University of Helsinki
Dissertationes Universitatis Helsingiensis 124/2025

Folkhälsan Research Center

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Helsinki, Finland

CEREBRAL SMALL VESSEL DISEASE AND STROKE IN TYPE 1 DIABETES

The impact of blood pressure, haptoglobin genotype, and diabetic retinopathy

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DOCTORAL DISSERTATION
To be presented, with the permission of the Faculty of Medicine of the University of Helsinki, for public examination in Hall 2, Haartman Institute
on the 4th of April 2025, at 1 p.m.

Helsinki 2025

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The Faculty of Medicine uses the Urkund system (plagiarism recognition) to examine all doctoral dissertations.

Publisher: University of Helsinki
Series: Dissertationes Universitatis Helsingiensis 124/2025

ISBN 978-952-84-0840-6 (print)
ISBN 978-952-84-0839-0 (online)
ISSN 2954-2898 (print)
ISSN 2954-2952 (online)

PunaMusta,
Joensuu 2025

“One has to discover everything for oneself. And get over it all alone.”

Tove Jansson

Abstract

Background In comparison to the general population, individuals with type 1 diabetes are prone to cerebrovascular disease and have up to a sixfold increased risk of stroke. Both asymptomatic cerebral small vessel disease (CSVD) and overt stroke of small vessel origin have been associated with type 1 diabetes and appear to develop by similar mechanisms as other microvascular complications of diabetes. Yet, the research on CSVD in type 1 diabetes remains sparse.

Aims This study sought to examine factors associated with asymptomatic CSVD in type 1 diabetes. Based on our hypothesis that particularly diabetic retinopathy is a marker of CSVD, we aimed to further explore how these factors translate into overt stroke.

Participants and methods The present study was carried out as part of the nationwide Finnish Diabetic Nephropathy (FinnDiane) Study. This observational study aims to identify genetic, environmental, and clinical risk factors for vascular complications in individuals with type 1 diabetes. Studies I–III included FinnDiane participants recruited for brain magnetic resonance imaging (MRI). The images were assessed for signs of CSVD (microbleeds, white matter hyperintensities, lacunes). The findings were analysed in association with the participants' blood pressure in Study I (n = 73), haptoglobin genotype (*HP1-1, HP1-2,* and *HP2-2*) in Study II (n = 179), and diabetic retinopathy severity in Study III (n = 189 + 29 diabetes-free controls). In Study IV, 1,268 FinnDiane participants with data on retinal health were assessed for the incidence of strokes during a median follow-up time of 18 years, and incident strokes were assessed in relation to the severity of the participants' diabetic retinopathy and macular oedema at baseline. Diabetic retinopathy and macular oedema severity were graded in accordance with the Early Treatment Diabetic Retinopathy (ETDRS) scale in Studies III and IV.

Results CSVD was observed in approximately a third of the participants in Studies I–III. In contrast to diurnal blood pressure, both nocturnal systolic and diastolic blood pressure, as well as mean arterial pressure, were associated with CSVD after adjustment for clinical confounders. Masked and nocturnal hypertension were prevalent among the participants and associated with CSVD. Haptoglobin genotype distribution and minor allele (*HP1*) frequency did not differ in participants with versus without CSVD, and *HP1-1* was not associated with any manifestation of CSVD in adjusted analysis. Participants with CSVD had more severe diabetic retinopathy compared to those without signs of small vessel disease on MRI. This observation was limited to type 1 diabetes. After adjustment, proliferative retinopathy (ETDRS ≥ 61) was associated with CSVD, particularly microbleeds. The number of cerebral microbleeds increased with the severity of diabetic retinopathy. Having > 2 microbleeds was associated with an increasing ETDRS score and with proliferative retinopathy after adjustment. Among participants in Study IV, 130 strokes occurred during follow-up: 96 ischaemic (lacunar and non-lacunar) and 34 haemorrhagic (intracerebral and subarachnoid haemorrhages) strokes. The incidence of

any ischaemic strokes, both ischaemic subtypes, and haemorrhagic strokes increased by diabetic retinopathy severity. Compared to no or very mild retinopathy (ETDRS score ≤ 20), both non-proliferative (ETDRS 35–53) and proliferative retinopathy were associated with an increased stroke risk after adjustment for confounders, including diabetic kidney disease. Non-proliferative or worse retinopathy (ETDRS ≥ 35) was associated with ischaemic stroke, although not significant after adjustment for kidney disease. Proliferative retinopathy was associated with a fourfold risk of haemorrhagic stroke in a model with diabetic kidney disease. The incidence of stroke was increased in participants with clinically significant macular oedema, but macular oedema of this severity was not a significant risk marker in the adjusted analysis.

Conclusions CSVD in type 1 diabetes is associated with a general increase in nocturnal blood pressure and masked hypertension. Although the prevalence of small vessel disease was high among our participants, we detected no association between haptoglobin genotype and CSVD in contrast to previous reports. The severity of diabetic retinopathy is associated with covert CSVD, as well as with the incidence of overt stroke, independent of comorbid conditions. Our observations suggest that diabetic retinopathy may serve as a marker of cerebrovascular complications in individuals with type 1 diabetes. Furthermore, our findings warrant future attention on circadian variation in blood pressure control.

Abstrakt (Abstract in Swedish)

Bakgrund I jämförelse med den allmänna befolkningen är personer med typ 1-diabetes benägna att insjukna i cerebrovaskulära sjukdomar och har upp till sex gånger förhöjd risk att insjukna i stroke. Både symptomfri cerebral småkärlssjukdom, samt småkärlsrelaterad stroke har förknippats med typ 1 diabetes och de har föreslagits uppstå via samma mekanismer som andra diabetiska mikrovaskulära komplikationer. Trots det finns endast sparsamt med forskning om cerebral småkärlssjukdom hos personer med typ 1-diabetes.

Målsättningar Ändamålet med denna studie var att undersöka faktorer associerade med symptomfri cerebral småkärlssjukdom vid typ 1-diabetes. Baserad på hypotesen att diabetes retinopati i synnerhet är förknippat med cerebral småkärlssjukdom, strävade vi till att vidare utvärdera hur detta potentiella samband hänger ihop med stroke.

Studiedeltagare och Metoder Denna studie förverkligades som en del av det nationellt omfattande forskningsprojektet FinnDiane – en observationsstudie med mål att identifiera genetiska, miljöbetingade och kliniska riskfaktorer förknippade med vaskulära komplikationer vid typ 1-diabetes. Studierna I-III inkluderade FinnDiane deltagare som rekryterats att delta i hjärnavbildning med magnetisk resonanstomografi (MRI). MRI-bilderna utvärderades för tecken på cerebral småkärlssjukdom (mikroblödningar, vitsubstansförändringar, lakuner) och fynden analyserades i relation till deltagarnas blodtryck i Studie I (n = 73), haptoglobingenotyp (*HP1-1, HP1-2,* och *HP2-2*) i Studie II (n = 179), och svårighetsgraden av diabetesretinopati i Studie III (n = 189 + 29 diabetesfria kontrollpersoner). Studie IV undersökte förekomsten av stroke hos 1 268 FinnDiane deltagare med tillgänglig information gällande ögonbottenfynd. Strokefall under uppföljningstiden (median 18) utvärderades i relation till svårighetsgraden av retinopati och makulärt ödem hos deltagarna vid uppföljningstidens början. Svårighetsgraden av deltagarnas diabetsretinopati och makulopati graderades i enlighet med Early Treatment Diabetic Retinopathy (ETDRS) skalan i Studierna III och IV.

Resultat Cerebral småkärlssjukdom observerades hos cirka en tredjedel av deltagarna i Studie II-III. I motsats till blodtrycket under dagtid, var systoliskt blodtryck, diastoliskt blodtryck, och arteriellt medeltryck nattetid associerat med förekomsten av cerebral småkärlssjukdom efter beaktande av kliniska förväxlingsfaktorer. Dold och nattlig hypertoni var vanliga bland deltagarna och bägge var förknippade med cerebral småkärlssjukdom. Fördelningen av haptoglobingenotyper, samt allelfrekvensen av *HP1* skilde sig inte signifikant mellan deltagare med *versus* utan cerebral småkärlssjukdom och *HP1-1* var inte associerad med någon MRI-markör då förväxlingsfaktorer beaktats. Studiedeltagare med cerebral småkärlssjukdom hade svårare diabetesretinopati jämfört med deltagare med normala MRI-fynd. Fyndet var begränsat till individer med typ-1 diabetes. Proliferativ retinopati (ETDRS ≥ 61) var associerat med cerebral småkärlssjukdom, speciellt med mikroblödningar, efter korrigering för förväxlingsfaktorer. Antalet cerebrala mikroblödningar ökade med stigande svårighetsgrad

av diabetesretinopati. Förekomsten av > 2 mikroblödningar var associerat med ökande ETDRS-poäng och med proliferativ retinopati efter korrigering för förväxlingsfaktorer. Under uppföljningstiden inträffade 130 fall av stroke bland deltagarna i Studie IV: 96 fall av ischemisk stroke (lakunära och icke-lakunära) och 34 hjärnblödningar (intracerebrala och subaraknoidala blödningar). Incidensen av ischemisk stroke över lag och bägge undertyper, samt incidensen av hjärnblödning ökade med svårighetsgraden av retinopati. Både icke-proliferativ (ETDRS 35–53) och proliferativ retinopati var förknippat med en förhöjd strokerisk vid jämförelse med lindrigare ögonbottenfynd (ETDRS ≤ 20) och beaktande av förväxlingsfaktorer, inklusive diabetisk njursjukdom. Icke-proliferativ och svårare retinopati (ETDRS ≥ 35) var associerat med ischemisk stroke, dock inte signifikant efter beaktande av diabetisk njursjukdom. Risken för hjärnblödning var fyrfaldig i anslutning till proliferativ retinopati då diabetisk njusjukdom beaktats. Strokeincidensen var förhöjd bland deltagare med makulärt ödem, men makulärt ödem utgjorde inte en riskmarkör i de korrigerade analyserna.

Slutsatser Cerebral småkärlssjukdom vid typ 1-diabetes är associerat med ett förhöjt blodtryck nattetid och med dold hypertoni. Till skillnad från tidigare rapporter upptäckte vi inte något samband mellan haptoglobingenotyp och cerebral småkärlssjukdom, trots att förekomsten av småkärlssjukdom var hög bland våra deltagare. Svårighetsgraden av diabetesretinopati är associerad med symptomfri cerebral småkärlssjukdom och med incidensen av stroke oberoende av parallellt förekommande sjukdomstillstånd. Våra observationer ger belägg för att diabetesretinopati kan fungera som en markör för cerebrovaskulära komplikationer vid typ 1-diabetes, och understryker dessutom vikten av att uppmärksamma cirkadiska variationer i blodtrycksregleringen.

Tiivistelmä (Abstract in Finnish)

Tausta Tyypin 1 diabetesta sairastavat henkilöt ovat alttiita aivoverenkiertohäiriölle ja heidän riskinsä sairastua aivohalvaukseen on jopa kuusinkertainen taustaväestöön verrattuna. Sekä oireeton aivojen pienten suonten tauti, että pienten suonten taudin aiheuttama aivohalvaus on yhdistetty tyypin 1 diabetekseen ja näiden on ehdotettu kehittyvän muita mikrovaskulaarisia komplikaatioita vastaavalla tavalla. Siitä huolimatta aivojen pienten suonten taudista on toistaiseksi vain rajoitetusti tutkimustietoa tyypin 1 diabeteksessa.

Tavoitteet Tämän tutkimuksen tavoite oli selvittää oireettomaan aivojen pienten suonten tautiin liittyviä tekijöitä tyypin 1 diabetesta sairastavilla. Perustuen hypoteesiin, että erityisesti diabeettinen retinopatia on yhteydessä aivojen pienten suonten tautiin, pyrimme lisäksi arvioimaan, kuinka tämä mahdollinen yhteys liittyy aivohalvaukseen.

Tutkimusaineisto ja menetelmät Tutkimus toteutettiin osana valtakunnallista FinnDiane-tutkimusta. FinnDiane on havaintotutkimus, jonka tavoitteena on tunnistaa tyypin 1 diabeteksen vaskulaaristen komplikaatioiden perinnöllisiä, ympäristöön liittyviä ja kliinisiä riskitekijöitä. Tutkimusten I-III osallistujat olivat kaikki osa aivojen magneettikuvantamiseen (MRI) rekrytoitua kohorttia. MRI:ssä arvioitiin aivojen pienten suonten tautiin viittaavia löydöksiä (mikrovuodot, valkean aineen muutokset, lakunat) ja löydökset analysoitiin suhteessa verenpaineeseen Tutkimuksessa I (n = 73), haptoglobiinin genotyyppiin (*HP1-1, HP1-2,* ja *HP2-2*) Tutkimuksessa II (n = 179), ja diabeettiseen retinopatiaan Tutkimuksessa III (n = 189 + 29 verrokkia ilman diabetesta). Tutkimuksessa IV tarkasteltiin aivohalvausten ilmaantuvuutta 1 268 FinnDiane-osallistujalla, joilla oli saatavilla tietoa silmänpohjien tilanteesta. Seurantajakson aikana (mediaani 18 vuotta) ilmeneviä aivohalvauksia arvioitiin suhteessa retinopatian ja makulaturvotuksen vaikeusasteeseen seuranta-ajan alkaessa. Osallistujien diabeettinen retinopatia arvoitiin Early Treatment Diabetic Retinopathy (ETDRS) asteikon mukaisesti Tutkimuksissa III ja IV.

Tulokset Tutkimuksessa I aivojen pienten suonten tauti havaittiin noin kolmanneksella Tutkimuksien II-III osallistujista. Yönaikainen systolinen ja diastolinen, sekä keskimääräinen valtimopaine olivat yhteydessä aivojen pienten suonten taudin esiintyvyyteen, kun sekoittavat kliiniset tekijät huomiotiin. Päivänaikaisessa verenpaineessa ei havaittu vastaavaa yhteyttä pienten suonten tautiin. Osallistujilla esiintyi usein piilevä- sekä yön aikainen hypertensio, ja nämä olivat yhteydessä aivojen pienten suonten tautiin. Haptoglobiini genotyyppien jakauma, tai *HP1* alleelitaajuus eivät eronneet merkittävästi aivojen pienten suonten tautia sairastavilla verrattuna muihin, eikä *HP1-1* ollut yhteydessä mihinkään pienten suonten taudin ilmentymään, kun sekoittavat tekijät huomioitiin. Aivojen pienten suonten tautia sairastavilla todettiin vaikea-asteisempi diabeettinen retinopatia verrattuna osallistujiin, joiden MRI oli normaali. Löydös rajoittui tyypin 1 diabetekseen. Korjatuissa analyyseissä proliferatiivinen retinopatia (ETDRS ≥ 61)

oli yhteydessä aivojen pienten suonten tautiin, etenkin aivojen mikrovuotoihin. Aivojen mikrovuotojen määrä suureni retinopatian vaikeusasteen myötä ja > 2 mikrovuodon ilmenemä oli yhteydessä ETDRS-pisteiden nousuun, sekä proliferatiiviseen retinopatiaan myös, kun sekoittavat tekijät huomioitiin. Tutkimuksen IV seuranta-ajan aikana 130 osallistujaa sairastuivat aivohalvaukseen: 96 infarktia (lakunaariset ja ei-lakunaariset infarktit) ja 34 aivoverenvuotoa (intrasebraali- ja lukinkalvonalaiset vuodot). Kaikkien aivohalvausten ilmaantuvuus lisääntyi retinopatian vaikeusasteen myötä. Verrattuna retinopatian puuttumiseen tai erittäin lievään retinopatiaan (ETDRS ≤ 20), ei-proliferatiivinen (ETDRS 35–53), sekä proliferatiivinen retinopatia olivat yhteydessä kohonneeseen riskiin sairastua aivohalvaukseen, kun sekoittavat tekijät, diabeteksen munaistauti mukaan lukien, huomioitiin. Ei-proliferatiivinen tai vaikeampi retinopatia oli yhteydessä aivoinfarktien esiintyvyyteen, mutta yhteys ei ollut tilastollisesti merkittävä, kun diabeteksen munaistauti huomioitiin. Aivoverenvuotojen riski oli nelinkertainen proliferatiivista retinopatiaa sairastavilla, kun diabeteksen diabeteksen munuaistauti huomioitiin. Kliinisesti merkittävä makulaturvotus oli yhteydessä kohonneeseen aivohalvausten ilmaantuvuuteen, mutta makulaturvotus ei ollut merkittävä riskimarkkeri, kun sekoittavat tekijät huomioitiin.

Päätelmät Aivojen pienten suonten tauti on yhteydessä yönaikaiseen kohonneeseen verenpaineeseen sekä piilevään hypertensioon. Vaikka aivojen pienten suonten taudin esiintyvyys oli korkea osallistujissamme, emme havainneet mitään yhteyttä haptoglobiinin genotyypin ja aivojen pienten suonten taudin välillä. Diabeettisen retinopatian vaikeusaste on yhteydessä oireettomaan aivojen pienten suonten tautiin, sekä aivohalvausten ilmaantuvuuteen, muista samanaikaisista sairauksista huolimatta. Löydöksemme viittaavat siihen, että diabeettinen retinopatia saattaisi toimia avoverenkiertoon liittyvien komplikaatioiden markkerina tyypin 1 diabeteksessa. Lisäksi tuloksemme osoittavat verenpaineen vuorokausivaihtelun huomioimisen tärkeyden.

List of abbreviations and symbols

α	Significance level
ACCORD Study	Action to Control Cardiovascular Risk in Diabetes Study
ADA	American Diabetes Association
AGEs	Advanced glycation end-products
anti-VEGF	Anti-vascular endothelial growth factor
CI	Confidence interval
CSME	Clinically significant macular oedema
CSVD	Cerebral small vessel disease
CT	Computer tomography
DCCT/EDIC	Diabetes Control and Complications Trial / Epidemiology of Diabetes Interventions and Complications
eGFR	Estimated glomerular filtration rate
ETDRS	Early Treatment Diabetic Retinopathy Study
FinnDiane Study	Finnish Diabetic Nephropathy Study
GADA	Glutamic acid decarboxylase autoantibodies
HbA_{1c}	$Haemoglobin_{A1c}$ (Glycated haemoglobin)
HDL	High-density lipoprotein
HP	Haptoglobin coding gene
HP1	Haptoglobin coding allele 1
HP1-1	Haptoglobin genotype 1-1
HP1-2,	Haptoglobin genotype 1-2
HP2	Haptoglobin coding allele 2
HP2-2	Haptoglobin genotype 2-2
HR	Hazard ratio
HUS	Helsinki University Hospital
HUSLAB	Central Laboratory of the Helsinki University Hospital
ICD-10	International Code of Disease, Tenth Revision
IAA	Insulin autoantibodies
KDIGO	Kidney Disease: Improving Global Outcomes
LADA	Latent autoimmune diabetes of adulthood
LDL	Low-density lipoprotein
MAF	Minor allele frequency
mmHg	Millimetre mercury

MRI	Magnetic resonance imaging
NPDR	Non-proliferative diabetic retinopathy
NPV	Negative predictive value
OCT	Optical coherence tomography
OR	Odds ratio
PCR	Polymerase chain reaction
PDR	Proliferative diabetic retinopathy
PPV	Positive predictive value
TOAST	Trial of Org 10172 in Acute Stroke Treatment
WHO	World Health Organization

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List of original publications

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I Eriksson MI, Gordin D, Shams S, Forsblom CM, Summanen P, Liebkind R, Tatlisumak T, Putaala J, Groop P-H, Martola J, Thorn LM, on behalf of the FinnDiane Study Group. Nocturnal blood pressure is associated with cerebral small-vessel disease in type 1 diabetes. *Diabetes Care* 2020; 43(8):e96–e98.

II Eriksson MI, Syreeni A, Sandholm N, Dahlström EH, Gordin D, Tatlisumak T, Putaala J, Groop P-H, Martola J, Thorn LM, on behalf of The FinnDiane Study. Haptoglobin genotype and its relation to asymptomatic cerebral small-vessel disease in type 1 diabetes. *Acta Diabetologica* 2023; 60(6):749–756.

III Eriksson MI, Summanen P, Gordin D, Forsblom C, Shams S, Liebkind R, Tatlisumak T, Putaala J, Groop P-H, Martola J, Thorn LM, on behalf of the FinnDiane Study Group. Cerebral small-vessel disease is associated with the severity of diabetic retinopathy in type 1 diabetes. *BMJ Open Diabetes Research & Care* 2021;9:e002274.

IV Eriksson MI, Hietala K, Summanen P, Harjutsalo V, Putaala J, Ylinen A, Hägg-Holmberg S, Groop P-H, Thorn LM, on behalf of the FinnDiane Study. Stroke incidence increases with diabetic retinopathy severity and macular edema in type 1 diabetes. *Cardiovascular Diabetology* 2024; 23:136.

The publications are referred to in the text by their Roman numerals.

1 Introduction

Over 530 million adults around the world are estimated to have diabetes. This global health crisis has not yet seen its end, as the number is predicted to reach 783 million by 2045, making diabetes one of the most rapidly increasing disease burdens of the 21st century (1). In Finland, nearly half a million people – that is, almost 10% of the population – have diabetes (2). Of these, roughly 50,000 have type 1 diabetes (2), which is an autoimmune disease that renders a person dependent on insulin treatment. The incidence of type 1 diabetes has been increasing globally since the 1950s (3), and although the incidence in Finland has plateaued during the last 20 years (4,5), Finland remains the country with the world's highest incidence of childhood-onset type 1 diabetes (6).

Diabetes is defined by chronically elevated blood glucose levels and is characterised by a global disruption of normal metabolism of nutrients, such as fats, in addition to glucose. Beyond the acute effects of hyperglycaemia, disturbed energy homeostasis leads to long-term diabetes-related complications, such as dyslipidaemia, deterioration of blood vessel walls, altered regulation of blood pressure and tissue perfusion, and tissue damage in susceptible organs (1,7). Organs typically affected are those with high metabolic activity and rich capillarisation but no means to actively downregulate the local glucose concentration; that is, glucose transportation is not directly insulin-dependent (8). This type of organ damage is classically categorised as microvascular complications, typically manifesting as diabetic retinopathy, diabetic kidney disease, and diabetic neuropathy (9). The capillary deterioration in diabetes is similar to that in normal ageing, but it happens prematurely (10). Signs of early vascular ageing also manifest as stiffening of the larger arteries, hypertension and, eventually, cardiovascular disease, such as coronary artery disease (11–15). The involvement of large arteries is typically referred to as macrovascular complications, although the distinction between micro- and macrovascular disease is not always clear or applicable.

The vascular complications of diabetes have grim consequences, as they are all severely burdensome diseases, and particularly diabetic kidney disease and cardiovascular diseases are, unfortunately, often mortal. These devastating micro- and macrovascular diseases often coincide and worsen parallelly (10,16,17). It is not fully clear how micro- and macrovascular complications impact each other, as either has been suggested to accelerate the other (18–20). A recognised hypothesis

is that they are “of common soil” and represent different manifestations of a mutual underlying cause (21).

Among the vascular complications of diabetes, cerebrovascular disease remains a less well-characterized entity, regardless of the outcomes being severely debilitating. On a global scale, neurological disorders, particularly stroke and cognitive impairment, are among the leading causes of disability and the second most common cause of death (22,23). Compared to the general population, individuals with type 1 diabetes have a three- to sixfold risk of stroke and the prognosis after a stroke is, furthermore, poorer (24–27). Yet, the aetiology of cerebrovascular disease in type 1 diabetes, and the processes leading up to stroke, are poorly understood. Diabetes is suggested to cause microvascular end-organ damage to the central nervous system similar to other microvascular complications (28,29), and although more well-studied in type 2 diabetes (30–32), cerebral small vessel disease (CSVD) has been linked to type 1 diabetes as well (33–35). Even if this microvascular disease of the brain can be asymptomatic, with only radiological markers (cerebral micro- and macro bleeds, white matter hyperintensities, lacunes, cortical superficial siderosis, and atrophy of the brain) indicating its entry, the clinical picture of CSVD is diverse, associated with overt stroke and cognitive impairment (36,37).

Although CSVD is more common in type 1 diabetes compared to healthy controls, little is known about the risk factors for apparently asymptomatic small vessel disease in type 1 diabetes. In these individuals, CSVD has been associated with increased blood pressure, signs of arterial stiffening, and diabetic retinopathy (35,38,39), which has led to the suggestion that CSVD is part of a generalised state of vascular dysfunction (39), fuelling the common soil hypothesis. Certain genetic factors, e.g., variation in the gene coding for haptoglobin has been prominently discussed, have further been suggested to play a role in both silent CSVD and overt stroke (40,41).

The reported link between diabetic retinopathy and CSVD, particularly cerebral microbleeds, is of special interest for many reasons: Firstly, the retina is an extension of the brain, and they share both structural and functional properties, wherefore mechanisms of microvascular insult to the retina are thought to reflect parallel processes in the brain (42). Secondly, severe diabetic retinopathy is shown to be a risk marker of stroke caused by cerebral haemorrhage (43). Therefore, the retina may provide vital information about cerebrovascular health in individuals with type 1 diabetes. As the retina is the only structure of the central nervous system accessible for direct non-invasive imaging, the eye is, indeed, considered to be the window to the brain (44,45).

2 Review of the literature

2.1 Diabetes Mellitus

2.1.1 Background

Diabetes mellitus, or simply *diabetes*, is a group of metabolic diseases in which the characteristic common attribute is elevated blood glucose levels (hyperglycaemia). Immediate symptoms include thirst, excessive urination, hunger, weight loss, blurred vision, and eventually, if untreated, death. These hallmark symptoms were described as early as 1500 BC and have given the disease its name, "diabetes", which is derived from the Greek and indicates abundant amounts of urine. The adjective *mellitus* (honeyed) was added to the name in the 18th century, although the sweet properties of diabetic urine had already been known prior to that (7,46).

At the beginning of the 19th century, concepts of glucose metabolism started to unfold, as it was discovered that glucose was normally present in the blood and stored in the liver. By the end of the 19th century, a perception of the pathophysiology behind these hallmark symptoms had begun to form, as the endocrine role of the pancreas was discovered. Diabetes was subdivided into *diabéte maigre* (diabetes in the lean) and *diabéte grass* (diabetes in the obese) on clinical grounds, and later into insulin-sensitive and insulin-insensitive types, as insulin's role in glucose metabolism became recognised. These were the forerunners of today's classification between type 1 diabetes and type 2 diabetes (46).

The true revolution of diabetes research and treatment, however, occurred in 1921–1922, when insulin was first isolated by F. Banting and C. Best in collaboration with J.J.R. Macleod and J.B. Collip and successfully administered to an individual with diabetes (46). The second big breakthrough was in 1993, when the Diabetes Control and Complications Trial / Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) showed that intensive insulin therapy, with multiple injections guided by frequent glucose monitoring, significantly reduces the risk of diabetes-related complications (47). Ever since, intensive treatment targeting physiological levels of blood glucose has become the gold standard of type 1 diabetes management (6).

The diagnostic criteria for diabetes are based on blood glucose measurements and/or measurements of glycated haemoglobin (HbA_{1c}) (7,9,48). The four different diagnostic tests and the diagnostic criteria currently recommended are presented in Figure 1.

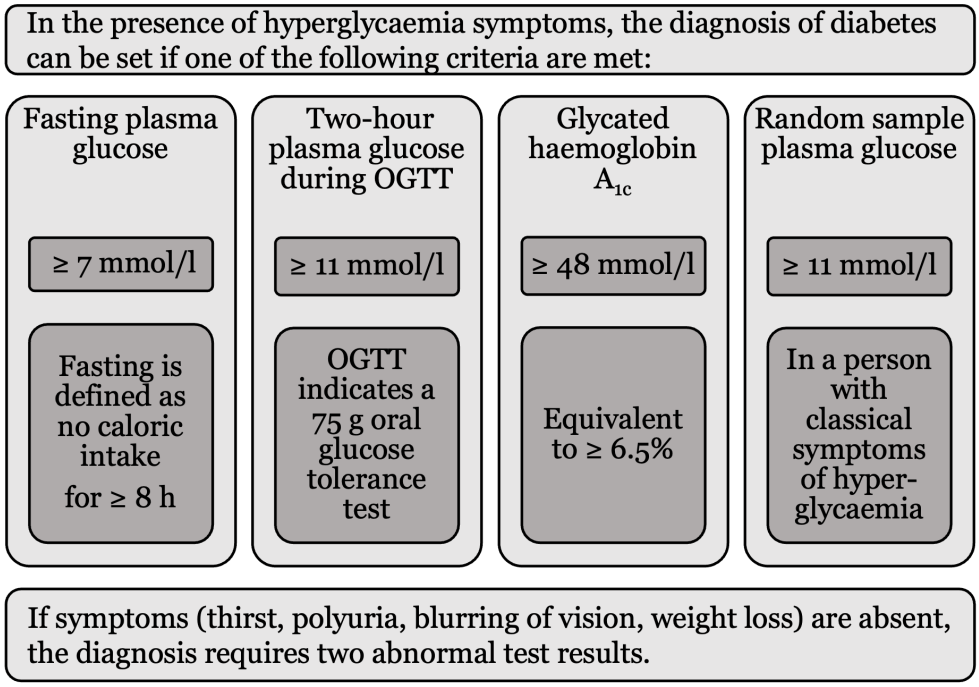

Figure 1 The diagnostic tests and corresponding threshold values for the diagnosis of diabetes as recommended by the World Health Organization (7), the American Diabetes Association (48), and the Finnish Current Care Guidelines (49,50). OGTT indicates oral glucose tolerance test. Gestational diabetes is defined by different criteria not presented in this figure.

2.1.1.1 Classification of diabetes

Diabetes can be divided into different subtypes based on the aetiology and clinical features of the disease. The most prevalent subtypes are type 1 and type 2 diabetes, of which type 2 is predominant, accounting for over 90% of all diabetes worldwide (1). Insulin is the key factor in diabetes, as hyperglycaemia results either from insufficient secretion of insulin in the body or from insufficient action of the insulin in the body's tissues. Insulin, a hormone produced by the pancreas, mediates uptake of the blood glucose into the cells, where it can be converted into energy for use or storage (7,9).

Type 1 diabetes is characterised by insulin deficiency and, if untreated, also presents with immediate symptoms of hyperglycaemia (weight loss, excessive urination, and eventually keto acidosis), whereas insufficient insulin action, i.e.,

insulin resistance, and obesity are typical in type 2 diabetes (9). The subtypes are heterogenous diseases, and the clinical presentation within a subgroup may vary considerably (48). Moreover, the World Health Organization (WHO) recognises hybrid forms of diabetes among the different subtypes (7). The classification presented in Table 1 also contains diabetes diagnosed during pregnancy and other specific types of diabetes defined by the WHO (7).

Table 1 Classification of diabetes

Category of diabetes	Characteristic features
Type 1 diabetes	- Autoimmune beta cell destruction - Usually causes absolute insulin deficiency - Category includes LADA
Type 2 diabetes	- Progressive loss of adequate beta cell insulin secretion - Underlying insulin resistance is typical
Specific types of diabetes due to other causes	- Monogenic diabetes syndromes (such as maturity-onset diabetes of the young or neonatal diabetes) - Secondary diabetes due to diseases of the exocrine pancreas - Drug- or chemical-induced diabetes
Gestational diabetes mellitus	- Diabetes diagnosed in the 2nd or 3rd trimester of pregnancy - Not clearly overt diabetes prior to gestation

The table shows the classification of diabetes presented by the American Diabetes Association in 2023 (48). LADA indicates latent autoimmune diabetes of adulthood.

Due to the heterogeneity of the disease, classification may vary somewhat depending on the context (7,51). Forerunners to the categories of today were described already in 1880, when diabetes was subdivided into diabetes of the lean and diabetes of the obese, based on the clinical picture (46). Historically, type 1 diabetes has also been defined as childhood-onset diabetes due to it being the most common diabetes type in children and young adults (52). After the age of 30, the incidence of type 2 diabetes exceeds that of type 1 diabetes (53–55); hence, type 2 diabetes was previously referred to as adult-onset diabetes (51). The definition of different subtypes is dynamic and continuously evolving (7,48).

To this date, there is still debate about whether slow-progressing autoimmune diabetes in adults, named latent autoimmune diabetes of adulthood (LADA), should be classified as type 1 diabetes. For example, the WHO still classifies LADA as a hybrid form of diabetes (7), whereas the American Diabetes Association (ADA) lists all forms of autoimmune-mediated diabetes under the category of type 1 diabetes (51). Moreover, the distinction between type 1 and type 2 diabetes becomes increasingly difficult with age, as type 2 is more common in adults, and the clinical features of adult-onset type 1 diabetes differ somewhat from childhood-onset disease. In adults, for example, the progression of type 1 diabetes to overt disease is often slower than in children. (56)

2.1.2 Pathogenesis of type 1 diabetes

2.1.2.1 Beta-cell destruction causing insulin deficiency

Type 1 diabetes is caused by immune-mediated destruction of the insulin-producing beta cells of the pancreas (57,58). The destruction of the beta cells leads to a total lack or a very small production of insulin. The disease is chronic, leaving affected individuals with a lifelong daily need for treatment with exogenous insulin for survival (6,9). The cause of this destructive process is not completely known. A likely explanation is that it results from a combination of genetic susceptibility mediated by multiple genes and several potential environmental triggers, such as a low microbial load (also referred to as the hygiene hypothesis) (58–60). The immune process leading to insulin deficiency may last for a period of months to years, during which autoantigens can be detected as markers of ongoing beta-cell destruction. The presence of measurable autoantibodies against beta-cell autoantigens, such as insulin autoantibodies (IAA) and glutamic acid decarboxylase autoantibodies (GADA), is a key factor in distinguishing type 1 diabetes from other types, such as type 2 diabetes. (58)

2.1.2.2 Hyperglycaemia and systemic metabolic changes

The acute effects of insulin deficiency are hyperglycaemia with simultaneous glucose deficiency in the cells. Diabetes is furthermore characterised by a global disturbance in the metabolism of nutrients, such as dyslipidaemia, as insulin is also essential for fat and protein metabolism in addition to glucose metabolism (7,9). Insulin promotes storage of energy in the tissue. In muscle and adipose cells particularly, insulin increases glucose uptake by activating insulin-dependent glucose transportation into the cells. The immediate effect is a decrease in the blood glucose levels concentration. Moreover, insulin promotes glucose conversion and storage as glycogen in the muscles as well as lipids in the adipose tissue, which is a crucial energy source during fasting (61). Insulin further reduces hyperglycaemia by suppressing cleavage of glycogen (glycogenolysis) and conversion of fat to glucose (gluconeogenesis) in the liver. In adipose tissue, insulin simultaneously decreases the breakdown of stored fat, decreasing lipids in the circulation. Insulin is thereby crucial not only for post-prandial blood glucose regulation but also for energy homeostasis overall (61).

2.1.3 Epidemiology of type 1 diabetes

2.1.3.1 Global prevalence

There are an estimated 537 million adults with diabetes in the world (1). Whereas type 1 diabetes accounts for up to 10% of all diabetes cases in some regions, such as Finland (2,6), the estimated worldwide prevalence of type 1 diabetes is lower, around 6/10,000 individuals (62), corresponding to around 5 million people in total. The prevalence of type 1 diabetes, however, has been suggested to exceed that, but the lack of data on the incidence of adult-onset type 1 diabetes limits the accurate assessment of the global burden of type 1 diabetes (9). The number of children and adolescents with type 1 diabetes is 1.2 million by estimation (9), making this group a considerable proportion of all type 1 diabetes globally.

Growing incidence of childhood-onset disease

The estimated global incidence rate of type 1 diabetes is 15 new cases per 100,000 person-years (62). Nevertheless, reports show large variations across regions (9,63) and age groups (64–68). The incidence of type 1 diabetes has been increasing globally since the 1950s (3). During the last 30 years, the incidence among children aged 0–14 years has been increasing by 3% annually (52). Studies limited to the European population have shown even higher increase rates (69,70), with the largest increase occurring in countries with previously relatively low incidence (70). Finland has the world's highest incidence rate of type 1 diabetes in children and adolescents (63). After achieving the dubious world record of the all-time high incidence of type 1 diabetes in 2006, the number of new cases seems to have plateaued or even decreased somewhat (4,5). During the last ten years, the annual incidence has been around 51–55 new cases per 100,000 (4,5), with a seemingly temporary peak of 61/100,000/year during the COVID-19 pandemic (60).

Incidence in different age groups

In most epidemiological studies having reviewed the incidence rates of childhood-onset type 1 diabetes, an often disregarded fact is that type 1 diabetes can develop at any age (56). Although the peak incidence of the disease is around puberty (65,66), adult-onset type 1 diabetes is actually more common than childhood-onset type 1 diabetes (67,68,71). The incidence rate in adults has been reported to equal that of young children; however, these observations are limited to a few, almost 20-year-old studies (67,72). As adult-onset type 1 diabetes constitutes a heterogenic group, clinically often different from childhood-onset disease (56), one should exercise caution in drawing conclusions based on a few studies, or results drawn from childhood-onset populations. Also in Finland, the latest epidemiological report dates to 2007, and there appears to be a noticeable lack of data on type 1 diabetes incidence in adults.

2.2 Blood pressure

The pumping of the heart generates pressure against the arterial walls as blood surges into circulation. The blood pressure level oscillates with the cardiac cycle, being at its highest peak in systole, the contraction phase of the cardiac cycle, and decreasing during diastole, the refill phase. Blood pressure can additionally be characterised by a pulsatile component and a steady component: the pulse pressure generated by the oscillation between systole and diastole and the mean arterial pressure, which is maintained throughout the cardiac cycle (73,74).

In a clinical context, blood pressure refers to the systolic and diastolic pressure in the brachial artery in the upper arm of the non-dominant limb, as this is the standard point for measuring blood pressure. This is also the measure generally used to assess the prognostic role of blood pressure (75–77).

The blood pressure level is determined by the cardiac output, blood volume, and properties of the vessel walls, which all depend on a vast set of regulatory mechanisms. The blood pressure level normally varies in response to the physiological demand (73). Elevated blood pressure, however, indicates a prolonged state of high pressure, which is associated with an increased risk of cardiovascular disease, such as stroke, and mortality. Elevated blood pressure above a specified critical level, is defined as hypertension (75–77).

2.2.1 Blood pressure measurement

2.2.1.1 Measurement techniques

Non-invasive methods for measuring blood pressure all rely on a device composed of an inflatable cuff to compress and decompress the artery in a controlled manner and a measuring unit to measure the pressure based on the compression. Measurements can be performed manually with an auscultatory technique or with an automatic oscillometer. In the auscultatory method, decompression of the artery at systole and diastole is assessed by auscultation over the artery. Based on auscultatory observations, the systolic and diastolic pressure is measured with an aneroid sphygmomanometer, or formerly with a mercury device (78,79).

The sphygmomanometer gives a direct and reliable measurement of blood pressure when measurements are performed correctly (80). The use of automated oscillometers, however, has become increasingly common for measuring blood pressure (76), as it overcomes many of the limitations of the auscultatory method (75,76,80,81).

The automatic device detects oscillations in the pulsing blood as volume changes in the cuff during compression and decompression of the artery. The device usually uses the maximum change in volume as an indicator of mean arterial pressure. By

automated calculations, the systolic and diastolic blood pressure is then estimated from the mean arterial pressure based on changes in the pulse amplitude (79,82). The blood pressure estimating algorithm is based on population data, and the automatic devices therefore require clinical validation for reliability (78,83). In addition, there are semiautomated or hybrid devices that employ features from either of these techniques (78).

2.2.1.2 Office blood pressure measurements

Office measuring refers to measurements performed by a health care professional at a clinic, and it is the most common method globally for the evaluation and management of blood pressure levels. Office blood pressure can be measured either by the auscultatory method or with an automatic oscillometer (78). A disadvantage of office blood pressure measurements is that sporadic measurements may not reflect the everyday situation of the individual. It is therefore recommended that in-office results should be confirmed by repeated blood pressure measurements, preferably also in an out-of-office setting (75–78).

2.2.1.3 Self-monitoring of blood pressure

Unattended blood pressure measurements have become accessible through the introduction of automatic blood pressure measuring devices. Self-monitoring, also called home blood pressure measurements, refers to repeated measuring of blood pressure by an individual at home or in another setting outside the clinic. Blood pressure measurements outside the clinic are recommended to confirm one-time observations in office measurements and is the primary recommended method for long-term follow-up of blood pressure (75–77). Home measurements can also be useful to identify hypertension that goes undetected in office measurements (75,76,84).

2.2.1.4 Ambulatory blood pressure monitoring

Ambulatory blood pressure monitoring is particularly useful for assessing alterations in blood pressure not detectable in an office setting (84,85). Ambulatory blood pressure has been shown to provide a better prognostic value regarding mortality and cardiovascular morbidity than office measurements (85–87), and for these reasons, ambulatory blood pressure monitoring is often considered the gold standard for assessing blood pressure (75,76).

Technical features of ambulatory blood pressure monitoring

In ambulatory blood pressure monitoring, the individual is equipped with a portable automatic device that measures the blood pressure at set intervals during a period, typically 24 hours (75,78,88). The monitor is usually programmed to measure the blood pressure at 15–30-min intervals for a period of 24 hours (88,89). Ambulatory blood pressure monitoring provides multiple readings during the day, measured during the usual daily circumstances of an individual and during daytime activities and sleep. Ambulatory blood pressure readings can be aggregated to yield a 24-hour mean systolic and diastolic blood pressure and further grouped into specific time intervals, such as daytime and nighttime for separate assessment. In addition to detecting elevated blood pressure, it is thereby also possible to detect alterations in physiological patterns, such as circadian blood pressure patterns, and variability of blood pressure (88,89).

Nocturnal blood pressure and nocturnal hypertension

Nocturnal blood pressure refers to the blood pressure level at a predefined nighttime interval or during sleep. Nocturnal hypertension means that the mean systolic or diastolic blood pressure is elevated over a critical level during nighttime or sleep (75–77,88,89). Compared to blood pressure measured in the office and during the day, nocturnal blood pressure has been shown as the most potent predictor of cardiovascular outcome (85,87).

Nocturnal dipping

During nighttime, the blood pressure level typically decreases by 10–20% compared to the daytime level (90,91). This phenomenon has been widely referred to as *nocturnal dipping* since the introduction of the term in the late 1980s (92). Non-dipping indicates a lack or decrease of the normal dipping pattern (92). Although originally determined by absolute units of blood pressure (mmHg) (92), dipping and non-dipping are usually defined by the relative decrease in blood pressure, or lack thereof (93,94). Typically, a less than 10% decrease in blood pressure during nighttime is defined as non-dipping (88,89,94), as this has been associated with increased cardiovascular risk (93,95,96).

Other alterations to normal blood pressure patterns include extreme dipping, referring to an over 20% decrease from daytime, and reverse dipping, meaning that the nocturnal blood pressure is increased in reference to the daytime pressure. Abnormal dipping patterns can occur within the limits of normal blood pressure, and they thereby differ from nocturnal hypertension, which refers to the absolute level of blood pressure being elevated (89). Both nocturnal hypertension and non-dipping are, however, associated with increased cardiovascular morbidity, including stroke (89,93,96–98). In addition to being a risk factor for cardiovascular disease, non-dipping has been suggested as a marker of pre-existing disease (99),

and it is associated with dysfunction in the mechanisms regulating the blood pressure, such as the autonomous nervous system (92).

2.2.2 Definition of hypertension

Although the blood pressure level increases the risk of cardiovascular disease in a continuous manner (100,101), elevated blood pressure and hypertension are defined by distinct cut-off values to facilitate clinical decision-making and targeting of individuals at high risk for cardiovascular disease (75). The cut-off values and guidelines for blood pressure management may therefore vary based on the population, measurement method, and/or the setting in which hypertension is defined.

Blood pressure measurements taken out of office typically yield slightly lower values compared to the office measurements, and hypertension is therefore defined by lower cut-offs in home measurements and ambulatory monitoring. For ambulatory blood pressure measurements, the thresholds defining hypertension further depend on the time of day (75–77). Table 2 summarises threshold values for hypertension in different measuring settings according to European, American, and Finnish guidelines (75–77).

If hypertension goes undetected in office measurements but is ascertained by the ambulatory or home measurements, it is called masked hypertension. White-coat hypertension indicates the opposite: isolated high blood pressure readings in office measurements in an individual with normal blood pressure outside the office (75–77).

Table 2 Threshold values for hypertension according to European, American, and Finnish guidelines

	European Society of Cardiology/ European Society of Hypertension		American College of Cardiology/ American Heart Association		Finnish Current Care Guidelines	
	Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic
Office blood pressure	≥ 140	≥ 90	≥ 130	≥ 80	≥ 140	≥ 90
Home measurements	≥ 135	≥ 85	≥ 130	≥ 80	≥ 135	≥ 85
Daytime mean of ambulatory measurements	≥ 135	≥ 85	≥ 130	≥ 80	≥ 135	≥ 85
Nighttime mean of ambulatory measurements	≥ 120	≥ 70	≥ 110	≥ 65	≥ 120	≥ 70
24-hour mean of ambulatory measurements	≥ 130	≥ 80	≥ 125	≥ 75	≥ 130	≥ 80

The table presents blood pressure cut-off values (in mmHg) that define hypertension according to the guidelines by the European Society of Cardiology / European Society of Hypertension (76), the American College of Cardiology / American Heart Association (75), and the Finnish Current Care Guidelines (77).

2.2.3 Blood pressure and diabetes

2.2.3.1 Hypertension in diabetes

The threshold for initiating blood pressure-lowering treatment can sometimes differ from the general definitions of hypertension, for example in individuals with a high risk of cardiovascular disease, such as in diabetes (76,77). Compared to the general population, hypertension is more common in individuals with diabetes (11,13) and is a prominent risk factor for cardiovascular disease (102,103). Cardiovascular disease, addressed in further detail in section 2.4.6, is the leading cause of morbidity and death in individuals with diabetes (104). Blood pressure is an independent risk factor for cardiovascular disease, including coronary artery disease and stroke, in both type 1 and type 2 diabetes (103,105,106). The combination of diabetes and hypertension confers a higher risk of cardiovascular disease and mortality than hypertension alone (107). Individuals with both hypertension and diabetes have been estimated to have a threefold increased risk of coronary artery disease compared to individuals with neither condition (108).

As the main objective of managing blood pressure is to reduce the risk of associated diseases, some individuals, such as those with diabetes, benefit from treatment at lower blood pressure levels than the cut-off defining hypertension (109,110). Antihypertensive treatment reduces the risk of diabetic vascular complications (12,111), wherefore lower thresholds for initiating hypertensive treatment and lower treatment targets are often recommended for these individuals (12,76,77). The European Society of Cardiology and The European Society of Hypertension, for example, recommend an office systolic blood pressure of 130 mmHg or lower, if tolerated, when an individual has diabetes (76).

Blood pressure-lowering treatment is particularly beneficial for protecting the kidneys in type 1 diabetes (18,112,113). The kidneys play a critical role in blood pressure regulation (73) and, like in the normal physiological state, there is a complex interaction between the kidneys and blood pressure. It is of note that blood pressure-lowering treatment slows the progression of diabetic kidney disease (112), but elevated blood pressure also increases with the progression of diabetic kidney disease (14,114). Other diabetic complications as well as diabetic kidney disease are suggested to be driven by the common culprit (115,116).

2.2.3.2 Arterial stiffening and altered blood pressure pattern

Diabetes is associated with early ageing and stiffening of the arteries (11,12), and hyperglycaemia increases the risk of developing hypertension (13,14). Arterial stiffening can be detected before any signs of micro- or macrovascular disease occur

(117), and this stiffening, marked by elevated pulse pressure, has been associated with increased risk of cardiovascular disease in type 1 diabetes (16,118,119).

In addition to deterioration of the arterial structure and function, blood pressure regulation may be affected by autonomic neuropathy (120,121). This can be displayed as alterations in physiological blood pressure patterns, such as non-dipping (122). Both non-dipping and nocturnal hypertension are common in individuals with diabetes (123–126). Particularly in type 1 diabetes, an elevation in the nocturnal blood pressure is often pronounced before any other clinical signs of hypertension are detected (123,125), which also explains the high prevalence of masked hypertension in these individuals (126,127). Elevated nocturnal blood pressure and non-dipping are additionally associated with overt microvascular complications (114,125,128) and cardiovascular mortality (129), that is, macrovascular disease, in diabetes.

2.3 Haptoglobin

Haptoglobin is a plasma protein with haemoglobin-binding properties. It is synthesised mainly in the liver, and it is an acute-phase protein, meaning that the synthesis and release into the blood are stimulated by acute infections and inflammation. The action of the protein is complex and modified by genetic variation (130). Moreover, certain genetic variants have been suggested to be associated with increased risk of cardiovascular disease (131–133).

2.3.1 Haptoglobin protein

Haptoglobin is involved in reducing the harmful effects of free haemoglobin released into the blood by damaged red blood cells (haemolysis). Additionally, it has some regulatory functions (130). Briefly, free haemoglobin has oxidative properties, which can cause damage to the body's tissues. Haptoglobin binds free haemoglobin and, thus, reduces its oxidative activity. The formation of this haemoglobin-haptoglobin complex promotes clearance of free haemoglobin by facilitating its uptake in the white blood cells, that is, monocytes and macrophages, from the extracellular space. Haemoglobin is then metabolically detoxified whereby the end products of the complex can be excreted and the iron in the haemoglobin complex can be recycled (134,135). Haptoglobin is also thought to play a role in the regulation of blood flow and vascular homeostasis because of its antioxidative properties as it maintains the bioavailability of nitric oxide. Nitric oxide is a free radical that is synthesised by the endothelial cells and has a principal role in capillary blood flow regulation (130,134).

2.3.2 Haptoglobin genotype and polymorphism

Haptoglobin consists of two different polypeptide chains, α and β, whose synthesis involves two genetic sites (loci). In humans, there are two major variant alleles of the haptoglobin gene (*HP*), *HP*1 and *HP*2, causing genetic variation in the α-chain. The two alleles give rise to three genotypes, *HP1-1*, *HP2-1*, and *HP2-2* (136,137), as demonstrated in Figure 2. The protein synthesis of the so-called α-chain differs between *HP1* and *HP2,* resulting in differences in the structure of the haptoglobin protein, as illustrated in Figure 3.

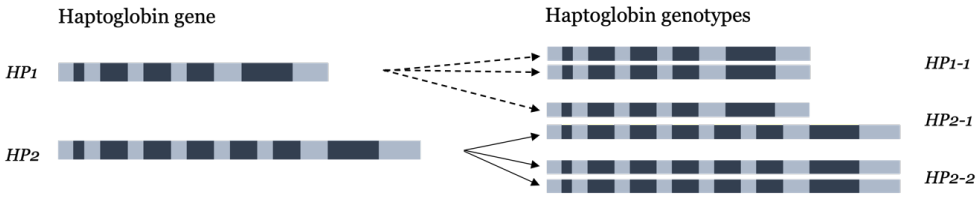

Figure 2 The schematic drawing illustrates the *HP1* and *HP2* alleles, of which *HP2* is longer due to having two additional exons (exons 5 and 6) compared to *HP1*. The arrows indicate how the haptoglobin (HP) coding alleles combine, resulting in three possible genotypes: *HP1-1*, *HP2-1*, and *HP2-2*.

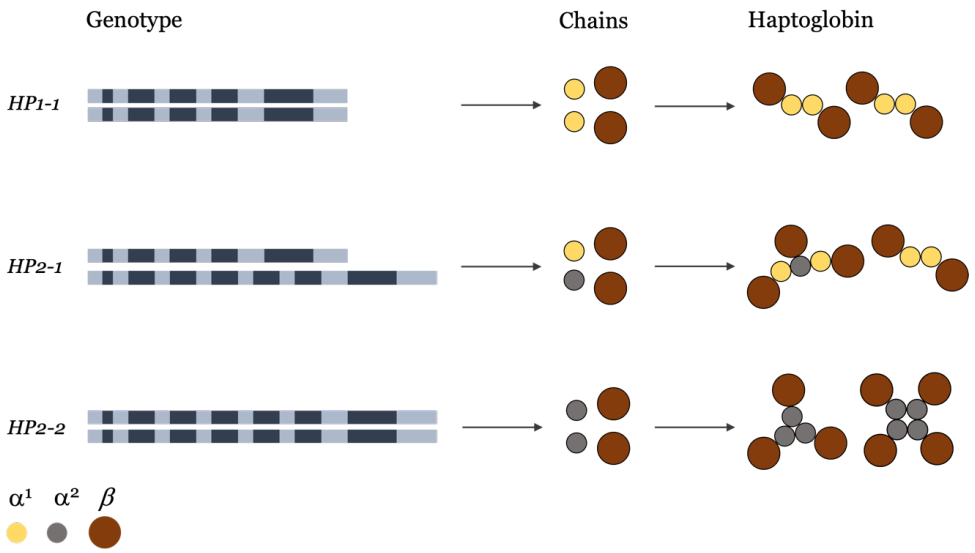

Figure 3 The haptoglobin gene (*HP*) codes for two different protein chains, α- and β-chains, forming the haptoglobin protein. The α-chain's properties differ between the two *HP* alleles, so that the α^1-chains coded by *HP1* only binds one other α-chain, whereas the *HP2* variant α^2 can bond with two α^1-chains, forming polymers. As a result, haptoglobin is secreted as dimers in genotype HP1-1, as a mixture of dimers and polymers in *HP2-1*, and as polymers in *HP2-2* (136).

These genetically defined properties of haptoglobin, together with its serum concentration, directly affect haptoglobin's haemoglobin binding capacity with a complex impact. For example, the haptoglobin coded by the *HP2* allele is associated with weaker haemoglobin binding, and the genotype *HP2-2* thus possesses the weakest haemoglobin binding and antioxidative properties (134,136). Simultaneously, however, *HP1* is associated with a weaker angiogenic effect (136,137).

The frequencies of different genotypes vary geographically. In European, North American, and Asian populations, *HP2-2* is the most commonly occurring genotype, followed by *HP2-1*, with *HP1-1* being the least frequent one. On the contrary, in many African populations, the *HP1* allele is the more dominant, and *HP2-2* is the least frequent (136).

2.3.3 Haptoglobin genotype and diabetes

Haematologic and genetic factors, including haptoglobin, have been suggested to play a role in cardiovascular disease associated with diabetes, although this association is not yet clear (133). The *HP2-2* genotype has been associated with increased risk of cardiovascular disease in type 2 diabetes (138,139). In type 1 diabetes, *HP2-2* has been linked to increased risk of coronary artery disease and cardiovascular mortality (140,141). On the other hand, the haptoglobin genotype *HP1-1* has been linked to cerebrovascular disease in type 1 diabetes (40,41), which will be described in further detail later (in section 2.4.7.4). It is thereby unclear whether the genotype poses a different impact depending on factors attributable to or associated with diabetes, such as hypertension, or whether the haptoglobin's action differ in different types of cardiovascular disease.

2.4 Complications of diabetes

Insulin deficiency and altered insulin action, such as insulin resistance, cause metabolic alterations, which may eventually lead to acute or chronic complications. The most prevalent long-term complications of diabetes are circulatory or vascular (46). Diabetes is also associated with an increased excess mortality (104). As the burden of vascular complications has decreased and the life expectancy in diabetes has improved, other chronic conditions associated with diabetes have been recognised. These include, for example, metabolic dysfunction-associated steatotic liver disease, sleep apnoea, cognitive decline, and an increased incidence of various infections (142,143).

2.4.1 Acute complications

Possible acute complications of diabetes are diabetic ketoacidosis, hyperglycaemic hyperosmolar state, lactic acidosis, and hypoglycaemia (142). The first three mentioned are caused by hyperglycaemia sprung from a state of insulin deficiency and the accompanying dysfunction in glucose downregulation. When there is an insufficient amount of insulin signalling in the body, the body starts to cleave its energy deposits, particularly fat. The process generates acidic metabolites that, when produced in excess, cannot be buffered, and this causes a state of global acidosis. Hyperglycaemia and acidosis cause osmotic diuresis, leading to volume depletion which worsens the acidosis. Ketoacidosis is predominantly seen in unmanaged type 1 diabetes and is a life-threatening medical emergency. A hyperosmolar state caused by hyperglycaemia typically occurs in the absence of ketosis and acidosis and is more common in type 2 diabetes than in type 1 (144).

Hypoglycaemia is an iatrogenic emergency caused by insulin overdosing relative to glucose intake and diminished counterregulatory response to low blood glucose. Hypoglycaemia generates an autonomic response and, in mild cases, palpitation and sweating may be the only manifestation. In severe cases, hypoglycaemia can cause convulsions and coma (145).

2.4.2 Long-term vascular complications

2.4.2.1 Classification of vascular complications

Vascular long-term complications of diabetes are commonly categorised as either micro- or macrovascular. Microvascular complications refer to diseases caused by pathologies in the capillaries and arterioles, also called microangiopathy, and their associated end-organ damage. These complications classically include diabetic retinopathy, diabetic kidney disease, and diabetic neuropathy (46). Diabetes-related pathologies in larger arteries such as coronary artery disease, cerebrovascular disease, and peripheral arterial disease are generally categorised as macrovascular complications (46). Outside the context of diabetes, these diseases are typically referred to as cardiovascular diseases, and this term is also frequently used in the literature for diabetic macrovascular complications (146).

There are diabetic complications featuring both micro- and macrovascular components, such as diabetic foot problems, and the distinction between micro- and macrovascular disease is not always clear or applicable. Moreover, micro- and macrovascular disease are strongly linked, they often coincide, and have been noticed to worsen in parallel (10,16,17). Either has been suggested to drive the other (18–20), and it is not fully unambiguous how micro- and macrovascular disease

impact each other. A recognised hypothesis is that they are “of common soil” and represent different manifestations of a common underlying cause (147,17,39,21).

2.4.2.2 Aetiology and pathogenesis of vascular complications

Microangiopathy and microvascular disease

Hyperglycaemia has a particularly harmful effect on cells that cannot regulate their glucose uptake in response to an increased glucose concentration in their surroundings, such as endothelial cells. Prolonged exposure to hyperglycaemia and altered metabolism causes structural and functional changes to the capillaries, which are key elements in microangiopathy. This eventually leads to deterioration of the vessels and impaired tissue perfusion, which in turn causes damage to the vascularised tissue, called end-organ damage (8).

Oxidative stress

Hyperglycaemia is associated with increased oxidative stress at the cellular level (8). This causes deterioration of the capillary walls in a process similar to normal ageing, but in diabetes, ageing and deterioration of the vessels happens prematurely (10). When there is a longstanding abundance of glucose in the blood, glucose adheres to the plasma proteins in a non-enzymatic process called glycation. This process additionally contributes to microvascular damage. The compounds formed in the process, so-called advanced glycation end-products (AGEs), interfere with normal protein function, such as receptor binding, and crosslink with other molecules, such as lipids, altering their action and metabolism both inside and outside the cell (148). The development of end-organ damage is further modified by genetic and other factors, such as coexisting diabetic complications, e.g., diabetic kidney disease and hypertension (8,30).

Endothelial dysfunction and ischaemia

Endothelial damage is characterised by the thickening of the basement membrane and impaired vessel integrity followed by structural changes, such as narrowing of the endothelial lumen. Accompanied by the disruption of normal endothelial regulation, these changes cause vessel occlusion and loss of perfusion, leading to ischaemia. On the other hand, ischaemia triggers a response to increase blood flow and vessel permeability in the area, causing hyperperfusion and leakage of the vessels (30,149,150).

In the eye, microvascular damage manifests as diabetic retinopathy, involving structural alteration of the blood vessels, signs of vessel bleeding and leakage, and eventually damage to the sensory tissue (150). In the kidneys, filtering of the blood is affected by structural and functional alterations in the microvasculature, causing proteins to leak from the blood into the urine and later, after further damage, also causing kidney function to decline (149).

Hyperglycaemia has also been suggested to induce damage by similar mechanisms in other microvascular beds, such as in the brain (29,30). Long-standing diabetes is associated with impaired regulation of cerebral blood circulation and brain atrophy (33,151). Although more well-known in type 2 diabetes (30–32), an increasing amount of evidence suggests that type 1 diabetes causes microvascular end-organ damage to the central nervous system in similarity with other microvascular complications (28,29,33–35).

Vessel leakage

Vessel damage induces inflammation, which in turn increases vessel permeability, i.e., leakage, further amplifying the process and creating a vicious cycle (10). Hyperglycaemic damage also disrupts the normal interaction between the endothelium and adjacent supporting cells. Supporting cells play a crucial role in controlling the environment in the tissue supplied by the capillaries. In the eye's retina, supporting cells include neural and glial cells that, together with pericytes, signal to the endothelium. They contribute to the proper functioning of the so-called inner blood-retinal barrier provided by the interendothelial tight junctions, and they form a functional unit that provides tight control of the retina's neural environment. Inflammation causes a breakdown of the blood-retinal barrier, and as the retinal environment becomes less favourable, the inflammation response is amplified, further feeding the cascade (150,152). Similarly, in diabetic kidney disease, supporting cells in the glomerulus of the kidney (podocytes) are damaged in association with microangiopathy. Like in retinopathy, the loss of podocytes accelerates the progression of the disease (149).

In the brain, the blood-brain barrier is the unit controlling the environment and interplay with the blood circulation. The blood-brain barrier resembles its counterpart in the eye. It consists of glial cells (astrocytes) and pericytes that, together with the endothelium, form a barrier between the blood and the brain's neurons, supplying them with nutrients, including energy, while protecting the neurons from harmful agents (30,153). The glucose uptake in the blood-brain barrier cells is insulin-independent and thereby not downregulated by hyperglycaemia (153). This renders the cells prone to hyperglycaemic damage. Disruption of the blood-brain barrier and the associated inflammatory response increases the permeability of the vessels. Like microvascular end-organ damage in the eyes and kidneys, altered regulation of the microvascular blood flow, vessel leakage, and inflammation eventually lead to central nervous system damage (30).

Arterial ageing and macrovascular disease

Diabetic macrovascular disease develops through similar mechanisms as microangiopathy (8,10). In the larger vessels, premature vascular ageing manifests as thickening and stiffening of the arterial wall, which leads to impaired haemodynamic regulation and atherosclerosis (10,11,21). In addition to

hyperglycaemia, insulin resistance and associated atherogenic conditions, e.g., dyslipidaemia, are strongly linked to the development of diabetic macrovascular disease (8,154). Macrovascular disease has also been associated with inflammatory markers (16,155,156), low-grade inflammation, and haematologic factors such as haptoglobin, and these are thought to play a pivotal role in the development of macrovascular complications (133).

Moreover, vascular diseases tend to cluster, which further affects the development of macrovascular disease. The presence of diabetic kidney disease and hypertension, for example, affects the impact of hyperglycaemia, demonstrating a complex interaction between micro and macrovascular disease. (157,158)

2.4.3 Diabetic eye disease

Long-term complications of diabetes include those affecting the eyes, particularly diabetic retinopathy and macular oedema. Other eye complications associated with diabetes include cataracts and glaucoma (159). Diabetic retinopathy is a neurovascular end-organ complication highly specific to diabetes (160). It degenerates the vasculature and the neurons of the retina, causes swelling called macular oedema, and, if left untreated, progressively leads to vision impairment and blindness (150,161).

2.4.3.1 Prevalence of diabetic retinopathy and macular oedema

Diabetic retinopathy is one of the most prevalent complications of diabetes. It has been estimated that around 20–30% of all individuals with diabetes have some degree of retinopathy (162–164). The proportion of individuals developing severe, vision-threatening diabetic retinopathy is smaller, around 2–6% globally by approximation (163,164).

The prevalence of diabetic retinopathy varies across different regions (163,164), ethnicity, and diabetes type (162,163). In the Western world, diabetic retinopathy and macular oedema are more prevalent in type 1 diabetes compared to type 2 (162,163), and it has been reported that more than half of individuals with type 1 diabetes show some degree of diabetic retinopathy after 10 years of diabetes, and almost 90% after 20 years of diabetes (162). Severe diabetic retinopathy affects less than a third of individuals with type 1 diabetes, with the prevalence ranging from around 2–30% depending on the duration of diabetes. In type 2 diabetes, however, severe diabetic retinopathy is more common (162,163).

2.4.3.2 Pathogenesis of diabetic retinopathy and macular oedema

The retina is a sensory organ with high metabolic activity, rich vascularisation, and complex interaction with this supporting vasculature – the so-called blood-retinal barrier. As such, the retina is sensitive to metabolic disturbances, such as hyperglycaemia (150). The anatomy of the eye and retina are illustrated in Figure 4.

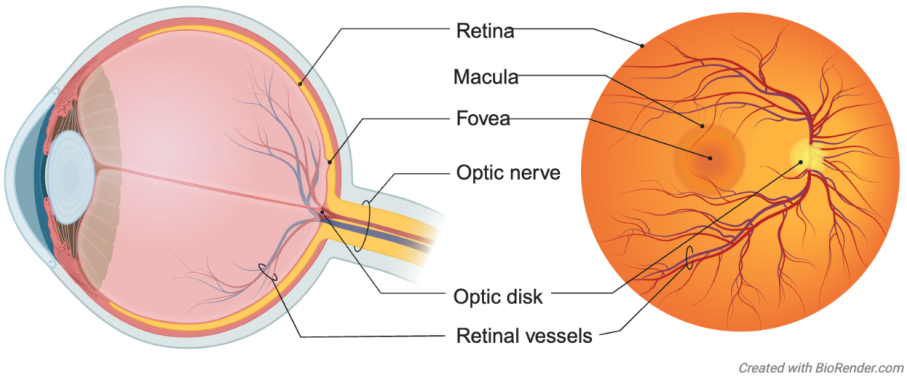

Figure 4 Schematic illustration of a sagittal cross section of the eye (on the left) and the interior surface of the posterior part of the eye, the fundus, viewed from the lens (on the right).

The first clinically noticeable signs of microvascular damage in the retina are local outpouchings of the capillary walls (microaneurysms). Later, ischaemia triggers a response to grow new vessels (neovascularization), which is the characteristic clinical trait for proliferative diabetic retinopathy. Ischaemia and vessel leakage result in the accumulation of extracellular fluid and thickening of the normally compact tissue of the macula, which is the area for sharp central colour vision on the retina (150,152). Macular oedema, which is swelling, can occur at any stage of diabetic retinopathy, but as diabetic retinopathy progresses, the risk of macular oedema also increases (150). Figure 5 on the following page illustrates the pathological pathways leading up to typical manifestations of diabetic retinopathy and macular oedema.

2.4.3.3 Risk factors

Risk factors for diabetic retinopathy

The development of diabetic retinopathy is most strongly linked to the duration of diabetes and poor glycaemic control (162,165–167). Hyperglycaemia and diabetes duration increase the risk of diabetic retinopathy overall (162,165,166) and are associated with the severity of the disease (165–168).

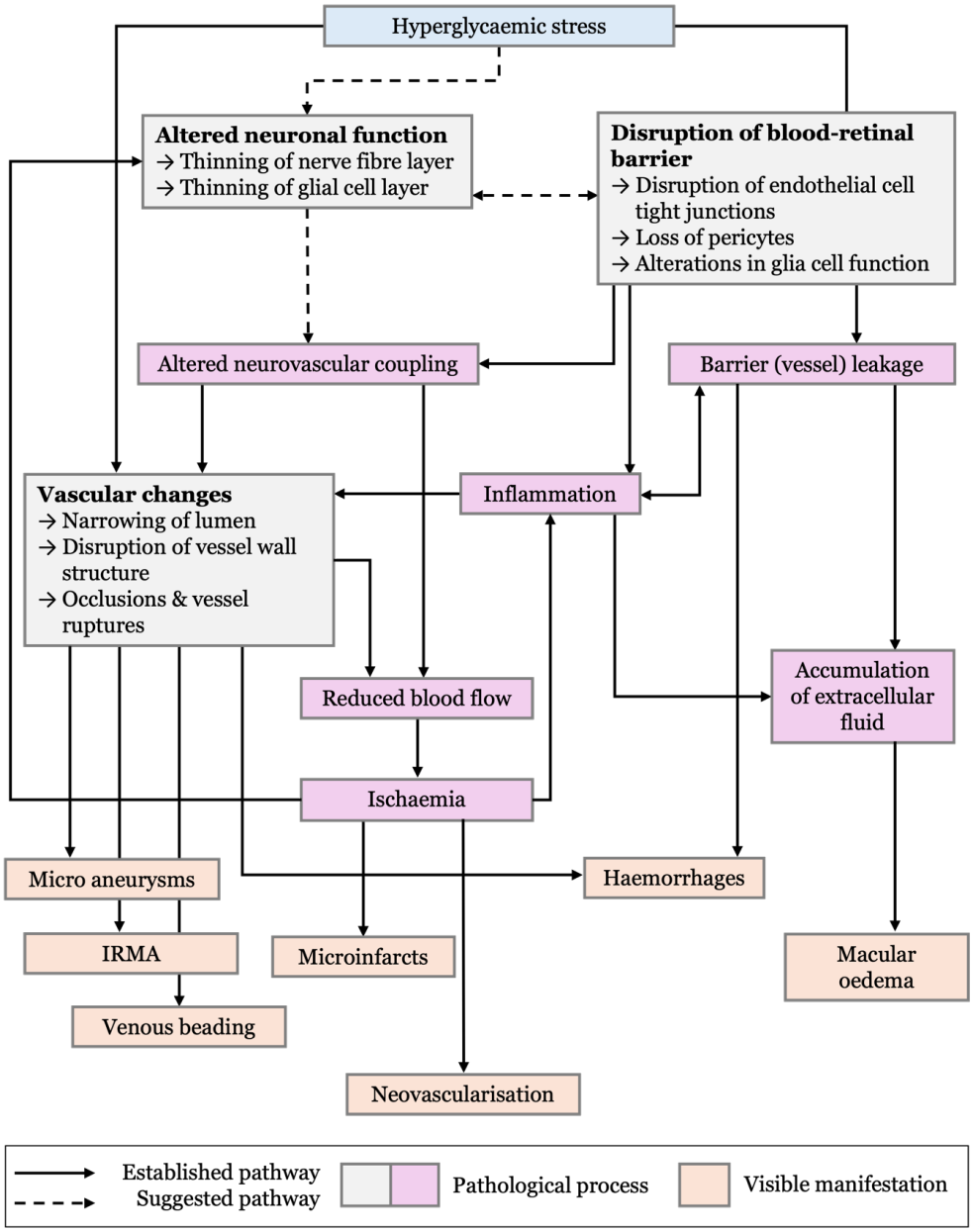

Figure 5 Long-term hyperglycaemia disrupts the selective permeability of the blood-retinal barrier, causing barrier leakage and altered neurovascular coupling that is neuronal regulation of blood flow and vessel maintenance. Breakdown of the blood-retinal barrier leads to a self-amplifying cascade causing structural and functional changes to the vessels, retinal ischaemia, and neuronal damage. This triggers a response to reoxygenate the tissue by increasing permeability of the vessel walls and growing new vessels (neovascularization), which are of poor quality and prone to occlusion and rupture, hence worsening the ischaemia. Hyperglycaemia also directly alters the function of the retinal neurons independent of vascular damage, although the interaction between early nerve fibre loss and vascular damage is not fully understood (150,152,169).

Elevated blood pressure also seems to increase the risk of developing diabetic retinopathy (20), particularly proliferative diabetic retinopathy (167,170,171). Most studies linking blood pressure to any diabetic retinopathy, including non-proliferative disease, however, are cross-sectional (172,173), and the causal relationship is not fully clear. It has been suggested that blood pressure is primarily a risk factor for progression of diabetic retinopathy (167,171), yet when studied in parallel, blood pressure was associated with proliferative disease but not with the progression itself (170).

Similarly, diabetic kidney disease has been described as a risk factor for diabetic retinopathy (166–168), although an association has been absent (172) or limited to a subset of individuals, such as those with longer duration of diabetes, in some studies (165).

Risk factors for macular oedema

Macular oedema shares risk factors with diabetic retinopathy, and hyperglycaemia and diabetes duration are the main driving factors (167,174). Other risk factors linked to macular oedema include elevated blood pressure (174) and diabetic kidney disease (165).

On the other hand, the risk profile for macular oedema has, in some studies, differed slightly from the vascular manifestations of diabetic retinopathy. In type 1 diabetes, the impact of kidney disease, for example, has been observed to be weaker for macular oedema than for diabetic retinopathy (170,174). Further, some studies have found dyslipidaemia to be associated with an increased risk of macular oedema but not of diabetic retinopathy (153,167). Even so, there is divergence in the reports regarding cholesterol. Whereas some studies have associated diabetic retinopathy with increased cholesterol (162,175) or triglyceride concentrations (168,176), others have even found the prevalence of diabetic retinopathy to decrease with increasing cholesterol (172).

2.4.3.4 Fundus photography and examination of the retina

As the eye is structured to let the retina see the outside world, the retina is also directly visible and accessible for examination by non-invasive techniques, such as slit-lamp biomicroscopy and fundus photography (44,177). Fundus photographs and/or biomicroscopy reveal signs of an ongoing disease process through findings in the form of microvascular abnormalities, for example, microaneurysms, haemorrhages, signs of vessel leakage (hard exudates) and, at an advanced stage, neovascularisation (150).

Screening for diabetic retinopathy

Fundus photography is used to screen for diabetic retinopathy to identify individuals at risk of retinopathy progression, prevent vision impairment, and

recognise when treatment interventions should be implemented to prevent or reverse the loss of vision (178,179). The principle of the screening involves retinal examinations performed at regular intervals of one to two years and referral to further ophthalmologic examinations, treatment, and/or intensified follow-up based on findings in the screening (177). Figure 6 demonstrates observations of diabetic retinopathy in fundus photography.

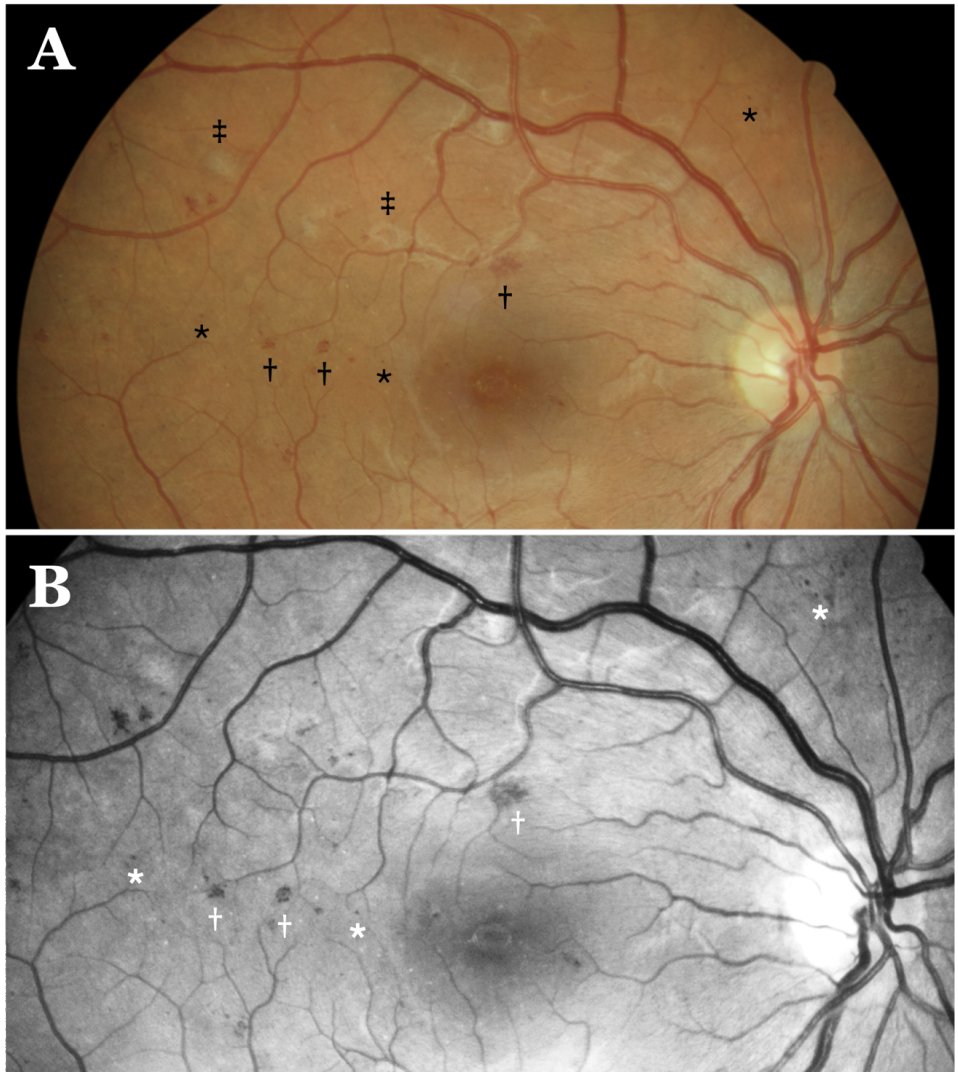

Figure 6 Manifestations of diabetic retinopathy are visible in fundus photography. Images A (in colour) and B (black and white) represent the same right eye with visible microaneurysms (*), haemorrhages (†), and microinfarcts (‡). The images are modified from an anonymised FinnDiane participant's fundus photography.

For example, the American Diabetes Association recommends annual screening both for individuals with type 1 and type 2 diabetes. If no signs of retinopathy are present in the annual examinations, then increasing the interval to two years may be considered. If there is evidence of progressing retinopathy, examinations are recommended to be performed more frequently than once a year (160,178).

The Finnish national screening guidelines differ slightly from the American, as they recommend screening individuals with type 1 diabetes every other year and those with type 2 diabetes every third year, for as long as there are no signs of retinopathy or macular oedema. If diabetic retinopathy is present, individuals with type 1 diabetes are referred to annual or more frequent screening based on retinopathy severity. The same protocol is used for type 2 diabetes unless the retinopathy is very mild, in which case screening every second year is advised (179). Although screening protocols show some regional differences, fundus photography is, to date, considered the most efficient method for diabetic retinopathy screening (177).

Retinal examination for other purposes

While the primary goal of diabetic retinopathy screening is the prevention of visual impairment, eye examinations may additionally give information about disease and conditions affecting other tissues as well, particularly circulation and the brain, as they can manifest themselves in the retina (44,180,181). The retina is an extension of the brain, and they share developmental, functional, and metabolic features. Both are glucose-dependent in a similar way, and the blood-brain barrier acts similar to its counterpart in the retina (45,182). Pathologies of the retina are, hence, suggested to reflect similar, parallelly occurring processes in the brain (39,44,45). As the retina is the only structure of the central nervous system accessible for direct non-invasive imaging, the eye is, indeed, considered to be the window to the brain (44,45).

2.4.3.5 Classification of diabetic retinopathy and macular oedema

The tell-tale sign of diabetic retinopathy, marking the presence of the disease, is retinal microaneurysms. Neovascularisation is the defining marker of proliferative diabetic retinopathy. In the assessment of fundus photographs, retinal thickening, and hard exudates are signs of macular oedema. In addition to these definitions of the disease, different classification protocols are used to describe the severity of diabetic retinopathy and macular oedema, depending on the setting (183).

The Early Treatment Diabetic Retinopathy Severity Study

In 1991, the Early Treatment Diabetic Retinopathy Severity Study (ETDRS) proposed a protocol for grading diabetic retinopathy from fundus photographs by scores ranging from 10–90 (184). The ETDRS was a collaborative clinical trial

designed to assess measures to reduce the progression of diabetic retinopathy (185). An update and revision of the originally proposed grading scale was later published in 1998 by the same group (168), and that final version of the grading scale has also been widely used outside the ETDRS ever since.

The ETDRS grading scale was developed in a research setting to indicate the risk of diabetic retinopathy progression (168) and is not commonly used in clinical practice or when screening for diabetic retinopathy. Although the ETDRS scale is considered the "gold standard" for grading diabetic retinopathy severity in clinical research, it is unpractical in a clinical everyday setting. The Proposed International Clinical Severity Scale has therefore been developed by C.P. Wilkinson and his colleagues in the Global Diabetic Retinopathy Project Group (183). Wilkinson's proposed scale contains fewer levels than the ETDRS scale (168,183); however, it is still more detailed than the diagnosis codes in the International Code of Disease, Tenth Revision (ICD-10) (186). A comparison of these different classifications is presented in Table 3.

The ETDRS also assessed macular oedema from fundus stereo photographs and used a classification of three categories based on the presence/absence of oedema, if present, and proximity of the retinal thickening to the centre of the macula, the fovea (see Figure 4). Briefly, retinal thickening or hard exudates close to the fovea are taken as evidence of macular oedema, and macular oedema is considered clinically significant if the thickening involves the centre of the macula (187).

The Proposed International Clinical Severity Scale recommends a two-tiered system for classification of macular oedema (183). The initial grading categorises macular oedema as apparently absent or present. If present, macular oedema can further be graded as mild, moderate, or severe. The ETDRS classification is stricter in defining the presence versus absence of macular oedema and defines fewer levels (187), whereas the Proposed International Clinical Classification contains more levels for describing the severity (183). Table 4 shows a comparison of the severity scales.

Table 3 Comparison of classifications of diabetic retinopathy severity

	ETDRS severity scale (168)		**Proposed International Clinical Severity Scale** (183)	**ICD-10** (186)
Observable findings	Score	Description	Description	Description, diagnose code
No abnormalities	10	DR absent	No apparent DR	
Isolated abnormalities, no microaneurysms	14/15	DR questionable		
Microaneurysms only	20	Very mild NPDR	Mild NDPR	Background DR, H36.00
More than only microaneurysms	35	Mild NPDR	Moderate NPDR	
	43	Moderate NPDR		
	47	Moderately severe NPDR		
Extensive intraretinal haemorrhages / definite venous beading / prominent intraretinal microvascular abnormalities	53	Severe NPDR	Severe NPDR	Pre-proliferative DR, H36.02
	55	Very severe NPDR		
Neovascularization or vitreous/preretinal haemorrhage	61	Mild PDR	PDR	PDR, H36.03
	65	Moderate PDR		
	71/75	High-risk PDR		
	81/85	Advanced PDR		
	90	Cannot grade		

The table shows how the Early Treatment Diabetic Retinopathy Study (ETDRS) retinopathy severity grading scale (168) corresponds to the Proposed International Clinical Diabetic Retinopathy Severity Scale (183) and the International Code of Disease, Tenth Revision (ICD-10) (186), in reference to a brief description of retinal abnormalities in fundus examination.

DR indicates diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; and PDR, proliferative diabetic retinopathy.

Table 4 Comparison of classifications of macular oedema severity

Observations of retinal thickening or hard exudates	**ETDRS severity scale** (187)	**Proposed International Clinical Severity Scale** (183)	
		Initial grading	2nd level grading
Absent	No DME	No apparent DME	No apparent DME
Apparent in posterior pole*		DME apparently present	Mild DME
Within 1 disc diameter of the fovea	DME		Moderate DME
Retinal thickening ≤ 500 µm from the centre of the macula or hard exudates ≤ 500 µm from the centre of the macula and associated with critical retinal thickening (not specified here)	Clinically significant macular oedema		Severe DME

The table presents the key retinal observations described by the Early Treatment Diabetic Retinopathy Study (ETDRS) (187) and corresponding severity level according to the ETDRS and Proposed International Clinical Classification of macular oedema (105). DME indicates diabetic macular oedema.

*Description by Proposed International Clinical Classification and absent in ETDRS classification

2.4.3.6 Retinal photocoagulation

Diabetic retinopathy associated with high risk of vision loss, such as proliferative and severe non-proliferative diabetic retinopathy can be treated by laser photocoagulation therapy. The treatment aims to halt neovascularisation and alleviate the symptoms of microangiopathy, thereby reducing the risk of vision loss. Pharmacological treatments, such as intravitreous injections of anti-vascular endothelial growth factor (anti-VEGF), are also used for proliferative retinopathy and are the first-line treatment in vision threatening macular oedema (178,179).

2.4.4 Diabetic kidney disease

Diabetic kidney disease is a microvascular complication damaging the kidney tissue, including the functional units, or nephrons, with their urine-filtering glomerulus. This damage causes proteins, albumin being the most abundant one, to leak into the urine. Consequently, diabetic kidney disease is characterised by an increased urinary albumin excretion rate (albuminuria), which progressively increases as the disease worsens. As the disease progresses, the glomerular filtration rate decreases, indicating that the kidney function decreases, which may eventually lead to kidney failure and the need for kidney replacement therapy in the form of dialysis or a kidney transplant (188,189).

2.4.4.1 Epidemiology

Between 30–60% of all individuals with diabetes have diabetic kidney disease. The prevalence varies regionally and by diabetes type, duration of diabetes, and the definition and/or classification of diabetic kidney disease (190). Diabetic kidney disease is more common in type 2 diabetes compared to type 1 diabetes (191–193). While over 40% of individuals with type 2 diabetes develop diabetic kidney disease within 25–30 years of diabetes, the corresponding number in type 1 diabetes is closer to 20% (191,194). The incidence of kidney failure leading to kidney replacement therapy, however, is lower, and around 10% develop kidney failure (194,195). Diabetic kidney disease in type 1 diabetes does not typically develop during the first years of type 1 diabetes and is mostly preceded by diabetic retinopathy and mild albuminuria. In type 2 diabetes, on the other hand, kidney disease may already be present at diagnosis and without simultaneous diabetic retinopathy (196). Kidney disease in type 2 diabetes is more heterogeneous than in type 1 diabetes, and declining kidney function often presents without albuminuria (197), which is rather uncommon in type 1 diabetes (198).

2.4.4.2 Screening and diagnosis

Screening for diabetic kidney disease takes place by measuring urinary albumin excretion and estimated glomerular filtration rate. Screening is recommended yearly starting at the year of diabetes diagnosis in individuals with type 2 diabetes and starting at five years from diagnosis in those with type 1 diabetes (196,199).

Clinical diagnosis

The diagnosis of diabetic kidney disease is usually clinical. Persistent albuminuria or/and decreased estimated glomerular filtration rate is taken as evidence of diabetic kidney disease when overt signs or symptoms of other causes of kidney damage are absent (196,199). Diabetic kidney disease typically occurs after a long

duration of diabetes and in association with diabetic retinopathy. The classical presentation includes albuminuria without evident haematuria and a gradually decreasing glomerular filtration rate. Yet, reduced glomerular filtration rate without albuminuria occurs in both type 1 and type 2 diabetes (196).

Classification

Diabetic kidney disease is categorised following the classification of chronic kidney disease recommended by the consensus conference *Kidney Disease: Improving Global Outcomes* (KDIGO) (200). The KDIGO classification defines chronic kidney disease and the associated prognosis by persistent (lasting over three months) albumin excretion rate and kidney function according to the estimated glomerular filtration rate, as shown in Table 5.

Table 5 KDIGO classification of chronic kidney disease

Prognosis of chronic kidney disease by albuminuria categories and eGFR, as presented by KDIGO (200)			Categories by albuminuria		
			Normal to mildly increased	Moderately increased	Severely increased
			Albumin/Creatinine ratio		
			< 3 mg/mmol	3–30 mg/mmol	> 30 mg/mmol
			24-hour urine collection		
			< 30 mg/24h	30–300 g/24h	> 300 mg/24h
			Timed urine collection*		
			< 20 µg/min	20–200 µg/min	> 200 µg/min
Categories by eGFR (ml/min/1.73 m^2)	Normal or high	≥ 90			
	Mildly decreased	60–89			
	Mildly to moderately decreased	45–59			
	Moderately to severely decreased	30–44			
	Severely decreased	15–29			
	Kidney failure	<15			

Green indicates no chronic kidney disease, low risk; yellow: chronic kidney disease, moderately increased risk; orange: chronic kidney disease, high risk; red: chronic kidney disease, very high risk. eGFR indicates estimated glomerular filtration rate.

*Compared to the original table published by the *Kidney Disease: Improving Global Outcomes* (KDIGO) consensus conference, the table has been modified to include threshold values for albumin excretion rate in timed urine samples corresponding to those of 24-hour urine collection according to the Finnish guidelines (199).

2.4.4.3 Risk factors and management

The risk of developing diabetic kidney disease increases with age and duration of the diabetes. Other non-modifiable factors impacting the diabetic kidney disease risk include age at onset of diabetes, sex, and genetic factors (195,201). To improve kidney and cardiovascular outcomes, the treatment of individuals with diabetic kidney disease should be comprehensive, accounting for lifestyle factors and comorbidities (188,202).

Risk factors

The incidence rate of severe albuminuria increases with the duration of type 1 diabetes (194). This, however, is a quite recent phenomenon: In individuals diagnosed with type 1 diabetes before 1980, the incidence rate of kidney disease has been at its highest peak around 20 years after the diabetes diagnosis, whereafter it has decreased (194,203). When comparing individuals diagnosed with diabetes before versus after 1980, the 25-year cumulative incidence of severe albuminuria has decreased. As the rate of new severe albuminuria cases is growing by diabetes duration in these individuals, however, it is not yet clear if the management strategies are truly preventing the disease or only postponing it (194).

Poor glycaemic control is the major modifiable risk factor for diabetic kidney disease (47,204). Other modifiable risk factors include high blood pressure (18,110) and dyslipidaemia (175,205). Diabetic kidney disease is also linked to lifestyle factors, including low physical activity, and poor mental well-being, such as depression (206,207). Low physical activity is associated with both initiation and progression of the disease (208). Other lifestyle-related risk factors include abdominal obesity (209–211) and smoking (212). Clustering of these unfavourable metabolic attributes and insulin resistance, as in the metabolic syndrome, further adds to the risk (205,213–215).

Management of diabetic kidney disease

The five key elements for managing diabetic kidney disease include 1) maintaining optimal glycaemic control with an HbA_{1c} below 53 mmol/mol; 2) ensuring optimal blood pressure control, aiming for a reading below 130/80 mmHg; 3) achieving the best possible lipid control, with LDL cholesterol levels under 1.4 mmol/L; 4) encouraging lifestyle modifications, particularly smoking cessation; and 5) utilizing prognosis-improving medications. Glycaemic control in type 1 diabetes relies on insulin, while in individuals with type 2 diabetes, sodium-glucose cotransporter-2 inhibitors and semaglutide have been shown to improve the prognosis. The first-line pharmaceutical therapy for blood pressure management in all individuals with diabetic kidney disease is renin-angiotensin system inhibitors, and statins are the recommended lipid-lowering treatment (196,202).

Response to treatment should be followed up after three to six months, whereafter yearly evaluations, at a minimum, are recommended. If the kidney disease progresses to kidney failure, the individual will require kidney replacement therapy, meaning dialysis or kidney transplantation, for their immediate survival (199,202).

2.4.5 Diabetic neuropathy

Metabolic dysfunction damages the peripheral neurons and their supporting tissues, such as the glia and vasculature, leading to diabetic neuropathy. Diabetic neuropathy includes manifestations in both somatic and autonomic parts of the peripheral nervous system and involves both sensory and motor nerves. In the lower limbs, this may, for example, cause neuropathic pain or severe foot problems because of sensory loss, loss of reflexes and postural awareness, and reduced sweat production, leading to dry skin (216).

2.4.5.1 Epidemiology

Diabetic neuropathies are perceived to be the most common complications of diabetes. Around 30–35% of individuals with diabetes have been reported to have diabetic neuropathy (217–219); however, due to the symptoms being diverse and unspecific, this may be an underestimation. For example, in the DCCT/EDIC Study with a well-characterised group of individuals with type 1 diabetes receiving intensive insulin therapy, 34% of the participants developed clinically evident distal peripheral neuropathy during 20 years of follow-up after their first study visit. Yet, within the same 20 years, 54% of the studied individuals had abnormal findings in nerve conduction studies. Similarly, the 20-year incidence of cardiovascular autonomic and peripheral distal neuropathy ranged from 20–30%, whereas the incidence of abnormal nerve conduction was higher, at 45% (219).

2.4.5.2 Diagnosis and classification

Diagnosis

Neuropathy is assessed based on medical history, symptoms, and clinical tests, such as testing of the reflexes and pinprick sensation to assess distal neuropathy. The diagnosis relies on clinical evaluation and exclusion of other comorbidities that may present with similar symptoms (178).

Classification

Among the various manifestations of diabetic neuropathy, distal symmetric polyneuropathy such as foot problems described in the example above and diabetic

autonomic neuropathy such as cardiovascular autonomic neuropathy and gastrointestinal neuropathy are the most well-described (216,220). Table 6 lists diabetic neuropathies according to the classification by the American Diabetes Association.

Table 6 American Diabetes Association classifications of diabetic neuropathies (220)

Diabetic neuropathies	Examples of symptoms / clinical manifestations
A. Diffuse neuropathy	
Distal symmetrical polyneuropathy	
• Primary small-fibre neuropathy	• Pain, pinprick sensations reduced/absent
• Primary large-fibre neuropathy	• Numbness, poor balance, reduced/ absent ankle reflexes
• Mixed small- and large-fibre neuropathy (most common)	
Autonomic neuropathy	
• Cardiovascular	• Resting tachycardia, orthostatic hypotension
• Gastrointestinal	• Diabetic gastroparesis (gastropathy), enteropathy (diarrhoea), colonic hypomobility (constipation)
• Urogenital	• Bladder and/or sexual dysfunctions
• Sudomotor dysfunction	• Reduced/increased distal sweat production
• Hypoglycaemia unawareness	
• Abnormal pupillary function	
B. Mononeuropathy (atypical forms)	
C. Radiculopathy or polyradiculopathy (atypical forms)	

The table lists different types of diabetic neuropathy by the classification proposed by the American Diabetes Association (ADA). The table is an abbreviated version of the one published by the ADA.

2.4.5.3 Risk factors and management

Poor glycaemic control increases the risk of diabetic neuropathy (221), and the primary prevention of diabetic neuropathy focuses on glucose control (49,50,220). Additionally, a multifactorial approach accounting not only for comorbidities, such as hypertension, but also lifestyle factors is recommended both for the prevention and management of neuropathy (178,220).

For individuals with type 2 diabetes, neuropathy assessment should be performed yearly after diabetes onset. Similarly, in type 1 diabetes, yearly assessments are recommended, but starting at five years of diabetes duration.

Treatment of diabetic neuropathies includes secondary prevention through intensive diabetes management, as well as treatment of the symptoms of neuropathy (49,50,178,220).

2.4.6 Cardiovascular disease

Cardiovascular disease typically refers to conditions affecting the heart and blood vessels, such as coronary artery disease, stroke, and peripheral arterial disease. Heart failure associated with diabetes is often also reviewed in the context of cardiovascular complications (146). This section provides an outline of the epidemiology, risk factors, and management of cardiovascular complications of diabetes. A more thorough review of cerebrovascular complications and stroke will follow in the next section (section 2.4.7).

2.4.6.1 Epidemiology

Although cardiovascular and peripheral arterial disease are not specific to diabetes, individuals with diabetes have a fivefold increased risk of cardiovascular disease compared to the general population and, similarly, a substantially increased risk of peripheral arterial disease (24,222,223,25). As for men in the general population, those with diabetes are more likely to develop cardiovascular disease compared to women with diabetes (24,224,225). Women with diabetes, however, have a higher relative risk of cardiovascular disease than men. Compared to women without diabetes, women with either type 1 or type 2 diabetes are estimated to have a fourteen times higher risk of cardiovascular disease. In men, the corresponding risk increase is eightfold in type 1 diabetes and threefold in type 2 diabetes (25,224). This indicates that although men with diabetes are more likely to get cardiovascular disease, the hazard conferred by diabetes is greater in women than in men (24,25,224).

Coronary artery disease and peripheral arterial disease

Coronary artery disease constitutes approximately 30% of macrovascular incidents and, at least in type 1 diabetes, most are acute myocardial infarctions (24,223). Although similar numbers have been reported for peripheral arterial disease, there seems to be more dispersion between reports, which might be explained by differences in the diagnostic methods and the definitions of the disease (24,223,226).

Heart failure

Heart failure accounts for around 15–20% of cardiovascular morbidity in diabetes and may occur in association with or in the absence of coronary heart disease

(24,223). Although commonly considered a macrovascular disease, heart failure is a collective name for many states of cardiac dysfunction with different aetiologies and presentations, e.g., preserved or reduced ejection fraction, and the pathophysiology in diabetes is not fully understood (227). With reference to the *common soil* hypothesis, it has been suggested that heart failure may be a manifestation of microvascular disease and metabolic abnormalities (228).

2.4.6.2 Risk factors and management

Primary prevention

Screening of a particular cardiovascular disease, such as coronary artery disease, has not been shown to prevent the disease; rather, a comprehensive assessment of risk factors is recommended (229). Age and duration of diabetes are among the most prominent risk factors for cardiovascular disease in diabetes. Except for these non-modifiable risk factors, other well-characterised risk factors include poor glycaemic control, diabetic kidney disease, hypertension, and dyslipidaemia (103,106,158,230), and prevention of cardiovascular disease largely focuses on these (229). Intensive glucose control and blood pressure-lowering treatment reduces cardiovascular incidents in both type 1 and type 2 diabetes (231,230,232,111).

Other aspects to consider in primary prevention are socioeconomic or lifestyle factors, such as low income and smoking, that significantly impact the risk of cardiovascular disease (103,106,158). Considering non-modifiable risk factors, such as diabetes duration, is highly relevant for prevention. As both micro- and macrovascular complications increase with the duration of diabetes (225,233,234), so does the interaction between them (234), and albuminuria is among the strongest risk markers for both myocardial infarction, heart failure, and stroke. The piling of complications linked to metabolic dysfunction further adds to the cardiovascular risk, wherefore primary prevention is of essence (154,235).

Secondary prevention

In individuals who develop macrovascular disease, the emphasis is on preventing the progression of the disease and collateral morbidities, in addition to managing the specific complication (49,50,229,236). Antiplatelet therapy, primarily aspirin therapy, is recommended for all individuals with a history of cardiovascular or peripheral arterial disease (229,236). Whenever appropriate, agents with both cardiovascular and kidney benefits, such as renin-angiotensin system inhibitors and angiotensin receptor antagonists, should be preferred (229). In type 2 diabetes, glucose-lowering treatment with sodium-glucose cotransporter 2 inhibitors and glucagon-like peptide 1 glucose has proven particularly beneficial for lowering the

risk of cardiovascular disease and heart failure. However, these agents are not recommended for individuals with type 1 diabetes (229,236).

2.4.7 Cerebrovascular disease

The central nervous system, including the brain, is in constant need of oxygen and glucose, which is supplied by blood circulation. Disruption of this circulation rapidly leads to permanent damage to the central nervous system. Cerebrovascular disease encompasses an array of pathological states with the common feature of impaired blood circulation in the brain. This group of circulatory disorders ranges from states of no clinical neurological symptoms or slowly progressing cognitive symptoms to rapidly onset loss of neurological function, as is typically seen, for example, in stroke (237). These neurological disorders, particularly stroke and dementia, are among the leading causes of disability and the second most common cause of death (23,238).

2.4.7.1 Classification of cerebrovascular disease

Cerebrovascular disease is categorised by aetiology and clinical manifestations. The diagnosis typically relies on features of both, as it is based on neuropathological, neuroimaging, and/or clinical evidence of injury (239). As with cerebrovascular diseases themselves, their classification is complex and overlapping, as will be demonstrated in the upcoming sections.

Classification by aetiology

Infarction or haemorrhage

An infarction in the central nervous system indicates cell death in the brain, spinal cord, or retina that is attributable to ischaemia. Damage to the central nervous system may also result from arterial disruption and haemorrhage in the brain tissue, the subarachnoid space, or the ventricle system, which is not caused by trauma (239).

Classification by vessel type and/or origin

Both infarctions and haemorrhages can be further subdivided according to the blood vessel type involved, e.g., large artery or small vessel occlusion, or site of the vessels, e.g., within the brain parenchyma (intracerebral haemorrhage) or on the surface of the brain (subarachnoid haemorrhage). Cerebrovascular disease and events are further categorised by other aetiological features if or when the distinction can be made, such as differentiation between stenosis and/or thrombosis at the site or occlusion caused by embolization (239,240).

Classification by clinical symptoms

Overt or covert events

Central nervous system infarctions occur over a spectrum of clinical symptoms. Ischaemia may be of a chronic, subclinical nature with slowly developing lesions or incomplete infarctions. Or it may be acute, ranging from silent infarctions that cause no known symptoms to overt disease like stroke, which causes neurological symptoms such as paresis (36,239). Similarly, haemorrhages may be silent, without acute neurological dysfunction attributable to the lesion, or they may present as a stroke (239).

Cognitive impairment

Although brain infarctions and haemorrhages may not cause immediate symptoms, both overt and silent events may be associated with cognitive decline. Cerebrovascular disease is associated with an increased risk of problems with information processing, and it plays a leading role in cognitive decline in the elderly (23,237). Cognitive impairment may be linked to critical focal injury (post-stroke) or to a more diffuse degradation of the brain vessels and tissue, such as cerebral small vessel disease, which we will review in detail in the following section (36,237).

2.4.7.2 Cerebral small vessel disease

Cerebral small vessel disease (CSVD) is a collective noun for disease of the microvasculature of the central nervous system. It encompasses all pathological processes that affect the small vessels of both the arterial and the venous compartment, that is, the arterioles, venules, and capillaries (36,37). As a term, *cerebral small vessel disease* takes on various meanings and will be reviewed in this thesis in the context of standardised features visible by neuroradiological imaging (37).

The clinical manifestations of CSVD are diverse. The disease may be asymptomatic or associated with overt stroke, and it often coexists with dementia (36,37). In young individuals without neurological symptoms, CSVD has been observed to be more common in individuals with both type 1 or type 2 diabetes compared to controls (31,33,35).

Definition and diagnosis by MRI

CSVD leads to heterogeneous alterations in the brain parenchyma, including lesions in the white and/or grey matter and atrophy that are visible in magnetic resonance imaging (MRI) of the brain. As the small size of the affected vessels poses a challenge for *in vivo* imaging, distinctive features visible by neuroimaging with MRI are used as markers of the disease (36,37).

Definition of cerebral small vessel disease

A unifying neuroimaging standard and terminology for the definition of CSVD was proposed in 2013 by Wardlaw and colleagues (37). The authors suggested an analysis standard for MRI that accounts for the presence and quality of the markers listed below and illustrated in Figure 7:

- Recent subcortical infarcts
- Lacunes of presumed vascular origin
- White matter hyperintensities
- Cerebral microbleeds
- Enlarged perivascular spaces
- Brain atrophy

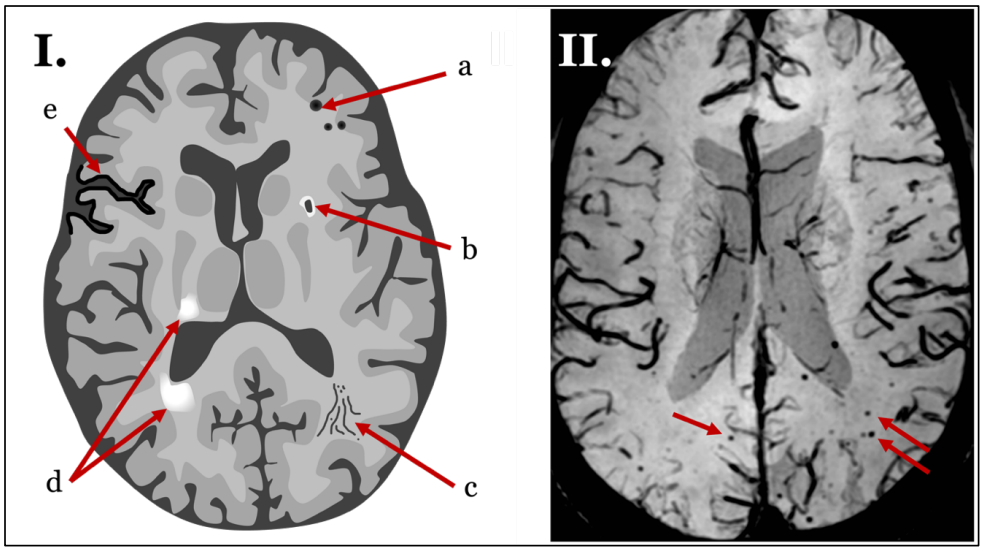

Figure 7 I. An animated illustration of cerebral small vessel disease markers visible in magnetic resonance (MR) imaging where a) represents cerebral microbleeds, b) a lacune, c) enlarged perivascular spaces, d) white matter hyperintensities, and e) superficial siderosis. II. A MR image of a FinnDiane Study participant's brain presenting with cerebral microbleeds, indicated by the red arrows.

Ischaemic lesions

CSVD is accompanied by ischaemic damage to the central nervous system, which may manifest as recent small subcortical infarctions when the lesion has occurred within the previous week. Later, these infarctions may be seen as small cavities, called lacunes, in the territory of the affected perforating arteriole. Lacunes of presumed vascular origin, as in small vessel disease, may also stem from cerebral haemorrhages (37).

Lesions in the white matter are visible as hyperintensities on MRI. These white matter hyperintensities of presumed vascular origin are suggested to reflect small

vessel disease-related ischaemia in the central nervous system (36,37) and have been linked to cerebral arterial stiffness (241). CSVD is also accompanied by lower brain volume, or atrophy, which is not associated with a specific focal injury (37,242). Brain atrophy is a nonspecific marker, also associated with ageing; however, volume loss is faster in individuals with CSVD compared to age-matched controls (243). Furthermore, brain atrophy and white matter hyperintensities have been observed to progress in parallel (244).

Perivascular spaces are fluid-filled microscopic spaces around the brain vessels. In association with CSVD, they may enlarge, and this enlargement is visible on MRI (37,241).

Vessel leakage and bleeding

Microbleeds are microscopic hypointense lesions on MRI, resulting from extravasation of blood reflecting bleeding-prone microangiopathy, or vessel leakage (245). Haemorrhagic manifestations of CSVD also include intracerebral haemorrhages and chronic blood products in the superficial cortex (cortical superficial siderosis), which can result from superficial cortical bleeding, or spontaneous subarachnoid bleeding (37). It is not fully understood why some presumed small-vessel haemorrhages present as microbleeds, whereas others appear as larger haemorrhages or lacunes (36).

Aetiology and pathogenesis

CSVD is thought to represent a cluster of vascular disorders with different pathological backgrounds (246). Figure 8 summarises the hypothesised pathogenesis behind brain damage associated with small vessel disease and corresponding markers in MRI.

Risk factor-related disease

One suggested aetiology is age- and risk-factor-related small vessel disease, where the disease is characterised by thickening of the vessel walls and narrowing of the lumen (36). CSVD of this presumed aetiology has been associated with systemic manifestations, also affecting the kidneys and the retinal vessels, and particularly with hypertension (36,247). CSVD accompanied by these features has also been associated with diabetes (36), namely type 2 diabetes (30); however, in type 1 diabetes, likewise, CSVD has been suggested to represent a generalised state of microangiopathy (39).

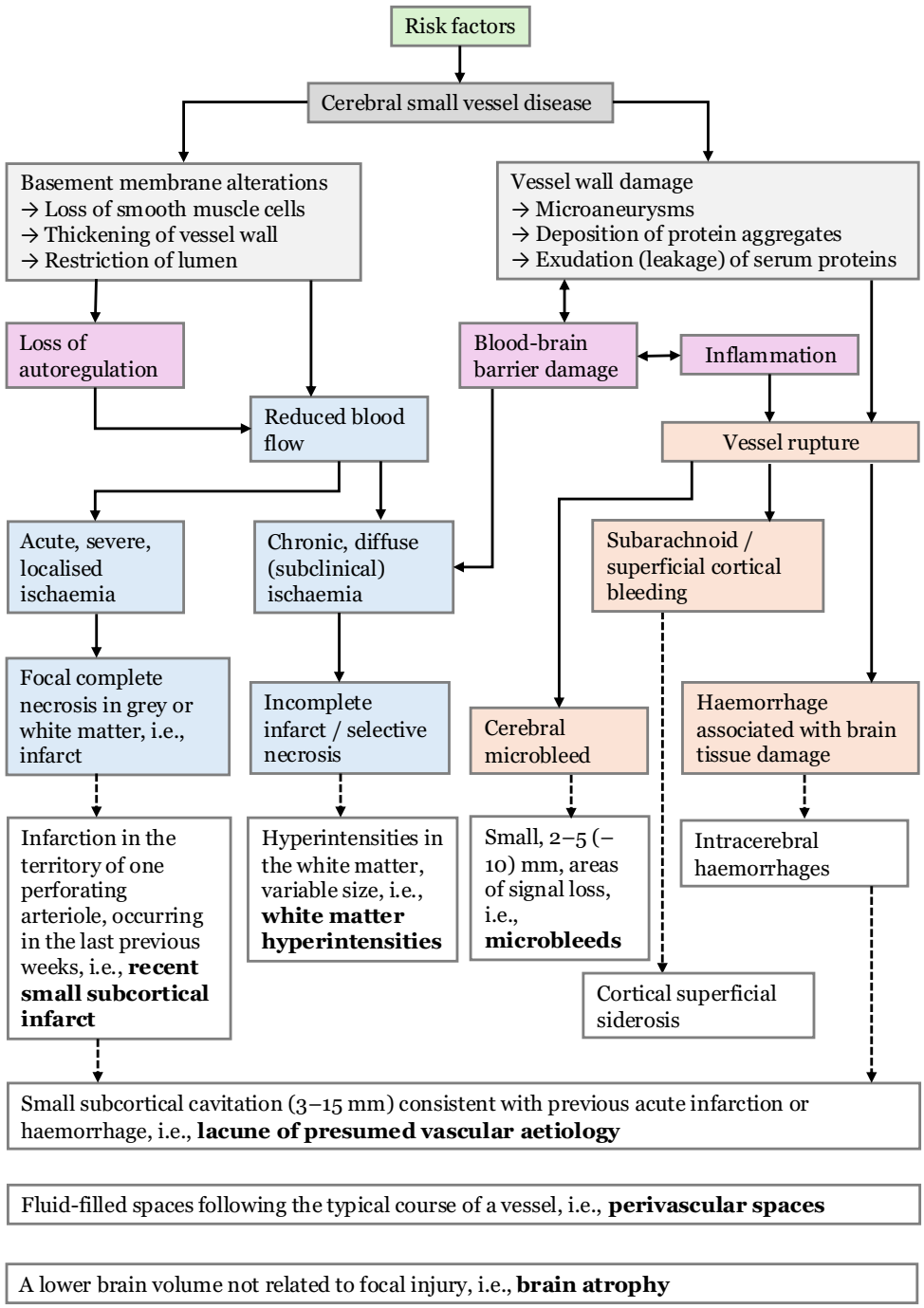

Figure 8 Diagram of the pathological processes associated with cerebral small vessel disease (boxes in colour) and corresponding markers on MRI (boxes in black and white). The diagram is a modified version of the one published by L. Pantoni in 2010 (36), including mechanisms described by Litak and co-workers (246) and MRI features according to Wardlaw and co-workers (37).

Aetiology in diabetes

In type 2 diabetes, the microvascular dysfunction is thought to be driven by hyperglycaemia, insulin resistance, and hypertension (30). So far, there are only a few studies assessing CSVD comprehensively in individuals with type 1 diabetes, including all MRI markers of the disease (35,38,39). To the best of our knowledge, the Finnish Diabetic Nephropathy (FinnDiane) Study, of which this thesis is also part, is so far one of only a few studies that can assess CSVD in type 1 diabetes with a control group with no diabetes as a reference (35). When previously studied in the MRI cohort of this thesis, CSVD in type 1 diabetes was associated with elevated blood pressure but not with markers of poor glycaemic control. It is therefore not known whether the pathophysiology in type 1 diabetes follows that in type 2 diabetes. Insulin plays a critical role in the brain's energy supply, for example as a mediator of vasodilatation (30). Nevertheless, how the altered insulin metabolism associated with type 1 diabetes and its treatment affects the brain is unclear (29). Furthermore, both hyper- and hypoglycaemia in type 1 diabetes have disruptive effects on the central nervous system (248).

Microbleeds are a prevalent feature of CSVD in type 1 diabetes (35,39) and, as illustrated in Figure 8, they are thought to reflect blood-brain barrier dysfunction. In type 2 diabetes, rather than microbleeds, white matter hyperintensities and lacunes are more frequently observed on MRI (32,249). White matter hyperintensities can represent both ischaemia and vessel leakage, while lacunes are thought to result either from small infarctions or haemorrhages (see Figure 8). Breakdown of the blood-brain barrier impairs normal vascular regulation, inducing ischaemia (30). In young neurologically asymptomatic adults with type 2 diabetes, microbleeds, but not white matter hyperintensities or lacunes, have been associated with diabetes in similarity with observations in type 1 diabetes (31,35), and it is unclear whether they represent different aetiologies of different stages of small vessel disease.

Alzheimer's-related disease

CSVD is perceived to play an important role in cognitive decline. Even so, the determining pathophysiological features of this association are poorly understood (36,37,250). CSVD is associated with Alzheimer's disease, but the pathophysiology is thought to differ from the risk factor-driven small vessel disease. Although diabetes is associated with cognitive decline (29,143,251), and Alzheimer's disease is perceived to share pathological features with type 2 diabetes (252), Alzheimer's disease-related neuropathology has not been linked to diabetes in autopsy studies (251,253). Also, when studied by MRI, observations of advanced brain ageing have been associated with reduced cognitive performance in individuals with type 1 diabetes, yet these individuals did not display signs of Alzheimer's-related neurodegeneration (254).

2.4.7.3 Stroke

Diabetes was established as a risk factor for stroke 30 years ago (255,256) and has since been shown to apply to all stroke types (222,257,258). Diabetes is associated with early vascular ageing of the brain (259), and individuals with diabetes suffer strokes at a younger age than the general adult population (258). Stroke in diabetes has generally been addressed in the context of macrovascular disease; however, as described in the previous sections, an increasing amount of evidence suggests that microangiopathy plays an important role in the brain pathology related to type 1 diabetes-related brain pathology (28,29,35).

Definition and classification of stroke

Stroke as a term refers to central nervous system infarction or haemorrhage accompanied by overt symptoms attributable to the infarction or non-traumatic haemorrhage. The symptoms present with rapidly developing signs of neurological dysfunction and evidence of permanent injury, meaning that they last ≥ 24 hours or until death (239).

Ischaemic stroke

Ischaemic stroke is defined as an episode of neurological dysfunction caused by an infarction in the central nervous system (239). Ischaemic strokes can additionally be subtyped by vessel type (large or small vessel) and by features emerging in the clinical examination, imaging, and diagnostic tests (239,240). Table 7 presents the commonly used Trial of Org 10172 in Acute Stroke Treatment (TOAST) categorisation (240), i.e., subtypes based on the aetiology causing the ischaemic strokes.

Haemorrhagic stroke

Stroke caused by cerebral haemorrhage refers to rapidly developing neurological symptoms attributable to intracerebral or subarachnoid haemorrhage, which are not caused by trauma. Intracerebral haemorrhage is defined as a focal collection of blood within the brain parenchyma or ventricular system and subarachnoid haemorrhage as bleeding into the subarachnoid space (239). Although less prevalent than ischaemic stroke, cerebral haemorrhages account for 9–27% of all strokes in the general population and up to 30% in individuals with diabetes (260,261). The term *haemorrhagic stroke* is typically used to indicate stroke caused by cerebral haemorrhage (26,43,261,262) and will also be used with that definition in this thesis. In the literature, however, in contrast to this thesis, the term *haemorrhagic stroke* is sometimes also defined to include other non-traumatic intracranial haemorrhages associated with stroke, such as haemorrhages occurring after an infarction, also referred to as *haemorrhagic transformation* or

haemorrhagic conversion of an infarction. It has therefore been suggested that the use of the term should be discontinued for the sake of clarity (239).

Table 7 TOAST classification of Subtypes of Acute Ischaemic Stroke (240)

Subtypes of ischaemic stroke possible or probable depending on results of ancillary studies
Large-artery atherosclerosis (embolus or thrombosis)
Cardioembolism (high-risk or medium-risk)
Small-vessel occlusion (lacune)
Stroke of other determined aetiology
Stroke of undetermined aetiology

TOAST indicates Trial of Org 10172 in Acute Stroke Treatment (240).

Clinical features and diagnosis of stroke

The classical clinical manifestation of an ischaemic stroke or an intracerebral haemorrhage is acute onset unilateral impairment of voluntary movement, either as a weakening of the motor functions (hemiparesis) or total paralysis (hemiplegia). The symptoms vary depending on stroke aetiology and the affected site in the central nervous system. Subarachnoid haemorrhages typically present with headache and stiffness of the neck. As the neural damage progresses, a stroke may affect one's level of consciousness and may lead to death (237).

Clinical diagnosis

Clinical diagnosis aims to establish if the neurological symptoms are 1) caused by a vascular process, and if so, 2) where the site of damage is and which blood vessel supplies that area, and 3) the disease mechanism, ischaemia or haemorrhage, for example (239,263).

Radiological diagnosis

Computed tomography (CT) scanning and MRI of the brain is used to confirm the clinical diagnosis. The primary objective of brain imaging in the acute phase is to evaluate if a lesion is caused by ischaemia or haemorrhage, or if it is related to some other non-vascular aetiology. Radiological differentiation between infarction and haemorrhage is crucial, as reperfusion therapies indicated for acute ischaemia could have detrimental effects by worsening intracerebral bleeding. Brain imaging can localise the affected region and display the extent of the ischaemia or haemorrhage. Ischaemic lesions are, in contrast to haemorrhages, typically not visible in the acute phase, however. The nature of a vascular lesion and how/if it relates to perfusion abnormalities in the brain can also be assessed by imaging. Vascular imaging by CT or MRI can identify occlusion and malformation of the (large) vessels. Additional imaging modalities, such as ultrasound, can be used for additional evaluation of the vessels supplying the region of vascular injury (239,263).

Neuropathological diagnosis

Although indicators of ischaemia are visible by CT scanning and MRI, the only definitive method for detecting ischaemic necrosis is invasive neuropathological evaluation of the brain. Stroke diagnostics do not comprise neuropathological samples, except in post-mortem evaluation (239,263).

Management of stroke

The primary prevention of stroke follows the principles of cardiovascular primary prevention described earlier (see section 2.4.6.2). The treatment of stroke depends on the stroke type and subtype. The acute phase treatment in ischaemic stroke is reperfusion of the tissue, whereas the aim in haemorrhagic stroke is to prevent haematoma growth (262,263). After initial treatment, the management includes the prevention of stroke-related complications and recurrent stroke (262,264).

Management of ischaemic stroke

The key element of acute management is rapid reperfusion of the ischaemic tissue before damage becomes irreversible. The prognosis for recovery decreases with the time elapsed since onset of symptoms; the time window for reperfusion is narrow, and treatment should be initiated within 4.5 hours from onset. The base of the treatment in both large- and small-vessel ischaemic strokes is intravenous thrombolysis with a recombinant tissue plasminogen activator (alteplase). Large artery occlusions can be treated with mechanical thrombectomy – a catheter-based endovascular procedure – if the site of occlusion is established. Depending on the aetiology of the stroke, treatment also involves attending to underlying causes, such as carotid stenosis or atrial fibrillation leading to embolisms (263,265,266).

Secondary prevention aims to reduce the risk of recurrent stroke and eventually mortality (264). Individuals with type 1 diabetes have a distinctively poor prognosis after stroke with more than 20% of the individuals dying within a year of their first stroke (27). Long-term treatment includes antiplatelet therapy, effective management of blood pressure, cholesterol concentrations, hyperglycaemia, and other known risk factors, such as smoking (263,264).

Management of haemorrhagic stroke

In intracerebral haemorrhages, the haematoma size is among the main prognostic factors, and rapid interventions preventing haematoma expansion are crucial (262,267). The mainstay strategy is early blood pressure lowering. Due to unclarity regarding the outcome – disability and mortality – administration of clot-promoting agents is not recommended to date (261,262,267). In some situations, such as with space-occupying haematomas, surgical interventions may be needed (262,267).

The treatment of spontaneous subarachnoid haemorrhages is primarily surgical, as the risk of rebleeding is substantial even if the haemorrhage stops spontaneously.

Treatment options include endovascular (coiling) occlusion of the ruptured vessel or neurosurgical (clipping) occlusion (268,269).

Secondary prevention of haemorrhagic stroke includes addressing underlying risk factors, such as anticoagulation treatment and aggressive blood pressure management (a level below 130/80 mmHg is recommended for all). Cholesterol-lowering treatment is not indicated for secondary prevention of intracerebral haemorrhages (262).

2.4.7.4 Risk factors for cerebral small vessel disease and stroke

The risk profile differs somewhat between stroke types both in type 1 diabetes and in the general population (43,270). Together with hypertension, modifiable risk factors such as smoking, abdominal obesity, unhealthy diet, and physical inactivity have been estimated to account for around 80% of the population-attributable risk of stroke (270). Additional risk factors include dyslipidaemia, psychosocial factors, genetic factors, and predisposing comorbidities, such as diabetes and cardiac disease (270–272). The following section will provide a more thorough overview of the risk factors particularly relevant in the context of this current thesis.

Type 1 diabetes

Type 1 diabetes and cerebral small vessel disease

Although CSVD is more common in asymptomatic individuals with type 1 diabetes compared to healthy controls (35), the impact of this or its relationship to stroke is yet unclear. Only a few studies have assessed cerebral small vessel disease by all previously defined markers in brain MRI (35,38,39).

In contrast to asymptomatic CSVD, the literature on overt disease is more comprehensive, but the type of diabetes is often not defined. Overt lacunar strokes have frequently been linked to diabetes (34,273–278). In individuals suffering a stroke before middle adulthood, both individuals with type 1 and type 2 diabetes are more likely to have a stroke of microvascular origin, although they are more frequent in type 1 diabetes (34). In elderly individuals, strokes of small-vessel aetiology have been observed more frequently in individuals with diabetes compared to those without diabetes (276,277). Other studies have found that single lacunes do not associate with diabetes, whereas multiple lacunes do (273,275). Regardless, the profile of overt CSVD seems to differ in individuals with versus without diabetes (277).

Type 1 diabetes and ischaemic and haemorrhagic stroke

Diabetes increases the risk of all types of strokes (222,271). Although individuals with type 2 diabetes have a markedly higher risk of stroke compared to the general population, the stroke risk associated with type 1 diabetes is particularly prominent

as the risk is three- to sixfold higher than in the general population (24–26). Additionally, comorbidities and complications of type 1 diabetes, such as hypertension and diabetic kidney disease, further increase the risk of both ischaemic and haemorrhagic stroke (43,260).

Type 1 diabetes is associated with an almost fourfold increased risk of ischaemic stroke (25). Although the proportion of haemorrhagic strokes seems higher in diabetes compared to the general population (260,261), there are only a few studies assessing different stroke types and subtypes in type 1 diabetes (25). While type 1 diabetes has been shown to increase the risk of haemorrhagic strokes (25,26), the number of studies on haemorrhagic strokes in type 1 diabetes is still too low to make conclusions about the general magnitude of that risk increase (25). It thereby follows that even less is known about subarachnoid haemorrhages. This smaller entity among haemorrhagic strokes has been suggested to have a distinct microvascular aetiology in type 1 diabetes, as non-aneurysmal subarachnoid haemorrhages have been observed more frequently than aneurysmal haemorrhages, which is the more typical manifestation in the general population (268,279). Up to every third cerebral haemorrhage in type 1 diabetes is a subarachnoid, and in the Finnish population, individuals with type 1 diabetes have a ten times higher incidence of subarachnoid haemorrhages compared to the general population (260,279,280).

Blood pressure

Blood pressure and cerebral small vessel disease

There are few longitudinal studies assessing risk factors for CSVD, yet elevated blood pressure, particularly systolic pressure, is the most consistently reported factor associated with the disease in both the general population and in individuals with diabetes (35,277,278,281,282). In individuals without overt symptoms of cerebrovascular disease, markers of CSVD have, in addition to hypertension, been associated with other alterations in normal blood pressure, such as short-term blood pressure variability and reduced nocturnal dipping (282–285).

A reduced dipping pattern has also been associated with the progression of CSVD after a symptomatic lacunar infarction (286). These blood pressure quantities are only detectable by ambulatory blood pressure monitoring, and ambulatory blood pressure monitoring has indeed been shown to correlate more strongly with CSVD compared to office measurements (284). This is partly explained by the strong association between nocturnal blood pressure and CSVD, as high nocturnal blood pressure is more strongly linked to CSVD than diurnal pressure (283,284).

In type 1 diabetes, CSVD has been associated with increased systolic office blood pressure and arterial stiffness (35,38). Although hypertension, altered blood

pressure patterns, and blood pressure regulation are common in type 1 diabetes (123–126), the role of blood pressure in CSVD remains unknown.

Blood pressure and overt stroke

Elevated blood pressure, however, is an indisputable risk factor for all types of strokes (158,270). In type 1 diabetes, the stroke risk increases linearly with the level of systolic blood pressure, and this applies to both ischaemic and haemorrhagic stroke (105,158). Diastolic blood pressure level is also associated with an increased risk of stroke in type 1 diabetes, but the association is less clear, as diastolic blood pressure tends to decrease with age and arterial stiffening (158,260). Diastolic blood pressure and increasing pulse pressure, as a marker of arterial stiffness, have both been linked to an increased risk of ischaemic stroke. Diastolic blood pressure and pulse pressure have further been associated with an increased risk of haemorrhagic stroke (105). Nevertheless, the connection is somewhat ambiguous: Pulse pressure is a particularly strong predictor of other, typically more frequent, cardiovascular events associated with diabetes (109,119), and when all-cause mortality is considered, diastolic blood pressure and pulse pressure do not seem to be significant markers of haemorrhagic stroke risk (105).

Haptoglobin genotype in cerebral small vessel disease and stroke

As haptoglobin plays an important role in microvascular homeostasis by maintaining normal regulation of capillary blood flow and impacting angiogenesis (130,134), it has been speculated that certain genotypes are linked to cardiovascular disease proneness in diabetes (133).

The genotype *HP1-1* has been linked to cerebrovascular disease in the general population (287). Nevertheless, when studied in the Finnish general population, the *HP1-1* genotype was not associated with any subtype of ischaemic stroke, including subtypes attributable to CSVD, nor to white matter hyperintensities (288,289). Meanwhile, *HP2* was reported to promote premature cardiovascular death in individuals with a history of stroke in the same cohort (289).

In type 1 diabetes, on the other hand, haptoglobin genotype *HP1-1* has been linked to stroke in hypertensive individuals (41), and to white matter hyperintensities, that is, CSVD (40). The association between haptoglobin genotype and other manifestations of CSVD in type 1 diabetes has not been reported, nor has the role of haptoglobin in comparison to other potential risk factors for CSVD.

Diabetic retinopathy

Diabetic retinopathy and cerebral small vessel disease

Proliferative diabetic retinopathy has been associated with cerebral microbleeds in type 1 diabetes (39). As discussed in previous sections, the retina shares

developmental, histological, and functional properties with the brain, and the mechanisms of microvascular insult to the retina are thought to reflect parallel processes in the brain (42). In individuals with type 1 diabetes, diabetic retinopathy is suggested to indicate a more widespread microangiopathy, also present in the brain (39).

Likewise, in type 2 diabetes, CSVD is associated with diabetic retinopathy and retinal microvascular abnormalities (290–292). Retinopathy in type 2 diabetes is associated with more severe CSVD (290), and CSVD severity has further been observed to increase with the severity of diabetic retinopathy (291). These studies, however, have predominantly associated diabetic retinopathy with white matter hyperintensities (290,291), whereas in type 1 diabetes, retinopathy has been linked to cerebral microbleeds (39). How other manifestations of CSVD or the burden of the disease link to diabetic retinopathy or its severity in type 1 diabetes remains to be assessed.

Diabetic retinopathy and overt stroke

The risk of stroke in type 1 diabetes is increased in individuals with severe diabetic retinopathy (43,260). Notably, the risk of haemorrhagic stroke associated with severe retinopathy is significant also in the presence of other known risk factors, such as diabetic kidney disease (43). Yet, when it comes to other common retinal complications, such as non-proliferative diabetic retinopathy and macular oedema, their role as potential markers for stroke risk in type 1 diabetes is unrecognised.

In individuals with type 2 diabetes, the risk of stroke, either ischaemic or haemorrhagic, has reportedly been associated with diabetic retinopathy ranging from mild non-proliferative to proliferative disease (293). So far, it is not clear if this link is limited to some specific subtype or if it is applicable to all types of strokes.

Whether macular oedema might serve as a tell-tale sign of cerebrovascular risk is not clear. To the best of our knowledge, there are no studies evaluating macular oedema in the context of stroke risk in type 1 diabetes. In type 2 diabetes, diabetic macular oedema has been identified as a predictor of adverse cardiovascular outcomes, including stroke (294). Even so, it remains undisclosed whether macular oedema is specifically linked to cerebrovascular events, and what the nature of the association would be in type 1 diabetes.

2.4.8 Mortality in diabetes

Both type 1 and type 2 diabetes are associated with excess mortality (104,295). The relative mortality risk in individuals with diabetes has often been estimated as three to four times higher than in the general population (104,295,296). There is, however, considerable regional variation in the mortality associated with both type 1 and type 2 diabetes (104,233). Moreover, the risk of death also varies depending

on the age at onset of diabetes (295,296), calendar year of diagnosis of type 1 diabetes (233), and the age of the individuals at risk (295,297). Nevertheless, in some cohorts, the mortality has been closer to eightfold higher than the general population (104,295).

During the last 30 years, there has been a significant reduction in mortality associated with type 1 diabetes (24,295,298,299). Although the decrease in mortality has been greater in type 1 diabetes compared to type 2 diabetes (298), the age-standardised mortality risk is still higher in type 1 than in type 2 diabetes (104,299). The excess mortality linked to diabetes is largely attributable to chronic vascular complications of diabetes and cardiovascular disease, most prominently coronary artery disease, which is the leading cause of death (104,233,299,300). While cardiovascular disease is also the leading cause of death globally (238), the mortality rate for cardiovascular disease is still higher in diabetes compared to the general population (296,297). Moreover, survival after a cardiovascular or cerebrovascular event is worse (301,27,302,303). Again, the impact of diabetes is greater in women, and they have a higher excess mortality risk (104,299).

The mortality profile varies somewhat between type 1 and type 2 diabetes. During the first 10 years of type 1 diabetes, acute diabetes-related complications account for most of the deaths. Later, death related to cardiovascular disease, kidney disease, and infections become the leading causes, and after 20 years of type 1 diabetes, cardiovascular disease is the most common cause of death. (300) Although also prevalent in type 2 diabetes, death caused by kidney disease is less common in type 2 than in type 1 diabetes, and cardiovascular deaths account for a larger proportion of deaths in type 2 diabetes (104). However, diabetic kidney disease is an important driver of cardiovascular disease, and individuals with type 2 diabetes in particular succumb to the consequences of cardiovascular disease before they reach the stage of kidney failure (304).

3 Aims

The objective of this thesis was to study CSVD and stroke in association with blood pressure, haptoglobin genotype, and diabetic retinopathy in individuals with type 1 diabetes. The study aimed to increase the knowledge of cerebrovascular disease and its aetiology in type 1 diabetes. Another aim of this thesis was to identify factors associated with cerebrovascular disease at an early stage, before the occurrence of overt stroke, which together with an increased understanding of the disease mechanism would enable more efficient prevention of cerebrovascular diseases in individuals with type 1 diabetes in the future.

The study specific aims were as follows:

I To assess the role of ambulatory blood pressure for CSVD (MRI markers) in individuals with type 1 diabetes and to evaluate covert (in an office setting) alterations in blood pressure.

II To assess associations between a certain haptoglobin genotype and CSVD and to evaluate the impact of the haptoglobin genotype in relation to other suggested risk factors for CSVD.

III To study associations between CSVD (MRI markers) and the severity of diabetic retinopathy according to the ETDRS grading scale, such as the association between cerebral microbleeds (prevalence and number of bleeds) and the severity of diabetic retinopathy. Furthermore, to determine whether retinopathy assessment could add to the identification of individuals at high risk of cerebral haemorrhages?

IV To study the association between diabetic retinopathy severity (by ETDRS grading) and overt stroke and to assess potential associations between diabetic retinopathy and stroke of microvascular aetiology, that is, CSVD.

4 Participants and study designs

4.1 The FinnDiane Study

All individuals participating in Studies I–IV are part of the Finnish Diabetic Nephropathy (FinnDiane) Study. FinnDiane is a nationwide multicentre study that was initiated in 1997. The study aims to uncover clinical, environmental, and genetic risk factors for micro- and macrovascular complications of type 1 diabetes.

FinnDiane includes 78 study centres, all within the public health care sector. All 5 university hospitals in Finland, 16 central hospitals, and 31 primary health care units included in the study centres are listed in the Appendix. Since 1998, adult participants have been consecutively recruited for a baseline visit when entering a regular diabetes-related visit at one of the study centres. Since 2004, the participants have entered follow-up visits, and follow-up data have been collected for the participants in the study.

By the end of 2023, the FinnDiane comprised of 5,500 regular participants who have type 1 diabetes, are living in Finland, and have entered a clinical study visit and been assessed by the FinnDiane Study protocol, which is described in detail in section 5.1. As there are roughly 50,000 individuals with type 1 diabetes in Finland (2), the regular participants of the FinnDiane Study cover approximately 10% of this population. With all available data combined, FinnDiane covers over 20% of all adults with type 1 diabetes in Finland. Corresponding to the geographical distribution of the general population of Finland, with the population density being highest in the south of Finland, the majority of the FinnDiane participants also reside in the southern parts of the country. Although the study design is not strictly population-based, the FinnDiane participants represent the Finnish population with type 1 diabetes well, as illustrated in Figure 9.

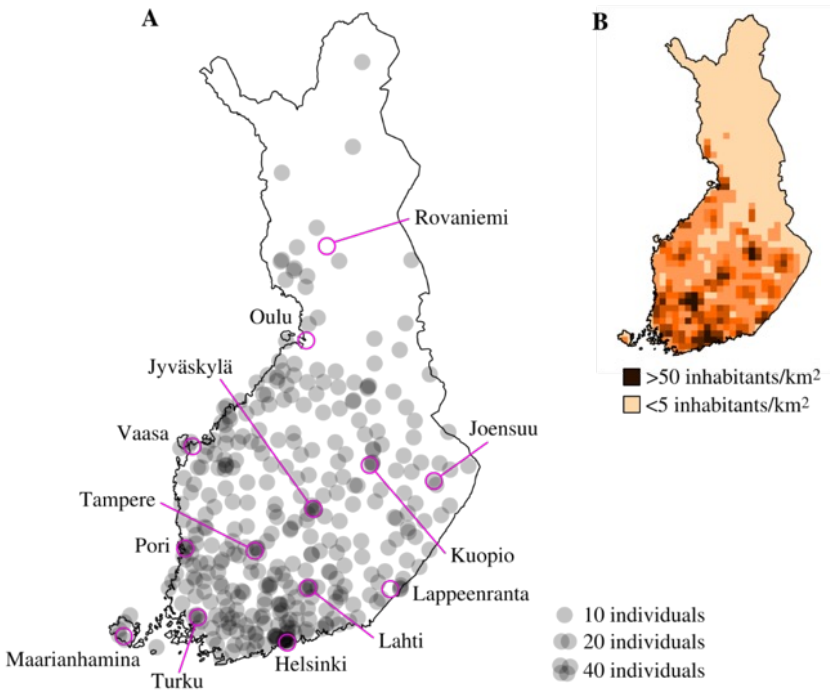

Figure 9 Chart **A** shows the site of residence of individuals with type 1 diabetes participating in the FinnDiane Study, with the circular markers representing a set of 10 local participants. **B** shows the regional population density, indicated by colour, in Finland according to data from Statistics Finland. An increased population density is represented by a darker indicator colour (305).

4.2 Brain MRI cohort

In 2010, a substudy titled *Risk factors for covert and overt cerebrovascular disease in patients with T1D: A longitudinal long-term brain MRI follow-up study* was initiated as a collaboration between the FinnDiane Study and the Helsinki University Hospital (HUS). Aims of the study were to characterise asymptomatic vascular lesions on brain MRI and to determine clinical factors associated with such lesions. Participants were to undergo a first brain MRI during 2011–2017 and to be re-examined during 2020–2023. This thesis includes cross-sectional data from the participants' first brain MRI and the associated FinnDiane Study visit.

FinnDiane participants entering the Helsinki study centre during 2011–2017 were consecutively recruited for a brain MRI in connection to the FinnDiane Study visit. A total of 191 individuals with type 1 diabetes, aged 18–50 years, were enrolled. Exclusion criteria were kidney replacement therapy, contraindications for MRI, or previous signs of stroke or transient ischaemic attack, evaluated with a validated questionnaire (Questionnaire for Verifying Stroke-Free Status, QVSFS) (306,307). As a control group, 30 healthy individuals matched for age and sex were included

and studied by the same protocol as those with type 1 diabetes. The control subjects did not have diabetes in a first-degree relative, were free of signs of cerebrovascular disease, and did not have any contraindications for MRI. All control subjects were normoglycemic with a mean fasting glucose of 4.4 ± 0.4 mmol/l.

4.3 Selection of participants for Studies I–III

Participants included in Studies I–III are all from the FinnDiane brain MRI cohort, and data are cross-sectional. The flowchart in Figure 10 presents a schematic overview of participants included in each of these studies.

4.3.1 Participants in Study I

All participants entering the FinnDiane MRI substudy were given the option of participating in a 24-hour ambulatory blood pressure monitoring in conjunction with the FinnDiane Study visit. A total of 73 (38%) of the 191 participants undergoing brain MRI also volunteered for the 24-hour ambulatory blood pressure monitoring. Study I comprised of these 73 participants with type 1 diabetes, for whom both MRI and 24-hour ambulatory blood pressure data were available.

4.3.2 Participants in Study II

Study II included all participants originally enrolled in the MRI substudy, for whom Hp genotyping had been determined by polymerase chain reaction (PCR) and analysis on a gel. Hp genotyping was successfully determined for 179 (94%) of the participants with type 1 diabetes. Study III comprised of these individuals.

4.3.3 Participants in Study III

For study III, we included all participants in the MRI substudy who had available data from fundus images. A total of 114 participants with type 1 diabetes and 29 controls had undergone fundus imaging at the FinnDiane facilities, and for 75 other participants with type 1 diabetes, we collected images taken at the participant's own health care unit. Thus, 189 (99%) of the participants with type 1 diabetes within the MRI cohort and 29 (97%) of the controls were eligible for this study.

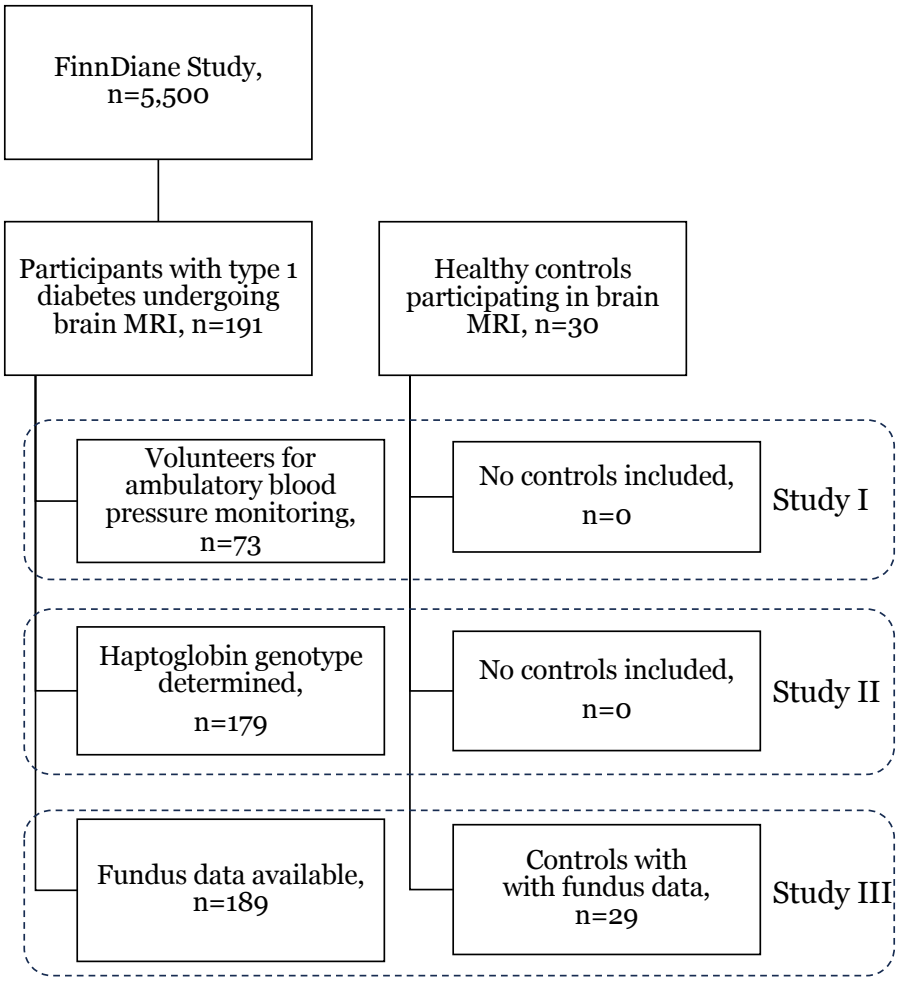

Figure 10 The flow chart illustrates the selection process of the participants included in Studies I–III.

4.4 Selection of participants for Study IV

4.4.1 Retinopathy subset

For participants entering the FinnDiane Study before January 2012, a subset of 1,983 individuals was drawn for a comprehensive assessment of diabetic eye disease. Fundus images taken for screening and clinical purposes and/or records

(medical files, verbal records) on fundus examinations performed by an ophthalmologist were obtained for all participants.

Retinopathy severity was evaluated and scored using the ETDRS grading scale (168) by a specialist in ophthalmology (Kustaa Hietala, Jyväskylä Central Hospital, Finland). Additionally, macular oedema was evaluated (K. Hietala) in 1,354 of the participants in the subset included for retinopathy assessment. The retinopathy subset has also been presented previously in K. Hietala's dissertation (308).

4.4.2 Participants in Study IV

The flow chart in Figure 11 displays the selection process. From the original subset (*N* = 1,983), we excluded 495 individuals that lacked information on retinopathy severity by the time of the baseline study visit and 57 individuals with insufficient clinical baseline data (data on glycaemic control, serum lipids, or blood pressure missing). A total of 45 individuals were excluded due to having suffered a stroke before baseline, and 5 due to insufficient information on stroke or stroke type during follow-up. This rendered us a cohort of 1,268 participants with type 1 diabetes. For a total of 1,074 (84.7%) participants, data on macular oedema were available in addition to data on retinopathy severity.

4.5 Ethical aspects and data integrity

All studies were carried out in accordance with the Declaration of Helsinki, a statement of ethical principles for medical research involving human subjects (309). The study protocol has been approved by the Ethics Committee of HUS (processing number 293/13/03/01/2010 and HUS/2184/2017 in Studies I–III, and 491/E5/2006, 238/13/03/00/2015, and HUS-3313-2018, July 3rd, 2019, in Study IV), and all study participants have given their written informed consent.

Data are stored at the FinnDiane research facilities and in the FinnDiane database. Each participant was given an identification number which is used when encoding their personal data in the database. Only FinnDiane researchers have access to this pseudo-anonymised information, and access requires signing non-disclosure and privacy agreements, as well as compliance with General Data Protection Regulation. Sensitive personal information (such as the participant's name and social security number) is accessed only when necessary and by a limited number of FinnDiane researchers. All data are handled on user-specific local servers, with protected access.

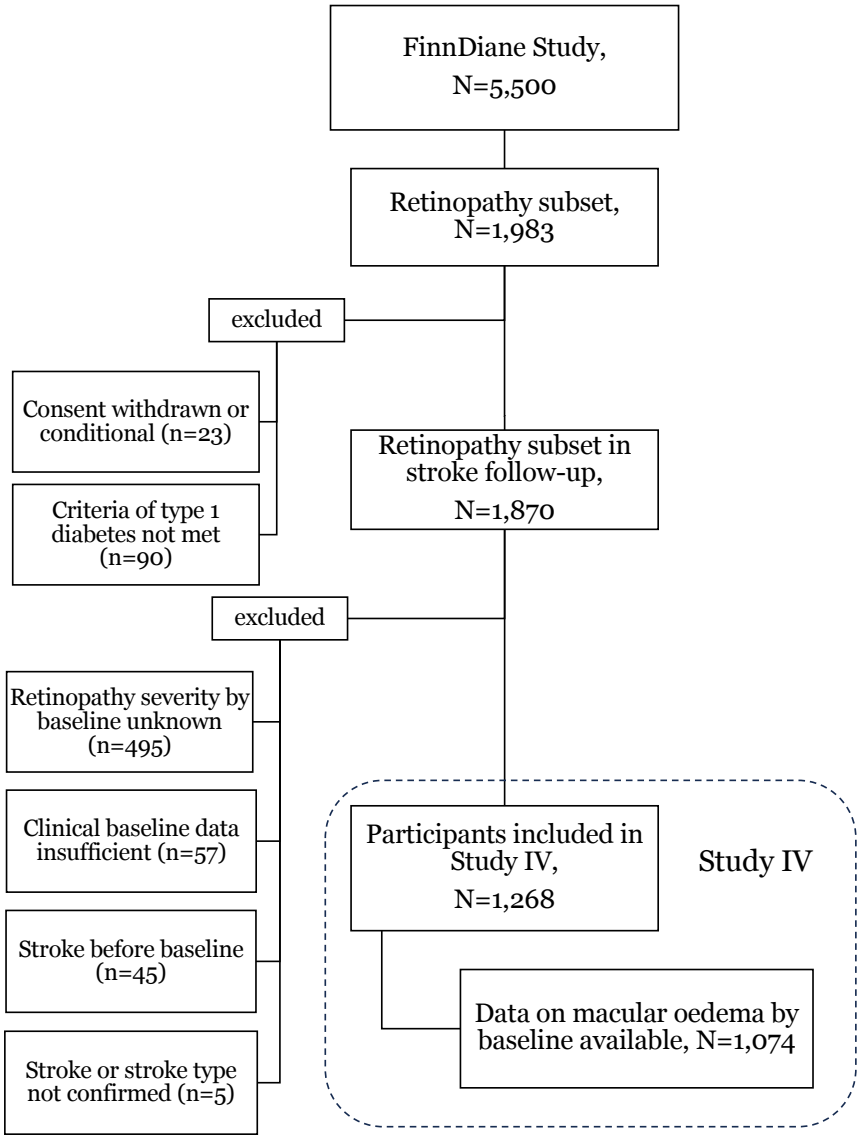

Figure 11 The flow chart illustrates the selection process of the 1,268 participants included in Study IV. All participants were part of the Finnish Diabetic Nephropathy Study. From the subset of participants with detailed retinopathy data, we excluded participant by the criteria presented under the *excluded* boxes in the flowchart. Of the 1,268 participants included for Study IV, a total of 1,074 had available information on macular oedema in addition to retinopathy severity.

5 Methods

5.1 The FinnDiane Study protocol

5.1.1 Study visit

All participants entering the FinnDiane Study have attended a study visit for a thorough clinical evaluation. Baseline examinations were carried out at a regular visit to the participants' attending physician at one of the study centres (see FinnDiane Study Centres in Appendix 1). A subset of the participants resided in the HUS region, and they visited the FinnDiane research centre at the Helsinki University Hospital for a more extensive visit. Most of the follow-up visits, as well as visits for specific substudies, such as the MRI substudy, were carried out at the FinnDiane centre in Helsinki.

The MRI substudy (Studies I–III) included both newly recruited participants and individuals already enrolled in the FinnDiane Study, i.e., those who had participated in a baseline study visit and entered the FinnDiane research clinic for a follow-up visit. All the study visits followed the same protocol. Study IV focused on baseline visits (first FinnDiane visit) only. Standardised forms regarding the participant's medical condition, medical history, and medication were filled out by the attending physician. In addition, the participants filled out questionnaires about their lifestyle, such as smoking habits.

The clinical evaluation included anthropometric measurements (height, weight, and hip and waist circumference) and blood pressure measurement. Blood and timed urine samples were collected for laboratory analyses. The timed urine samples were performed either as a 24-hour or an overnight collection. Samples were sent to the central laboratory of the FinnDiane Study and to the central laboratory of Helsinki University Hospital (HUSLAB).

5.1.2 Definition of type 1 diabetes

Participants were defined as having type 1 diabetes if they had received a diabetes diagnosis before 40 years of age and if permanent insulin medication was initiated within a year of receiving the diagnosis.

5.1.3 Anthropometric measurements

Body weight readings were registered with 0.1 kg precision. Height, hip, and waist circumference were measured with 1 cm precision. Waist and hip circumference were measured horizontally midway between the lowest rib and the iliac crest and at the widest part of the gluteal region, respectively. To calculate the waist-to-hip ratio, the waist measure was divided by the hip measure. Body mass index was calculated as weight divided by height, in metres squared (kg/m^2).

5.1.4 Medication

Medication in regular use was charted at the FinnDiane Study visit. The use of any statins, fibrates, or ezetimibe was denoted as lipid-lowering medication. Any blood pressure-lowering agent, such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor (AT2) blockers, beta-adrenergic blocking agents, calcium channel blockers, diuretics, or other antihypertensive agents such as moxonidine were referred to as antihypertensive medication. Aspirin indicated low-dose acetylsalicylic acid and/or clopidogrel, and anticoagulants included warfarin, low-molecular-weight heparin, or direct oral anticoagulants.

5.1.5 Smoking

Smoking was defined as regularly smoking a minimum of one cigarette per day for a continuous period of at least one year. Based on information in the lifestyle questionnaires, participants where categorised according to smoking status in Studies I, II, and IV. Smoking data were not used in Study III.

In Study I, participants fulfilling the criteria of smoking by the time of the visit were categorised as current smokers, whereas participants who had ceased smoking or had never smoked were categorised as non-smokers. In Study II, participants were categorised as having a history of smoking if they were currently smoking or had ceased smoking; otherwise, they were listed as having no history of smoking. In Study IV, current smokers, participants who had ceased smoking, and participants with no history of smoking were separated into three categories.

5.2 Blood pressure measurements and definition of hypertension

5.2.1 Office measurements

Blood pressure measured at the FinnDiane Study visit by the attending health care professional was referred to as office blood pressure. Blood pressure was measured twice with the study participant in a sitting position after a 10-minute period of rest, and the average of the systolic and diastolic blood pressure values from the two measurements were calculated and recorded.

For participants in Studies I–III, office blood pressure was measured with an automated oscillometric blood pressure measuring device (Omron M6, Omron Heath Care, Hoofddorp, Netherlands). For participants in Study IV, measurements were performed either with a manual mercury sphygmomanometer or with a standardised automated device.

5.2.2 Ambulatory blood pressure monitoring (Study I)

5.2.2.1 Blood pressure recordings

Ambulatory blood pressure monitoring was performed on a subset of participants attending the FinnDiane Study Centre at Helsinki University Hospital and were available for 73 of the MRI substudy participants. The systolic and diastolic blood pressure was recorded every 15 minutes during daytime (07:00–22:00) and every 30 minutes during nighttime (22:00–07:00) for 24 hours by ambulatory blood pressure monitoring (Mobil-O-Graph, I.E.M., Stolberg, Germany). Information about time spent awake or asleep was obtained through a study diary kept by the participant (see Appendix 2).

The quality of the 24-hour ambulatory blood pressure monitoring for each participant was validated by standardised criteria (88,310). Participants were categorised as having good quality measurements versus not. Good quality was defined as follows:

- A duration of over 20 hours with at least 70.0% successfully completed measurements
- A minimum of 20 diurnal blood pressure readings with a minimum frequency of two measurements per hour
- A minimum of seven nocturnal blood pressure readings with a minimum frequency of one measurement per hour

5.2.2.2 Calculation of blood pressure variables

Blood pressure and mean arterial pressure

The average systolic and diastolic blood pressure during time awake (diurnal), time asleep (nocturnal), and the entire 24-hour ambulatory follow-up was calculated for each participant. Mean arterial pressure (2/3 diastolic + 1/3 systolic blood pressure) and pulse pressure (systolic – diastolic blood pressure) were derived from systolic and diastolic blood pressure.

Average real variability

We calculated blood pressure variability (average real variability) of both systolic and diastolic blood pressure during ambulatory monitoring as suggested by L. Mena et al. (311):

$$ARV = \frac{1}{N-1}\sum_{k=1}^{N-1}|BP_{k+1} - BP_k|$$

where N denotes the number of valid blood pressure (BP) measurements in the ambulatory blood pressure monitoring data corresponding to a given participant.

Nocturnal dipping

Nocturnal dipping of systolic and diastolic blood pressure (BP) was calculated as follows (94):

$$Nocturnal\ dipping = 1 - \frac{Nocturnal\ BP}{Diurnal\ BP} \times 100$$

Participants were categorised as non-dippers if their mean nocturnal systolic blood pressure decreased less than 10% from the diurnal mean blood pressure level (94); otherwise they were categorised as dippers.

5.2.3 Definition of hypertension

Hypertension was defined based on the threshold values recommended by the European Society of Cardiology and the European Society of Hypertension (76). The cut-off values are presented in Table 8.

Participants using antihypertensive medication were considered as having hypertension regardless of their blood pressure level at the study visit or during ambulatory blood pressure monitoring. If a participant had an office blood pressure within the normal range (< 140/90 mmHg) and no antihypertensive medication in use, while simultaneously fulfilling the criteria of hypertension on ambulatory blood pressure monitoring, they were defined as having masked hypertension.

White-coat hypertension indicated having an elevated office blood pressure but no hypertension during ambulatory blood pressure monitoring and no use of antihypertensive medication.

Table 8 Cut-off values to define hypertension

	Cut-off values defining hypertension in office measuring and ambulatory blood pressure monitoring	
	Systolic (mmHg)	Diastolic (mmHg)
Office blood pressure	≥ 140	≥ 90
Ambulatory monitoring:		
Diurnal mean	≥ 135	≥ 85
Nocturnal mean	≥ 120	≥ 70
24-hour mean	≥ 130	≥ 80

5.3 Laboratory measurements and analysis

5.3.1 Lipids and lipoproteins

The serum lipid and lipoprotein concentrations were determined from blood samples drawn in the non-fasting state at the FinnDiane Study visit. The measurements were performed in Professor Marja-Riitta Taskinen's research laboratory at Helsinki University Central Hospital, Helsinki, Finland.

5.3.1.1 Total cholesterol and triglycerides

Total cholesterol and triglycerides were first measured enzymatically with a Cobas Mira analyser (Hoffman-La Roche, Basel, Switzerland) using commercially available kits (Hoffman-La Roche until November 2001, then ABX Diagnostics, HORBIRA ABX, Montpellier, France, until January 2006), and later with a Konelab 60i analyser (Thermo Fisher Scientific Inc. Waltham, MA, USA) using kits from the same manufacturer.

5.3.1.2 HDL cholesterol

HDL cholesterol was determined enzymatically using a HTS 7000 Plus Bio Assay Reader (Perkin Elmer, Waltham, MA, USA), with a commercially available kit from Roche Diagnostics Hitachi (Hitachi, Tokyo, Japan).

5.3.1.3 LDL cholesterol

LDL cholesterol concentration was calculated using Friedewald's formula (312) in Studies I–III and by Sampson's equation (313) in Study IV. Like Friedewald's formula, Sampson's equation estimates LDL cholesterol based on enzymatically measured total cholesterol and HDL cholesterol (312,313). Sampson's equation, however, has appeared more accurate in determining LDL cholesterol concentration, especially in patients with high triglyceride concentrations.

Friedewald's formula

$$LDL = C_{tot} - HDL - \frac{TG}{2.2}$$

Friedewald's formula (312) for estimating LDL cholesterol concentration (mmol/l), where LDL indicates the estimated concentration of LDL cholesterol (mmol/l), C_{tot} the concentration of total cholesterol (mmol/l), HDL the concentration of HDL cholesterol (mmol/l), and TG the concentration of triglycerides (mmol/l).

Sampson's equation

$$LDL = \frac{C_{\text{tot}}}{0.948} - \frac{HDL}{0.971} - \left(\frac{TG}{8.56} + \frac{TG \times non\text{-}HDL}{2140} - \frac{TG^2}{16100}\right) - 9.44$$

Sampson's equation (313) for estimation of LDL cholesterol concentration (mmol/l). In Sampson's formula, non-HDL is given by subtracting HDL from C_{tot}.

5.3.2 Glycated haemoglobin (HbA_{1c})

Glycated haemoglobin (HbA_{1c}) was measured locally at the study centres with standardised assays. In Studies I–III, the HbA_{1c} of all participants was measured at the same laboratory (HUSLAB). The reference range was 4–6% in accordance with the NGSP (formerly the National Glycohemoglobin Standardization Program) (314) and 20–42 mmol/mol as recommended by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (315) from 2010 onwards. Measurement obtained before versus after 2010 have been harmonised by conversion between the two methods through the NGSP's formula $NGSP = (0.09148 \times IFCC) + 2.152$ (316).

5.3.3 Serum creatinine

Serum creatinine concentration (µmol/l) was measured at HUSLAB on an automated analyser (Hitachi 911 E, Boehringer Mannheim, Mannheim, Germany) based on Jaffé's chemical method until January 2002. Later, a more precise enzymatic method involving measurements by isotope dilution mass spectrometry was employed using automated analysers (Hitachi 917 or Modular Analyser,

Boehringer Mannheim). The correlation coefficient between the enzymatic and the chemical methods was 0.988, and measurements obtained using different methods were harmonised using the following formula:

$$Creatinine_{corrected} = (0.953 \times Creatinine_{chemical\ method}) - 7.261$$

5.3.4 Haptoglobin genotyping

DNA was extracted from white blood cells for haptoglobin genotyping. Laboratory analyses were performed at the Finnish Institute of Molecular Medicine by Anna Syreeni (Folkhälsan Research Center, Helsinki, Finland).

A copy number variation affects the haptoglobin gene length. As there are five exons in the *HP1* allele, whereas the *HP2* allele contains seven exons, the difference is detectable through amplification of DNA and gel electrophoresis.

To determine haptoglobin genotype, DNA was amplified through two polymerase chain reactions (PCRs) and two different pairs of forward and reverse primers were used to generate DNA strings of distinct length, depending on the presence versus the absence of exons 5 and 6 on the α-chain coding loci, that is, *HP2* versus *HP1*. The schematic drawing in Figure 12 illustrates the process. The DNA fragments were analysed in an agarose gel electrophoresis using a Caliper LabChip GX instrument (PerkinElmer, MA, USA). A similar method has previously been described by Ijäs et al. (132).

	PCR protocol	
	1st PCR	2nd PCR
	PCR with primers A & B	PCR with primers C & D
Genotype	Size of PCR products	
HP1-1	Medium chain	No product
HP2-1	Medium chain & Large chain	Small chain
HP2-2	Large chain	Small chain

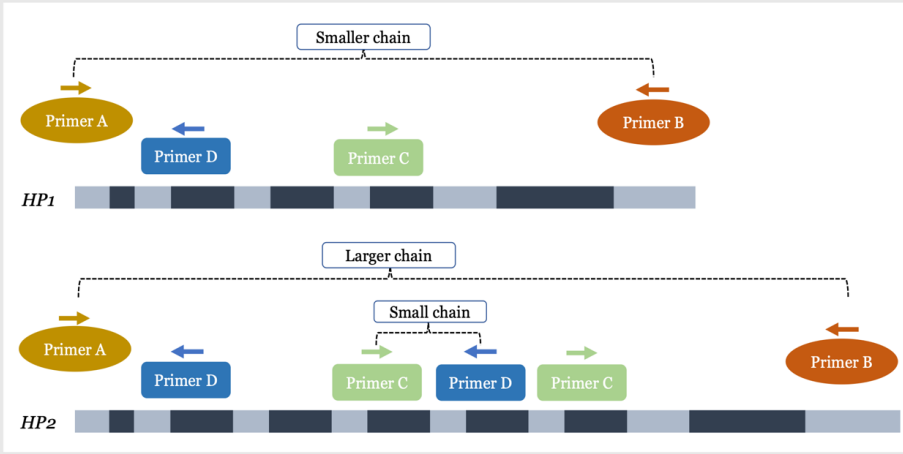

Figure 12 The 1st PCR used forward primer A and reverse primer B, replicating both alleles at their entire length. *HP2* allele, with its copy number variation, generates large chain copies, compared to the medium chain copies of *HP1*. The 2nd PCR used forward primer C and reverse primer D, promoting amplification of the copy number site at Hp2 only and yielding small chain copies. Chain sizes were analysed by electrophoresis, and by combining results from the 1st and 2nd PCR, Hp genotype could be determined.

5.4 Vascular complications

5.4.1 Diabetic kidney disease and assessment of kidney function

5.4.1.1 Classification based on albumin excretion rate

The assessment of the participants' kidney status was based on their urinary albumin excretion rate in at least two out of three consecutive and timed (over night or 24h collections) urine samples. A urinary albumin excretion rate of < 20 µg/min or < 30 mg/24h was considered normal. Moderately increased albumin excretion was defined as ≥ 20 and < 200 µg/min or ≥ 30 and < 300 mg/24h, and severely increased as ≥ 200 µg/min or ≥ 300 mg/24h. Participants on dialysis or those who

had received a kidney transplant were considered as having kidney replacement therapy (200).

In Studies I–III, the participants were categorised as having either a normal albumin excretion or albuminuria if the excretion was above normal. Participants on kidney replacement therapy were excluded in studies I–III.

In Study IV, diabetic kidney disease was defined as either having a severe albuminuria or kidney replacement therapy.

5.4.1.2 Assessment of glomerular filtration rate

Estimated glomerular filtration rate (eGFR) was calculated based on a single serum creatinine measurement using the formula presented by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (317).

The CKD-EPI formula

$$eGFR = 141 \times \min\left(\frac{Crea}{\kappa}, 1\right)^{\alpha} \times \max\left(\frac{Crea}{\kappa}, 1\right)^{-1.209} \times 0.993^{Age} \times 1.018[\text{If woman}]$$

In the CKD-EPI formula (317) above, *Crea* is serum creatinine, κ is 0.7 for women and 0.9 for men, α is -0.329 for women and -0.411 for men, min indicates the minimum of $Crea/\kappa$ or 1, and max the maximum of $Crea/\kappa$ or 1. For African American individuals, the expression is further multiplied by 1.159.

5.4.2 Diabetic retinopathy and macular oedema

5.4.2.1 Definition of diabetic retinopathy in Study I

At the study visit, the participants' medical history was examined and assessed for clues of diabetic retinopathy. A previous diagnosis of diabetic retinopathy and/or history of retinal photocoagulation were noted. In Study I, participants who had received retinal photocoagulation, regardless of the indication or width of the treatment, were considered to have diabetic retinopathy. Participants with diabetic retinopathy and/or increased urinary albumin excretion rate (≥ 20 μg/min or ≥ 30 mg/24h) were further categorised as having microvascular diabetic complications.

5.4.2.2 Retinopathy severity grading in Studies II–IV

In Studies II–IV, retinopathy severity was assessed based on the diabetic retinopathy severity scale published by the ETDRS (168). Table 3 (see section 2.4.3) shows a summary of the grades and corresponding retinal manifestations of

retinopathy. Macular oedema was also evaluated following the classification protocol by the ETDRS (187).

Images of both fundi (left and right eye) of the participants were evaluated, and the grade of the eye presenting more severe diabetic retinopathy was used for further categorisation and analysis. For those having undergone scatter photocoagulation, the severity of diabetic retinopathy that indicated the treatment was used as the grade, i.e., the most severe lifetime diabetic retinopathy.

ETDRS retinopathy severity grading in Studies II–III

For Studies II–III, diabetic retinopathy severity was graded by Paula Summanen (Department of Ophthalmology, Helsinki University Hospital, Helsinki, Finland), who is an experienced ophthalmologist specialised in diabetic retinopathy. The ophthalmologist was blinded to the clinical data, but not to whether the participant had diabetes or not. Retinopathy-like abnormalities in the healthy control's fundi were graded by the same protocol as the fundi of participants with type 1 diabetes.

For 114 participants with type 1 diabetes (60.3%) and all control individuals (n = 29) included, the assessment was based on fundus images taken at the FinnDiane Study visit. For 65 participants (34.4%), images taken for screening or clinical purposes at the participants' own health care unit were collected and used for the grading. Reliable evaluations could be made based on the participants' medical (ophthalmological) records for an additional 10 participants who did not have available fundus images. When participants had images available from several time points, the images taken closest to the brain MRI were used for grading. Of the graded images, 87% were taken within a year from the brain MRI.

ETDRS retinopathy grading and classification of macular oedema in Study IV

In Study IV, diabetic retinopathy and macular oedema were assessed by ophthalmologist Kustaa Hietala (Hospital Nova of Central Finland, Jyväskylä, Finland) based on fundus images and medical records collected retrospectively. The scoring procedure has previously been described in detail in Hietala's doctoral thesis (308) and are summarised in brief below.

Fundus photographs taken for screening and clinical purposes and/or records (medical files, verbal records) on fundus examinations performed by an ophthalmologist were obtained for all participants. All available data (both images and verbal records) were used for the scoring. When data were available from several different occasions of eye examination, a separate ETDRS score was given for each date in question. Similarly, the classification of macular oedema at each different occasion was registered.

For this study, we included only ETDRS scoring and macular classification before or at baseline, ascertaining the minimum severity grade possible at baseline. When data from several time points were available, the score dated closest to the baseline visit was chosen for analysis.

5.4.2.3 Definition of diabetic retinopathy and macular oedema in Studies II–IV

Any diabetic retinopathy

In Studies II and III, participants with an ETDRS score of 20 or higher, which indicates the presence of at least retinal microaneurysms, were defined as having any diabetic retinopathy.

Proliferative diabetic retinopathy

In Studies II and III, participants displaying neovascularisation or those who had received treatment for neovascularisation with retinal photocoagulation were categorised as having proliferative diabetic retinopathy (ETDRS score ≥ 61), which will hereafter be abbreviated as PDR.

Diabetic retinopathy groups

Study III

In study III, participants were further assigned into one of three categories based on ETDRS score (Table 9): Participants with an ETDRS score ≤ 35 were categorised as having no or mild retinopathy, ETDRS score 43–57 as moderate–severe non-PDR, and ETDRS score ≥ 61 as PDR.

Study IV

In study IV, participants with an ETDRS score < 35 were categorised as having no–very mild diabetic retinopathy, and ETDRS scores ≥ 35 and < 61 as having mild–severe non-PDR. Similar to Study III, ETDRS scores ≥ 61 was categorised as PDR.

Table 9 Comparison between the retinopathy categories used in Studies III and IV

	No or mild / no–very mild diabetic retinopathy	Mild / moderate–severe non-proliferative diabetic retinopathy	Proliferative diabetic retinopathy
Study III			
ETDRS score	10–35	43–53	61–85
Description	DR absent or microaneurysms alone or combined with other non-extensive lesions	Extensive lesions (severe–very severe) with/without intraretinal microvascular abnormality and, e.g., vitreous bleeding	Retinal neovascularisation with/without complications
Study IV			
ETDRS score	10–20	35–53	61–85
Description	DR absent or microaneurysms only	Microaneurysms combined with other lesions/ extensive manifestation	Retinal neovascularisation with/without complications

Diabetic retinopathy (DR) grouping based on severity score according to the Early Treatment Diabetic Retinopathy Study (ETDRS) grading scale (168).

5.4.3 Diabetic neuropathy

At the study visit, the participants were assessed for signs of diabetic neuropathy. This information, however, is not included in this present study due to a lack of standardised data.

Signs of cardiovascular autonomic neuropathy, such as non-dipping, and abnormal blood pressure variability were derived from the 24-hour ambulatory blood pressure monitoring in Study I described previously (see section 5.2.2).

5.4.4 Macrovascular complications

5.4.4.1 Coronary artery disease and peripheral arterial disease

The participant's medical history of macrovascular complications was reviewed at the study visit. Coronary artery disease was defined as a previous diagnosis of ischaemic heart disease, a history of myocardial infarction or coronary revascularisation, or the use of long-acting nitrates. Participants with a history of peripheral revascularisation or limb amputation were categorised as having peripheral arterial disease.

In Study I, the umbrella term *macrovascular diabetic complications* was used to indicate coronary artery disease and/or peripheral arterial disease.

5.4.4.2 Cerebrovascular disease including stroke

The participants' medical history regarding stroke (any type of stroke before visit: yes/no) was also investigated at the study visit. None of the included participants had a history of stroke, as this was an exclusion criterion for all studies (Studies I–IV). For participants in Studies I–III, previous signs of stroke or transient ischaemic attack were evaluated with a validated questionnaire (Questionnaire for Verifying Stroke-Free Status) (306,307) (Meschia 2000, Jones 2001). The assessment of stroke in participants in Study IV is described in detail in section 5.6.1.

5.5 Brain MRI and definition of cerebral small vessel disease

Participants in Studies I–III underwent brain MRI within a year from the clinical FinnDiane Study visit. The MRI was performed at the Helsinki Medical Imaging Center, HUS, with the same 3.0T scanner (Achieva, Philips, Best, The Netherlands). The scans included T1, T2, T2*, diffusion-weighted imaging (DWI), and susceptibility-weighted imaging (SWI), as well as fluid attenuated inversion recovery (FLAIR), magnetisation-prepared rapid gradient echo (MPRAGE), and time-of-flight magnetic resonance angiography (TOF MRA) sequences.

The images were assessed by an experienced neuroradiologist (Juha Martola, Department of Radiology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland), and the neuroradiologist was blinded to the participants' clinical data, but not to whether they had type 1 diabetes or not.

Markers of cerebral small vessel disease were evaluated by standardised criteria (37,318), including the Fazekas scale for categorisation of white matter hyperintensities (245). Presence of any cerebral microbleeds, cortical superficial siderosis, lacunar infarctions, or white matter hyperintensities of Fazekas category 1 or higher were taken as evidence of cerebral small vessel disease.

5.6 Prospective follow-up data

5.6.1 Definition and classification of stroke

For participants in Study IV, we identified cerebrovascular events occurring during the follow-up time from the baseline visit until the end of 2017. The information about strokes was assembled from the National Care Register for Health Care (Hilmo) and the Finnish Cause of Death Register, identified by the 10th revision of the International Classification of Disease (ICD-10) codes I60–64, and the 9th revision (ICD-9) codes 430–434 (186).

After identifying cerebrovascular events from registers, the participant's medical files (verbal records from clinical examinations, computer tomography, and/or MRI) were ordered from the hospital where the participant was treated, or/and death certificates were ordered from Statistics Finland. From medical files, all stroke cases were verified and categorised based on aetiology by medical doctors. The categorisation was confirmed by a stroke neurologist (Jukka Putaala, HUS Neurocenter, Helsinki, Finland), who confirmed all strokes occurring before 2010 and all differentiations between large- and small-vessel aetiology. Evaluation of large-vessel haemorrhages and occlusion occurring in 2011–2017 was done by a general practitioner and a specialist in general medicine and additionally confirmed by the stroke neurologist (J.P.) if there was any uncertainty or disagreement in the initial categorisation.

Overt cerebrovascular events that presented with persisting clinical symptoms and a radiologically differentiated aetiology (ischaemic lesions, or in the absence of detectable lesions, haemorrhages excluded or confirmed) were defined as stroke. Transient ischaemic attacks and events caused by trauma were not considered as stroke. Strokes consistent with vessel occlusion were defined as ischaemic stroke. This included cerebral infarctions with haemorrhagic transformation. Ischaemic strokes were further subtyped as lacunar strokes if they were of small-vessel origin (occlusion of a single perforating artery) or non-lacunar if they had a large-vessel aetiology. Intracerebral haemorrhage and subarachnoid haemorrhages were defined as haemorrhagic strokes.

5.6.2 Mortality

Information about death during follow-up was obtained from the Finnish Cause of Death Register. Death of any cause during follow-up was noted, but no further classification of cause of death was done.

5.6.3 Follow-up time

Participants remained in follow-up until the date of their first stroke, death, or censoring by the end of follow-up time, 31 December, 2017.

5.7 Statistical analyses

5.7.1 Exploratory data analysis and hypothesis tests

After evaluating the main characteristics of the data, such as distribution, central tendency, and dispersion, different groups of participants were compared using statistical hypothesis testing.

In all studies, continuous variables with normally distributed data were analysed using the t-test when comparing two groups and one-way ANOVA when comparing three groups. Results are presented as mean ± standard deviation. For non-normally distributed continuous variables, we used the Wilcoxon rank-sum test (or Mann-Whitney U-test) when comparing two groups and the Kruskal-Wallis H test for three groups. The central tendency is presented as median [interquartile range]. To compare categorical variables between groups, we used the chi-squared test, and Fisher's exact test when observed frequencies were < 5. A p-value < 0.05 was considered statistically significant in all studies.

Together with variables that differed between the outcome groups in the initial analyses, the study-specific traits of interest were analysed in association with the studied outcome in additional statistical analyses. These study specific statistical analyses are described in detail in the following sections.

We used IBM SPP Statistics 22 software (International Business Machines Corporation, Armonk, NY) to perform all analyses in Study I and part of the analysis in Study III. R version 4.0.0 (ref) was used for the rest of the analyses in Study III and for all analyses in Study II. Study IV was performed using R version 4.2.3 (2023-03-15 ucrt) (319).

5.7.2 Statistical analyses in Study I

After comparing participants with versus without cerebral small vessel disease in the exploratory data analyses, the association between blood pressure and cerebral small vessel disease was assessed by logistic regression. Cerebral small vessel disease was analysed as a dependent variable with the blood pressure variable of interest as independent in a logistic regression model adjusted for age, use of antihypertensive medication (yes/no), and ambulatory blood pressure monitoring quality. All blood pressure variables of interest were analysed in separate models. The results are presented as odds ratios (OR) with a 95% confidence interval (CI).

5.7.3 Statistical analyses in Study II

To test for potential bias or effects of genetic selection in the cohort, haptoglobin genotype frequencies were tested against the Hardy-Weinberg equilibrium using the chi^2 test.

We used logistic regression analysis to assess the association between cerebral small vessel disease and the haptoglobin genotype in relation to clinical confounders. Cerebral small vessel disease was analysed as the dependent variable, with haptoglobin genotype and clinical confounders as covariates. Cerebral small vessel disease (any manifestation) and all different manifestations (cerebral microbleeds, white matter hyperintensities, and lacunar infarctions) were analysed in separate models. The clinical confounders were chosen as covariates based on their significance in the initial hypothesis testing.

We performed post hoc power analysis to evaluate the smallest detectable effect size in our cohort. The detectable OR for cerebral small vessel disease as a binary outcome (present versus absent) was calculated based on minor allele frequency, the proportion of participants with cerebral small vessel disease, and the number of participants in the study. The analysis was performed with the R package pwr (320). A significance level of α=0.05 was chosen for the analysis. The results showed that effect sizes of OR 1.91 or larger would be detected with 80% probability (α=0.05) for the included number of participants. The estimated sample size required to detect effect sizes smaller than OR 1.5 (80% probability of detection, α=0.05) was 4,295 participants.

5.7.4 Statistical analyses in Study III

We used logistic regression to analyse the association between cerebral small vessel disease and diabetic retinopathy. Cerebral small vessel disease and its different manifestations were analysed as dependent variables in separate models, with diabetic retinopathy and potential clinical confounders as independent variables. Age, systolic office blood pressure, increased albumin excretion rate (yes/no), and HbA_{1c} were chosen as clinical confounders based on significance in initial comparison of participants with versus without cerebral small vessel disease and presumed clinical significance. Results are presented as ORs with 95% CI.

We calculated positive and negative predictive values, which express the conditional probability of the presence or absence of cerebral small vessel disease at a given severity level of diabetic retinopathy.

The positive predictive value (PPV) was calculated as follows:

$$\text{PPV} = \frac{\text{Number of true positives}}{\text{Number of positive calls}}$$

PPV expresses the conditional probability of having cerebral small vessel disease (*A)* given that diabetic retinopathy severity is observed (*B*) when the conditional probability is expressed as follows:

$$P(A|B) = \frac{P(A \cap B)}{P(B)}$$

The negative predictive value (NPV) was calculated as follows:

$$\text{NPV} = \frac{\text{Number of true negatives}}{\text{Number of negative calls}}$$

which represents the case of $P(A|B)$ when A is the absence of cerebral small vessel disease and B the absence of diabetic retinopathy.

5.7.5 Statistical analyses in Study IV

The stroke incidence rate was calculated as the number of events per 100,000 person-years with a 95% CI using a Poisson distribution. Using the R package survminer (321), we plotted cumulative events by the Kaplan-Meier method, with log-rank testing for deviations between the groups. All outcomes of interest, i.e., all stroke types and subtypes, were analysed separately by Cox regression models. Potential prognostic variables, which were significant when comparing individuals with versus without stroke, were entered as covariates in the Cox regression models. If several variables represented the same characteristic and/or there was multicollinearity between variables, only one of the corresponding variables was included in the model. Age at onset of diabetes was included based on assumed clinical relevance, regardless of significance in initial analysis. Observations with missing values were omitted in the initial comparison of the groups. Variables containing observations with missing values, e.g., smoking status, were excluded from the adjusted analysis. Cox regression and testing of the assumption of proportional hazards were performed with the survival package (322,323).

To evaluate stroke in the presence of death as competing risk, we analysed the cumulative incidence of stroke and death as competing risks in the same cumulative incidence function. We also calculated sub distribution hazards by covariate in a Fine-Gray regression model. The Fine-Gray model included the same covariates as in the Cox regression models. The competing risk analysis was performed with the R package cmprsk (324).

6 Results

This chapter presents results from the original publications (Studies I–IV) and some previously unpublished data.

6.1 Cerebral small vessel disease in association with ambulatory blood pressure (Study I)

6.1.1 Participants' characteristics

The study included 73 participants who had undergone MRI and 24-hour ambulatory blood pressure monitoring. Table 10 presents a comparison of clinical characteristics for the participants included in this study and the excluded (n = 118) participants from the brain MRI cohort. When comparing the included and excluded brain MRI participants (previously unpublished data), there was no significant difference in age or sex, nor in HbA1c. Office systolic blood pressure and use of antihypertensive medication was similar across the groups, whereas the included participants had lower office diastolic blood pressure. The included participants were less frequently smokers. Of the included, 15 (20.8%) had a history of microvascular complications, whereas none had macrovascular complications.

6.1.2 Prevalence of cerebral small vessel disease

Of the 73 participants studied, 20 (27.4%) had cerebral small vessel disease, and the prevalence did not differ compared to the excluded participants (29.8%, p = 0.080). Microbleeds were the most frequently observed manifestation, followed by white matter hyperintensities, as presented in Figure 13.

Table 10 Characteristics of the included versus excluded participants of the brain MRI cohort

	Included	Excluded	*P*
N	73	118	
Men, *n* (%)	40 (55.8)	50 (42.4)	0.095
Age, years	40.6 [33.7,45.7]	39.2 [32.4, 44.9]	0.184
Diabetes duration, years	21.2 [18.9, 24.0]	23.7 [13.4, 32.4]	0.397
Body mass index, kg/m^2	27.5 ± 3.8	26.2 ± 4.4	0.193
HbA_{1c}, mmol/mol	65 ± 12	66 ± 13	0.181
Lipid-lowering medication, *n* (%)	18 (24.7)	24 (20.3)	0.484
Total cholesterol, mmol/l	4.3 (3.9, 5.0)	4.5 (4.0, 5.0)	0.043
LDL cholesterol, mmol/l	2.4 ± 0.7	2.5 ± 0.8	0.935
HDL cholesterol, mmol/l	1.5 [1.2, 1.9]	1.5 [1.3, 1.8]	0.895
Triglycerides, mmol/l	0.9 [0.7, 1.4]	0.9 [0.7, 1.4]	0.801
BP-lowering medication, *n* (%)	29 (39.7)	39 (33.1)	0.349
Office SBP, mmHg	131 [123, 142]	128 [117, 138]	0.091
Office DBP, mmHg	81 [74, 85]	73 [69, 80]	<0.001
Albuminuria (history of), *n* (%)	8 (11.0)	22 (18.6)	0.156
eGFR, ml/min	109 [102, 116]	107 [93, 114]	0.047
Current smoker, *n* (%)	1 (1.4)	14 (11.9)	0.009

Data are presented as median [quartiles] and mean ± standard deviation. BP indicates blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; and eGFR, estimated glomerular filtration rate.

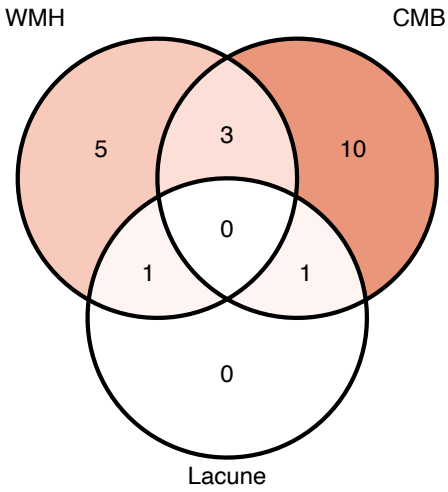

Figure 13 The Venn diagram displays the number of participants with a given manifestation of cerebral small vessel disease and the overlap of manifestations in participants. WMH indicates white matter hyperintensity of Fazekas ≥ 1, and CMB cerebral microbleed.

Participants with versus without cerebral small vessel disease did not differ with regards to sex distribution (50.0% vs. 56.6% men, $p = 0.613$) or age (median 42.2 [lower quartile 39.3, upper quartile 46.2] vs. 39.6 [33.4, 45.2] years, $p = 0.113$). The same was true for diabetes-related characteristics such as duration of diabetes (20.9 [19.2, 32.1] vs. 21.2 [19.9, 23.9] years, $p = 0.748$), age at onset of diabetes (19.9 [10.7, 24.1] vs. 15.5 [11.0, 22.8] years, $p = 0.638$), history of albuminuria (20.0% vs. 7.5%, $p = 0.203$), eGFR (106 [95, 115] vs. 111 [104, 117] ml/min, $p = 0.122$), and history of retinal photocoagulation (15.0% vs. 17.0%, $p > 0.999$).

6.1.3 Blood pressure measurements

6.1.3.1 Office blood pressure

A total of 40.0% ($n = 8$) of the participants with cerebral small vessel disease and 39.6% ($n = 21$) of those without disease used antihypertensive medication at the time of assessment ($p = 0.977$). The use of antihypertensive medication was not associated with cerebral small vessel disease in a logistic regression model adjusted for age and quality of ambulatory blood pressure recordings ($p = 0.791$). Office measurements of blood pressure did not differ between the groups; the results are presented in Table 11.

Table 11 Office blood pressure according to presence of cerebral small vessel disease

	Cerebral small vessel disease	No cerebral small vessel disease	*P*	*P**
N	20	53		
Office SBP, mmHg	133 [124,139]	130 [123,142]	0.916	0.270
Office DBP, mmHg	79 [83, 75]	81 [74, 85]	0.697	0.503
Office pulse, /min	69 ± 12	69 ± 12	0.961	0.778

Data are presented as median [quartiles] and mean ± standard deviation. SBP indicates systolic and DBP diastolic blood pressure. *Analysed in logistic regression adjusted for age, antihypertensive medication, and ambulatory blood pressure monitoring quality.

6.1.3.2 Ambulatory measurements

The median time passed between the 24-hour ambulatory blood pressure monitoring and the MRI was 2.89 [0.88, 5.02] months, and the success rate of the measurements averaged 90.8% [82.2%, 94.3%]. Average duration of ambulatory blood pressure monitoring was 23.4 ± 4.1 hours; the average number of diurnal readings was 52 ± 13 and 16 ± 6 for nocturnal readings.

6.1.3.3 Ambulatory systolic and diastolic blood pressure

During 24 hours of ambulatory blood pressure monitoring, the participants' median systolic blood pressure was 124 [119, 131] mmHg, and the diastolic blood pressure was 81 [75, 84] mmHg. Hypertension was noted in 41 (56.2%) of the participants and masked hypertension in 22 (30.3%). The proportion of blood pressure recordings of good quality (45.0% vs. 58.5%, p = 0.302) did not differ between individuals with versus without cerebral small vessel disease.

Table 12 displays the 24-hour blood pressure results during the different intervals of the monitoring period. Participants with cerebral small vessel disease had a higher nocturnal systolic and diastolic blood pressure. The same was true after adjustments for age, antihypertensive medication, and ambulatory blood pressure monitoring quality. No differences in diurnal or 24-hour average systolic blood pressure and diastolic blood pressure were observed (Table 12).

Table 12 24-hour systolic and diastolic blood pressure

	Cerebral small vessel disease	No cerebral small vessel disease	*P*	*P**
N	20	53		
24-hour SBP, mmHg	127 [124,135]	122 [118, 129]	0.058	0.078
Diurnal SBP, mmHg	130 [127, 140]	127 [122, 132]	0.115	0.173
Nocturnal SBP, mmHg	117 [111, 124]	110 [107, 116]	0.013	0.010
24-hour DBP, mmHg	79 [76, 86]	79 [76, 83]	0.586	0.356
Diurnal DBP, mmHg	82 [79, 89]	82 [79, 87]	0.096	0.648
Nocturnal DBP, mmHg	72 [68, 76]	67 [64, 72]	0.018	0.009

Data are presented as median [quartiles]. SBP indicates systolic and DBP diastolic blood pressure. *Analysed in logistic regression adjusted for age, antihypertensive medication, and ambulatory blood pressure monitoring quality.

6.1.3.4 Nocturnal dipping and heart rate

Participants with cerebral small vessel disease displayed an 11.0% [6.7%, 14.0%] nocturnal dip in systolic blood pressure, whereas the nocturnal dip in those without cerebral small vessel disease was 13.4% [9.3%, 16.9%] (p = 0.094). The proportion of nocturnal dippers was 60.0% (n = 12) in participants with cerebral small vessel disease and 67.9% (n = 36) in those without the disease (p = 0.719). Only four participants had a decrease ≥ 20.0% in nocturnal blood pressure, wherefore no additional analysis were implemented for this group separately.

In logistic regression analysis, nocturnal dipping was borderline associated with lower odds for cerebral small vessel disease (OR 0.90 [95% CI 0.80–1.00] per 1.0% increase in dip). The model additionally included age, use of antihypertensive medication, and quality of measurements.

The 24-hour median heart rate (73 [70, 86] vs. 75 [70, 84] /min, p = 0.509), diurnal heart rate (76 [70, 86] vs. 75 [70, 84] /min, p = 0.504), or nocturnal heart rate (63 [58, 69] vs. 62 [56, 68] /min, p = 0.658) did not differ between the groups.

6.1.3.5 Mean arterial, pulse pressure, and blood pressure variability

Mean arterial pressure and pulse pressure are presented in Table 13. Participants with cerebral small vessel disease had higher nocturnal mean arterial pressure and higher diurnal pulse pressure compared to those without small vessel disease. Nocturnal mean arterial pressure remained significantly associated with cerebral small vessel disease in the logistic regression model, whereas pulse pressure was no longer significant after adjusting for age, antihypertensive medication, and quality of recordings. No association between average real variability of the blood pressure and cerebral small vessel disease was observed (Table 4).

Table 13 Mean arterial pressure, pulse pressure, and blood pressure variability

	Cerebral small vessel disease	No cerebral small vessel disease	P	P*
N	20	53		
24-hour MAP, mmHg	95 [90, 101]	94 [90, 98]	0.429	0.416
Diurnal MAP, mmHg	97 [93, 104]	97 [93, 102]	0.911	0.852
Nocturnal MAP, mmHg	86 [83, 92]	81 [78, 86]	0.005	0.006
24-hour PP, mmHg	47 [44, 52]	44 [41, 48]	0.054	0.074
Diurnal PP, mmHg	47 [45, 53]	44 [41, 48]	0.032	0.091
Nocturnal PP, mmHg	45 [41, 4]	44 [40, 47]	0.356	0.176
24-hour SBP ARV, mmHg	10 ± 3	10 ± 3	0.742	0.604
24-hour DBP ARV, mmHg	8 [7, 9]	7 [6, 9]	0.536	0.351

Data are presented as median [quartiles] and mean ± standard deviation. MAP mean arterial pressure; PP, pulse pressure; and ARV, average real variability. *Analysed in logistic regression adjusted for age, anti-hypertensive medication, and ambulatory blood pressure monitoring quality

6.1.3.6 Hypertension

Elevated office blood pressure was equally prevalent in both groups (20.0% vs. 34.0%, p = 0.246). A total of 56.2% (n = 41) of the participants fulfilled the criteria for hypertension during at least one of the studied time intervals (24-hour, nighttime, or daytime). Figure 14 presents the prevalence of hypertension during the different time intervals in participants with and without cerebral small vessel disease.

Nocturnal hypertension and masked hypertension were more common in individuals with cerebral small vessel disease compared to participants without the

disease. The prevalence of elevated 24-hour blood pressure and diurnal blood pressure did not differ between the groups. White coat hypertension was not observed in any of those with cerebral small vessel disease (Figure 14).

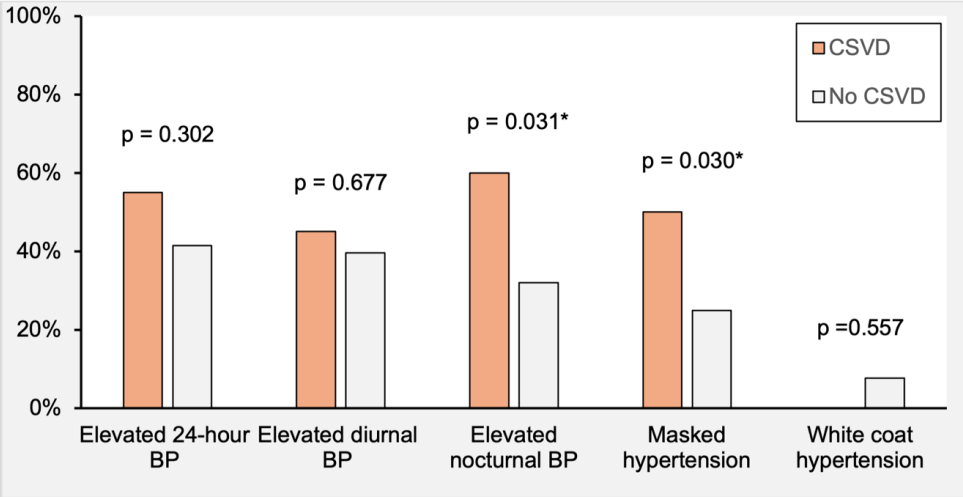

Figure 14 Nocturnal hypertension was more prevalent (60.0% vs. 41.5%) among participants with cerebral small vessel disease (CSVD) compared to no CSVD. The same was true for masked hypertension (50.0% vs. 25.0%).

Associations between hypertension and cerebral small vessel disease after adjustments are presented in Table 14. In the adjusted model, nocturnal hypertension and masked hypertension were independently associated with cerebral small vessel disease. No associations were seen for elevated office blood pressure, 24-hour mean blood pressure, or diurnal blood pressure.

Table 14 Association between hypertension and cerebral small vessel disease

	OR (95% CI) for cerebral small vessel disease	*P*
Elevated office BP	0.398 (0.106–1.499)	0.174
Elevated 24-hour mean BP	1.718 (0.537–5.492)	0.361
Diurnal hypertension	1.101 (0.361–3.363)	0.866
Nocturnal hypertension	4.086 (1.265–13.196)	0.019
Masked hypertension	3.740 (1.170–11.954)	0.020

Results are presented as odds ratios (OR) with 95% confidence intervals (95% CI). BP indicates blood pressure. All parameters are analysed separately in a logistic regression model adjusted for age, antihypertensive medication, and 24-hour ambulatory blood pressure monitoring quality.

When stratifying the data based on antihypertensive medication use, differences in 24-hour ambulatory blood pressure monitoring were seen. Participants with cerebral small vessel disease and antihypertensive medication

showed higher median systolic blood pressure (127 [119, 132] vs. 113 [108, 117] mmHg, p = 0.001) and diastolic blood pressure (75 [70, 78] vs. 67 [64,73] mmHg, p = 0.021), in unadjusted analyses. Participants with antihypertensive medication and small vessel disease also had a higher prevalence of nocturnal and masked hypertension (78.5% vs. 35.0%, p = 0.033 and 75.0% vs. 23.8%, p = 0.028, respectively). In participants without antihypertensive medication, no difference in blood pressure was observed (systolic 113 [106, 118] vs. 109 [104, 114] mmHg, p = 0.314 and diastolic 69 [66, 73] vs. 67 [61, 71] mmHg, p = 0.288). There was also no difference in nocturnal hypertension (33.3% vs. 41.7%, p = 0.491) nor masked hypertension (33.3% vs. 23.3, p = 0.699) between the groups.

6.2 Cerebral small vessel disease in relation to haptoglobin genotype (Study II)

6.2.1 Participants' characteristics

The study included 179 participants, with a mean age of 38.8 ± 7.3 years and 53.1% (n = 95) were women. The average duration of diabetes was 23.3 ± 10.1 years among the participants, and their mean HbA_{1c} was 65 ± 12 mmol/mol. None of the participants had peripheral arterial disease.

6.2.1.1 Prevalence of cerebral small vessel disease

A total of 62 (34.6%) participants had at least one marker of cerebral small vessel disease on MRI. Cerebral microbleeds were observed in 40 (22.3%), white matter hyperintensities of Fazekas ≥ 1 in 31 (17.3%), and lacunar infarctions in 4 (2.2%) participants. Cortical superficial siderosis was not observed in any of the participants. Nine participants displayed both white matter hyperintensities and cerebral microbleeds on brain MRI. All four participants with lacunar infarctions had some additional manifestations: two had white matter hyperintensities and the other two had cerebral microbleeds.

6.2.1.2 Haptoglobin genotypes

HP2 was the most frequent allele among the participants, with 40.8% being homozygotes (*HP2-2*), and 43.6% heterozygotes (*HP2-1)*. A total of 15.6% participants had the *HP1-1* genotype, and the minor allele (*HP1*) frequency within the study cohort was 37.4%. The distribution was similar to frequencies previously observed in the Finnish population (289) and within the Hardy-Weinberg equilibrium (p = 0.647).

Table 15 presents clinical characteristics by genotype. The clinical characteristics did not differ significantly across participants with different genotypes, except for median diastolic blood pressure, which was the highest in participants with *HP1-1* (Table 15).

Table 15 Clinical characteristics by haptoglobin genotype

	HP1-1	***HP2-1***	***HP2-2***	***P***
N	28	78	73	
Sex (women), *n* (%)	19 (67.9)	41 (52.6)	35 (47.9)	0.198
Age, years	38.5 ± 6.4	38.4 ± 7.9	39.2 ± 6.9	0.792
Diabetes duration, years	22.2 ± 7.8	23.8 ± 10.7	23.2 ± 10.2	0.794
Age at diabetes onset, years	14.7 [8.1, 24.8]	12.3 [7.6, 20.7]	14.2 [8.9, 21.9]	0.563
Body mass index, kg/m2	27.4 ± 4.2	27.1 ± 4.24	25.8 ± 3.7	0.089
Systolic blood pressure, mmHg	132 ± 15	131 ± 14	129 ± 15	0.523
Diastolic blood pressure, mmHg	81 [74, 83]	75 [70, 80]	75 [72, 81]	0.019
Antihypertensive medication, *n* (%)	14 (50.0)	28 (35.9)	20 (27.4)	0.097
HbA1c, mmol/mol	64 [60, 70]	65 [56, 72]	66 [57, 73]	0.540
Haemoglobin, g/l	139 [133, 144]	137 [128, 146]	139 [130, 145]	0.936
Total cholesterol, mmol/l	4.4 [4.1, 5.4]	4.4 [4.0, 5.0]	4.5 [4.0, 4.9]	0.770
LDL cholesterol, mmol/l	2.6 [1.9, 3.0]	2.3 [2.1, 2.7]	2.5 [2.0, 3.0]	0.456
HDL cholesterol, mmol/l	1.5 ± 0.4	1.5 ± 0.4	1.6 ± 0.4	0.854
Triglycerides, mmol/l	0.9 [0.7, 1.5]	1.0 [0.7, 1.5]	0.9 [0.6, 1.2]	0.254
Lipid-lowering medication, *n* (%)	5 (17.9)	17 (21.8)	15 (20.5)	0.907
Creatinine, µmol/l	66 [59, 78]	71 [62, 82]	67 [61, 78]	0.346
eGFR, ml/min/1.73 m2	108 [96, 113]	106 [95, 114]	109 [97, 115]	0.538
Albuminuria, *n* (%)	5 (17.9)	14 (17.9)	11 (15.1)	0.881
History of smoking, *n* (%)	11 (39.3)	20 (25.6)	26 (35.6)	0.276
Any diabetic retinopathy, *n* (%)	27 (96.4)	61 (79.2)	57 (79.2)	0.094
Proliferative diabetic retinopathy, *n* (%)	6 (21.4)	12 (15.4)	9 (12.3)	0.517
Coronary artery disease, *n* (%)	0 (0.0)	1 (1.3)	0 (0.0)	0.521

Results are presented as *n* (%), mean ± standard deviation, and median [quartiles], with *P* given for comparison across all groups. HbA1c indicates glycated haemoglobin and eGFR estimated glomerular filtration rate.

6.2.2 MRI features by different genotypes

6.2.2.1 Any markers of cerebral small vessel disease

Figure 15 illustrates genotype frequencies and minor allele frequencies for participants with versus without cerebral small vessel disease. In the comparison of genotype frequencies by presence versus absence of markers of cerebral small vessel disease, the genotype frequencies did not differ significantly. Likewise, there was no difference in the minor allele frequencies by presence (*MAF 39.5%*) versus absence (*MAF 36.3%*) of cerebral small vessel disease markers (Figure 15).

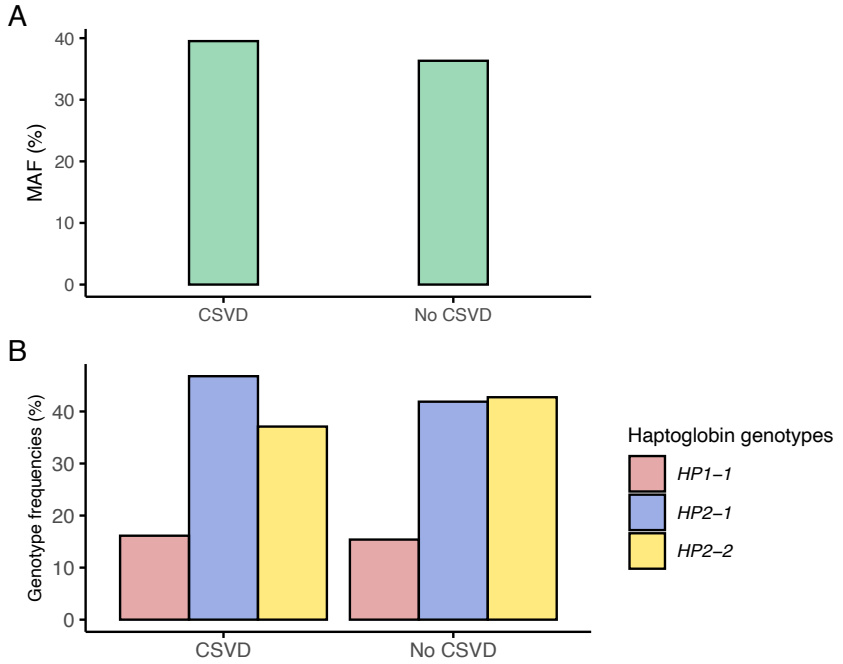

Figure 15 Allele and genotype frequencies in individuals with versus without cerebral small vessel disease (CSVD). Plot A displays minor allele frequency (MAF) in individuals with versus without CSVD (p = 0.533). Plot B displays frequencies of genotypes *HP1-1, HP2-1*, and *HP2-2* in participants with CSVD versus no CSVD (p = 0.758).

6.2.2.2 Markers of cerebral small vessel disease

The prevalence of white matter hyperintensities or cerebral microbleeds did not vary across different genotypes, as presented in Table 16. The same was true regarding lacunar infarctions (Table 16).

Table 16 Prevalence of cerebral small vessel disease by genotype

	HP1-1	*HP2-1*	*HP2-2*	*P*
N	28	78	73	
Cerebral small vessel disease (any manifestation)	10 (35.7)	29 (37.2)	23 (31.5)	0.758
White matter hyperintensities	4 (14.3)	17 (21.8)	10 (13.7)	0.428
Cerebral microbleeds	7 (25.0)	16 (20.5)	17 (23.3)	0.860
Lacunar infarctions	0 (0)	1 (1.3)	3 (4.1)	0.563

Results are presented as *n* (%).

We additionally assessed the minor allele (*HP1*) frequencies in the participants with versus without the respective manifestations of cerebral small vessel disease and were not able to detect any differences across the groups: The minor allele frequency was 37.4% in participants with cerebral microbleeds compared to 37.5% in those without microbleeds, $p = 0.988$. In participants with versus without white matter hyperintensities, the minor allele frequency was 40.3% versus 36.8%, $p = 0.605$. As we had only four observations of lacunar infarctions, we could not perform statistical testing for those.

6.2.2.3 HP1 in association with cerebral small vessel disease

To assess the potential effect of *HP1,* we analysed the additive effect of the *HP1* allele by simple logistic regression. We did not detect any significant association between cerebral small vessel disease and the number of *HP1* alleles (OR 1.14 95% CI 0.73–1.75, $p = 0.566$).

Next, we strode to evaluate if the absence of HP2 might be associated with an increased probability of cerebral small vessel disease or any specific marker thereof. When comparing individuals with *HP1-1* ($n = 28$) to *HP2* carriers (*HP2-1* or *HP2-2*, $n = 151$), homozygotes for *HP1* did not significantly differ from *HP2* carriers regarding clinical characteristics (data not shown), except for diastolic blood pressure (*HP1-1* average 80 ± 6 mmHg vs. *HP2* carriers 76 ± 9 mmHg, $p = 0.027$).

Table 17 presents observations of cerebral small vessel disease in individuals with genotype *HP1-1* versus *HP2* carriers. The cerebral small vessel disease prevalence did not vary significantly between *HP1* homozygotes and *HP2* carriers. Genotype *HP1-1* was not associated with cerebral small vessel disease after adjustment for age, systolic and diastolic blood pressure, and any diabetic retinopathy in a logistic regression model. We did not detect an association between *HP1-1* and white matter hyperintensities or cerebral microbleeds when analysing

each manifestation separately, adjusted for the same clinical confounders (Table 17).

Table 17 Association between cerebral small vessel disease and haptoglobin genotype Hp1-1

	Unadjusted analysis			Adjusted model		
	HP1-1	*HP2* carriers	*P*	OR	95% CI	*P*
N	28	151				
Cerebral small-vessel disease, *n* (%)	10 (35.7)	52 (34.4)	>0.999	1.19	0.46–2.94	0.712
White matter hyperintensities, *n* (%)	4 (14.3)	27 (17.9)	0.790	0.79	0.21–2.44	0.703
Cerebral microbleeds, *n* (%)	7 (25.0)	33 (21.9)	0.904	1.23	0.43–3.25	0.679
Lacunar infarctions, *n* (%)	0 (0.0)	4 (2.6)	>0.999	–	–	–

P-values for unadjusted analysis are calculated with chi-square testing from univariable analyses, and results are presented as *n* (%). In adjusted analysis, *HP1-1* compared to *HP2* carriers are analysed in a logistic regression model adjusted for age, systolic and diastolic blood pressure, and any diabetic retinopathy. Results are presented as odds ratios (OR) with 95% confidence intervals (CI).

6.3 Cerebral small vessel disease in association with diabetic retinopathy (Study III)

6.3.1 Characteristics of participants and controls

The 189 study participants with type 1 diabetes had a median age of 40 (quartiles 33, 45) years, median duration of diabetes of 21.6 (18.2, 30.7) years, and 101 (53%) were female. None of the participants had a history of coronary heart disease or peripheral arterial disease.

The controls without diabetes were sex- and age-matched to the participants with diabetes. Clinical characteristics of the controls are presented in Table 18.

6.3.1.1 Prevalence and severity of diabetic retinopathy

Diabetic retinopathy was present in 155 (82%) of the participants with type 1 diabetes. Of the controls, one individual (3%) had a microaneurysm in one eye (corresponding to an ETDRS score of 20), whereas 28 individuals exhibited no retinopathy-like abnormalities. Figure 16 presents the distribution of ETDRS scores in participants with type 1 diabetes (previously unpublished data).

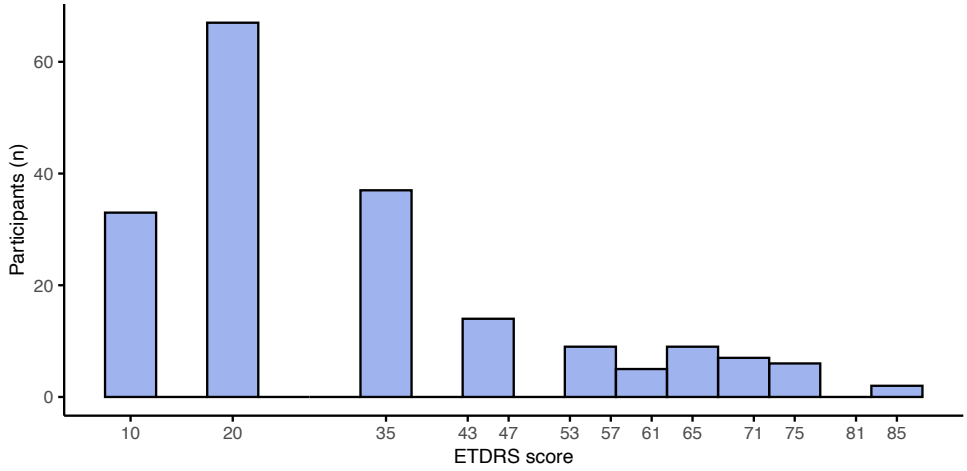

Figure 16 Histogram displaying the distribution of Early Treatment Diabetic Retinopathy Study (ETDRS) scores in all participants with type 1 diabetes.

No–mild diabetic retinopathy (ETDRS score ≤ 35) was observed in 137 (72%) participants, moderate–severe non-PDR (ETDRS score 43–57) in 23 (12%), and PDR (ETDRS score ≥ 61) in 29 (15%) participants. The clinical characteristics for the retinopathy groups are also shown in Table 9.

Table 18 Clinical characteristics of healthy controls and individuals with type 1 diabetes by retinopathy severity

	Healthy controls, *N* = 29	No or mild DR, *N* = 137	*P**	Moderate–severe NPDR, *N* = 23	*P*†	PDR, *N* = 29	*P*‡
Female sex, *n* (%)	16 (55.2)	75 (54.7)	0.966	8 (34.8)	0.076	17 (58.6)	0.703
Age, years	37.7 (31.3, 43.4)	39.8 (32.6, 44.3)	0.602	39.4 (34.4, 45.6)	0.493	41.5 (35.3, 47.6)	0.158
Diabetes duration, years	–	20.4 (14.4, 26.7)	-	22.3 (19.9, 29.2)	0.061	35.8 (27.9, 37.8)	<0.001
Body mass index, kg/m^2	24.4 (22.1, 26.1)	25.7 (23.6, 28.5)	0.032	28.9 (25.8, 31.0)	0.004	27.1 (23.9, 30.1)	0.087
Systolic blood pressure, mmHg	121 (112, 130)	129 (118, 138)	0.004	136 (122, 144)	0.062	132 (121, 148)	0.195
Diastolic blood pressure, mmHg	76 (73, 85)	77 (70, 82)	0.448	81 (74, 87)	0.035	72 (74, 80)	0.708
HbA_{1c}, mmol/mol	33 (31, 34)	64 (56, 71)	<0.001	65 (61, 70)	0.198	73 (60, 83)	0.002
Creatinine, µmol/l	74 (67, 81)	66 (61, 77)	0.019	69 (60, 79)	0.537	77 (63, 90)	0.009
Total cholesterol, mmol/l	4.6 (4.2, 5.4)	4.3 (4.0, 4.9)	0.145	4.4 (3.8, 5.5)	0.649	4.8 (4.0, 5.2)	0.055
LDL cholesterol, mmol/l	2.6 (2.3, 3.3)	2.4 (2.0, 2.8)	0.028	2.5 (2.0, 3.0)	0.714	2.4 (2.0, 3.0)	0.629
HDL cholesterol, mmol/l	1.45 (1.23, 1.66)	1.48 (1.25, 1.82)	0.303	1.30 (1.15, 1.80)	0.153	1.56 (1.40, 1.78)	0.174
Triglycerides, mmol/l	0.84 (0.68, 1. 27)	0.84 (0.64, 1.21)	0.997	1.18 (0.88, 2.29)	0.003	1.01 (0.82, 1.51)	0.021
Antihypertensive medication, *n* (%)	0	32 (23.4)	0.004	13 (56.5)	0.001	22 (75.9)	<0.001
Lipid-lowering medication, *n* (%)	0	20 (14.6)	0.028	11 (47.8)	<0.001	10 (34.5)	0.011
Aspirin therapy, *n* (%)	0	8 (5.8)	0.182	2 (8.7)	0.638	4 (14.8)	0.226
Albuminuria, *n* (%)	0	8 (5.8)	0.182	9 (39.1)	<0.001	12 (41.4)	<0.001

Data are presented as median (quartiles) [maximum–minimum] or number (%). No or mild diabetic retinopathy (DR) indicates an Early Treatment Diabetic Retinopathy Study (ETDRS) score ≤ 35; moderate–severe non-proliferative diabetic retinopathy (NPDR), ETDRS score 43–57; and proliferative diabetic retinopathy (PDR), ETDRS score ≥ 61. *Participants with no or mild DR compared to controls. † Participants with moderate–severe NPDR compared to no or mild DR, controls excluded. ‡ Participants with PDR compared to no or mild DR, controls excluded.

All 29 participants with PDR had undergone retinal photocoagulation treatment. A further 11 (48%) participants with moderate–very severe non-PDR and 4 (4%) with very mild or mild diabetic retinopathy had undergone photocoagulation. There were 44 participants altogether.

The participants with the most severe retinopathy (ETDRS score ≥ 61) had a longer duration of diabetes, worse glycaemic control, worse lipid profile, and more use of lipid-lowering medication, as well as a higher prevalence of albuminuria. The highest office blood pressure was observed in those with moderate–very severe non-PDR (ETDRS score 43–57), and participants with PDR did not differ significantly from those with no or mild retinopathy (score ≤ 35). In subanalyses including only participants with ambulatory blood pressure monitoring (n = 73), we observed no significant difference in diurnal or nocturnal blood pressure across the three retinopathy groups (diurnal blood pressure $p_{systolic}$=0.567 and $p_{diastolic}$=0.814; and nocturnal blood pressure $p_{systolic}$=0.438 and $p_{diastolic}$=0.649; data previously unpublished).

6.3.1.2 Observations of cerebral small vessel disease

Of the participants with type 1 diabetes, 67 (35.4%) had some marker of cerebral small vessel disease on MRI. Cerebral microbleeds were observed in 45 (23.8%), white matter hyperintensities in 44 (23.3%), and lacunes in 4 (2.1%) participants. None of the participants showed superficial siderosis on MRI. Only three (10.3%) of the controls had cerebral small vessel disease.

6.3.2 Cerebral small vessel disease by retinopathy severity

6.3.2.1 Any marker of cerebral small vessel disease

The distribution of ETDRS scores by presence and absence of cerebral small vessel disease are presented in Figure 17 (previously unpublished data). Participants with type 1 diabetes and cerebral small vessel disease had higher ETDRS scores (35 [20–61] vs. 20 [20–35], p = 0.022) and a higher prevalence of PDR (25.4 vs. 9.0%, p = 0.002) than those without small vessel disease.

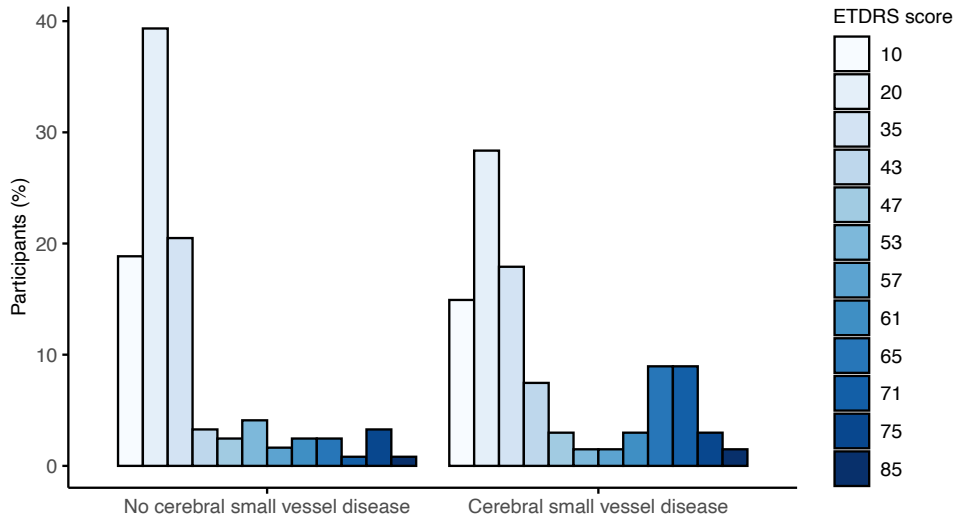

Figure 17 Distribution of Early Treatment Diabetic Retinopathy Study (ETDRS) score by presence or absence of cerebral small vessel disease in participants with type 1 diabetes.

Table 19 presents MRI findings of cerebral small vessel disease for participants with type 1 diabetes by diabetic retinopathy severity. We found PDR to be associated with cerebral small vessel disease (OR 2.57 [95% CI 1.04–6.35]) after adjustment for age, systolic blood pressure, albuminuria, and HbA_{1c} in logistic regression analysis. When analysing the whole discrete ETDRS scale as a covariate in a similar model, ETDRS score was not associated with cerebral small vessel disease after adjustment.

Table 19 Cerebral small vessel disease markers by retinopathy severity

	No or mild DR, *N* = 137	Moderate–severe NPDR, *N* = 23	*P**	PDR, *N* = 29	*P†*
Cerebral small vessel disease, *n* (%)	41 (29.9)	9 (39.1)	0.378	17 (58.6)	0.003
White matter hyperintensities, *n* (%)	31 (22.6)	3 (13.0)	0.298	10 (34.5)	0.179
Microbleeds, *n* (%)	24 (17.5)	8 (34.8)	0.087	13 (44.8)	0.001
Number of microbleeds	0 [0, 0] (0–16)	0 [0,1] (0–75)	0.035	0 [0, 3] (0–105)	<0.001
Lacunar infarcts, *n* (%)	2 (1.5)	0	>0.999	2 (6.9)	0.141

Data are presented as median [quartiles] (maximum–minimum) or number (%). No or mild diabetic retinopathy (DR) indicates an Early Treatment Diabetic Retinopathy Study (ETDRS) score ≤ 35; moderate–severe non-proliferative diabetic retinopathy (NPDR), ETDRS score 43–57; and proliferative diabetic retinopathy (PDR), ETDRS score ≥ 61. All control participants are excluded from the table. *Participants with moderate–severe NPDR compared to no or mild DR.

† Participants with PDR compared to no or mild DR.

MRI markers of small vessel disease observed in controls are presented in Table 20. None of the controls with cerebral small vessel disease showed any signs of pathologies in the fundus images. Therefore, the potential associations between small vessel disease and retinopathy were not further analysed in healthy controls.

Table 20 Markers of cerebral small vessel disease in controls

	Healthy controls, *N* = 29	No or mild DR, *N* = 137	*P*
Cerebral small vessel disease, *n* (%)	3 (10.3)	41 (29.9)	0.030
White matter hyperintensities, *n* (%)	2 (6.9)	31 (22.6)	0.054
Microbleeds, *n* (%)	1 (3.9)	24 (17.5)	0.030
Number of microbleeds	0 [0, 0] (0–1)	0 [0, 0] (0–16)	0.053
Lacunar infarcts, *n* (%)	0	2 (1.5)	0.513

Data are presented as median [quartiles] (maximum–minimum) or number (%). No or mild diabetic retinopathy (DR) indicates an Early Treatment Diabetic Retinopathy Study (ETDRS) score ≤ 35.

We observed no difference in the prevalence of cerebral small vessel disease in those with versus without any sign of diabetic retinopathy (32.4% in participants with ETDRS < 20 vs. 36.1% in ETDRS ≥ 20, $p = 0.677$). When dividing participants at ETDRS score 35 (mild non-PDR), cerebral small vessel disease was more prevalent in participants with ETDRS score > 35 compared to those with ETDRS score ≤ 35 (26 [50.0%] vs. 41 [29.9%], $p = 0.010$). The same was true when grouping by ETDRS > 47 (moderate non-PDR or milder) vs. ETDRS ≤ 47 (19 [50%] vs. 48 [32%], $p = 0.036$). When calculating the predictive values of ETDRS > 35 for cerebral small vessel disease within the cohort, PPV was 50% (95% CI 36–64%) and NPV 70% (62–78%).

6.3.2.2 Cerebral microbleeds in association with diabetic retinopathy

We further assessed separate manifestations of cerebral small vessel disease and observed a higher prevalence (21 [40%] vs. 24 [18%], $p = 0.001$) and number ($p = 0.008$) of cerebral microbleeds in those with ETDRS score > 35 than in those with ≤ 35. Likewise, when grouping by ETDRS score > 47 compared to ETDRS ≤47, both the prevalence (15 [40%] vs. 30 [20%], $p = 0.011$) and number of microbleeds was increased in participants with the worse retinopathy ($p = 0.004$). PPV of ETDRS score > 35 was 40% (95% CI 27–54%) and NPV 82% (76–89%).

No association with diabetic retinopathy emerged for white matter hyperintensities (prevalence 25% vs. 23%, $p = 0.730$ when ETDRS > 35 vs. ≤ 35 and 29% vs. 22%, $p = 0.355$ when ETDRS > 47 vs. ≤ 47) or for lacunes (prevalence 4% vs. 2%, $p = 0.304$ when ETDRS > 35 vs. ≤ 35, and 5% vs. 1%, $p = 0.181$ when ETDRS > 47 vs. ≤ 47).

In participants with cerebral microbleeds, we observed a higher median ETDRS score (35 [20–65] vs. 20 [20–35], $p = 0.024$) and a higher prevalence of PDR (29% vs. 13%, $p = 0.002$) compared to those without cerebral microbleeds. ETDRS scores increased by the number of cerebral microbleeds: The median ETDRS score was 20 (interquartile range: 20–35) in those with null cerebral microbleeds, 20 (20–45) in those with 1–2 cerebral microbleeds, and 63 (43–71) in those with > 2 microbleeds ($p = 0.001$). Figure 18 presents the distributions of ETDRS scores by quantities of microbleeds.

A finding of > 2 microbleeds in a participant's MRI was independently associated with an increased ETDRS score (OR 1.05 [95% CI 1.02–1.09] by each discrete score increase) in a logistic regression model adjusted for age, systolic blood pressure, albuminuria, and HbA_{1c}. PDR was similarly associated with having > 2 cerebral microbleeds (OR 8.52 [95% CI 1.91–37.94]) when analysed separately in a similar model.

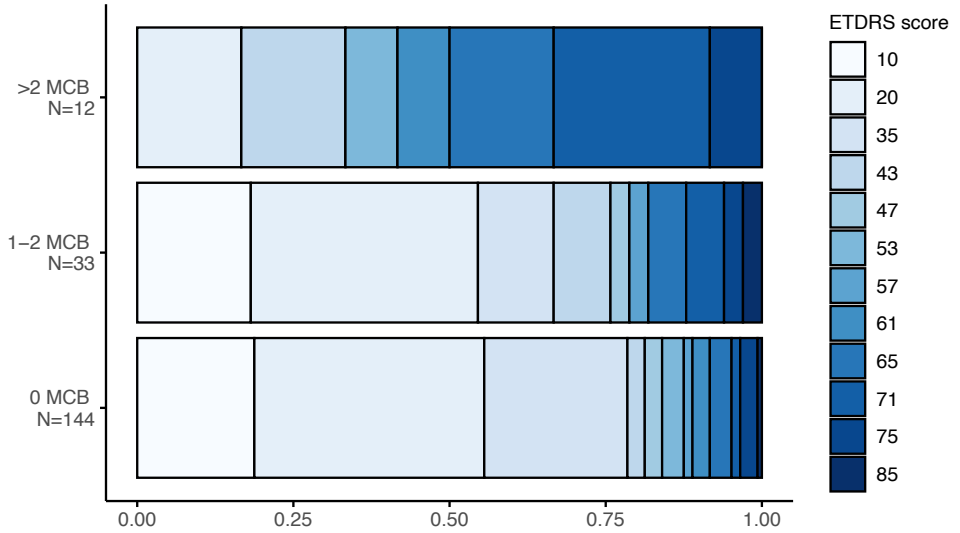

Figure 18 Stacked bar plot of Early Treatment Diabetic Retinopathy Study (ETDRS) score distribution grouped by number of cerebral microbleeds (CMB).

When calculating the predictive values of ETDRS > 35 for the prevalence of > 2 microbleeds within the cohort, the PPV was 19% (95% CI 9–30%) and the NPV 99% (97–100%). The PPV and NPV of PDR were 25% (95% CI 8–42%) and 96% (94–99%), respectively. We observed no association between the number of white matter hyperintensities and ETDRS score or PDR (data not shown). Due to the low number of cases with lacunes (4 participants), we were not able to study the association between lacunar infarctions and ETDRS score further.

6.4 Incidence of overt stroke in association with diabetic retinopathy and macular oedema (Study IV)

6.4.1 Participants' characteristics

The 1,268 participants with type 1 diabetes included in this follow-up study had a mean age of 38.7 years and half were men, which corresponds to the rest of the FinnDiane cohort (excluded participants, n = 3,687). Clinical characteristics of those included in comparison to the excluded participants are presented in Table 21. The included participants had a longer diabetes duration, higher HbA_{1c}, and diabetic kidney disease more often (Table 21). Their follow-up time was longer (17.9 [14.0, 19.3] vs. 15.3 [7.6, 17.5] years, $p < 0.001$) and their strokes more prevalent (10.3% vs. 8.2%, $p < 0.001$). Ischaemic and haemorrhagic strokes were equally common in both included and excluded participants ($p = 0.168$ and $p = 0.051$).

Table 21 Clinical characteristics of participants included vs. excluded from our study

	***n* in analysis**	**Included**	**Excluded**	***P***
N		1,268	3,687	
Men	4,955	656 (51.7)	1,906 (51.7)	1.000
Age, years	4,955	38.7 ± 10.8	38.0 ± 12.7	0.095
Duration of diabetes, years	4,955	25.5 ± 9.7	21.4 ± 13.2	<0.001
Age at onset of diabetes, years	4,955	12.0 [7.3, 17.5]	14.7 [9.6, 23.4]	<0.001
Body mass index, m^2/kg	4,817	25.4 ± 3.6	25.0 ± 3.7	0.001
HbA_{1c}, mmol/mol	4,787	71 ± 16	67 ± 16	<0.001
Total cholesterol, mmol/l	4,874	5.1 ± 1.0	4.8 ± 1.0	<0.001
LDL cholesterol, mmol/l	4,867	3.2 ± 0.9	2.9 ± 0.9	<0.001
HDL cholesterol, mmol/l	4,870	1.3 ± 0.4)	1.4 ± 0.4	<0.001
Triglycerides, mmol/l	4,872	1.1 [0.8, 1.6]	1.0 [0.8, 1.4]	<0.001
Use of lipid-lowering medication	4,952	147 (11.6)	566 (15.6)	0.001
Systolic blood pressure, mmHg	4,803	136 ± 19	134 ± 19	0.001
Diastolic blood pressure, mmHg	4,802	81 ± 10	79 ± 10	<0.001
Antihypertensive medication	4,898	661 (52.2)	1304 (35.9)	<0.001
Diabetic kidney disease	4,948	425 (33.5)	679 (18.5)	<0.001
Coronary artery disease	4,923	78 (6.2)	244 (6.7)	0.567
Peripheral arterial disease	4,922	75 (5.9)	156 (4.3)	0.021

Data are presented as mean ± standard deviation, median [quartiles], and *n* (%).

6.4.2 Diabetic retinopathy at baseline

A total of 1,119 (88.2%) participants had an ETDRS score of 20 or higher, indicating diabetic retinopathy of any grade. When comparing the retinopathy groups, the participants' age, duration of diabetes, and systolic and diastolic blood pressure increased with the severity of diabetic retinopathy, as presented in Table 22. Similarly, the prevalence of kidney disease and kidney replacement therapy were the highest, and estimated glomerular filtration rate the lowest in participants with PDR (ETDRS ≥61). The lipid profile differed between the groups, and the use of lipid-lowering medication was most prevalent in the PDR group (Table 22).

6.4.3 Macular oedema at baseline

Compared to the 1,074 participants with available macular data, those lacking data (n = 194) were younger, more often women, and had a lower prevalence of diabetic kidney disease. The data on clinical baseline characteristics in reference to macular oedema are presented in Table 23. Participants with and without available macular data did not differ regarding the prevalence of stroke (p = 0.920), ischaemic stroke (p = 0.726) or ischaemic subtypes (p = 0.802), or haemorrhagic stroke (p = 0.886) or subtypes (p > 0.999). The proportion of deaths during follow-up was also similar in those with and without available macular data (p = 0.176).

Of the participants with macular data, 534 (50.6%) had some degree of macular oedema at baseline, and 386 (35.9%) had clinically significant macular oedema (CSME). Of the participants with CSME, 67.6% also had PDR compared to 27.3% when CSME was absent (p < 0.001). Mild–severe non-proliferative diabetic retinopathy, i.e., non-PDR, was observed in 80 (20.7%) of the participants with CSME, and no–very mild retinopathy in 45 (11.7%). Participants with CSME were older, had a longer diabetes duration, and the use of lipid-lowering medication was higher in participants with CSME (Table 23). Participants with CSME had higher LDL cholesterol, higher systolic blood pressure, and more frequent use of antihypertensive medication, and diabetic kidney disease was more prevalent (Table 23).

Table 22 Clinical characteristics presented by diabetic retinopathy severity at baseline

	***n* in analysis**	**No–very mild DR**	**Non-proliferative DR**	**Proliferative DR**	***P***
N		575	203	490	
Women	1,268	312 (54.3)	77 (37.9)	223 (45.5)	<0.001
Age, years	1,268	33.7 [26.1, 43.4]	39.6 [32.6, 47.7]	41.7 [34.4, 48.8]	<0.001
Duration of diabetes, years	1,268	21.1 ± 9.3	25.8 ± 8.1	30.5 ± 8.1	<0.001
Age at onset of diabetes, years	1,268	12.9 [7.9, 19.1]	12.8 [8.7, 19.7]	10.2 [6.6, 15.2]	<0.001
Body mass index, m^2/kg	1,266	25.1 ± 3.2	25.5 ± 4.0	25.7 ± 4.0	0.052
HbA_{1c}, mmol/mol	1,268	70 ± 16	71 ± 16	71 ± 16	0.672
Total cholesterol, mmol/l	1,268	4.9 ± 1.0	5.2 ± 1.0	5.3 ± 1.0	<0.001
LDL cholesterol, mmol/l	1,268	3.1 ± 0.9	3.3 ± 0.8	3.4 ± 0.9	<0.001
HDL cholesterol, mmol/l	1,268	1.3 ± 0.4	1.3 ± 0.4	1.2 ± 0.4	<0.001
Triglycerides, mmol/l	1,268	1.0 [0.8, 1.5]	1.1 [0.8, 1.6]	1.3 [0.9, 1.9]	<0.001
Use of lipid-lowering medication, *n* (%)	1,267	32 (5.6)	24 (11.8)	91 (18.6)	<0.001
Systolic blood pressure, mmHg	1,268	130 ± 16	137 ± 19	143 ± 21	<0.001
Diastolic blood pressure, mmHg	1,265	80 [72, 85]	81 [76, 88]	82 [76, 90]	<0.001
Use of antihypertensive medication, *n* (%)	1,267	146 (25.4)	123 (60.6)	392 (80.2)	<0.001
Diabetic kidney disease, *n* (%)	1,268	65 (11.3)	61 (30.0)	319 (65.1)	<0.001
eGFR, ml/min/1.73 m^2	1,267	101 ± 21	90 ± 28	70 ± 33	<0.001
Coronary artery disease, *n* (%)	1,268	13 (2.3)	11 (5.4)	56 (11.4)	<0.001
Peripheral arterial disease, *n* (%)	1,268	8 (1.4)	8 (3.9)	59 (12.0)	<0.001
Smoking status, *n* (%)	1,246				<0.001
Current smoker		141 (24.9)	50 (25.1)	112 (23.3)	
History of smoking		109 (19.3)	53 (26.6)	149 (31.0)	
No history of smoking		316 (55.8)	96 (48.2)	220 (45.7)	

Data are presented as mean ± standard deviation, median [quartiles], and *n* (%). Normally distributed continuous variables have been tested with one way ANOVA, and non-normal variables with the Kruskal-Wallis test. Categorical data were analysed with the chi-squared test or Fisher's exact test if observations were ≤ 5 in one of the groups. DR indicates diabetic retinopathy.

Table 23 Clinical characteristics presented by the status of macular oedema

	n	No macular data	*P*	*n*	no CSME	CSME	*P**
N	1268	194		1074	688	386	
Women	1268	118 (60.8)	<0.001	1074	337 (49.0)	157 (40.7)	0.011
Age, years	1268	37.0 ± 11.3	0.015	1074	37.16 ± 10.8	42.4 ± 9.7	<0.001
Duration of diabetes, years	1268	24.3 ± 10.5	0.058	1074	24.3 ± 9.8	28.2 ± 8.4	<0.001
Age at onset of diabetes, years	1268	12.7 ± 7.6	0.314	1074	12.8 ± 8.0	14.2 ± 8.2	0.009
Body mass index, m^2/kg	1266	24.8 ± 3.2	0.009	1072	25.4 ± 3.8	25.7 ± 4.0	0.181
HbA1c, mmol/mol (%)	1268	70 ± 16	0.748	1074	70 ± 16	71 ± 16	0.407
Total cholesterol, mmol/l	1268	5.0 ± 1.0	0.082	1074	5.0 ± 1.0	5.3 ± 0.9	<0.001
LDL cholesterol, mmol/l	1268	3.1 ± 0.9	0.014	1074	3.2 ± 0.9	3.4 ± 0.8	<0.001
HDL cholesterol, mmol/l	1268	1.4 ± 0.4	0.001	1074	1.3 ± 0.4	1.2 ± 0.4	0.006
Triglycerides, mmol/l	1268	1.0 [0.8, 1.5]	0.011	1074	1.1 [0.8, 1.6]	1.3 [1.0, 1.9]	<0.001
Lipid-lowering medication	1267	9 (4.6)	0.002	1073	62 (9.0)	76 (19.7)	<0.001
Systolic blood pressure, mmHg	1268	133 ± 18	0.022	1074	133 ± 18	142 ± 21	<0.001
Diastolic blood pressure, mmHg	1266	80 ± 9	0135	1072	81 ± 10	82 ± 11	0.099
Antihypertensive medication	1267	73 (37.0)	<0.001	1074	295 (42.9)	293 (76.1)	<0.001
Diabetic kidney disease	1268	51 (26.3)	0.007	1074	175 (25.4)	219 (56.7)	<0.001
Coronary artery disease	1268	6 (3.1)	0.066	1074	38 (5.5)	36 (9.3)	0.025
Peripheral arterial disease	1268	8 (4.1)	0.325	1074	27 (3.9)	40 (10.4)	<0.001
Smoking status	1246		0.009	1054			0.015
Current smoker		53 (27.6)			159 (23.6)	91 (24.0)	
History of smoking		31 (16.1)			161 (23.9)	119 (31.4)	
No history of smoking		108 (56.2)			355 (52.6)	169 (44.6)	

N denotes the number of participants in the analysis, *P* the p-value in tests comparing participants with vs. without macular data, and P* the p-value in tests comparing participants with vs. without clinically significant macular oedema (CSME). Data are presented as mean ± standard deviation, median [quartiles], and number (percent). Normally distributed continuous variables have been analysed with one way ANOVA, non-normal variables with the Kruskal-Wallis test, and categorical data with the chi-squared test or Fisher's exact test if observations were ≤ 5 in one of the groups.

6.4.4 Endpoints during follow-up

We recorded 130 (10.3%) incident strokes (96 ischaemic and 34 haemorrhagic) during a median follow-up time of 18.0 (quartiles: 14.1, 19.3) years. In a subclassification of the ischaemic strokes, 42 (43.8%) were lacunar strokes, 27 (28.1%) were non-lacunar strokes, and 27 (28.1%) were of undetermined aetiology. Of the haemorrhagic strokes, 27 (79.4%) were intracerebral haemorrhages, and 7 (20.6%) were subarachnoid haemorrhages. Endpoints by diabetic retinopathy severity grouping are presented in Table 24.

The incidence rate for stroke of any type was 648 (95% CI 542–770) per 100,000 person-years among all participants. The incident rate for ischaemic stroke was 479 (95% CI 366–585) and for haemorrhagic stroke 170 (95% CI 117–237). A total of 244 participants (19.2%) died during follow-up, and mortality was lowest in participants with no–very mild retinopathy and highest in participants with PDR (Table 24).

Table 24 Endpoints during follow-up by diabetic retinopathy severity

	***n* in analysis**	**No–very mild diabetic retinopathy**	**Non-proliferative diabetic retinopathy**	**Proliferative diabetic retinopathy**	***P***
Any stroke, *n* (%)	1,268	26 (4.5)	25 (12.3)	79 (16.1)	<0.001
Ischaemic stroke	1,268	23 (4.0)	20 (9.8)	53 (10.8)	<0.001
Type of ischaemic stroke	**96**				0.492
Lacunar stroke		8 (34.8)	10 (50.0)	24 (45.3)	
Non-lacunar stroke		5 (21.7)	6 (30.0)	16 (30.2)	
Undefined ischaemic stroke		10 (43.5)	4 (20.0)	13 (24.5)	
Haemorrhagic stroke, *n* (%)	1,268	3 (0.5)	5 (2.5)	26 (5.3)	<0.001
Type of haemorrhagic stroke	**34**				0.146
Intra cerebral haemorrhage		1 (33.3)	4 (80.0)	22 (84.6)	
Subarachnoid haemorrhage		2 (66.7)	1 (20.0)	4 (15.4)	
Death during follow-up, *n* (%)	1,268	43 (7.5)	34 (16.7)	167 (34.1)	<0.001

Data are presented as *n* (%). *P* was calculated with the chi-squared test or with Fisher's exact test when observations were ≤ 5 in any group.

6.4.5 Stroke in association with retinopathy severity

6.4.5.1 Any stroke

The incidence rate of stroke during follow-up and adjusted HRs are presented by retinopathy severity group in Table 25. Participants with PDR at baseline had the highest incidence rate of stroke, followed by those with non-PDR. The incidence rate was the lowest in no–very mild retinopathy (Table 25).

Table 25 Incidence and hazard ratio for stroke by retinopathy severity

	Stroke of any type
No or very mild DR (ETDRS < 35)	
Incidence rate	260 (170–382)
Hazard ratio	*REF*
Hazard ratio*	*REF*
Non-proliferative DR (35 ≥ ETDRS < 61)	
Incidence rate	798 (516–1,178)
Hazard ratio	2.16 (1.24–3.77)
Hazard ratio*	1.79 (1.02–3.15)
Proliferative DR (ETDRS ≥ 61)	
Incidence rate	1,140 (902–1,420)
Hazard ratio	2.67 (1.66–4.31)
Hazard ratio*	1.69 (1.02–2.82)

Data are presented as incidence rate/100,000 person-years with (95% confidence interval). DR indicates diabetic retinopathy, and ETDRS Early Treatment Diabetic Retinopathy Study score. The hazard ratios (with 95% confidence interval) were calculated in a Cox proportional hazards model adjusted for sex, age, diabetes onset age, LDL cholesterol, and systolic blood pressure. *Diabetic kidney disease added to the model.

The cumulative incidence of stroke by 20 years was 5.8% in no–very mild retinopathy, 14.6% in non-PDR, and 20.5% in PDR in the Kaplan-Meier analysis (Figure 19). When including death as a competing risk in the cumulative incidence function, the probability of stroke by 20 years of follow-up was 5.5% in no–very mild retinopathy, 13.2% in non-PDR, and 16.7% in PDR ($p < 0.001$). The probability of death for the corresponding time was 7.5%, 16.9%, and 35.1% in no–very mild retinopathy, non-PDR, and PDR, respectively ($p < 0.001$).

In comparison to participants with no–very mild retinopathy, the risk of any stroke was increased by 79% in non-PDR when adjusting for sex, age, diabetes onset age, LDL cholesterol, systolic blood pressure, and presence of diabetic kidney disease in a Cox model. The corresponding risk increase associated with PDR was 69% in the same model (Table 25).

When analysing the subdistribution HRs in a Fine-Gray model with the same covariates and death as the competing event, the subdistribution HR was 1.80 (95% CI 1.02–3.18) in non-PDR and 1.65 (95% CI 0.971–2.79) in PDR. In that same model, the subdistribution HR for death was 1.72 (95% CI 1.16–2.56) in PDR, and non-PDR was not a significant predictor of death (p = 0.230).

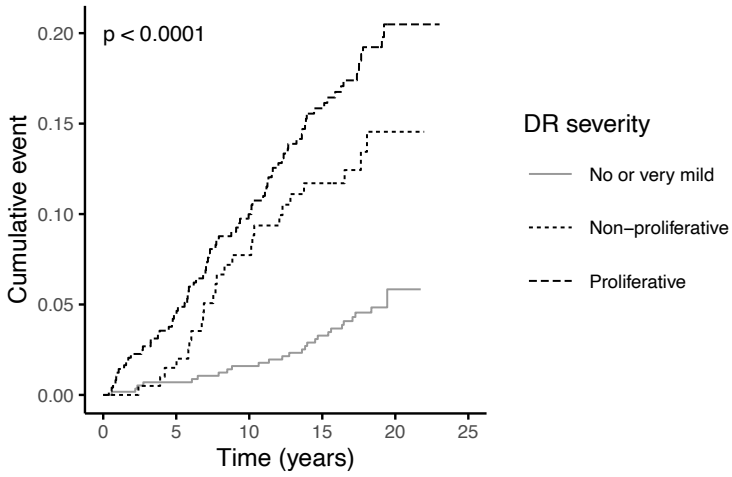

Figure 19 Kaplan-Meier plot showing the cumulative incidence of stroke stratified by diabetic retinopathy severity at baseline. *P* represents the p-value of log-rank testing.

6.4.5.2 Ischaemic stroke

The incidence rates and adjusted HRs for the distinct stroke types and subtypes are presented by retinopathy severity group in Table 26. The ischaemic stroke incidence rate increased by the severity of diabetic retinopathy at baseline (Table 16). Figure 20 presents the cumulative incidence of ischaemic stroke and its subtypes in the Kaplan-Meier analysis stratified by retinopathy severity. The proportion of ischaemic strokes during follow-up increased by retinopathy severity. This also applies when assessing lacunar and non-lacunar strokes separately (Figure 20). Compared to no–mild retinopathy, the risk of any ischaemic stroke and lacunar stroke was increased both in non-PDR and PDR when adjusting for age, diabetes onset age, LDL cholesterol, and systolic blood pressure in a Cox model. When diabetic kidney disease was added to the model, retinopathy severity was no longer significant. Non-lacunar stroke was not associated with any grade of diabetic retinopathy in the adjusted Cox model (Table 26).

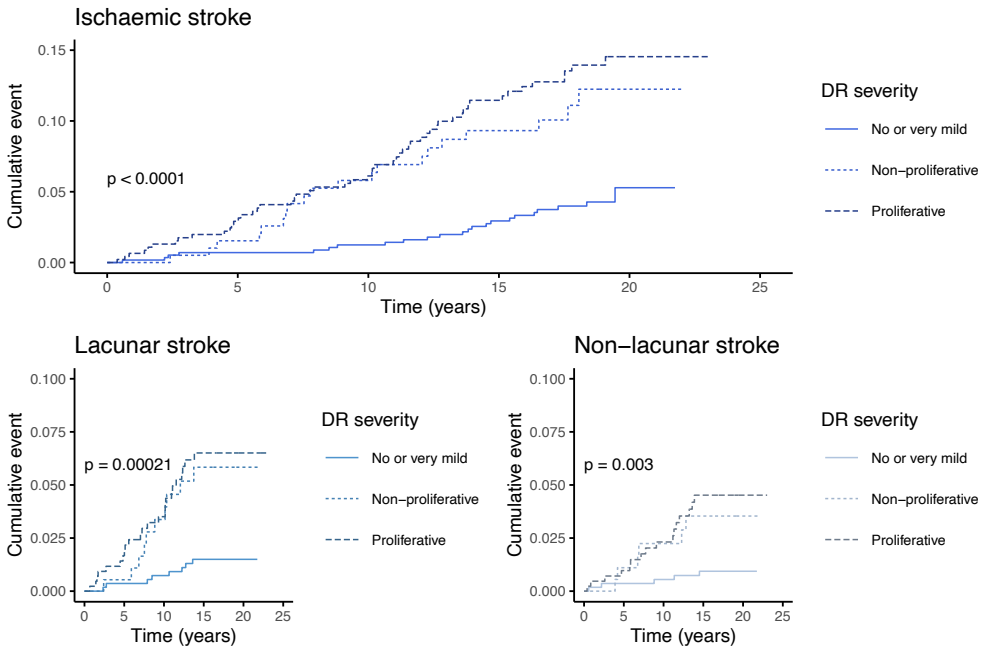

Figure 20 Cumulative incidence of ischaemic, lacunar, and non-lacunar stroke in Kaplan-Meier analyses, stratified by diabetic retinopathy severity at baseline. *P* represents the p-value of log-rank testing.

6.4.5.3 Haemorrhagic stroke

Participants with PDR had the highest haemorrhagic stroke incidence rate (Table 26). The cumulative incidence increased stepwise by retinopathy severity category, as displayed in Figure 21. Intracerebral and subarachnoid haemorrhages were not analysed separately due to the low number of the latter (n = 7). PDR, compared to no–very mild retinopathy, was associated with a fourfold increase in HR for haemorrhagic stroke when adjusting for sex, age, age at onset, triglycerides, systolic blood pressure, and diabetic kidney disease. We did not find non-PDR to be associated with an increased risk of haemorrhagic stroke in that model (Table 26).

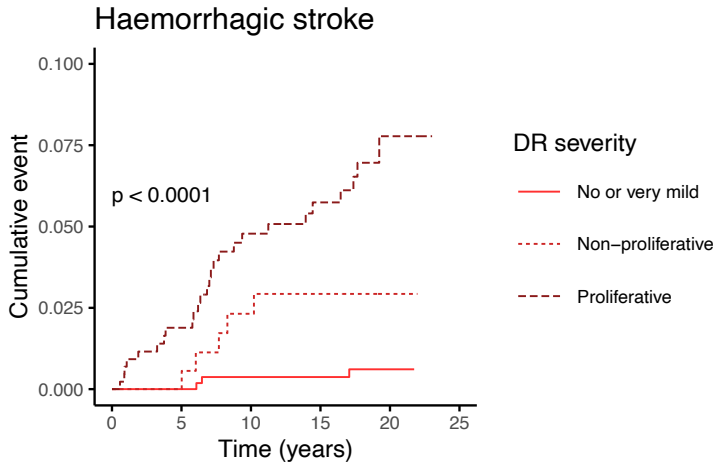

Figure 21 Cumulative incidence of haemorrhagic stroke in Kaplan-Meier analyses, stratified by diabetic retinopathy severity at baseline. *P* represents the p-value of log-rank testing.

Table 26 Incidence rates and hazard ratio for stroke types by diabetic retinopathy severity

DR severity	Any ischaemic stroke	Lacunar stroke	Non-lacunar stroke	Any haemorrhagic stroke
No or very mild (ETDRS < 35)				
Incidence rate	230 (146–345)	80 (35–158)	50 (16–117)	30 (6–88)
Hazard ratio*	REF	REF	REF	REF
Hazard ratio†	REF	REF	REF	REF
Non-proliferative (35 ≥ ETDRS < 61)				
Incidence rate	638 (390–986)	319 (153–587)	192 (70–417)	160 (52–372)
Hazard ratio*	2.03 (1.11–3.71)	2.77 (1.09–7.07)	2.68 (0.81–8.80)	3.54 (0.83–15.04)
Hazard ratio†	1.68 (0.91–3.10)	2.39 (0.93–6.14)	2.36 (0.71–7.85)	2.84 (0.66–12.28)
Proliferative (ETDRS ≥61)				
Incidence rate	765 (573–1000)	346 (222–515)	231 (132–375)	375 (245–550)
Hazard ratio*	2.13 (1.26–3.60)	2.55 (1.09–5.96)	2.23 (0.78–6.33)	6.88 (1.94–24.42)
Hazard ratio†	1.35 (0.77–2.36)	1.72 (0.70–4.22)	1.48 (0.51–4.35)	4.31 (1.16–16.10)

Data are presented as incidence rate/100,000 person-years with (95% confidence interval). DR indicates diabetic retinopathy, and ETDRS Early Treatment Diabetic Retinopathy Study score. The hazard ratios (with 95% confidence interval) were calculated in Cox proportional hazards models.

*Analyses of any ischaemic lacunar, and non-lacunar strokes were adjusted for age, diabetes onset age, LDL cholesterol, and systolic blood pressure. The analysis of haemorrhagic stroke was adjusted for sex, age, diabetes onset age, triglycerides, and systolic blood pressure.

†Diabetic kidney disease added to the models.

6.4.6 Stroke incidence by CSME

6.4.6.1 Stroke of any type

Endpoints during follow-up for participants with versus without CSME are presented in Table 27. When analysing any stroke by presence versus absence of CSME, the cumulative incidence was 20.8% (95% CI 15.8–25.5) in those with CSME and 8.8% (95% CI 5.6–14.3) in those without CSME ($p < 0.001$). The HR in CSME was 1.92 (95% CI 1.31–2.82, $p = 0.001$) compared to no CSME. The analysis was adjusted for sex, age, age at onset, LDL cholesterol, and systolic blood pressure. After further adjustment for diabetic kidney disease, CSME was no longer associated with any stroke despite the nominally increased hazard (HR 1.44 [95% CI 0.97–2.15], 0=0.073).

Table 27 Endpoints during follow-up in participants with versus without CSME

	n in analysis	no CSME	CSME	*P**
Any stroke	1074	49 (7.1)	62 (16.1)	<0.001
Ischaemic stroke	1074	38 (5.5)	45 (11.7)	<0.001
Type of ischaemic stroke	83			0.573
Lacunar stroke		18 (47.4)	17 (37.8)	
Non-lacunar stroke		11 (28.9)	13 (28.9)	
Undefined ischaemic stroke		9 (23.7)	15 (33.3)	
Haemorrhagic stroke		11 (1.6)	17 (4.4)	0.010
Type of haemorrhagic stroke	28			0.653
Intracerebral haemorrhage		8 (72.7)	14 (82.4)	
Subarachnoid haemorrhage		3 (27.3)	3 (17.6)	
Death during follow-up	1074	89 (12.9)	125 (32.4)	<0.001

Data are presented as *n* (%). *P* was calculated with the chi-squared test, or Fisher's exact test when observations were ≤ 5 in either group. CSME indicates clinically significant macular oedema.

6.4.6.2 Ischaemic and haemorrhagic stroke

The cumulative incidence of ischaemic and haemorrhagic stroke stratified by CSME are presented in Figure 22. Compared to participants without CSME, those with CSME had a higher cumulative incidence of ischaemic stroke (Figure 22). When adjusting for age, diabetes onset age, LDL cholesterol, and systolic blood pressure, the HR for ischaemic stroke was 1.85 (95% CI 1.19–2.88, $p = 0.007$) in CSME compared to no CSME. CSME was neither associated with lacunar ($p = 0.335$) nor non-lacunar stroke ($p = 0.140$) in a similar Cox model. When adding diabetic kidney

disease to the model of any ischaemic stroke, CSME was no longer significant (p = 0.181).

CSME was associated with a higher cumulative incidence (Figure 22) of haemorrhagic stroke, and the HR in CSME was 2.37 (95% CI 1.10–5.14) after adjusting for sex, age, diabetes onset age, triglycerides, and systolic blood pressure. CSME was not associated with haemorrhagic stroke after adding diabetic kidney disease to the model (p = 0.132).

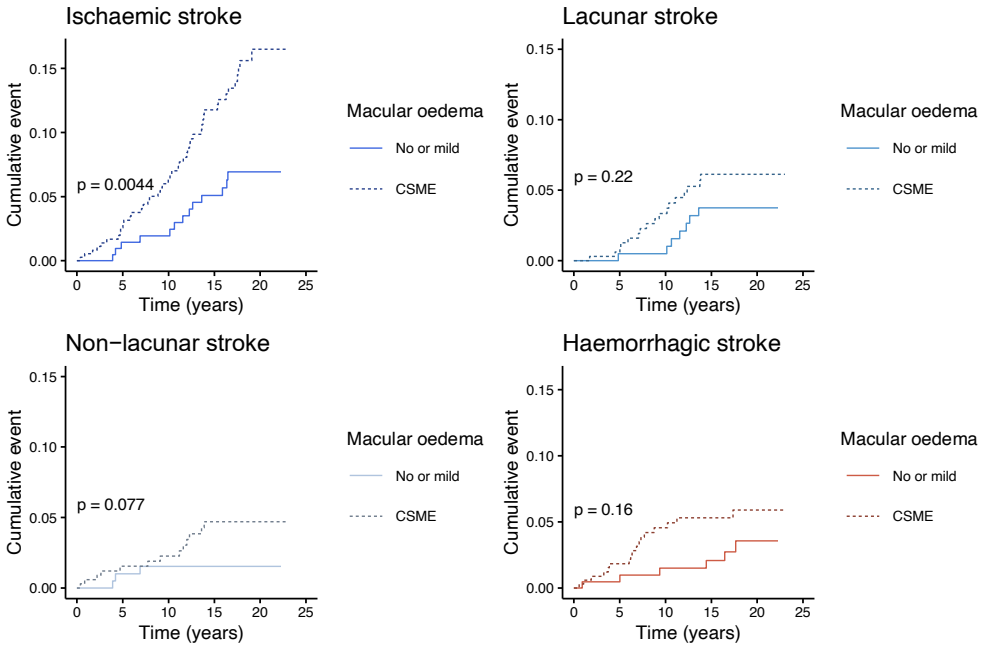

Figure 22 Cumulative incidence of ischaemic, lacunar, non-lacunar, and haemorrhagic stroke, stratified by the presence vs. absence of clinically significant macular oedema (CSME) in Kaplan-Meier analyses. *P* represents the p-value of log-rank testing.

7 Discussion

There has been a considerable knowledge gap regarding early or asymptomatic stages of cerebrovascular disease in individuals with type 1 diabetes. No previous studies have comprehensively characterised CSVD while accounting for all known MRI markers in type 1 diabetes, and our understanding of CSVD remains limited, even within the general population. For the first time, in Study I, we examine ambulatory blood pressure in association with CSVD in individuals with type 1 diabetes and show that nocturnally increased blood pressure is associated with CSVD. The novelty of Study II lies in the thorough characterisation of all markers of CSVD in relation to the haptoglobin genotype. Furthermore, here, we present the largest haptoglobin-genotyped brain MRI study population to date. This thesis further introduces diabetic retinopathy severity as a potential marker of silent and overt manifestations of CSVD, as well as overt stroke. The results, however, need to be interpreted with respect to the strengths and limitations of this thesis.

7.1 Strengths and limitations

7.1.1 Study participants and the FinnDiane Study protocol

One of our main strengths is the well-characterised study populations in which all participants have entered a study visit following the standardised FinnDiane Study protocol, by which the same examinations are performed for every individual at each visit. Although the cross-sectional study design limits any interpretation of causality, the MRI cohort of Studies I–III is unique in its size, being one of the largest study populations with type 1 diabetes and brain MRI to date. The subcohort volunteering for ambulatory blood pressure monitoring, to the best of our knowledge, constitutes the first and only study population with brain MRI and 24-hour blood pressure monitoring in the context of type 1 diabetes. Regardless, a larger study cohort, particularly when it comes to the healthy controls, would have increased the statistical power to detect more subtle differences in blood pressure between the groups. It is worth acknowledging that, as participants received a free-of-cost report of the MRI findings, as well as consultation with a neurologist, if needed, this may have attracted individuals particularly eager to learn more about

their brain health, such as those with a family history of cerebrovascular disease. Moreover, Study II did not include healthy controls, and although the distribution of genotypes among the participants does not indicate any selection bias, the healthy controls might have further affirmed our negative findings.

Similarly, participants in the follow-up cohort in Study IV have all attended a FinnDiane Study visit and undergone the standardised clinical evaluation at baseline. The FinnDiane Study is one of the most extensive and well-characterised cohorts of individuals with type 1 diabetes in the world. Participants have been recruited for almost 30 years, allowing for a long follow-up time, but there is also valuable temporal heterogeneity, such as diabetes diagnosis at different decades, as new participants of different ages are constantly recruited. Yet, the format of recruiting adult volunteers may bring about a self-selection bias and the health behaviour of the participants may deviate from what would be seen in a random sample. Despite the observational study design, attending a clinical study visit may also impact the participants' health behaviour. Indeed, these phenomena have been pointed out when attendance and the effects of volunteer health check-ups have previously been studied in population-based settings (325,326).

The FinnDiane Study protocol's classification of type 1 diabetes is a key strength. Only individuals with typical type 1 diabetes are recruited for the study. The requirement of participants to have a diabetes diagnosis before age 40 and to have started insulin treatment within a year ensures consistency in the classification and reduces ambiguity. As our participants are, by design, not recruited at the time of diabetes diagnosis, we do not include autoantibody measurements. Moreover, a substantial proportion of our participants received their diabetes diagnosis before autoantibody measurements were in clinical practice. This may complicate the classification of those diagnosed between the ages of 30–40 years. Despite this, genetic profiling of a subpopulation of FinnDiane participants diagnosed between 30 and 40 years of age shows that participants diagnosed in this age range have genetic markers typical of childhood-onset type 1 diabetes, not latent autoimmune diabetes in adults (327).

7.1.2 Assessed exposures and markers

7.1.2.1 Ambulatory blood pressure monitoring

The strengths of the ambulatory monitoring performed in Study I include using devices of the same model for all participants; defining and calculating nocturnal and diurnal blood pressure by the sleep time reported by the participant; and a thorough evaluation of the quality of measurements for all time periods in each participant individually. It should be acknowledged, however, that single-day monitoring may not represent the typical blood pressure level or patterns of a

participant. Monitoring itself may also affect the blood pressure level by changing a participant's behaviour, such as compliance with medication, or by causing discomfort and/or affecting sleep quality. To reduce the misinterpretation of data due to said confounders, the participants were asked to go about their daily routines as usual and make notes of anything out of the ordinary. All readings for an individual participant were interpreted regarding the participants' notes and were carefully considered as successful or unsuccessful.

7.1.2.2 Haptoglobin genotyping

In Study II, haptoglobin genotyping was carried out by PCR. A considerable alternative would have been the imputation method developed by Boetteger and co-workers (328), which can also detect the "*S*" and "*F*" alleles (*HP1S*, *HP1F*, *HP2FF*, *HP2FS*, and *HP2SS*) (134). We did, however, use this method in a separate FinnDiane Study assessing overt cerebrovascular disease in relation to haptoglobin genotype (329) and obtained results similar to those in Study II. A limitation of this study of haptoglobin genotype is that the statistical power does not allow us to reject the possibility of a subtle association between haptoglobin genotype and CSVD. Yet, our analysis of statistical power showed that effect sizes of OR 1.91 or larger would be detectable with 80% probability (α=0.05) for this number of participants, indicating that the study is well-powered to detect more prominent associations.

7.1.2.3 Retinopathy severity grading

The ETDRS grading scale used in Studies III and IV was originally developed to assess diabetic retinopathy as an endpoint, and it represents the escalating risk of vision-threatening diabetic retinopathy on an ordered alphanumerical scale (168). Due to its high reproducibility and detail, it has since been established as the gold standard for retinopathy grading and, although its position has been debated during the last years, it remains the benchmark method of today (330,331). When interpreting our results, it should be kept in mind that the ETDRS levels represent the risk of retinopathy progression, which does not follow retinopathy severity linearly, although the ETDRS levels can be converted from the alphanumeric levels to a linear score for analysis.

In Study III, most of the participants' fundi were imaged during the study visit at the FinnDiane Study Centre. However, for some participants, we used images taken for clinical use at other institutions – with different imaging equipment – which may have affected the interpretation of the images. The possibility of linking visit data from the FinnDiane Study with data derived from national registers and health care units through the personal identification code used in Finland is unique. As all Finnish residents are offered medical care in the public health care system,

most of our participants regularly attend public sector medical units, which enables the acquisition of medical records for follow-up data and retrospective characterisation, such as fundus images utilised for evaluation of diabetic retinopathy and macular oedema in Study IV. Although the retinal data in Study IV are unique in size and detail, some limitations of this data acquisition method should be acknowledged. In addition to the risk of selection bias associated with this method, data are heterogeneous and there is, for example, variation in the time elapsed between the last retinal examination before baseline and the baseline visit. The given ETDRS scores should, therefore, be interpreted as the mildest possible diabetic retinopathy for an individual participant during follow-up. We furthermore acknowledge that, although well-characterised, our retinal data are limited to information available from fundus images and medical records and do not include optical coherence tomography (OCT).

7.1.3 Outcomes and endpoints

7.1.3.1 Cerebral small vessel disease

The assessment of CSVD by brain MRI is a major strength of Studies I–III. All participants' cerebrovascular status was assessed implementing the same methodology, including the MRI scanner, facilities, and the same experienced neuroradiologist assessing all participants and controls. The assessment protocol followed standardised criteria (37,318), and all markers of disease were accounted for. Although participants with any previous sign of cerebrovascular disease were excluded by a validated protocol (306,307), further neuropsychological evaluations were not performed. Retrospectively, with knowledge of the high prevalence of CSVD, we appreciate that assessments aiming to identify a milder decrease in cognitive performance might have provided interesting information for the study. On the other hand, as our rationale was to explore early markers and potential aetiological factors of cerebrovascular disease, information on the participants' cognitive performance is unlikely to add to our understanding of cerebrovascular pathophysiology. A potential advantage of disregarding symptoms other than overt cerebrovascular incidents is minimising the potential for observer bias. We acknowledge that preceptive errors are also possible in our study, yet we underline that the *tabula rasa* approach of studying seemingly asymptomatic participants is a strength of our study.

7.1.3.2 Overt stroke

In Study IV, we were able to thoroughly classify all incident strokes from medical records, providing a detailed and comprehensive case assignment. For those

without stroke, we did not confirm stroke-free status beyond information available in care registers, and some might have asymptomatic cerebrovascular disease, possibly diluting or confounding the results. We have, however, excluded all individuals for whom the clinical and radiological observations were not in agreement, the date of stroke or stroke type could not be determined, or when information regarding stroke was otherwise insufficient. Although verification of strokes from multiple data sources is a strength of this study, the aspect of the time-consumption of this effort may pose a limitation by delaying results and reproduction of similar data in other cohorts.

7.1.4 Statistical analyses

In the cross-sectional studies (Studies I–III), we used logistic regression models to assess the covariate of interest together with potential confounders, in association with CSVD as the outcome. After critical consideration, the final choices of covariates were based primarily on clinical relevance, as all significant covariates could not be included due to caution of overfitting. Disregarding a possible better fit may have increased the variability in outcomes not explained by the model, affecting the preciseness of the model prediction. Besides the risk of over- or underfitting, logistic regression modelling comes with some additional limitations, such as the assumption of a linear relationship between the covariates and the outcome, which should be considered when interpreting our results.

In Study IV, the cumulative incidence over time was estimated by the Kaplan-Meier method. A limitation of the Kaplan-Meier analysis is that it tends to overestimate incidence in the absence of competing events. To account for this, stroke, as the outcome of interest, was analysed in relation to death as a competing event in a cumulative incidence function. However, we could not do similar analyses with other cardiovascular events as the event competing with stroke. As myocardial infarction is the most prevalent cardiovascular manifestation in Finnish individuals with type 1 diabetes (24), the absolute incidence of any cardiovascular events should be interpreted with caution if analysed in an isolated context. The strength of the Kaplan-Meier analysis lies in its being a nonparametric estimator suited for the visualisation of changes in the probability of an event over time, contrary to the Cox proportional hazards model. The strength of the Cox model is that it allows for the inclusion of both discrete and continuous covariates, although using a single time point measure in this model may lead to a loss of information on how risk develops over time. In our study, it is possible that the stroke risk associated with retinopathy severity did vary over time, even though we did not detect a significant association with time for any of the covariates included in the final models.

7.1.5 Generalisability of the results

In addition to potential methodological issues that may impact the reliability of our results, it is worth acknowledging that firstly, the Finnish population is genetically homogeneous, and secondly, most of the population is White. Lastly, Finland has the highest incidence of childhood-onset type 1 diabetes in the world. These demographical factors may limit the generalisability of our findings.

7.2 Cerebral small vessel disease in association with ambulatory blood pressure

Study I assessed 24-hour ambulatory blood pressure in association with markers of CSVD on brain MRI in neurologically asymptomatic individuals with type 1 diabetes. We showed that systolic and diastolic blood pressure levels, mean arterial pressure, and pulse pressure differ in participants with versus without CSVD. We also reported the prevalence of alterations in normal blood pressure or blood pressure patterns, including hypertension during different intervals of the day and diminished nocturnal dipping.

7.2.1 Participants with cerebral small vessel disease had higher nocturnal blood pressure

We found that nocturnal systolic and diastolic blood pressure, though within the normotensive range, was significantly higher in individuals with CSVD compared to those without the disease. It thereby logically follows that the mean arterial pressure was increased as well. These associations also remained significant after adjustment for clinical confounders. Although ambulatory blood pressure in relation to CSVD has not previously been studied in diabetes, our results are in accordance with studies carried out in individuals without diabetes. Increased ambulatory blood pressure has been linked to CSVD, particularly white matter hyperintensities, in healthy elderly individuals in the PROOF Study (284). Also, in hypertensive individuals in their sixties, subclinical CSVD has been associated with higher blood pressure on ambulatory monitoring (282). Additionally, in contrast to our observations, elevated diurnal and 24-hour average blood pressure has been reported as significant in reference to CSVD (284). This divergence from our result might be ascribed to our study participants being younger and, diabetes aside, having a lower office blood pressure and less often blood pressure-lowering medication compared to the previous studies.

Although both extreme dipping and non-dipping blood pressure patterns have been associated with CSVD in other studies (285), we did not detect a significant difference in dipping patterns between participants with and without CSVD. There

was, however, a trend towards decreased nocturnal dipping in association with CSVD. It is possible that we were not able to detect an existing effect of non-dipping due to a fairly low number of participants, particularly as extreme dippers were too few (n = 4) for grouping independent from normal dippers. On the other hand, the previously mentioned PROOF Study with over 800 participants (284) observed no association between nocturnal dipping and CSVD.

Our participants with CSVD seemed to have a higher diurnal pulse pressure than those with normal MRI findings, but the association was not significant after adjusting for clinical confounders. After publishing our results from Study I, arterial stiffness has been thoroughly assessed by pulse wave velocity and the augmentation index in a larger portion (n = 186) of the FinnDiane MRI cohort (332). Like our results in Study I, individuals with CSVD were observed to have stiffer arteries than those with normal MRI. Yet, the association was not significant after adjustment for confounders.

Approximately a third (31%) of our study participants exhibited masked hypertension on ambulatory blood pressure monitoring. Previously reported frequencies of masked hypertension in type 1 diabetes range from 14–24% depending on the cohort and the definition of hypertension (125,128). The prevalence of masked hypertension could be explained by the even higher prevalence of nocturnal hypertension in our cohort, 47%, as isolated nocturnal hypertension would not be detected in an office setting. Our observations suggest underreporting of elevated blood pressure with CSVD, as we found both masked and nocturnal hypertension to be associated with CSVD. In type 2 diabetes, furthermore, the occurrence of masked hypertension has been linked to silent brain infarctions (333).

On the other hand, we may also have overestimated the prevalence of masked hypertension. It is considerable that individuals aware of their poor blood pressure control would have been less prone to volunteer for 24-hour ambulatory blood pressure monitoring. It should indeed be acknowledged that some degree of selection bias is possible in our study. Although the included participants did not differ largely from the rest of the MRI cohort, some differences could indicate a stronger inclination to participate among a certain group of people. For example, there were fewer smokers among the included participants, which could imply that individuals with a healthier lifestyle were more prone to volunteer for 24-hour blood pressure monitoring. The included participants also had a better lipid profile and lower office blood pressure regardless of similar uses of lipid- and blood pressure-lowering medications across the groups, as well as a higher eGFR. This could also reflect a healthier lifestyle or better health in general among the included participants. In this case, it is possible that we have underestimated or been unable to detect existing effects of blood pressure, such as the impact of nocturnal dipping or pulse pressure.

7.2.2 Does elevated blood pressure impact small vessel disease or is it a marker of vascular dysregulation?

Our results indicate that elevated nocturnal blood pressure is associated with early markers of cerebrovascular disease in type 1 diabetes. Although nocturnal blood pressure is recognised as a stronger predictor of cardiovascular disease than diurnal blood pressure (37), our cross-sectional design limits the interpretation of causality in the current study. However, the lack of association between arterial stiffness and CSVD observed in this particular study and a later FinnDiane report (332) provides some basis for the speculation that CSVD is not primarily driven by large-vessel dysfunction in type 1 diabetes; rather, it might represent a more wide-ranging state of pre-existing vessel pathology and circulatory dysregulation. With that said, the potential impact of blood pressure on CSVD should not be disregarded, as elevated nocturnal blood pressure has previously been reported to accelerate the progression of white matter hyperintensities in individuals without diabetes (334). Also in diabetes, treatment of hypertension is known to slow the progression of microvascular complications (112).

In this study, we observed more prominent findings in participants using blood pressure-lowering medication, while in participants without the medication, we observed no association between CSVD and blood pressure or hypertension. There was, however, no association between CSVD and the use of blood pressure medication as opposed to observations from earlier studies (282,284). In addition to endogenous dysregulation and/or long-term increase of the absolute blood pressure level, nocturnal hypertension could be associated with taking blood pressure-lowering medication in the morning or early daytime (335,336). Due to our study design, it is not possible to determine the underlying cause of nocturnal hypertension in our participants.

It should be mentioned that there may be further confounding factors affecting blood pressure and/or cerebrovascular health that we have not been able to account for. For example, when it comes to confounders of nocturnal blood pressure, we did not explicitly screen for sleep apnoea or other sleeping disorders. However, the participants were instructed to report their time awake and asleep, as well as any physical or mental stress while wearing the ambulatory blood pressure monitor, and these notes were considered when analysing and interpreting the results. Further, the nocturnal blood pressure was defined according to the sleeping time reported by the participants, rather than a set time interval, which we consider a strength of our study.

7.2.3 Futures prospects: Focus on chronotherapy and self-monitoring

The effect of chronotherapy – timing administration of medication to best fit the circadian rhythm – provides interesting research prospects for the future. Adjusting the dose of blood pressure-lowering agents to closer mimic the physiological variation in the blood pressure, particularly nocturnal dipping, has shown promising results both regarding the control of blood pressure levels and for the prevention of cardiovascular disease, including stroke (335,337,338). As nocturnal hypertension has been associated with multiple microvascular complications of diabetes (114,125,128), in addition to our observations regarding CSVD, a heavier emphasis on research and interventions targeting chronotherapy could also be a cost-effective strategy in diabetes care.

Although ambulatory monitoring is the gold standard of blood pressure measuring (75,76), its clinical use is limited by accessibility and the subjective discomfort it poses to the patient. It would, therefore, be relevant to evaluate if our results from this study apply to other out-of-office settings, such as self-monitoring.

A limitation of our study is the lack of measures of cardiovascular autonomic neuropathy beyond blood pressure patterns. Including other measurements indicating autonomic neuropathy in future studies could provide a more comprehensive portrayal of how vascular and neurological dysfunction are interrelated and how this links to cerebrovascular manifestations. Regardless, our current study shows that CSVD is linked to blood pressure dysregulation, namely elevated nocturnal blood pressure. Even if our results can neither confirm nor deny the *common soil* hypothesis, our observations fit the theory that complications of small and large vessels represent a common underlying cause.

7.3 Cerebral small vessel disease in relation to haptoglobin genotype

Study II assessed haptoglobin genotype in relation to asymptomatic CSVD in individuals with type 1 diabetes. We studied the prevalence of CSVD in participants of different genotypes, as well as minor allele frequencies in association with CSVD overall, and its different manifestations. We detected no significant association between a certain genotype or allele and CSVD, despite a rather high prevalence of the disease among the participants.

7.3.1 No prominent association between haptoglobin genotype and cerebral small vessel disease

To our knowledge, this is the first study to examine haptoglobin genotypes in relation to all manifestations of CSVD, i.e., microbleeds, white matter hyperintensities, and lacunar infarctions in type 1 diabetes. The negative findings in this current study extend to another recent study of ours regarding haptoglobin genotype and cerebrovascular disease in type 1 diabetes (329). We observed no associations between haptoglobin genotype and cerebrovascular disease in that study on overt stroke of different subtypes in 4,000 participants (329). Our results also agree with some previous studies in individuals without diabetes, which have reported no association between any of the haptoglobin genotypes and CSVD (288,289). Nevertheless, reports have been contradictory in individuals without diabetes, and one previous study of individuals without diabetes has linked the genotype *HP1-1* to larger volumes of white matter hyperintensities (339).

Our observations differ from a previous type 1 diabetes study by Costacou and co-workers (40), which demonstrated an association between white matter hyperintensities and the *HP1* allele. They found individuals with genotype *HP1-1* to have greater white matter hyperintensities. Furthermore, in their study, the genotype contributed significantly to white matter hyperintensity variation in the corpus callosum but not to white matter hyperintensities in the total brain volume (40). In contrast to Costacou and co-workers, we did not assess the localisation or volume of white matter hyperintensities; rather, the white matter hyperintensities were graded according to the Fazekas scale for analysis (318). Our study participants also differ from the Costacou and co-workers (40) cohort, which had fewer participants (n = 94), a markedly higher prevalence of white matter hyperintensities (99% compared to 17% in our study), and older participants with almost 20 years longer duration of diabetes. Even though we have not associated diabetes duration with white matter hyperintensities when studied previously (35), these differences might account for some of the divergence between our current study and the one by Costacou and co-workers (40). Notably, they do not mention cerebral microbleeds, which were the most common manifestation in our study, frequently coinciding with white matter hyperintensities.

As in the case of white matter hyperintensities, reports on the association between haptoglobin genotype and lacunar infarctions diverge in their observations (287,288), while to the best of our knowledge, no previous studies on cerebral microbleeds exist.

7.3.2 Could haptoglobin genotype still be linked to cerebral small vessel disease?

While the role of the haptoglobin genotype in humans, *in vivo*, is still unclear, studies in animal models and *in vitro* have described potential pathophysiological roles of haptoglobin for both cerebral haemorrhages and ischaemia (340,341). *HP1-1* is suggested to induce cerebral ischaemic damage, particularly lacunar strokes, as it has been linked to reduced endothelial repair (341). On the other hand, along with endothelial dysfunction, inflammation is hypothesised to be a central component of CSVD (37,342), and *HP2-2* has been suggested to promote inflammation after a haemorrhagic brain event (343). One could speculate that the impact of a certain haptoglobin genotype varies depending on the type of tissue damage. Whereas one genotype might lessen the ischaemic damage, another may protect from vessel leakage, including microbleeds. In theory, the link between *HP1-1* and CSVD could be limited to certain manifestations, such as white matter hyperintensities, as observed by Costacou and co-workers (40). Nevertheless, we did not detect any significant associations between a specific manifestation and haptoglobin genotype among our participants.

Some previous studies examining haptoglobin genotypes in CSVD have acknowledged the potential influence of survival bias (40). Survival bias is possible in our study as well, as we excluded individuals with previous cerebrovascular events or kidney failure. This might have masked a competing risk. Regardless, considering the consistent reports of *HP2-2* being associated with cardiovascular and kidney disease in diabetes (138,140,141), such a selection would likely have led to an overestimation of the effect of *HP1*.

Although the statistical power of this study does not allow us to reject the possibility of a subtle association between haptoglobin genotypes and CSVD, it is of note that we have access to the largest type 1 diabetes study population with brain MRI and haptoglobin genotyping to date. In addition, the negative findings regarding strokes, including overt lacunar strokes, and haptoglobin genotype in our later and larger study further support the observation that haptoglobin genotype does not have a prominent effect on cerebrovascular disease in individuals with type 1 diabetes.

7.3.3 Future prospects: Haptoglobin in longitudinal studies

Notably, we estimated the sample size required to detect more subtle effects (OR < 1.5) of the haptoglobin genotype to be 4,295 participants (80% probability of detection, α=0.05). However, a potential association between haptoglobin genotype and CSVD could become more prominent as the small vessel disease advances. Previously reported associations have indeed been observed in cohorts

smaller than ours but with more severe disease, e.g., overt lacunar infarction and a larger extent of white matter hyperintensities (40,287,339). We were not able to evaluate the severity of CSVD in our participants, as the MRI findings were moderate (none of the participants had a Fazekas score > 2) and, moreover, the cross-sectional design limits us from assessing the progression of the small vessel disease. Future studies with a longitudinal setting could provide information about the pathogenies of asymptomatic CSVD, such as clinical implications, and resolve questions regarding the actual relevance of factors potentially associated with the disease.

Some studies have suggested that the association between CSVD and *HP1-1* is more pronounced in individuals with hypertension both in type 1 diabetes and individuals without diabetes (41,339). In our study, participants with the *HP1-1* genotype had higher diastolic blood pressure than the Hp2 carriers and, though not significant, there was a trend towards more frequent use of antihypertensive medication associated with *HP1-1*. Due to the low number of individuals with a history of hypertension (62 participants used blood pressure-lowering medication), we were not able to perform further analyses stratified by hypertension. Future research focusing on how the haptoglobin genotype might interact with blood pressure and other modifiable potential risk factors could potentially add relevant insights to the pathophysiology of CSVD in type 1 diabetes.

7.4 Cerebral small vessel disease in association with diabetic retinopathy

In Study III, we evaluated the association between covert CSVD and diabetic retinopathy severity in individuals with type 1 diabetes and diabetes-free controls. We reported that CSVD, particularly cerebral microbleeds, was associated with the severity of diabetic retinopathy when graded by the ETDRS scale. No such association was detectable among the controls. We found the number of cerebral microbleeds to increase with the severity of retinopathy, and the NPV associated with absent or very mild retinopathy was high.

7.4.1 Cerebral microbleeds are associated with diabetic retinopathy severity

Participants with CSVD more often had moderate NPDR or more severe retinopathy compared to participants with normal MRI findings. In the analysis adjusted for confounders, CSVD was associated with an ETDRS score of ≥ 61 (PDR). All diabetes-free controls with CSVD had a normal retina, although retinopathy-like abnormalities were observed among some of the controls. PDR or severe diabetic

retinopathy indicated by a history of retinal photocoagulation has previously been assessed in relation to markers of covert and overt CSVD in individuals with type 1 diabetes (39,260). In accordance with our study, cerebral microbleeds have been associated with PDR in asymptomatic CSVD in a study by Woerdeman and co-workers (39). In our current study, as a novelty, we confirm that this observation also holds true when the diabetic retinopathy severity grade is confirmed by fundus assessment rather than by proxy.

Moreover, PDR was associated with CSVD also when age was considered, which has not been indicated by previous studies. CSVD has mostly been studied in elderly people without diabetes, and the condition seems to be related to ageing (36). In type 1 diabetes, however, CSVD is common already in younger individuals (35). In line with our observations, Woerdeman and co-workers also found PDR to be associated with CSVD in young individuals (39). Yet, the association between age and CSVD was not further elucidated in their study.

Although our study confirms an association between PDR and CSVD, we did not observe any association between diabetic retinopathy and manifestations other than cerebral microbleeds (white matter hyperintensities and/or lacunes) when analysed separately. This could result from distinct manifestations having different aetiologies. As described in the Review of the Literature of this thesis (chapter 2.4.7), CSVD is indeed thought to arise through an array of possible pathological pathways, possibly rendering different manifestations (36). Nonetheless, our negative results might also be explained by the relatively low prevalence of white matter hyperintensities and lacunes among our participants.

White matter hyperintensities have previously been assessed in association with diabetic retinopathy in individuals with type 2 diabetes by Sanahuja and co-workers (290). Although our participants were 21 years younger than the median from aforementioned study, our results agree with theirs: Sanahuja and co-workers reported that diabetic retinopathy is associated with the overall prevalence of CSVD but not with white matter hyperintensities or lacunes when analysed separately (290).

In this current study, we observed a significant increase in cerebral microbleeds at a moderate severity level of diabetic retinopathy. Participants with an ETDRS score > 35, compared to ≤ 35, had a higher prevalence and a higher number of microbleeds. Although an independent association between severe diabetic retinopathy and cerebral microbleeds as well as overt cerebral haemorrhages has previously been reported (39,260), to our knowledge, there are no reports on any associations between milder diabetic retinopathy and cerebrovascular disease in type 1 diabetes.

7.4.2 Diabetic retinopathy – a marker for cerebral small vessel disease?

The observed link between the number of microbleeds and diabetic retinopathy could indicate that progression from mild diabetic retinopathy (ETDRS score ≤ 35) to more severe forms is associated with an increased risk of cerebrovascular disease even before the manifestation of retinal neovascularisation (PDR): When diabetic retinopathy has progressed from very mild or mild (microaneurysms only or typically additional small haemorrhages) to moderate or more severe retinopathy (more widespread changes in the retina indicated by larger haemorrhages, intraretinal microvascular abnormalities, and/or venous beading), it already reflects permanent capillary damage. These markers of permanent vessel damage in the retina might correlate with an increased risk of cerebrovascular disease. Due to our cross-sectional study design, however, it can only be speculated, as Woerdeman and co-workers previously stated that diabetic retinopathy appears to represent a generalised state of microangiopathy (39).

Interestingly, although hyperglycaemia is among the main drivers of diabetic retinopathy progression (165,167,168), we have not found CSVD to be associated with either single-measurement HbA_{1c} or long-term HbA_{1c} levels in our cohort when studied outside the context of this thesis (35,344). In our current study, participants with moderate–severe NPDR (ETDRS > 35) did not have a significantly higher HbA_{1c} compared to those with milder retinopathy, yet they had a higher number of microbleeds. In participants with PDR, both HbA_{1c} and the number of microbleeds were higher compared to those with the mildest retinopathy. This could suggest that fundus examination, i.e., retinopathy severity, gives a more sensitive representation of glycaemic control than a single HbA_{1c} measure.

When we calculated the predictive values of given ETDRS scores as cut-offs, the PPV of an ETDRS score > 35 was 50% for the prevalence of CSVD overall and 19% for the prevalence of > 2 microbleeds in our cohort. This reflects that the likelihood of having multiple microbleeds was not high regardless of a participant having more than mild retinopathy. The NPV, on the other hand, was 70% for any CSVD and 99% for > 2 microbleeds, meaning that when retinopathy was absent or mild, participants also had 70% probability of being free from CSVD, and free from multiple microbleeds with a 99% probability. Although a low ETDRS score excluded the presence of multiple microbleeds within the current cohort, the study setting does not answer how well the ETDRS score could predict cerebral microbleeds or other manifestations of CSVD in a longitudinal setting.

7.4.3 Future prospects: Temporal association between cerebral small vessel disease and diabetic retinopathy

In Study I and a previous FinnDiane report (35), we reported an association between CSVD and systolic blood pressure. Diabetic retinopathy is also known to be linked to elevated blood pressure (20,167), yet in this study, we observed no significant variation in office nor ambulatory blood pressure levels when grouping by retinopathy severity. In the logistic regression analysis including PDR, age, office systolic blood pressure, HbA1c, and albuminuria, only age and PDR were associated with CSVD. Although this suggests that diabetic retinopathy is associated with CSVD beyond blood pressure, we did not further assess the possible interaction between these covariates. After finishing Study III, we further discovered that the prevalence of cerebral microbleeds is associated with a lower retinal arteriovenous ratio, indicating retinal arteriolar narrowing (345) in participants within our MRI cohort (346). In the same study, a higher blood pressure correlated with a decrease in the central retinal arteriole diameter, suggesting that retinal vascular abnormalities and blood pressure are linked. As our study design would not reveal non-linear nor temporal associations between retinopathy, blood pressure, and CSVD, their relationship remains yet to be scrutinised for better a understanding of the underlying pathogenesis. Assessing associations and possible interactions between these in a longitudinal study setting could resolve these queries in the future.

Although we found the severity of retinopathy to be associated with CSVD and the number of microbleeds, it remains unclear what information about the progression of small disease that fundus examination may offer. Thereby, future studies employing a longitudinal design are needed to answer those questions as well. Using information from retinal examinations to profile individuals with type 1 diabetes in regard to their probability of silent cerebrovascular disease is a tempting prospect. Fundus photography is non-invasive and would require few additional resources, as retinal examinations are already recommended to be performed routinely (177–179). Our results, however, would need to be confirmed and characterised with additional precision in other populations. Additionally, there is a need to better understand the implications of CSVD in type 1 diabetes – the long-term effects and preventive measures – for screening to be ethical, efficient, and beneficial for the individual as well as for society.

7.5 Overt stroke in association with diabetic retinopathy and macular oedema

In Study IV, we assessed the incidence of overt stroke by different stroke subtypes in association with diabetic retinopathy severity graded on the ETDRS scale. We

found non-proliferative diabetic retinopathy to be associated with an increased risk of stroke and that the risk increase was of the same magnitude as that associated with PDR. Interestingly, this main observation emerged regardless of the presence of diabetic kidney disease. We also reported that the incidence of both ischaemic and haemorrhagic stroke increases with the severity of diabetic retinopathy, and we observed a higher stroke incidence in participants with CSME.

7.5.1 Stroke incidence and risk increases with the severity of diabetic retinopathy

Our observations regarding a link between PDR and stroke risk are in line with previous data from the FinnDiane Study, reporting that severe diabetic retinopathy, indicated by a cruder evaluation, i.e., retinal photocoagulation, is a risk marker for stroke in type 1 diabetes (260). In Study IV, we showed for the first time that this holds true for a broader spectrum of diabetic retinopathy determined from fundus images. Furthermore, in the present study, the association was independent of diabetic kidney disease. This is somewhat surprising as diabetic kidney disease is currently one of the strongest known risk factors for stroke in type 1 diabetes (24,43).

When analysing the competing risk of stroke and death in the cumulative incidence function, the probability of death was higher than the probability of stroke in both non-proliferative retinopathy and PDR. Whereas PDR was associated with death in the Fine-Gray model, interestingly, non-proliferative retinopathy was not. The prevalence of any diabetic retinopathy has previously been reported to predict mortality in type 1 diabetes (157,347–349), although in most of these studies, the association has become insignificant when adjusting for diabetic kidney disease and/or age and sex (157,348,349), in contrast to our results. One previous study assessing mild and moderate NPDR as separate exposures (based on ETDRS grading) did report moderately severe NPDR and PDR to predict all-cause mortality after adjusting for age and sex, yet not after including kidney disease in the model (347). In our present study, the subdistribution hazards for stroke in the Fine-Gray model were largely unchanged compared to the Cox model, except that the association between stroke and PDR weakened slightly so that it was non-significant, but borderline, for PDR.

We further assessed ischaemic and haemorrhagic stroke, as well as subtypes of ischaemic stroke, as separate outcomes in marginal analyses. The cumulative incidence of ischaemic strokes in participants with non-proliferative retinopathy resembled that of the participants with PDR. The likeness between the groups was particularly prominent for lacunar strokes. Moreover, in the Cox analysis not adjusted for diabetic kidney disease, the HRs in NPDR and PDR were similar.

The incidence of lacunar strokes was higher than non-lacunar ischaemic strokes, but they followed a similar cumulative pattern in relation to retinopathy severity. In contrast to non-lacunar strokes, lacunar strokes were associated with retinopathy severity in the adjusted analysis, although not after adjusting for kidney disease. Previous studies have associated lacunar strokes with retinal vascular changes when studied in cross-sectional data of the general population (350), but reports from longitudinal studies are sparse in both the general population and type 1 diabetes.

We found the cumulative incidence of haemorrhagic stroke to increase stepwise with increasing retinopathy severity. To our surprise, the association between haemorrhagic stroke and non-proliferative retinopathy was not significant in the adjusted Cox model. This was somewhat unexpected considering our results in Study III, where we reported an association between asymptomatic cerebral microbleeds and moderate–severe non-proliferative retinopathy. In line with our previous reports (43), PDR also increased the HR for haemorrhagic stroke in all models in this current study. In contrast to the Study III cohort, this current study included participants receiving kidney replacement therapy, which we did not adjust for in our analysis. Moreover, we have previously reported that severe diabetic retinopathy and diabetic kidney disease in conjunction increase the risk of haemorrhagic stroke more than either complication alone (260), which may also explain our observations in this current study.

7.5.2 Stroke incidence increased when CSME was present

Like the observations made regarding diabetic retinopathy, the cumulative incidence of stroke was increased in participants with CSME. By 20 years of follow-up, the cumulative incidence had reached 21% in participants with CSME compared to 15% in non-proliferative retinopathy and 20% in PDR. In contrast to diabetic retinopathy, CSME was not associated with an increased stroke risk when adjusting for diabetic kidney disease in the Cox model. In separate analyses of ischaemic stroke, the cumulative incidence was 17% in CSME compared to 15% in PDR at 20 years. Both covariates were associated with an increased HR for ischaemic stroke when diabetic kidney disease was not included in the model. For haemorrhagic stroke, the cumulative incidence at 20 years was 6% in participants with CSME and 8% in PDR. In contrast to PDR, however, CSME was not associated with an increased risk of haemorrhagic stroke when kidney disease was considered.

We may have been unable to detect an existing association, as information on macular status was available only for a subset (85%) of our participants. The stroke prevalence, however, did not differ between participants with versus without macular data. Notwithstanding that macular oedema and severe diabetic retinopathy often coincide, their risk factors differ somewhat; for example, whereas

high systolic blood pressure increases the risk of CSME, PDR develops independently of systolic blood pressure (351). Thus, it should not be ruled out that macular oedema and diabetic retinopathy represent different risk profiles. In type 2 diabetes, cardiovascular disease appears more resolutely linked to macular oedema than to PDR, particularly concerning fatal coronary events. The link between macular oedema and stroke remains unknown in type 2 diabetes (294).

7.5.3 Can we identify individuals at risk of stroke by evaluating diabetic retinopathy severity?

A key finding of Study IV is that the stroke risk associated with non-proliferative retinopathy is of the same magnitude as the risk associated with PDR. One could hypothesise that non-proliferative retinopathy is an earlier marker of stroke in type 1 diabetes compared to PDR. Non-proliferative retinopathy may thus have a larger impact on the cause-specific hazard when the presence of other risk factors is low. In participants displaying several and/or more prominent risk factors for stroke, such as those with PDR who also had a high prevalence of diabetic kidney disease, the stroke-predicting role of diabetic retinopathy might become less.

In contrast to milder retinopathy, PDR was significantly associated with mortality in the Fine-Gray analysis. Based on this, PDR could be interpreted as an independent risk marker of mortality. However, as these participants also had more and worse kidney disease and a higher prevalence of other cardiovascular complications, PDR could just as easily be associated with these firmly established mortality contributors (104,233), which we did not assess further. Although it does not confirm our hypothesis, the Fine-Gray results support the notion that non-proliferative retinopathy is a more sensitive indicator of stroke, whereas PDR represents a less specific profile, but not less grave in terms of clinical prognosis. Nevertheless, both non-proliferative retinopathy and PDR were also significant predictors of stroke in the Cox model when adjusted for diabetic kidney disease.

There is relatively limited research evaluating stroke in individuals with type 1 diabetes outside the FinnDiane Study (25), and results from previous studies assessing diabetic retinopathy and stroke have been inconclusive (352). Based on previous reports, retinopathy severity seems to be a risk marker for stroke in type 2 diabetes (293,352). The Action to Control Cardiovascular Risk in Diabetes (ACCORD) Study (293) has reported that mild diabetic retinopathy, as well as moderate–severe, is associated with stroke in type 2 diabetes. Moreover, they found the stroke risk to increase by retinopathy severity. The ACCORD Study's methodology differs from ours in regards to cut-off values for retinopathy severity grouping, and the report does not mention diabetic kidney disease (293). Nonetheless, we obtained similar effect sizes as the aforementioned study and

likewise found the HR for stroke to increase by retinopathy severity in a Cox model, not including diabetic kidney disease.

One of our main research questions has been whether diabetic retinopathy severity can indicate microvascular disease of the brain. In an attempt to answer that question in this longitudinal study, lacunar and haemorrhagic strokes were analysed individually. Although our current observations, in addition to our previous report (43), suggests that lacunar strokes and diabetic retinopathy are linked, this relationship, particularly the hypothesized common aetiology, warrants further exploration. CSVD also underlies most spontaneous intracerebral haemorrhages (267), and the same is suggested for subarachnoid haemorrhages in type 1 diabetes (279). While we were unable to analyse subtypes of haemorrhagic stroke separately, we reproduced our previous results (43), now with a different methodology, and confirmed the statement that PDR is a strong risk marker for haemorrhagic stroke.

7.5.4 Future prospects: More to be learned from retinal screening?

Our current study does not assess the progression of diabetic retinopathy during follow-up. Similarities across different categories of diabetic retinopathy severity could be due to the progression of retinopathy, which our methodology would not reveal. Whether or not progression was present, our participants with non-proliferative retinopathy (more than only microaneurysms) at baseline were at significantly higher risk of stroke. Nevertheless, in the future, it would be interesting to assess if the progression of diabetic retinopathy is a more precise marker of cerebrovascular disease compared to the absolute retinopathy severity level.

We again acknowledge that a more detailed classification based on OCT or OCT angiography could have provided us with additional information for further or more precise categorisation of the participants. Since the time of our participants' baseline visit, OCT has become the primary method for assessing macular oedema (179,353). With the substantial positive change in accessibility to OCT and related examination techniques, one is eager to learn what new information it can present in upcoming studies.

All things considered, as screening by fundus imaging is the gold standard for detecting diabetic retinopathy to date (177), diabetic retinopathy evaluation from retinal images can still be considered highly relevant. Furthermore, there are promising methods for facilitating and improving the efficiency of diagnosing and grading diabetic retinopathy with the help of machine learning-based automation (354). Grading both diabetic retinopathy and macular oedema from digital fundus images as well as classifying diabetic retinopathy from medical text files using

artificial intelligence has shown good accuracy (355,356). Furthermore, combining information from images and medical text files using artificial intelligence, i.e., automating the same measures we have undertaken manually for this study, has been shown to further improve automated image-based classification (356). This type of automation of information retrieval implies a massive potential for further studies, but also for use in clinical practice – would it prove beneficial and effective to use retinopathy severity grading in cerebrovascular risk profiling? Retinal images could, thus, serve as an accessible tool for the assessment of cerebrovascular health in individuals with diabetes. Whether this holds true, and for which individuals and populations, remains to be explored.

7.6 Recapitulation of the results

This thesis has assessed 24-hour ambulatory blood pressure, haptoglobin genotype, and diabetic retinopathy severity in association with markers of CSVD in neurologically asymptomatic individuals with type 1 diabetes in three separate studies with a cross-sectional design (Studies I–III). Diabetic retinopathy and macular oedema were then further evaluated in association with incident stroke of different subtypes in a longitudinal setting in a separate cohort of participants with type 1 diabetes (Study IV).

We found participants with CSVD to have higher nocturnal blood pressure, but not higher diurnal blood pressure or pulse pressure, compared to those without CSVD (Study I). This suggests that CSVD in type 1 diabetes is not primarily driven by large vessel disease, and we rationalise that CSVD represents a more generalised state of vessel dysfunction and pathology.

Even though CSVD in type 1 diabetes has previously been linked to the haptoglobin genotype, no specific allele or genotype emerged as prominently associated with CSVD in our participants (Study II). Considering the complexity of CSVD, and its link to other markers of vascular dysregulation that we have observed in this thesis, we consider it reasonable to dismiss this monogenic variation as a prominently influential factor in CSVD.

Our observation that CSVD, particularly microbleeds, is positively associated with diabetic retinopathy severity (Study III) further strengthens the idea of CSVD representing a more widespread state of microangiopathy. Moreover, we found that a positive association between diabetic retinopathy severity and cerebrovascular disease extends to overt stroke in a longitudinal setting (Study IV). The results of this thesis support the hypothesis that cerebrovascular disease in type 1 diabetes is not to be ascribed to large-vessel pathology, that is, macrovascular disease. Rather, both silent markers as well as overtly manifesting cerebrovascular disease seem strongly linked to microangiopathy, supporting the hypothesis that they are of common soil.

8 Summary and conclusions

I In neurologically asymptomatic individuals with type 1 diabetes, cerebral small vessel disease is associated with nocturnal systolic and diastolic blood pressure, as well as nocturnal hypertension and masked hypertension on 24-hour ambulatory blood pressure monitoring. Ambulatory blood pressure monitoring provided insights into blood pressure alterations, such as nocturnal hypertension and masked hypertension, which could not be obtained through single office measurements. Whether cerebral small vessel disease and nocturnal blood pressure are casually linked or simply reflect vasculopathy and/or blood pressure dysregulation needs further investigation.

II There is no prominent association between cerebral small vessel disease and genetic variation of haptoglobin. Despite a rather high prevalence of cerebral small vessel disease in our participants, we detected no significant difference in genotype distribution or minor allele (*HP1*) frequencies between individuals with and without the disease. We assessed the variants in relation to separate manifestations of cerebral small vessel disease, and the results revealed no link between the minor or major allele (*HP2)* and a given manifestation of cerebral small vessel disease. Our results do not rule out a subtle association and/or one that may be relevant for the progression of small vessel disease, illustrating the need for further understanding of the pathophysiology of the disease.

III Cerebral small vessel disease, particularly cerebral microbleeds, is associated with diabetic retinopathy severity graded by the ETDRS, and the association was limited to participants with type 1 diabetes. The number of microbleeds increased with the severity of diabetic retinopathy in our participants with type 1 diabetes, and when the predictive values were calculated, the PPV of multiple microbleeds associated with no–very mild retinopathy was moderate. On the other hand, the corresponding NPV was high (99%), indicating that when diabetic retinopathy was absent or very mild, the likelihood of having multiple microbleeds was very low.

IV Diabetic retinopathy manifesting with more than only microaneurysms marks an increased risk of stroke in individuals with type 1 diabetes. The incidence of any stroke increased by retinopathy severity category, so that it was lowest in individuals with no or very mild retinopathy and highest in those with proliferative disease. This was also true for all subtypes of ischaemic strokes and for haemorrhagic strokes. The patterns of cumulative incidence in relation to retinopathy severity differed between ischaemic and haemorrhagic strokes, as did the hazard associated with diabetic retinopathy severity. Whereas diabetic retinopathy was not associated with the risk of ischaemic stroke when kidney disease was regarded, PDR was a significant risk marker for haemorrhagic stroke. Our observations suggest that diabetic retinopathy, already of moderate grade, is a marker of cerebrovascular disease and, in addition to other known risk factors, may be clinically useful for profiling individuals at risk.

Acknowledgements

This study was carried out between 2019 and 2024 at the Folkhälsan Research Center, at the University of Helsinki and Helsinki University Hospital, and at the Research Program for Clinical and Molecular Metabolism, Faculty of Medicine, University of Helsinki. I would like to express my gratitude to both past and present leaders of the institutes, Professor Anna-Elina Lehesjoki, Professor Emerita Kaisu Pitkälä, Associate Professor Lena Thorn, Associate Professor Merja Laine, Docent Agneta Ekstrand, and Professor Kirsi Pietiläinen for providing an excellent research environment and facilities.

I extend my sincere thanks for the personal grants given to me by the Medical Society of Finland, Wilhelm and Else Stockmann Foundation, *Svenska Kulturfonden* Foundation, the *Nylands Nation* Foundation, Otto Malm Foundation, The Diabetes Research Foundation, Aarne Koskelo foundation, The Finnish Medical Foundation, Finnish Brain Foundation, the Biomedicum Foundation, and Dorothea Olivia, Karl Walter and Jarl Walter Perklén Foundation. In addition, the FinnDiane Study was financially supported by the Folkhälsan Research Foundation, the Wilhelm and Else Stockmann Foundation, the *Liv och Hälsa* Society, the Sigrid Jusélius Foundation (220027), the Medical Society of Finland, and state funding for university-level health research by Helsinki University Hospital (TYH2023403, TYH2024216).

I have received a tremendous amount of support from my supervisors, and I wish to express my deepest gratitude to them:

To Associate Professor Lena Thorn, who introduced me to the fascinating world not only of diabetes research but also of clinical research in general. Lena was the supervisor of my “licentiate thesis” (advanced medical studies) on ambulatory blood pressure in cerebral small vessel disease, which later developed into Study I of this thesis. By that time, Lena was celebrating her freshly achieved title of docent, and I was immensely impressed and flattered by the invitation to celebrate this achievement with her. And Lena has continued to inspire me: She has since become Associate Professor of the Department of General Practice and Primary Healthcare, as well as principal investigator of the FinnDiane Study group. Lena’s mentorship has been very important for me during this crucial time of exploring my path and professional identity. She has always shown trust in my work and offered me the

encouragement and support I need. I am truly grateful for her dedication as a supervisor and, mostly, for her friendship.

To Professor Per-Henrik Groop for welcoming me into the FinnDiane Study group. This study group, founded and led by him, provides a reliable and inspiring environment to conduct research and learn. It is my belief that this is also the reason FinnDiane has become a significant contributor to the knowledge of diabetes complications over the past 25 years. Perra's enthusiasm together with his vast knowledge and guidance has been of utmost help in this thesis project.

To Docent Juha Martola, who is a neuroradiologist with extensive experience in both the clinical and research fields of neuroimaging. I want to express my gratitude for his invaluable advice, particularly in the MRI project. Whenever I was in need of advice, and with no question too small, Juha has always taken the time to share his knowledge, and I thank him for his support through this thesis.

I extend my sincerest thanks to Professor Emerita Sirkka Keinänen-Kiukaanniemi and docent Hannele Uusitalo-Järvinen, the official reviewers of this thesis, for their constructive feedback. I also wish to express my appreciation to the opponent, Professor Kåre Birkeland, and the official representative of the Faculty of Medicine, Docent Nico Wasenius, for their time and commitment. I further wish to acknowledge the members of my thesis committee Ilkka Tikkanen, Tero Kivelä, and Leo Niskanen. I also thank April Gallego, the language reviewer of this thesis, for her thorough work and feedback.

I also want to acknowledge the many skilled and inspiring co-researchers whom I have had the privilege of working with during this thesis project. I want to extend a particular word of gratitude to Paula Summanen who, with her long experience as an ophthalmologist and her extensive knowledge in diabetic retinopathy, has guided me in all aspects regarding this topic. Paula has been involved in this thesis project since the planning stage, through practical work such as data collection and diabetic retinopathy severity grading, to the finished product you are reading now. I could not have done this without her help.

I am also very thankful for the work by Kustaa Hietala, upon which the fourth study of this thesis is built, and for his advice and practical help regarding the data. I also wish to express my gratitude to Anna Syreeni, Adjunct Professor Niina Sandholm-Lafferre, and Emma Dahlström for their "official" contribution to the methodological aspects of Study II in particular and for the daily dialogues and insights that we have shared working together in the FinnDiane Study group. My warmest thanks also go to Stefan Mutter for his advice on statistical analysis and data management in my day-to-day work. I further want to thank Daniel Gordin, Turgut Tatlisumak, Sara Shams, and Ron Liebkin for their collaboration on the MRI project and their input. Moreover, a lot of work has gone into collecting and interpreting the stroke data, and I want to thank Jukka Putaala, Valma Harjutsalo,

Anni Ylinen, and Stefanie Hägg-Holmberg especially for their contribution to this task.

I wish to express my heartfelt thanks to the late Carol Forsblom (1964–2022), the international coordinator of the FinnDiane Study Group, for all the help and advice he has given me on this journey. I also want to recognise Pentti Pölönen, Markku Kaste, Oili Salonen, Maija Parkkonen, Emilia Franzén, and Janne Kytö for their help with data acquisition and interpretation.

The FinnDiane clinical visits are the backbone of the FinnDiane Study, and these would not be possible without the help of numerous physicians and nurses working at the local FinnDiane centres. A special acknowledgement goes to the skilled study nurses Anna Sandelin, Jaana Tuomikangas, Mira Koronalinen, Kirsi Uljala, and Anu Dufva at the FinnDiane centre in Helsinki, who have been carrying out the visits, as well as a lot of additional preparations and coordination related to the visits. Additionally, my sincerest gratitude goes to all the participants in the FinnDiane Study for their time and dedication. They are the ones truly enabling us to carry out this work.

As I previously mentioned, the FinnDiane Study group is an extraordinary community for development – both academic and personal – and I feel truly privileged to be part of the FinnDiane family. Alongside those already mentioned, I want to express my appreciation for my colleagues Anni A., Anniina P., Anniina T., Anmol, Carina, Erika, Erkka, Fanny, Hanna O., Hanne, Heli, Jani, Johanna, Kaarina, Krishna, Matias, Markku, Nadja, Patrik, Raija, Rasmus, Susanna, Torbjörn, and all other past and present members of the FinnDiane Study Group. I am particularly grateful for the spirit in room C329b, for all the brainstorming sessions within the FinnDiane clinical team, and for all the fruitful lunch discussions and the get-togethers, which I hope to continue enjoying even after this thesis project is finished.

I am lucky to have many wonderful friends whose support I can always count on. From the day we met in daycare, Laura has been a brilliant friend through primary and secondary school, engineering studies, medical school, and the journey of becoming an adult. Together with Martina and Alexandra, we have shared the highs and lows of balancing education and work with personal life, and I am grateful for the friendship within the *Drumsö tjejer* squad. I also wish to express my thanks to Daniel and Sebastian, who I can always count on to cheer me up with an adventure. It is also with fondness that I think of the countless days that we have spent studying medicine together. When it comes to peer support, I owe a particular word of gratitude to Fanny, who became a dear friend to me during medical school. Besides that, she is also the most loyal of research colleagues, and I have truly appreciated working together with her.

And last, but not least, to the most important people in my life, my family:

To my parents, Birgitta and Stefan, who have without doubt been my most influential and important teachers. Thank you for reading all those books for me as a child and for always encouraging me to read, study, and explore the world beyond what is already known and written in text. Many are the times you told me that "one does not study using the brain, rather, the gluteal muscles" – a statement in which I have found comfort, although regarding my topic of research, I feel compelled to also underline the importance of the brain!

To Patrik, my brother, friend, and "accomplice" since childhood. As your elder sister, I ought to have set a good example, but that never seemed necessary as you have always succeeded perfectly well on your own regardless. No, being equal "partners in crime" has been much more fun and rewarding and, in a way, you were my first peer reviewer in life. And to Monica and Julia: there is nothing like family, and I am so thankful to have you in mine.

Finally, and foremost, to Mathias, my wonderful, patient, and encouraging husband. You always support me in all my undertakings, and this thesis is no exception. I cannot describe how thankful I am for your way of pushing me to challenge myself and for your belief in my abilities when I lack confidence in myself. Thank you from the bottom of my heart. I could never have done this without you. You are my biggest supporter, my teammate, my best friend, and my greatest love.

Appendices

Appendix 1. FinnDiane Study Centres

FinnDiane Study Centres	Physicians and nurses
Anjalankoski Health Center	S.Koivula, T.Uggeldahl
Central Finland Central Hospital, Jyväskylä	T.Forslund, A.Halonen, A.Koistinen, P.Koskiaho, M.Laukkanen, J.Saltevo, M.Tiihonen
Central Hospital of Åland Islands, Mariehamn	M.Forsen, H.Granlund, A.-C.Jonsson, B.Nyroos
Central Hospital of Kanta-Häme, Hämeenlinna	P.Kinnunen, A.Orvola, T.Salonen, A.Vähänen
Central Hospital of Kymenlaakso, Kotka	R.Paldanius, M.Riihelä, L.Ryysy
Central Hospital of Länsi-Pohja, Kemi	H.Laukkanen, P.Nyländen, A.Sademies
Central Ostrobothnian Hospital District, Kokkola	S.Anderson, B.Asplund, U.Byskata, P.Liedes, M.Kuusela, T.Virkkala
City of Espoo Health Center:	
Espoonlahti	A.Nikkola, E.Ritola
Tapiola	M.Niska, H.Saarinen
Samaria	E.Oukko-Ruponen, T.Virtanen
Viherlaakso	A.Lyytinen
City of Helsinki Health Center:	
Puistola	H.Kari, T.Simonen
Suutarila	A.Kaprio, J.Kärkkäinen, B.Rantaeskola
Töölö	P.Kääriäinen, J.Haaga, A-L.Pietiläinen
City of Hyvinkää Health Center	S.Klemetti, T.Nyandoto, E.Rontu, S.Satuli-Autere
City of Vantaa Health Center:	
Korso	R.Toivonen, H.Virtanen
Länsimäki	R.Ahonen, M.Ivaska-Suomela, A.Jauhiainen
Martinlaakso	M.Laine, T.Pellonpää, R.Puranen
Myyrmäki	A.Airas, J.Laakso, K.Rautavaara

Rekola	M.Erola, E.Jatkola
Tikkurila	R.Lönnblad, A.Malm, J.Mäkelä, E.Rautamo
Heinola Health Center	P.Hentunen, J.Lagerstam
Helsinki University Hospital, Department of Medicine, Division of Nephrology	T.Claesson, A.Dufva, N.Elonen, M.Eriksson, J.Fagerudd, M.Feodoroff, D.Gordin, P.-H.Groop, O.Heikkilä, K.Hietala, S.Hägg-Holmberg, F.Jansson Sigfrids, M.Korolainen, J.Kytö, S.Lindh, H.Paajanen, K.Pettersson-Fernholm, K.Rimpeläinen, M.Rosengård-Bärlund, M.Rönnback, L.Salovaara, A.Sandelin, M.Saraheimo, S.Satuli-Autere, R.Simonsen, P.Smidtslund, L.Thorn, H.Tikkanen, J.Tuomikangas, A.Tynjälä, K.Uljala, T.Vesisenaho, J.Wadén, A.Ylinen
Herttoniemi Hospital, Helsinki	V.Sipilä
Hospital of Lounais-Häme, Forssa	T.Kalliomäki, J.Koskelainen, R.Nikkanen, N.Savolainen, H.Sulonen, E.Valtonen
Hyvinkää Hospital	L. Norvio, A.Hämäläinen
Iisalmi Hospital	E.Toivanen
Jokilaakso Hospital, Jämsä	A.Parta, I.Pirttiniemi
Jorvi Hospital, Helsinki University Central Hospital	S.Aranko, S.Ervasti, R.Kauppinen-Mäkelin, A.Kuusisto, T.Leppälä, K.Nikkilä, L.Pekkonen
Jyväskylä Health Center, Kyllö	K.Nuorva, M.Tiihonen
Kainuu Central Hospital, Kajaani	S.Jokelainen, K.Kananen, M.Karjalainen, P.Kemppainen, A-M.Mankinen, A.Reponen, M.Sankari
Kerava Health Center	H.Stuckey, P.Suominen
Kirkkonummi Health Center	A.Lappalainen, M.Liimatainen, J.Santaholma
Kivelä Hospital, Helsinki	A.Aimolahti, E.Huovinen
Koskela Hospital, Helsinki	V.Ilkka, M.Lehtimäki
Kotka Health Center	E.Pälikkö-Kontinen, A.Vanhanen
Kouvola Health Center	E.Koskinen, T.Siitonen
Kuopio University Hospital	E.Huttunen, R.Ikäheimo, P.Karhapää, P.Kekäläinen, M.Laakso, T.Lakka, E.Lampainen, L.Moilanen, S. Tanskanen, L.Niskanen, U.Tuovinen, I.Vauhkonen, E.Voutilainen
Kuusamo Health Center	T.Kääriäinen, E.Isopoussu
Kuusankoski Hospital	E.Kilkki, I.Koskinen, L.Riihelä
Laakso Hospital, Helsinki	T.Meriläinen, P.Poukka, R.Savolainen, N.Uhlenius
Lahti City Hospital	A.Mäkelä, M.Tanner
Lapland Central Hospital, Rovaniemi	L.Hyvärinen, K.Lampela, S.Pöykkö, T.Rompasaari, S.Severinkangas, T.Tulokas
Lappeenranta Health Center	P. Erola, L.Härkönen, P.Linkola, T.Pekkanen, I.Pulli, E.Repo
Lohja Hospital	T.Granlund, K.Hietanen, M.Porrassalmi, M.Saari, T.Salonen, M.Tiikkainen,

Länsi-Uusimaa Hospital, Tammisaari	I.-M.Jousmaa, J.Rinne
Loimaa Health Center	A.Mäkelä, P.Eloranta
Malmi Hospital, Helsinki	H.Lanki, S.Moilanen, M.Tilly-Kiesi
Mikkeli Central Hospital	A.Gynther, R.Manninen, P.Nironen, M.Salminen, T.Vänttinen
Mänttä Regional Hospital	I.Pirttiniemi, A-M.Hänninen
North Karelian Hospital, Joensuu	U-M.Henttula, P.Kekäläinen, M.Pietarinen, A.Rissanen, M.Voutilainen
Nurmijärvi Health Center	A.Burgos, K.Urtamo
Oulaskangas Hospital, Oulainen	E.Jokelainen, P-L.Jylkkä, E.Kaarlela, J.Vuolaspuro
Oulu Health Center	L.Hiltunen, R.Häkkinen, S.Keinänen-Kiukaanniemi
Oulu University Hospital	R.Ikäheimo
Päijät-Häme Central Hospital	H.Haapamäki, A.Helanterä, S.Hämäläinen, V.Ilvesmäki, H.Miettinen
Palokka Health Center	P.Sopanen, L.Welling
Pieksämäki Hospital	V.Sevtsenko, M.Tamminen
Pietarsaari Hospital	M-L.Holmbäck, B.Isomaa, L.Sarelin
Pori City Hospital	P.Ahonen, P.Merisalo, E.Muurinen, K.Sävelä
Porvoo Hospital	M.Kallio, B.Rask, S.Rämö
Raahe Hospital	A.Holma, M.Honkala, A.Tuomivaara, R.Vainionpää
Rauma Hospital	K.Laine, K.Saarinen, T.Salminen
Riihimäki Hospital	P.Aalto, E.Immonen, L.Juurinen
Salo Hospital	A.Alanko, J.Lapinleimu, P.Rautio, M.Virtanen
Satakunta Central Hospital, Pori	M.Asola, M.Juhola, P.Kunelius, M.-L.Lahdenmäki, P.Pääkkönen, M.Rautavirta
Savonlinna Central Hospital	T.Pulli, P.Sallinen, M.Taskinen, E.Tolvanen, T.Tuominen, H.Valtonen, A.Vartia, S-L.Viitanen
Seinäjoki Central Hospital	O.Antila, E.Korpi-Hyövälti, T.Latvala, E.Leijala, T.Leikkari, M.Punkari N.Rantamäki, H.Vähävuori
South Karelia Central Hospital, Lappeenranta	T.Ensala, E.Hussi, R.Härkönen, U.Nyholm, J.Toivanen
Tampere Health Center	A.Vaden, P.Alarotu, E.Kujansuu, H.Kirkkopelto-Jokinen, M.Helin, S.Gummerus, L.Calonius, T.Niskanen, T.Kaitala, T.Vatanen
Tampere University Hospital	P. Hannula, I.Ala-Houhala, R.Kannisto, T.Kuningas, P.Lampinen, M.Määttä,H.Oksala, T.Oksanen, A.Putila, H.Saha, K.Salonen, H.Tauriainen, S.Tulokas
Tiirismaa Health Center, Hollola	T.Kivelä, L.Petlin, L.Savolainen
Turku Health Center	A.Artukka, I.Hämäläinen, L.Lehtinen, E.Pyysalo, H.Virtamo, M.Viinikkala, M.Vähätalo
Turku University Central Hospital	K.Breitholz, R.Eskola, K.Metsärinne, U.Pietilä, P.Saarinen, R.Tuominen, S.Äyräpää

Vaajakoski Health Center	K.Mäkinen, P.Sopanen
Valkeakoski Regional Hospital	S.Ojanen, E.Valtonen, H.Ylönen, M.Rautiainen, T.Immonen
Vammala Regional Hospital	I.Isomäki, R.Kroneld, L.Mustaniemi, M.Tapiolinna-Mäkelä
Vasa Central Hospital	S.Bergkulla, U.Hautamäki, V-A.Myllyniemi, I.Rusk

Appendix 2. Diary for ambulatory blood pressure monitoring

FD nr

Verenpaineen vuorokausirekisteröinti **Seurantapäiväkirja**

NIMI _____ HENKILÖTUNNUS _____

Verenpaineen vuorokausirekisteröinti

alkoi 23.2.2016 klo 14.00 Työpäivä kyllä / (ei)

päättyi 24.2.2016 klo 11.00 Työn kuvaus: _____

Merkitse päiväkirjaan **nukkumaanmeno- ja heräämisaika** sekä mahdolliset henkiset ja ruumiilliset rasitukset. Palauta lomake tutkimushoitajalle verenpainelaitteen poiston yhteydessä.

KLO	TOIMINTO	KLO	TOIMINTO
esim. 7.15	herääminen		
esim.23.30	nukkumaan		
klo: 16-18	Lepo		
klo: 18-19	Leikkiä lasten kanssa		
19-20	Siivousta, imurointi		
0.49	nukkumaan		
09.00	herääminen		
noin 09.00-09.25	mittari pois → lapsen pesu & pukeminen		
11.00	Mittari pois		

Tutkimushoitajat
Anna Sandelin anna.sandelin@hus.fi tai
Jaana Tuomikangas jaana.tuomikangas@hus.fi
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AS 070113

Versio 2 7.1.2013

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