2 test p-value	Multivariate Odds Ratio (95%CI)[b]	χ^2 test p-value
Age (years), per 1 year	**1.15 (1.12-1.19)**	**<0.0001**	**1.15 (1.11-1.19)**	**<0.0001**
Education (years)				
≤ 9	1.00 Ref.		1.00 Ref.	
> 9	0.96 (0.62-1.49)	0.8592	0.95 (0.61-1.48)	0.8346
Sex				
Female	1.00 Ref.		1.00 Ref.	
Male	1.17 (0.82-1.66)	0.3915	1.20 (0.84-1.71)	0.3158
SBP (mmHg), per 1 mmHg	1.00 (0.99-1.01)	0.5886	1.00 (0.99-1.01)	0.6024
BMI (kg/m^2), per 1 kg/m^2	0.98 (0.94-1.02)	0.3241	0.98 (0.94-1.02)	0.3741
Total cholesterol (mmol/L), per 1 mmol/L	0.92 (0.80-1.06)	0.2402	0.91 (0.80-1.05)	0.2081
Physical activity[c]				
Inactive	1.00 Ref.		1.00 Ref.	
Active	0.78 (0.52-1.17)	0.2290	0.80 (0.53-1.21)	0.2887
***APOE* genotypes**				
ε4 non-carrier	-	-	1.00 Ref.	
ε4 carrier	-	-	**1.83 (1.28-2.63)**	**0.0010**

Note: Numbers printed in bold are statistically significant.

Abbreviations: CI, Confidence Interval; APOE, apolipoprotein E; SBP, systolic blood pressure; BMI, body mass index.

[a]The CAIDE model 1 includes age, education, sex, systolic blood pressure, body-mass index, total cholesterol and physical activity.

[b]The CAIDE model 2 includes the variables of CAIDE model 1 and *APOE* ε4 status.

[c]"Inactive" was defined by <1 hour of vigorous or <1 hour light physical activity per week. All other amounts of physical activity were grouped into the category "Active."

Supplemental Table 36. β-coefficients of variables included in prediction models for <u>all-cause dementia</u> in the <u>total cohort</u>

CAIDE model 1[a]		CAIDE model 2[b]	
Variables	**β-coefficient[c]**	**Variables**	**β-coefficient[c]**
(Intercept)	-13.99669	(Intercept)	-14.24841
Age (per 1 year)	0.13138	Age (per 1 year)	0.13716
Education (high)	-0.02589	Education (high)	-0.04391
Sex (male)	0.26020	Sex (male)	0.27305
SBP (per 1 mmHg)	0.00197	SBP (per 1 mmHg)	0.00164
BMI (per 1 kg/m²)	-0.02642	BMI (per 1 kg/m²)	-0.02412
Total cholesterol (per 1 mmol/L)	-0.00229	Total cholesterol (per 1mmol/L)	-0.00279
Physical activity (active)	-0.46176	Physical activity (active)	-0.42463
APOE genotypes(ε4 carrier)	-	*APOE* genotypes(ε4 carrier)	0.88692
Beta-NGF	-0.29713	Beta-NGF	-0.33123
CCL20	0.00430	-	-
CD244	0.16104	CD244	0.08373
CX3CL1	0.15650	CX3CL1	0.15356
CXCL5	0.06214	CXCL5	0.05145
EN-RAGE	0.24358	EN-RAGE	0.24156
FGF-23	-0.18144	FGF-23	-0.1941
-	-	IL-12B	-0.04106
-	-	IL-18	0.00793
LAP TGF-beta-1	0.21536	LAP TGF-beta-1	0.21721
LIFR	0.08872	LIFR	0.14818
OPG	0.03190	-	-
OSM	-0.18734	OSM	-0.16982
SCF	-0.02694	SCF	-0.0219
SLAMF1	-0.09525	SLAMF1	-0.07977
TGF-alpha	-0.01119	-	-
TNFB	-0.17436	TNFB	-0.09308
VEGF-A	0.18693	VEGF-A	0.17965

Abbreviations: *APOE*, apolipoprotein E; SBP, systolic blood pressure; BMI, body mass index. For biomarker abbreviations, see Supplemental Table 5.

[a]The CAIDE model 1 includes age, education, sex, systolic blood pressure, body-mass index, total cholesterol and physical activity.

[b]The CAIDE model 2 includes the variables of CAIDE model 1 and *APOE* ε4 status.

[c]ß-coefficients shown for continuous variables are expressed per 1 unit. All variables except education, sex, physical activity and APOE genotype were modelled continuously. The categorical variables were dichotomized as shown in Supplemental Table 33.

Supplemental Table 37. β-coefficients of variables included in prediction models for Alzheimer's disease in the total cohort

CAIDE model 1[a]		CAIDE model 2[b]	
Variables	**β-coefficient[c]**	**Variables**	**β-coefficient[c]**
(Intercept)	-13.89488	(Intercept)	-13.98410
Age (per 1 year)	0.12625	Age (per 1 year)	0.13242
Education (high)	-0.12614	Education (high)	-0.13515
Sex (male)	0.02286	Sex (male)	0.06503
SBP (per 1 mmHg)	0.00291	SBP (per 1 mmHg)	0.00277
BMI (per 1 kg/m²)	-0.05057	BMI (per 1 kg/m²)	-0.05078
Total cholesterol (per 1 mmol/L)	-0.00243	Total cholesterol (per 1 mmol/L)	-0.00268
Physical activity (active)	-0.59140	Physical activity (active)	-0.55151
APOE genotypes (ε4 carrier)	-	*APOE* genotypes (ε4 carrier)	1.22666
Beta-NGF	-0.10860	Beta-NGF	-0.13213
CCL19	0.05023	CCL19	0.02963
CCL28	0.18491	CCL28	0.10267
CXCL5	0.03057	-	-
EN-RAGE	0.18131	EN-RAGE	0.13902
FGF-19	0.01183	-	-
FGF-23	-0.27873	FGF-23	-0.26129
IL-7	-0.02734	-	-
LAP TGF-beta-1	0.46103	LAP TGF-beta-1	0.47298
MCP-3	-0.04798	MCP-3	-0.02825
SLAMF1	-0.06575	SLAMF1	-0.00448
-	-	ST1A1	0.00669

Abbreviations: *APOE*, apolipoprotein E; SBP, systolic blood pressure; BMI, body mass index. For biomarker abbreviations, see Supplemental Table 5.

[a]The CAIDE model 1 includes age, education, sex, systolic blood pressure, body-mass index, total cholesterol and physical activity.

[b]The CAIDE model 2 includes the variables of CAIDE model 1 and *APOE* ε4 status.

[c]ß-coefficients shown for continuous variables are expressed per 1 unit. All variables except education, sex, physical activity and APOE genotype were modelled continuously. The categorical variables were dichotomized as shown in Supplemental Table 33.

Supplemental Table 38. β-coefficients of variables included in prediction models for vascular dementia in the total cohort

CAIDE model 1[a]		CAIDE model 2[b]	
Variables	**β-coefficient[c]**	**Variables**	**β-coefficient[c]**
(Intercept)	-15.09097	(Intercept)	-15.20000
Age (per 1 year)	0.13329	Age (per 1 year)	0.13400
Education (high)	0.01567	Education (high)	0.00095
Sex (male)	0.17871	Sex (male)	0.19200
SBP (per 1 mmHg)	0.00306	SBP (per 1 mmHg)	0.00324
BMI (per 1 kg/m²)	-0.01988	BMI (per 1 kg/m²)	-0.01930
Total cholesterol (per 1 mmol/L)	-0.00106	Total cholesterol (per 1 mmol/L)	-0.00141
Physical activity (active)	-0.23865	Physical activity (active)	-0.20400
APOE genotypes(ε4 carrier)	-	*APOE* genotypes(ε4 carrier)	0.60200
Beta-NGF	-0.09165	Beta-NGF	-0.08250
CASP-8	-0.03019	CASP-8	-0.02410
CCL19	-0.06665	CCL19	-0.02990
CD244	0.15920	CD244	0.05120
CD5	0.07209	CD5	0.07270
CDCP1	0.06014	CDCP1	0.05550
CST5	-0.12628	CST5	-0.08700
CXCL5	0.08446	CXCL5	0.07050
CXCL6	0.04192	CXCL6	0.02390
CXCL9	0.05735	CXCL9	0.02360
EN-RAGE	0.28622	EN-RAGE	0.23000
FGF-23	-0.21114	FGF-23	-0.22800
IL-10	0.11384	IL-10	0.11100
IL-18	0.20724	IL-18	0.20300
LAP TGF-beta-1	0.01349	LAP TGF-beta-1	0.03040
MMP-1	-0.00406	-	-
MMP-10	0.01176	MMP-10	0.00263
NT3	0.16764	NT3	0.16800
OPG	0.11262	OPG	0.01870
OSM	-0.13282	OSM	-0.07970
SCF	-0.08219	SCF	-0.02440
SIRT2	-0.08191	SIRT2	-0.14200
SLAMF1	-0.15773	SLAMF1	-0.12400
STAMBP	-0.21429	STAMBP	-0.10100
TNFB	-0.20264	TNFB	-0.12700
VEGF-A	0.20117	VEGF-A	0.20000

Abbreviations: *APOE*, apolipoprotein E; SBP, systolic blood pressure; BMI, body mass index. For biomarker abbreviations, see Supplemental Table 5.

[a]The CAIDE model 1 includes age, education, sex, systolic blood pressure, body-mass index, total cholesterol and physical activity.

[b]The CAIDE model 2 includes the variables of CAIDE model 1 and *APOE* ε4 status.

[c]ß-coefficients shown for continuous variables are expressed per 1 unit. All variables except education, sex, physical activity and APOE genotype were modelled continuously. The categorical variables were dichotomized as shown in Supplemental Table 33.

Supplemental Table 39. β-coefficients of variables included in prediction models for <u>all-cause dementia</u> in the <u>mid-life cohort</u>

CAIDE model 1[a]		CAIDE model 2[b]	
Variables	**β-coefficient[c]**	**Variables**	**β-coefficient[c]**
(Intercept)	-25.10000	(Intercept)	-25.30000
Age (per 1 year)	0.19200	Age (per 1 year)	0.19000
Education (high)	-0.10800	Education (high)	-0.15400
Sex (male)	0.74800	Sex (male)	0.80500
SBP (per 1 mmHg)	-0.00310	SBP (per 1 mmHg)	-0.00431
BMI (per 1 kg/m²)	0.00034	BMI (per 1 kg/m²)	0.00072
Total cholesterol (per 1 mmol/L)	-0.00136	Total cholesterol (per 1 mmol/L)	-0.00230
Physical activity (active)	-0.43300	Physical activity (active)	-0.32900
APOE genotypes(ε4 carrier)	-	*APOE* genotypes(ε4 carrier)	1.01000
4E-BP1	0.00872	4E-BP1	0.00353
Beta-NGF	-0.58600	Beta-NGF	-0.63200
CCL11	0.05200	CCL11	0.03450
CCL20	-0.00002	CCL20	-0.00397
CCL3	-0.07000	CCL3	-0.07480
CD244	0.08280	-	-
CX3CL1	0.12700	CX3CL1	0.12300
CXCL1	-0.19000	CXCL1	-0.19100
CXCL10	-0.12300	CXCL10	-0.09960
CXCL11	-0.01530	CXCL11	-0.03900
CXCL5	0.39200	CXCL5	0.39100
CXCL9	0.24600	CXCL9	0.23000
EN-RAGE	0.13200	EN-RAGE	0.18200
FGF-19	0.04440	FGF-19	0.04420
FGF-21	0.08060	FGF-21	0.07220
FGF-23	-0.16800	FGF-23	-0.22900
IL-10	-0.09570	IL-10	-0.05930
IL-12B	-0.07140	IL-12B	-0.13900
IL-18R1	0.29000	IL-18R1	0.31400
IL-7	-0.12800	IL-7	-0.18400
LAP TGF-beta-1	0.05120	LAP TGF-beta-1	0.07180
LIFR	0.49600	LIFR	0.48300
MCP-1	-0.05420	MCP-1	-0.04250
-	-	MMP-1	0.00004
MMP-10	0.08380	MMP-10	0.07990
SIRT2	-0.07570	SIRT2	-0.07470
SLAMF1	-0.28300	SLAMF1	-0.24600
ST1A1	-0.07070	ST1A1	-0.09610
TNFB	-0.22800	TNFB	-0.13000
TRAIL	0.00204	TRAIL	0.05600
-	-	TRANCE	-0.01030
-	-	uPA	0.07730
VEGF-A	0.43200	VEGF-A	0.37700

Abbreviations: *APOE*, apolipoprotein E; SBP, systolic blood pressure; BMI, body mass index. For biomarker abbreviations, see Supplemental Table 5.

[a]The CAIDE model 1 includes age, education, sex, systolic blood pressure, body-mass index, total cholesterol and physical activity.

[b]The CAIDE model 2 includes the variables of CAIDE model 1 and *APOE* ε4 status. [c]ß-coefficients shown for continuous variables are expressed per 1 unit. All variables except education, sex, physical activity and APOE genotype were modelled continuously. The categorical variables were dichotomized as shown in Supplemental Table 33.

Supplemental Table 40. β-coefficients of variables included in prediction models for Alzheimer's disease in the mid-life cohort

CAIDE model 1[a]		CAIDE model 2[b]	
Variables	**β-coefficient[c]**	**Variables**	**β-coefficient[c]**
(Intercept)	-19.70000	(Intercept)	-18.23707
Age (per 1 year)	0.20100	Age (per 1 year)	0.19737
Education (high)	0.15200	Education (high)	0.17226
Sex (male)	0.41000	Sex (male)	0.54320
SBP (per 1 mmHg)	0.00635	SBP (per 1 mmHg)	0.00514
BMI (per 1 kg/m²)	-0.02470	BMI (per 1 kg/m²)	-0.02830
Total cholesterol (per 1 mmol/L)	0.00069	Total cholesterol (per 1 mmol/L)	-0.00138
Physical activity (active)	-0.95300	Physical activity (active)	-0.83288
APOE genotypes(ε4 carrier)	-	*APOE* genotypes(ε4 carrier)	1.49428
CCL23	0.10800	-	-
CCL28	0.33300	CCL28	0.27706
LAP TGF-beta-1	0.34600	LAP TGF-beta-1	0.25338
TRAIL	0.04200	TRAIL	0.12571

Abbreviations: *APOE*, apolipoprotein E; SBP, systolic blood pressure; BMI, body mass index. For biomarker abbreviations, see Supplemental Table 5.

[a]The CAIDE model 1 includes age, education, sex, systolic blood pressure, body-mass index, total cholesterol and physical activity.

[b]The CAIDE model 2 includes the variables of CAIDE model 1 and *APOE* ε4 status.

[c]ß-coefficients shown for continuous variables are expressed per 1 unit. All variables except education, sex, physical activity and APOE genotype were modelled continuously. The categorical variables were dichotomized as shown in Supplemental Table 33.

Supplemental Table 41. β-coefficients of variables included in prediction models for vascular dementia in the mid-life cohort

CAIDE model 1[a]		CAIDE model 2[b]	
Variables	**β-coefficient[c]**	**Variables**	**β-coefficient[c]**
(Intercept)	-26.21456	(Intercept)	-26.73380
Age (per 1 year)	0.22219	Age (per 1 year)	0.22072
Education (high)	0.00208	Education (high)	-0.00929
Sex (male)	0.62175	Sex (male)	0.68819
SBP (per 1 mmHg)	-0.00517	SBP (per 1 mmHg)	-0.00584
BMI (per 1 kg/m²)	0.03583	BMI (per 1 kg/m²)	0.03762
Total cholesterol (per 1 mmol/L)	0.00160	Total cholesterol (per 1 mmol/L)	0.00143
Physical activity (active)	0.02998	Physical activity (active)	0.05462
APOE genotypes(ε4 carrier)	-	*APOE* genotypes(ε4 carrier)	0.45865
Beta-NGF	-0.87501	Beta-NGF	-0.95800
CCL19	-0.01053	CCL19	-0.01109
CXCL5	0.31228	CXCL5	0.32092
CXCL9	0.15708	CXCL9	0.15326
EN-RAGE	0.12058	EN-RAGE	0.14020
FGF-19	0.05150	FGF-19	0.05875
FGF-21	0.10997	FGF-21	0.10171
FGF-23	-0.21879	FGF-23	-0.25444
IL-10RA	-0.05286	IL-10RA	-0.06032
IL-18R1	0.02391	IL-18R1	0.09612

CAIDE model 1[a]		CAIDE model 2[b]	
Variables	**β-coefficient[c]**	**Variables**	**β-coefficient[c]**
(Intercept)	-26.21456	(Intercept)	-26.73380
Age (per 1 year)	0.22219	Age (per 1 year)	0.22072
Education (high)	0.00208	Education (high)	-0.00929
Sex (male)	0.62175	Sex (male)	0.68819
SBP (per 1 mmHg)	-0.00517	SBP (per 1 mmHg)	-0.00584
BMI (per 1 kg/m²)	0.03583	BMI (per 1 kg/m²)	0.03762
Total cholesterol (per 1 mmol/L)	0.00160	Total cholesterol (per 1 mmol/L)	0.00143
Physical activity (active)	0.02998	Physical activity (active)	0.05462
APOE genotypes(ε4 carrier)	-	*APOE* genotypes(ε4 carrier)	0.45865
MMP-10	0.34340	MMP-10	0.32560
SIRT2	-0.08547	SIRT2	-0.11328

Abbreviations: *APOE*, apolipoprotein E; SBP, systolic blood pressure; BMI, body mass index. For biomarker abbreviations, see Supplemental Table 5.

[a]The CAIDE model 1 includes age, education, sex, systolic blood pressure, body-mass index, total cholesterol and physical activity.

[b]The CAIDE model 2 includes the variables of CAIDE model 1 and *APOE* ε4 status.

[c]ß-coefficients shown for continuous variables are expressed per 1 unit. All variables except education, sex, physical activity and APOE genotype were modelled continuously. The categorical variables were dichotomized as shown in Supplemental Table 33.

Supplemental Table 42. β-coefficients of variables included in prediction models for <u>all-cause dementia</u> in the <u>late-life cohort</u>

CAIDE model 1[a]		CAIDE model 2[b]	
Variables	**β-coefficient[c]**	**Variables**	**β-coefficient[c]**
(Intercept)	-9.38551	(Intercept)	-10.30000
Age (per 1 year)	0.12903	Age (per 1 year)	0.13400
Education (high)	0.07163	Education (high)	0.05720
Sex (male)	0.02614	Sex (male)	0.03350
SBP (per 1 mmHg)	0.00420	SBP (per 1 mmHg)	0.00441
BMI (per 1 kg/m²)	-0.04770	BMI (per 1 kg/m²)	-0.04340
Total cholesterol (per 1 mmol/L)	-0.00307	Total cholesterol (per 1 mmol/L)	-0.00342
Physical activity (active)	-0.45232	Physical activity (active)	-0.43400
APOE genotypes(ε4 carrier)	-	*APOE* genotypes(ε4 carrier)	0.79700
CX3CL1	0.00999	CX3CL1	0.00949
EN-RAGE	0.03885	EN-RAGE	0.04430
-	-	IL-18	0.00090
LAP TGF-beta-1	0.18015	LAP TGF-beta-1	0.20100

Abbreviations: *APOE*, apolipoprotein E; SBP, systolic blood pressure; BMI, body mass index. For biomarker abbreviations, see Supplemental Table 5.

[a]The CAIDE model 1 includes age, education, sex, systolic blood pressure, body-mass index, total cholesterol and physical activity.

[b]The CAIDE model 2 includes the variables of CAIDE model 1 and *APOE* ε4 status.

[c]ß-coefficients shown for continuous variables are expressed per 1 unit. All variables except education, sex, physical activity and APOE genotype were modelled continuously. The categorical variables were dichotomized as shown in Supplemental Table 33.

Supplemental Table 43. β-coefficients of variables included in prediction models for Alzheimer's disease in the late-life cohort

CAIDE model 1[a]		CAIDE model 2[b]	
Variables	**β-coefficient[c]**	**Variables**	**β-coefficient[c]**
(Intercept)	-9.70751	(Intercept)	-10.20694
Age (per 1 year)	0.10766	Age (per 1 year)	0.11519
Education (high)	-0.26357	Education (high)	-0.29841
Sex (male)	-0.21977	Sex (male)	-0.17284
SBP (per 1 mmHg)	0.00053	SBP (per 1 mmHg)	0.00150
BMI (per 1 kg/m²)	-0.06815	BMI (per 1 kg/m²)	-0.06351
Total cholesterol (per 1 mmol/L)	-0.00414	Total cholesterol (per 1 mmol/L)	-0.00388
Physical activity (active)	-0.42427	Physical activity (active)	-0.42146
APOE genotype (ε4 carrier)	-	*APOE* genotype (ε4 carrier)	1.05112
ADA	-0.08614	-	-
Beta-NGF	-0.02722	-	-
CCL19	0.05818	-	-
CCL20	0.06039	CCL20	0.01222
CCL28	0.04074	-	-
EN-RAGE	0.24888	EN-RAGE	0.15466
FGF-23	-0.60062	FGF-23	-0.50814
IL-7	-0.11421	-	-
LAP TGF-beta-1	0.31744	LAP TGF-Beta-1	0.27973
MCP-3	-0.03548	-	-
ST1A1	0.05413	ST1A1	0.07016

Abbreviations: *APOE*, apolipoprotein E; SBP, systolic blood pressure; BMI, body mass index. For biomarker abbreviations, see Supplemental Table 5.

[a]The CAIDE model 1 includes age, education, sex, systolic blood pressure, body-mass index, total cholesterol and physical activity.

[b]The CAIDE model 2 includes the variables of CAIDE model 1 and *APOE* ε4 status.

[c]ß-coefficients shown for continuous variables are expressed per 1 unit. All variables except education, sex, physical activity and APOE genotype were modelled continuously. The categorical variables were dichotomized as shown in Supplemental Table 33.

Supplemental Table 44. β-coefficients of variables included in prediction models for vascular dementia in the late-life cohort

CAIDE model 1[a]		CAIDE model 2[b]	
Variables	**β-coefficient[c]**	**Variables**	**β-coefficient[c]**
(Intercept)	-8.20520	(Intercept)	-9.73206
Age (per 1 year)	0.12217	Age (per 1 year)	0.10830
Education (high)	-0.00587	Education (high)	0.00675
Sex (male)	0.05431	Sex (male)	0.05333
SBP (per 1 mmHg)	0.00626	SBP (per 1 mmHg)	0.00706
BMI (per 1 kg/m²)	-0.05043	BMI (per 1 kg/m²)	-0.05109
Total cholesterol (per 1 mmol/L)	-0.00377	Total cholesterol (per 1 mmol/L)	-0.00333
Physical activity (active)	-0.37136	Physical activity (active)	-0.33378
APOE genotype (ε4 carrier)	-	*APOE* genotypes(ε4 carrier)	0.68664
-	-	IL-10	0.07642
-	-	IL-10RA	0.00784
-	-	IL-18	0.12688
-	-	VEGF-A	0.05217

Abbreviations: *APOE*, apolipoprotein E; SBP, systolic blood pressure; BMI, body mass index. For biomarker abbreviations, see Supplemental Table 5.

[a]The CAIDE model 1 includes age, education, sex, systolic blood pressure, body-mass index, total cholesterol and physical activity.

[b]The CAIDE model 2 includes the variables of CAIDE model 1 and *APOE* ε4 status.

[c]ß-coefficients shown for continuous variables are expressed per 1 unit. All variables except education, sex, physical activity and APOE genotype were modelled continuously. The categorical variables were dichotomized as shown in Supplemental Table 33.

Supplemental Table 45. Associations of diabetes, physical activity and APOE ε4 genotype with all-cause and common subtype dementia incidences in models with and without adjustment for 8-iso-prostaglandin $F_{2\alpha}$ levels

Baseline characteristics	All-cause dementia		Alzheimer's disease		Vascular dementia	
	Main model*	Main model* + 8-iso-$PGF_{2\alpha}$†	Main model*	Main model* + 8-iso-$PGF_{2\alpha}$†	Main model*	Main model* + 8-iso-$PGF_{2\alpha}$†
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
8-iso-$PGF_{2\alpha}$ levels per 1 SD‡	Not included	**1.47 (1.19-1.82)**	Not included	**1.55 (1.05-2.29)**	Not included	1.20 (0.83-1.73)
Physical activity§						
Inactive	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
Low	**0.68 (0.53-0.87)**	**0.68 (0.53-0.87)**	**0.52 (0.33-0.83)**	**0.52 (0.33-0.83)**	0.79 (0.52-1.20)	0.79 (0.52-1.20)
Medium or high	**0.59 (0.44-0.79)**	**0.60 (0.45-0.80)**	**0.57 (0.34-0.95)**	**0.58 (0.35-0.97)**	0.61 (0.37-1.01)	0.61 (0.37-1.01)
Diabetes						
No	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
Yes	**1.63 (1.27-2.10)**	**1.58 (1.23-2.03)**	**1.68 (1.05-2.67)**	**1.61 (1.01-2.57)**	**1.80 (1.18-2.73)**	**1.77 (1.16-2.69)**
***APOE* genotypes**						
ε4 non-carrier	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
ε2/ε4	1.35 (0.75-2.42)	1.36 (0.75-2.45)	1.03 (0.30-3.59)	1.04 (0.30-3.63)	**2.16 (1.00-4.65)**	**2.17 (1.01-4.69)**
ε3/ε4	**1.63 (1.28-2.07)**	**1.62 (1.28-2.06)**	**2.44 (1.61-3.70)**	**2.42 (1.60-3.68)**	**1.63 (1.06-2.51)**	**1.63 (1.06-2.50)**
ε4/ε4	**4.08 (2.39-6.95)**	**4.09 (2.40-6.99)**	**6.81 (3.07-15.08)**	**6.83 (3.08-15.15)**	1.01 (0.13-7.70)	1.00 (0.13-7.68)

Abbreviations: 8-iso-$PGF_{2\alpha}$, 8-iso-prostaglandin $F_{2\alpha}$; CI, confidence interval; hazard ratio.

NOTE: Numbers printed in bold are statistically significant ($p < 0.05$).

* The model was adjusted for age (continuously), sex, education, physical activity, BMI (categorical), diabetes, and *APOE* **ε**4 polymorphism.

† 8-iso-prostaglandin $F_{2\alpha}$ levels were logarithmized and used as a continuous variable in the model.

‡ 1 SD of 8-iso-prostaglandin $F_{2\alpha}$ levels = 0.278 nmol/mmol creatinine.

§ "Inactive" was defined by < 1 h of vigorous or < 1 h light physical activity per week. "Medium or high" was defined by ≥ 2 h of vigorous and ≥ 2 h of light physical activity/week. All other amounts of physical activity were grouped into the category "Low".

Curriculum vitae

PERSONALIEN

Vorname und Name:	Kira Trares
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SCHULISCHER WERDEGANG

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10/2013 – 09/2016	Bachelorstudiengang Bioinformatik, Goethe Universität Frankfurt am Main
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Eidesstattliche Versicherung

1. Bei der eingereichten Dissertation zu dem Thema
   „Improved risk prediction of all-cause dementia, Alzheimer's disease, and vascular dementia by biomarkers of oxidative stress and inflammation"

   handelt es sich um meine eigenständig erbrachte Leistung.

2. Ich habe nur die angegebenen Quellen und Hilfsmittel benutzt und mich keiner unzulässigen Hilfe Dritter bedient. Insbesondere habe ich wörtlich oder sinngemäß aus anderen Werken übernommene Inhalte als solche kenntlich gemacht.

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