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RISK FACTORS FOR RETINOPATHY IN TYPE 1 DIABETES

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Academic Dissertation

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“The main things which seem to me important on their own account, and not merely as means to other things, are knowledge, art, instinctive happiness, and relations of friendship or affection.”

Bertrand Russell

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1 LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following original publications which are referred to in the text by Roman numerals I–V:

I. Hietala K, Forsblom C, Summanen P, Groop PH (2008) Heritability of proliferative diabetic retinopathy. Diabetes 57: 2176-2180

II. Hietala K, Harjutsalo V, Forsblom C, Summanen P, Groop PH (2010) Age at onset and the risk of proliferative retinopathy in type 1 diabetes. Diabetes Care 33: 1315-1319

III. Hietala K, Wadén J, Forsblom C, Harjutsalo V, Kytö J, Summanen P, Groop P-H (2013) HbA_{1c} variability is associated with an increased risk of retinopathy requiring laser treatment in type 1 diabetes. Diabetologia 56: 737-745

IV. Hietala K, Forsblom C, Summanen P, Groop P-H, (2013) Higher age at onset of type 1 diabetes increases risk of macular edema, Acta Ophthalmologica, in press

V. Hietala K, Forsblom C, Summanen P, Groop P-H, (2012) The risk of proliferative retinopathy in siblings with type 1 diabetes. Diabet Med 29: 1567-1573

2 ABBREVIATIONS

ACCORD	Action to Control Cardiovascular Risk in Diabetes Trial
ADA	The American Diabetes Association
ADVANCE	The Action in Diabetes and Vascular Disease Trial
AGE	advanced glycation end-products
AHT	antihypertensive medication
ARIC	Atherosclerosis Risk in Communities Study
BIC	Bayesian information criteria
BMI	body mass index
BP	blood pressure
BRB	blood-retina barrier
CI	confidence interval
CRP	C-reactive protein
CSME	clinically significant macular edema
CV	coefficient of variation
CVD	cardiovascular disease
DCCT	Diabetes Control and Complications Trial
DIEP	Diabetes in Early Pregnancy Study
DME	diabetic macular edema
DRP	diabetic retinopathy
DRS	Diabetic Retinopathy Study
DRVS	Diabetic Retinopathy Vitrectomy study
eGDR	estimated glucose disposal rate
ESRD	end-stage renal disease
ETDRS	Early Treatment of Diabetic Retinopathy Study
FIELD	Fenofibrate Intervention and Event Lowering in Diabetes Trial
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GCK	glucokinase
h2	heritability of a trait
HbA_{1c}	glycosylated hemoglobin A1c
HDL	high density lipoprotein
HIF-1α	hypoxia-inducible factor 1-alpha
HLA	human leukocyte antigen

HNF1A, HNF4A	hepatocyte nuclear factors 1A and 4A
HR	hazard ratio
ICC	intraclass correlation
IFG	impaired fasting glycaemia
IGF	insulin like growht factors
IGT	impaired glucose tolerance
IQR	inter quartile range
IRMA	intraretinal microvascular abnormality
LADA	latent autoimmune diabetes of adulthood
LDL	low density lipoprotein
MAP	mean arterial blood pressure
MODY	maturity onset diabetes of the young
NO	nitric oxide
NPDR	non-proliferative diabetic retinopathy
NVD	neovascularisation at the disc
NVE	neovascularisation elsewhere
NVG	neovascular glaucoma
OR	odds ratio
PDR	proliferative diabetic retinopathy
PKC	protein kinase C
PRH	preretinal haemorrhage
ROS	reactive oxygen species
SD	standard deviation
TGF-β	transforming growth factor beta
TNF-α	tumor necrosis factor- α
TRD	tractional retinal detachment
UAER	urinary albumin excretion rate
UKPDS	United Kingdom Prospective Diabetes Study
VEGF	vascular endothelial growth factor
WESDR	The Wisconsin Epidemiologic Study of Diabetic Retinopathy
VH	vitreous haemorrhage
WHO	World Health Organisation
WHR	waist-to-hip ratio

3 ABSTRACT

Background

Diabetic retinopathy is the leading cause of acquired visual disability among people of working age in all industrialised countries. Established risk factors for diabetic retinopathy include the duration of diabetes, glycaemic control, blood pressure and dyslipidaemia. However, these risk factors explain less than half of the risk for diabetic retinopathy. It is, thus, obvious that a large proportion of the risk remains to be explored.

Aim

The aim of the present study was to investigate potential risk factors that could affect the development of severe forms of retinopathy in type 1 diabetes.

Patients and Methods

The study patients were drawn from the large FinnDiane (Finnish Diabetic Nephropathy study) database. The FinnDiane study is an observational cohort study which since 1997 has collected comprehensive data on patients with type 1 diabetes at 92 centers throughout Finland with the aim of identifying genetic and environmental risk factors for diabetic complications. The patients' retinopathy status were verified from ophthalmic and medical files and fundus photographs when available and graded with the ETDRS-scale. All patients underwent a thorough clinical characterisation of their clinical diabetes status by the attending physician at the participating study centers.

Results

Proliferative diabetic retinopathy showed significant familial clustering in siblings with type 1 diabetes and the heritability h^2 adjusted for conventional risk factors suggested a significant genetic contribution to the risk. The siblings first affected by type 1 diabetes had a lower risk of proliferative retinopathy as compared to the siblings later affected by type 1 diabetes. The risk of both proliferative retinopathy and clinically significant macular edema were modified by the patient's age at onset of type 1 diabetes. The patients with higher age at onset of type 1 diabetes had a lower risk of proliferative retinopathy but conversely, a higher risk of clinically significant macular edema. The HbA_{1c} variability was lower in those patients with higher age at onset of type 1 diabetes and the patients with lower HbA_{1c} variability had a

lower cumulative incidence and risk of laser treatment and proliferative retinopathy.

Conclusion

In addition to the conventional risk factors, such as diabetes duration, glycaemic control and blood pressure, familial factors, age at onset of type 1 diabetes and glycaemic profile may explain a significant proportion of the risk of severe forms of diabetic retinopathy.

4 INTRODUCTION

DRP (diabetic retinopathy) is the most common microvascular complication in type 1 diabetes (1). It was first described by Eduard Jäger in 1855, much before the discovery of the pathogenesis of type 1 diabetes itself (2). DRP changes were so distinct that they were described only a few years after the introduction of the first ophthalmoscope by Hermann von Helmholtz in 1852. At this time, diabetes was a feared and deadly disease. It was known that sugar worsened the condition and that the most effective treatment was to put the patients on very strict diets where sugar intake was kept to a minimum. In some cases, the harsh diets even caused patients to starve to death. In 1869, a German medical student, Paul Langerhans, found that within the pancreatic tissue there were clusters of cells which were eventually shown to be the insulin producing beta cells (3). In 1889, a German physiologist Oskar Minkowski and physician Joseph von Mering showed that if the pancreas was removed from a dog, the dog developed diabetes (4). In 1921, doctors Frederick Banting and John MacLeod, a medical student Charles Best and a biochemist Bertram Collip purified insulin from the pancreatic extract of cattle and in Toronto, Canada, in January 1922, a 14-year Leonard Thompson was chosen as the first person with diabetes to receive insulin (5). Soon, the industrial production of insulin made it possible to save the lives of thousands of patients with type 1 diabetes. However, it immediately became evident that diabetes was not a solved problem since patients developed a myriad of other health problems that decreased their quality of life and shortened their lifespans.

Almost a hundred years later, and with greatly improved medical care, the risk of co-morbidity is reduced, but it is still very much present and puts a considerable burden upon the patients. The sheer number of diabetes patients weighs heavily upon the whole society as well. There are roughly 500 000 diagnosed diabetes patients in Finland (6, 7) and nearly 366 million worldwide and, by 2030, this number will have soared to 552 million (8). Despite modern medical and surgical treatment, the relative risk of blindness in diabetes patients is still five times higher as compared to non-diabetic people (9). DRP is the most important preventable and treatable cause of blindness among working age people in Finland (10).

Fortunately, with the advent of modern laser and surgical treatment, very few patients become blind, but many more will have low vision as a result of diabetes. Visual impairment has a very significant adverse effect on the patients' quality of life (11). Not only is the quality of life lower, but visual impairment can also adversely affect the patients ability to manage their diabetes which may, in turn, have a negative impact on the incidence of other diabetic complications (11). Although hyperglycaemia is a prerequisite for DRP, a considerable proportion of the risk remains unexplained. The

individual's response to hyperglycaemia is likely to be influenced by genetic and environmental factors. There are well established risk factors for DRP such as the duration of diabetes, hyperglycaemia, dyslipidaemia and elevated BP (blood pressure). However, these risk factors explain much less than 50% of the risk (12, 13). The key to preventing the co-morbidity lies in the knowledge of the risk factors. Therefore, this thesis aims to discover and quantitate the contribution of other risk factors for DRP.

5 REVIEW OF THE LITERATURE

5.1 THE DIAGNOSIS OF DIABETES

Diabetes is a heterogeneous group of metabolic disorders characterised by elevated blood glucose concentrations (14). The elevated glucose concentrations, i.e. hyperglycaemia, may be caused by either insufficient insulin secretion by the β-cells in the pancreatic islets, or deficient biological action of insulin in target tissues, or both. Defective insulin action in the target tissues may also lead to disturbances in amino acid and lipid metabolism. The diagnosis of diabetes is primarily based on the measurement of fasting plasma glucose concentrations of ≥ 7.0 mmol/l (15). If the diagnosis is based solely on the fasting glucose measurements, roughly 30% of the patients may be left undiagnosed (15, 16). For this reason, a 75-g oral glucose tolerance test to detect the elevated plasma glucose concentrations is advisable for high risk patients. In this test, a 2-hour post-load plasma glucose concentration of at least 11.1 mmol/l is diagnostic of diabetes. In patients with the classical symptoms of diabetes (thirst, polyuria, weight loss), the diagnosis of diabetes may be confirmed by just one measurement of a plasma glucose concentration of at least 11.1 mmol/l. The glucose concentrations below this fasting cut-off value but above the normal range are referred to as IFG (impaired fasting glycaemia) which encompasses values which are above normal but below the diagnostic cut-off for diabetes (plasma ≥ 6.1 to <7.0 mmol/l), and if the 75-g oral glucose post-load concentration is between 7.8 mmol/l–11.0 mmol/l, it is referred to as IGT (impaired glucose tolerance) (15). According to WHO (World Health Organization), HbA_{1c} can be used as a diagnostic test for diabetes providing that stringent quality assurance tests are in place and assays are standardised to criteria aligned to the international reference values, and there are no conditions present which preclude its accurate measurement. An HbA_{1c} of ≥ 6.5% is recommended as the cut-off point for diagnosing diabetes. However, a value of less than 6.5% does not exclude diabetes or IGT as diagnosed by using the aforementioned tests (17).

5.2 DIFFERENT TYPES OF DIABETES

Diabetes has traditionally been classified into type 1 and type 2 diabetes (18) although this distinction is often not clinically obvious and many patients have typical features of both types (19). Despite the overlap of clinical representation, it appears that type 1 and type 2 diabetes are genetically distinct disease entities (20).

Type 1 diabetes, also known as “insulin dependent” or “juvenile onset” diabetes, is characterized by the destruction of the insulin-secretory pancreatic β-cells of the islets of Langerhans which usually leads to total insulin deficiency. The etiology of human type 1 diabetes remains elusive, but it is clear that both genetic and environmental factors are important in defining the risk (21). The importance of heritable factors was shown in a Finnish twin study in which the proband-wise concordance for monozygotic twins was estimated to be ~50% as compared with ~8% for dizygotic twins (22). The statistical estimates in the Finnish twin population suggested that as much as 88% (95% CI 78–94%) of the phenotypic variance in the liability to type 1 diabetes was due to additive genetic effects, and 12% (95% CI 6–22%) to unique environmental effects (22). The genetic factors may be influenced by a complex interaction of familial factors. It has been noted that the risk of diabetes may decrease in larger sibships, but studies have also suggested that this risk reduction is modified by an interplay between birth order and maternal age at delivery (23, 24). Age at onset and sex may affect the transmission of type 1 diabetes from diabetic fathers and mothers to their offspring. Young age at onset of diabetes in fathers, but not in mothers has been shown to increase the risk of type 1 diabetes in the offspring of diabetic parents (25). If there are many siblings with diabetes in the family, those siblings that are genetically or environmentally most susceptible to diabetes are likely to be the first to manifest type 1 diabetes. Hence, the siblings first affected by diabetes are usually younger than the later affected siblings (26-28).

Both the animal model and human studies have indicated that an autoimmune response to the β-cells of the pancreatic islets occurs in type 1 diabetes. The outcome of this response is substantially influenced by an unknown series of probably random environmental events or developmental factors. The autoimmune process, which is to a large degree determined by the patient’s genotype, then progresses through a preclinical phase, leading to the destruction of the β-cells and a stage of hyperglycaemia resulting from the reduced β-cell mass and insulin secretory capacity (20). The greatest genetic risk for type 1 diabetes is conferred by specific alleles, genotypes, and haplotypes of the HLA class II genes. The highly polymorphic HLA class II molecules, the DR and DQ α-β heterodimers, are the central players in the susceptibility to type 1 diabetes (29, 30). The mechanisms by which the HLA class II molecules confer susceptibility to immune-mediated destruction of the pancreatic islets is still not known, but the binding of the key peptides from autoantigens to HLA class II molecules in the thymus and in the periphery is likely to play an important role. There are currently roughly 50 non-HLA region loci that also influence the type 1 diabetes risk. Many of the assumed functions of the non-HLA genes suggest that the genetic variants at these loci act in the adaptive and innate immune systems to initiate, amplify, and sustain the β-cell destruction (20).

A steady increase in the incidence of type 1 diabetes has been going on worldwide (31). Finland has the highest incidence of type 1 diabetes in the world, reaching 64.2 per 100 000 people per year in 2005 (32). From 1980 to 2005 the overall incidence rate of type 1 diabetes in children under the age of 15 years has doubled in Finland. The increase has been the greatest in the youngest children under 4 years of age (32). It is not clear why this increase has taken place. The proposed theories include distorted immune responses due to improved hygiene and decreased number of childhood infections (33). Another plausible theory proposes that the increase in body weight is leading to the younger onset and, thus, higher incidence of type 1 diabetes (34). High birth weight and increased early weight gain have indeed been shown to be risk factors for type 1 diabetes (31).

Type 2 diabetes, also known as "non-insulin dependent" or "adult-onset" diabetes, is characterized by insulin resistance in the peripheral tissues and an insulin secretory defect of the β-cells. Type 2 diabetes is often associated with increasing age, obesity, and sedentary lifestyle (35). Genetic liability is also a risk factor for type 2 diabetes. In a Finnish twin study, the heritability estimates for type 2 diabetes were 73% in males and 64% in females. In this study, only one fifth of the covariance of BMI and type 2 diabetes was due to shared genetic influences (36).

Since the advent of the first genome-wide association studies, the knowledge of the genetic susceptibility to type 2 diabetes has increased dramatically. The number of susceptibility loci for type 2 diabetes started to grow significantly in 2007. The current total is approximately more than 40 confirmed type 2 diabetes loci. Most of these genetic susceptibility variants act by disturbing insulin secretion, rather than insulin action, with inherited abnormalities of β -cell function and/or mass as the critical components of the progression to type 2 diabetes (35). The incidence of type 2 diabetes has been on the rise in Finland, as the number of type 2 diabetes patients using antidiabetic medications has risen from 42 000 adults in 1970 to 169 000 adults in 2006 (7, 37).

The other subtypes of diabetes are diagnosed less frequently. Of the remaining diabetes types, the two most prevalent forms are MODY (maturity onset diabetes of the young) and LADA (latent autoimmune diabetes of adulthood). Among patients with phenotypic type 2 diabetes, LADA occurs in 10% of individuals older than 35 years and in 25% below 35 years of age (38). MODY-type diabetes is a well described, less common subtype of diabetes that consists of several monogenic β-cell disorders and is estimated to account for approximately 1% of all diabetes cases (39). This heterogeneous group is characterized by autosomal dominant inheritance, young age of onset, usually in the 2nd–4th decade, and continued secretion of insulin. The most frequent causes are mutations in genes encoding the transcription factors HNF1A (Mody 3) and HNF4A (Mody 1) and GCK enzyme (Mody 2). Mutations in a number of other genes can also present with a MODY phenotype but these are rare in clinical practice (40).

The onset of LADA occurs usually in adult life, and because this form is usually not initially insulin-requiring, the patients appear clinically to be affected by type 2 diabetes. Such patients probably have the same disease process as the patients with type 1 diabetes in that they have a similar HLA-genetic susceptibility, as well as autoantibodies to islet antigens, low insulin secretion, and a higher rate of progression to total insulin dependency (41).

Gestational diabetes refers to insulin resistance that manifests during pregnancy. There has been a steady increase in recent years in this form of diabetes as well. The prevalence of gestational diabetes in Finland was 10-11% between the period from 2004 to 2006 (42).

There are yet other forms of diabetes, such as secondary diabetes caused by insulin deficiency due to pancreatitis, trauma or sometimes even pancreatic cancer. Iatrogenic diabetes may occur due to immunosuppressive medications, such as glucocorticoids (43) and calcineurin inhibitors cyclosporine and tacrolimus (44) .

5.3 DIABETIC COMPLICATIONS

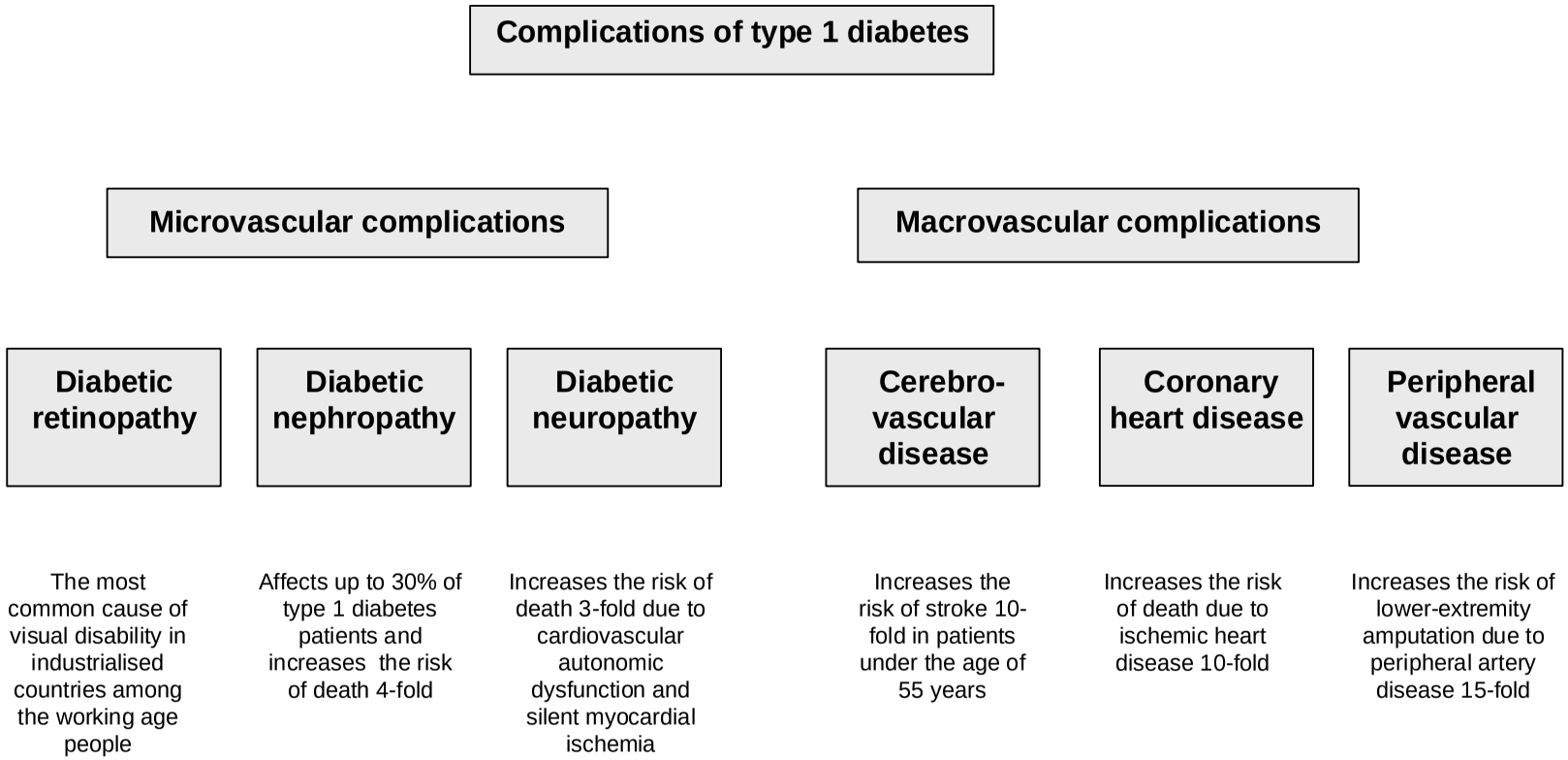
The complications of diabetes can be broadly classified as microvascular and macrovascular, although a number of complications do not easily fit either category, such as the increased risk of Alzheimer's disease, gingivitis and female infertility (Fig.1). Despite modern therapeutics, type 1 diabetes continues to be associated with the increased risk of premature death. The standardised mortality ratio was 3.6 in patients with diabetes diagnosed between 0-14 years of age and 2.8 in the patients with diabetes diagnosed between 15-29 years of age (45). The median age of death for type 1 diabetes patients in Finland was only 49 years in 2002 (46). Fortunately, the life expectancy for type 1 diabetes patients has been steadily improving. The results of a 30-year study by the University of Pittsburgh showed that patients with type 1 diabetes born after 1965 had a life expectancy of 69 years, whereas in participants diagnosed 1950–1964 it was only 53 years (47). A similar time trend has been observed in Finland as well. The standardised mortality ratio at 20 years' duration of diabetes in patients with diabetes onset between 0-14 years decreased from 3.5 in patients diagnosed in 1970-1974 to 1.9 in those diagnosed in 1985-1989. (45). Patients with type 2 diabetes also have an increased risk of death, with up to four times higher mortality as compared to non-diabetic people (48). A 50-year-old patient with type 2 diabetes dies, on average, 6 years earlier than a counterpart without diabetes (49). The complications of diabetes continue to place a great burden on the health care system. People with diagnosed diabetes, on average, have medical expenditures that are approximately 2.3 times higher than what the expenditures would be without diabetes (50). Diabetes affects all organs, including skeletal muscle, liver, adipose tissue, kidney, retina, and even the bones and skin. The impact of hyperglycaemia on the

tissues depends on the tissue's responsiveness to metabolic and/or inflammatory insults. Complications, such as retinopathy, nephropathy, neuropathy, and CVD (cardiovascular disease) manifestations are organ changes that cause direct clinical impairments.

5.3.1 DIABETIC NEPHROPATHY

Diabetic nephropathy, also known as Kimmelstiel-Wilson syndrome, nodular diabetic glomerulosclerosis or intercapillary glomerulonephritis, is characterised by a progressive increase in the urinary albumin excretion rate (51). The syndrome was discovered by the British physician Clifford Wilson (1906–1997) and the German-born American physician Paul Kimmelstiel (1900–1970), and was described for the first time in 1936 (52). Diabetic nephropathy is accompanied by an increase in the BP and a decline in the glomerular filtration rate with renal failure as the ultimate endpoint of nephropathy progression. Diabetic nephropathy is a common complication of type 1 diabetes, affecting up to 30% of patients (53). Previous studies have shown that diabetic renal disease is a significant risk factor for increased mortality in type 1 diabetes (54, 55). In the absence of diabetic nephropathy, the long-term survival of patients is similar to that of the general population (56, 57). The diabetic nephropathy is categorised into stages according to UAER (urinary albumin excretion rate). Normal values for UAER are <30 mg/24h or < 20 ug/min in an overnight urine collection. This is referred to as normoalbuminuria. Diabetic nephropathy, or macroalbuminuria, is present if UAER ≥300 mg/24h, or ≥ 200 ug/min is detected. The intermediate range of UAER ≥30 but < 300 mg/24h, or ≥20 but <200 ug/min, is called microalbuminuria (51). The end-stage renal disease refers to the failure of kidney function which requires dialysis, or renal kidney transplantation for the survival of the patient.

Figure 1 Diabetic complications



5.3.2 DIABETIC NEUROPATHY

Diabetic neuropathy is defined by the ADA (American Diabetes Association) as “the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes” (58).

Diabetic neuropathy may affect all peripheral nerves including pain fibers, motor neurons, and the autonomic nervous system. The most common among the neuropathies are chronic sensorimotor distal symmetric polyneuropathy, and the autonomic neuropathies, and, thus, diabetic neuropathy can affect all organs and systems, as all are innervated. Diabetic vascular and neural diseases are closely intertwined. The neuropathies are thought to result from diabetic microvascular injury involving small blood vessels that supply nerves (vasa nervorum) in addition to macrovascular conditions that can result in diabetic neuropathy.

Similar to other microvascular complications, the prevalence of diabetic neuropathy increases with duration of diabetes and poor glycaemic control (59). Severe diabetic polyneuropathy can develop in young adults even within a few months after the onset of type 1 diabetes if the diabetes is poorly controlled (60). Chronic sensorimotor distal symmetric polyneuropathy is, however, the most common form of neuropathy in diabetes, as more than 80% of patients with clinical diabetic neuropathy have this form of diabetic neuropathy (61). Typically, patients experience burning, tingling, and “electrical” pain, but sometimes they may experience simple numbness. Up to 50% may be asymptomatic, and these patients are at risk of having insensate injury to their feet (58). In clinical testing, the patients have a sensory loss to light touch, vibration, temperature, and they may have a loss of the ankle reflex. Combining more than one test increases the sensitivity to detect diabetic distal symmetric polyneuropathy to > 87% (58). Diabetic autonomic neuropathy is the second most common form of diabetic neuropathy (58). It causes significant morbidity and even mortality. Neurological dysfunction may occur in most organ systems and can be manifested by gastroparesis, constipation, diarrhoea, anhidrosis, bladder dysfunction, erectile dysfunction, exercise intolerance, resting tachycardia, silent ischemia, and even sudden cardiac death (62)(Fig. 1).

Cardiovascular autonomic neuropathy is the most studied and clinically most important form of diabetic autonomic neuropathy. It may be indicated by resting tachycardia (100 beats per minute), orthostasis (a fall in systolic BP > 20 mmHg upon standing), or other disturbances in autonomic nervous system functions involving the skin, pupils, or gastrointestinal and genitourinary systems (58). Cardiovascular autonomic dysfunction is associated with an increased risk of silent myocardial ischemia and mortality (63) (Fig. 1). Pure sensory neuropathy is relatively rare and associated with periods of poor glycaemic control or considerable fluctuation in diabetes control. It is characterized by isolated sensory findings without signs of motor neuropathy. The symptoms are typically most prominent at night (62).

Mononeuropathies typically have a more sudden onset and involve virtually any nerve, but most commonly the median, ulnar, and radial nerves are affected. Cranial neuropathies also occur, such as paresis of the abducens and trochlearis nerves which cause diplopia and are commonly seen by ophthalmologists. The patient's visual fields may be disturbed by nonarteritic anterior ischemic optic neuropathy, for which diabetes is also a risk factor (64). Diabetic amyotrophy may be a manifestation of diabetic mononeuropathy and is characterized by severe pain and muscle weakness and atrophy, usually in the large thigh muscles (62).

5.3.3 MACROVASCULAR COMPLICATIONS

Common macrovascular diseases are coronary heart disease, cerebrovascular disease, and peripheral vascular disease. The impact of these diabetic macrovascular complications is so great that they account for nearly 60% of the health care expenditures in people with diabetes (50).

The central pathological mechanism in macrovascular disease is the process of arteriosclerosis which leads to the loss of elasticity and narrowing of arterial walls in critical organs throughout the body. Diabetes is an important cause of arteriosclerosis in addition to hypertension and aging. Atherosclerosis is a specific type of arteriosclerosis that results from chronic inflammation and injury to the arterial wall. In response to endothelial injury and inflammation, oxidized lipids from LDL particles accumulate in the endothelial wall of arteries, namely intima media. Monocytes infiltrating the arterial wall differentiate into macrophages, which accumulate oxidized lipids to form foam cells. The foam cells stimulate macrophage proliferation and attraction of T-lymphocytes which, in turn, induce smooth muscle proliferation in the arterial walls and collagen accumulation. The end-result is the formation of a lipid-rich atherosclerotic lesion, atheroma, with a fibrous scar. The narrowing of the arterial wall by atheromas or the rupture of the atherosclerotic plaque results in vascular infarction (65). The risk of infarction by the arteriosclerotic process may be perpetuated by the enhanced thrombotic potential characteristic of diabetes. Diabetes increases the platelet activation and decreases the endogenous inhibitors of platelet activity. In addition to potentiating intrinsic platelet function, diabetes augments blood coagulability, making it more likely that atherosclerotic plaque rupture or erosion will result in thrombotic occlusion of the artery (66). As a consequence of this precipitated arteriosclerosis, diabetes patients are 15 times more likely to have a lower-extremity amputation due to peripheral artery disease as compared to people without diabetes (67). The peripheral artery disease is characterized by the occlusion of the lower-extremity arteries which causes intermittent claudication and pain and which may lead to lower limb amputations (68).

Diabetes increases the risk of CVD (cardiovascular disease) (69). Although the precise mechanisms through which diabetes increases the likelihood of arteriosclerosis and atherosclerotic plaque formation are not completely understood, the association between these and diabetes is clear. CVD is the primary cause of death in people with type 1 diabetes (70). Among macrovascular diabetes complications, coronary heart disease has been associated with diabetes in numerous studies beginning with the Framingham study (71). More recent studies have shown that the risk of myocardial infarction in people with type 2 diabetes is equivalent to the risk in non-diabetic patients with a history of previous myocardial infarction (72). Although the risk of CVD is not as great in type 1 diabetes as in type 2 diabetes, most likely due to the younger age of the type 1 diabetes patients, the CVD risk is still dramatically increased also in type 1 diabetes (69, 70, 73). The studies have shown that these patients have a higher mortality from ischemic heart disease at all ages compared to the general population. In individuals > 40 years of age, women with diabetes experience a higher mortality from ischemic heart disease than men, which is in contrast to the non-diabetic population (70). These discoveries have led to new recommendations by the ADA and American Heart Association that diabetes be considered a coronary artery disease risk equivalent rather than a risk factor for future CVD events (73).

Diabetes is also a strong independent predictor of the risk of cerebrovascular disease and a stroke (74). Diabetes increases the risk of atherosclerotic carotid artery occlusive disease which causes a thromboembolic threat to the central retinal artery and increases the risk of a stroke (75). Observational studies have shown that the cerebrovascular mortality rate is elevated at all ages in patients with type 1 diabetes (76). Diabetes particularly affects the risk of a stroke among younger patients. In a population with an age younger than 55 years, diabetes increased the risk of a stroke more than 10-fold (77). In addition to increased risk of a stroke itself, the sequalea of a stroke may also be worse in diabetes patients. The risk of a stroke-related dementia is increased threefold, and the risk of a stroke recurrence is increased twofold as compared to non-diabetic patients. Diabetes increases stroke-related mortality as well (66).

Fortunately, the prevention of macrovascular complications by improving the glycaemic control and lowering the blood pressure has been as successful in reducing the macrovascular complications as it has been in reducing the microvascular complications. Studies in type 1 diabetes have shown that an intensive diabetes control is associated with a lower resting heart rate, and that patients with higher degrees of hyperglycaemia tend to have a higher heart rate which is associated with higher risk of CVD (78). Even more convincingly, the DCCT demonstrated that during 17 years of prospective analysis, intensive treatment of type 1 diabetes, including lower HbA_{1c}, was associated with a 42% risk reduction in all cardiovascular events and a 57% reduction in the risk of nonfatal myocardial infarction, a stroke, or death

because of CVD (55). The risk of cardiovascular events can be further reduced by the optimal treatment of dyslipidaemia (79, 80).

5.3.4 THE HISTORY OF DIABETIC RETINOPATHY RESEARCH

The first reports on DRP were published in the mid-19th century. Diabetic macular changes in the form of lipid exudates and edema were observed for the first time by Eduard Jäger in 1855. He published a manuscript "Beiträge zur Pathologie des Auges" where he included 21 fundus paintings of macular changes (2). Albrecht von Graefe (1828-1870) was sceptical of his findings and claimed that there was no proof of a cause-effect relationship between diabetes and retinal changes (81). The opinion of von Graefe was adopted by many ophthalmologists, and it was not until 1872 when Edward Nettleship published "Oedema or cystic disease of the retina" which provided the first histological descriptions of "cystoid degeneration of the macula" in patients with diabetes (82). In 1876, Wilhelm Manz described the typical neovascularisations in PDR (proliferative diabetic retinopathy), TRD (tractional retinal detachment), and VH (vitreous haemorrhage) (83). In 1890, Julius Hirschberg (1843-1925) classified DRP into four types (retinitis centralis punctuate, haemorrhagic form, retinal infarction, and haemorrhagic glaucoma), thus describing the natural course of DRP and also creating the first clinical classification of DRP (84). Retinopathy caused by hypertension had been described by Markus Gunn at the end of the 19th century (85). Like diabetic retinopathy, it could cause visual impairment due to macular edema and neovascularisation. A four-grade-classification scale for hypertensive retinopathy was developed by Norman Keith, Henry Wagener, and Nelson Barker in 1939 which predicted the survival of hypertensive patients (86). The work of Arthur James Ballantyne in 1943 gave proof that DRP is indeed a unique form of vascular retinal disorder, which is distinct from hypertensive retinopathy, although they share many features (87). The knowledge of DRP has since greatly increased, and the definition and classification of DRP has evolved through many stages.

5.3.5 THE DEVELOPMENT OF RETINOPATHY SEVERITY GRADING

For a long time, the classification by Julius Hirschberg was the only one available, and there was an obvious need for a more detailed classification (84). The so called Airlie House classification for DRP was developed at a symposium organized by the US Public Health Service in 1968 held in Airlie House, Warrington, PA, hence the name (88). The principle of grading various retinal changes in the Airlie House classification was adopted from a study by a Finnish group describing DRP changes following hypophysectomy (89-91). The Airlie House classification used five standard photographic

fields and stereo photographs to assess the severity of the retinopathy. The DRS (Diabetic Retinopathy Study), the first randomised controlled trial in medicine, added two more photographic fields, inferonasal and superonasal, to the original Airlie House classification resulting in seven standard photographic fields (92). The original Airlie House grading classified fundus lesions into one of three categories: absent, mild to moderate, or severe (88). With only three levels of severity, this system was impractical for research purposes. The DRS, therefore, defined more severity grades with the aid of standard fundus photographs. The additional severity grades included severe background retinopathy, also known as preproliferative DRP, and PDR with high-risk characteristics for severe visual loss which was used as an indication for photocoagulation without delay in the DRS (93).

The most detailed classification was created in the ETDRS (Early Treatment of Diabetic Retinopathy Study) in 1980's. The ETDRS grading was based on the DRS modified Airlie House classification. The ETDRS was a prospective randomised controlled trial which was conducted to assess the use of aspirin and photocoagulation in the treatment of non-proliferative DRP and early PDR which did not fulfil the high-risk characteristics for severe visual loss as already defined in the DRS. It, therefore, needed to have a more sensitive scale for the early DRP changes. More steps and more fundus photographic risk factors were added to the scale at the mild and moderate end of the DRS modified Airlie House severity scale. The 12-step ETDRS final retinopathy severity scale was based on assessing the retinopathy features in seven standard 30^{o} photographic fields (94). The ETDRS-scale was later modified for use with fundus quadrants instead of seven standard photographic fields (95). The diabetes patients may have findings typically associated with DRP, such as venous irregularities, retinal microinfarcts, or macular edema, but only when microaneurysms are present, the retinal changes are considered to be DRP in the ETDRS scale. In addition to a more detailed DRP severity scale, a logarithmic visual acuity test was developed. The ETDRS visual acuity test incorporated specific design criteria to make it more accurate than the Snellen acuity test. Both the ETDRS retinopathy severity scale and the ETDRS visual acuity test have now become standards for defining the severity of DRP and measuring visual acuity in research and even in clinical settings.

5.3.6 PATHOGENESIS OF DIABETIC RETINOPATHY

The mechanism by which hyperglycaemia exerts its detrimental effects in the retina are many. All retinal cells, such as neural retina, glial cells, vascular walls and blood itself, are affected by the hyperglycaemic environment. This causes a multitude of functional and structural changes from very early on (96, 97). Some of the early changes may be reversible (98).

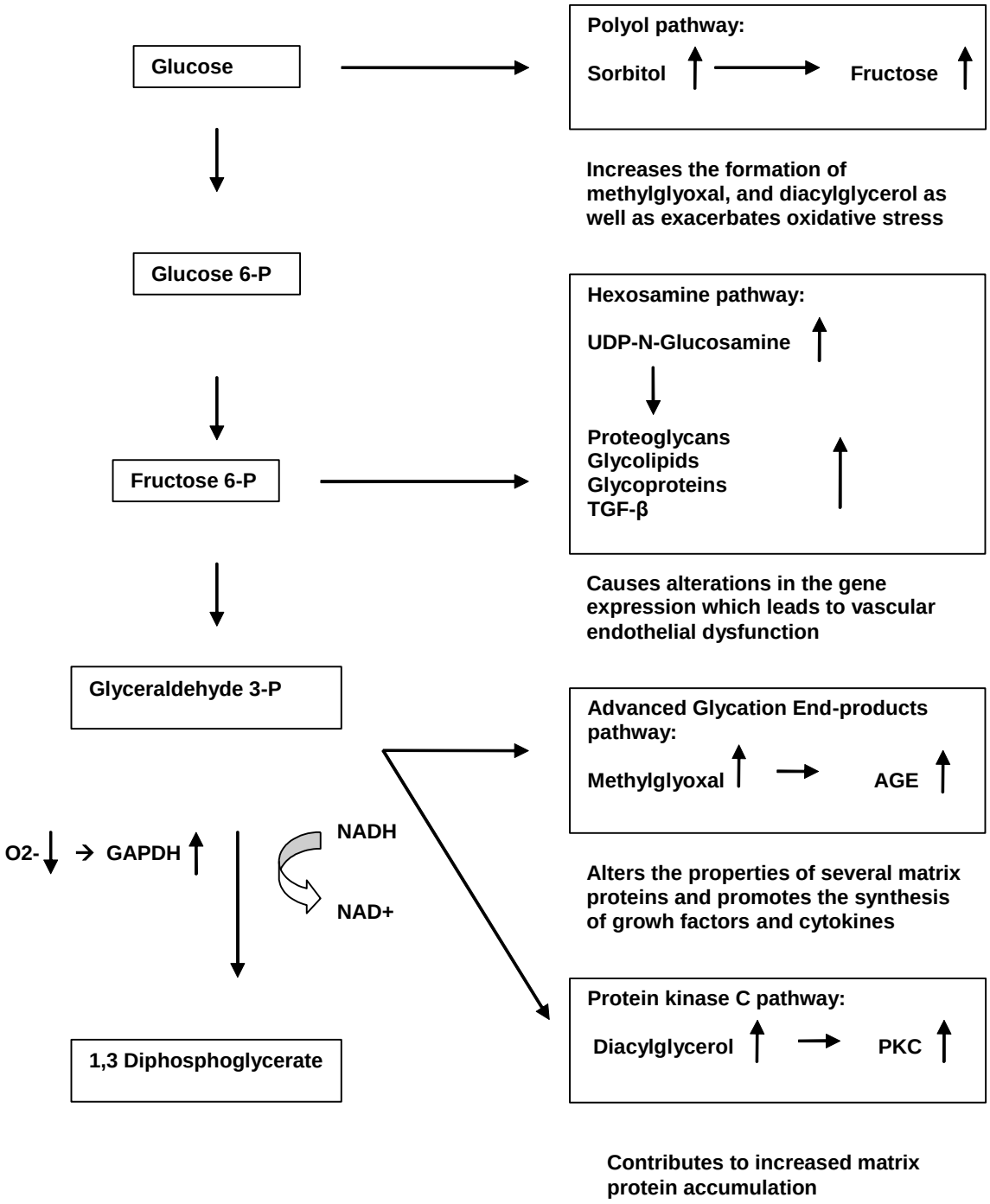


Figure 2 Important biochemical pathways in the pathogenesis of diabetic retinopathy. Hyperglycaemia induced superoxide production (O_2^-) inhibits glyceraldehyde 3-phosphate dehydrogenase (GAPDH) which in turn leads to the accumulation of upstream glucose metabolites. These are then diverted into four different metabolic pathways, each of which causes vascular and interstitial tissue damage.

Oxidative stress has been suggested as one of the most important pathophysiological factors that may explain the majority of the retinal changes. There are four biochemical pathways with considerable cross-over reactions that are alternatively activated as a consequence of hyperglycaemia and oxidative stress (Fig. 2) (99). These pathways are thought to explain many of the changes leading to DRP. Hyperglycaemia induced production of ROS which includes O_2-, H_2O_2, -OH^-, and singlet oxygen, decreases GAPDH activity which, in turn, increases the metabolites in the upstream glycolytic pathway. The metabolites are then diverted into four alternative pathways, each of which leads to vascular and interstitial tissue damage. The four metabolic pathways involved are: polyol pathway, AGE (advanced glycation end products), activation of PKC (polyol kinase C) and hexosamine pathway (100).

In the polyol pathway the affinity of aldose reductase to glucose is increased in hyperglycaemia. It has previously been thought that glucose is, therefore, converted into sorbitol and then to fructose. It would appear, however, that glycolytic metabolites of glucose such as glyceraldehyde 3-phosphate, for which aldose reductase has a much higher affinity, may be the physiologically relevant substrate, since the glucose concentrations within cells are probably too low in diabetes. Several mechanisms have been proposed to explain how the increase in polyol pathway flux could damage the tissues involved. The most probable is an increase in redox stress (101).

The activation of the hexosamine pathway causes alterations in gene expression which are known to lead to vascular endothelial cell dysfunction and other retinal changes that are commonly seen in diabetic retinopathy. The mechanism by which the increased hexosamine pathway flux causes these changes is not certain (101). The hexosamine pathway also produces glucosamine 6-P which leads to the increased synthesis of glycolipids, glycoproteins, proteoglycans and, TGF-β.

The AGEs interact with cells by three main routes. First, the AGE-modified serum proteins interact with vascular endothelium via AGE-receptors causing increased cytokine and adhesion molecule production. The serum-derived AGEs may reach vascular pericytes via transendothelial trafficking or as a result of blood–retinal barrier breakdown. The serum AGEs may also interact directly with cell surface glycoproteins with damaging effects on membrane integrity and function. Second, the AGEs can form directly within cells from reaction of glucose or methylglyoxal with amino groups. Third, the AGEs cause collagen crosslinking and impair matrix-cell interaction with a potentially significant detrimental effect on the cell function (97, 102).

In the PKC activation pathway, the intracellular diacylglycerol is increased in hyperglycaemia, and this activates PKC which contributes to increased matrix protein accumulation and also induces the expression of VEGF. Furthermore, hyperglycaemia activates many PKC isoforms which

mediate retinal blood flow abnormalities by depressing the NO production (101).

There are also other mechanisms which are likely to have a role in the development of DRP. Low intracellular oxygen tension elevates the intracellular concentration of ROS which consequently limits the cell's ability to hydroxylate HIF-1α, which is the key mediator of hypoxic responses. This leads to hypoxia induced cascade of protein synthesis which includes inflammatory cytokines, such as VEGF. Other intracellular molecules also affect the stability of HIF-1α. These include growth factors such as IGF-1 and IGF-2. Insulin induces the expression of VEGF and together with the stabilized HIF-1α may explain the initial worsening of DRP which is observed in improved glycaemic control (103, 104).

Inflammatory changes have also been suggested to contribute to the development of DRP, since many of the observed abnormalities are consistent with inflammation (105). As a consequence of the many disturbed biochemical pathways, there are functional changes in the retina which include increased blood flow causing shear stress, leukostasis, blood retina barrier breakdown, impaired vascular autoregulation, and decreased visual function (105).

5.3.6.1 Clinical signs of diabetic retinopathy

The first clinically visible lesions of DRP are vascular abnormalities. However, diabetes affects the entire retinal parenchyma causing structural and functional changes from very early on (96). There is evidence that suggests that the late stages of the retinopathy develop as a consequence of these earlier retinal changes (106). The patients with diabetes have reduced electrical responses as shown by electroretinography, lowered blue-yellow colour sensitivity, and a diminished contrast sensitivity even before the appearance of any microvascular lesions (96). Patients with an early ophthalmoscopically detectable DRP have retinal microaneurysms which appear as red dots on dilated funduscopic examination. The microaneurysms are localized dilatations of the capillaries which have been postulated to develop as a result of localized weaknesses in the vessel wall (pericytes), pressure disturbances, glial retraction/death, or endothelial cell proliferation in response to local capillary closure (Fig. 3)(107). Other retinopathy changes typical for the NPDR stages of the DRP (haemorrhages, retinal edema, lipid exudates, microinfarcts, IRMA) do not necessarily cause symptoms if outside the macular area (Fig 4). The increase in their presence and severity tends to predict progression towards the more advanced stages of the disease (95).

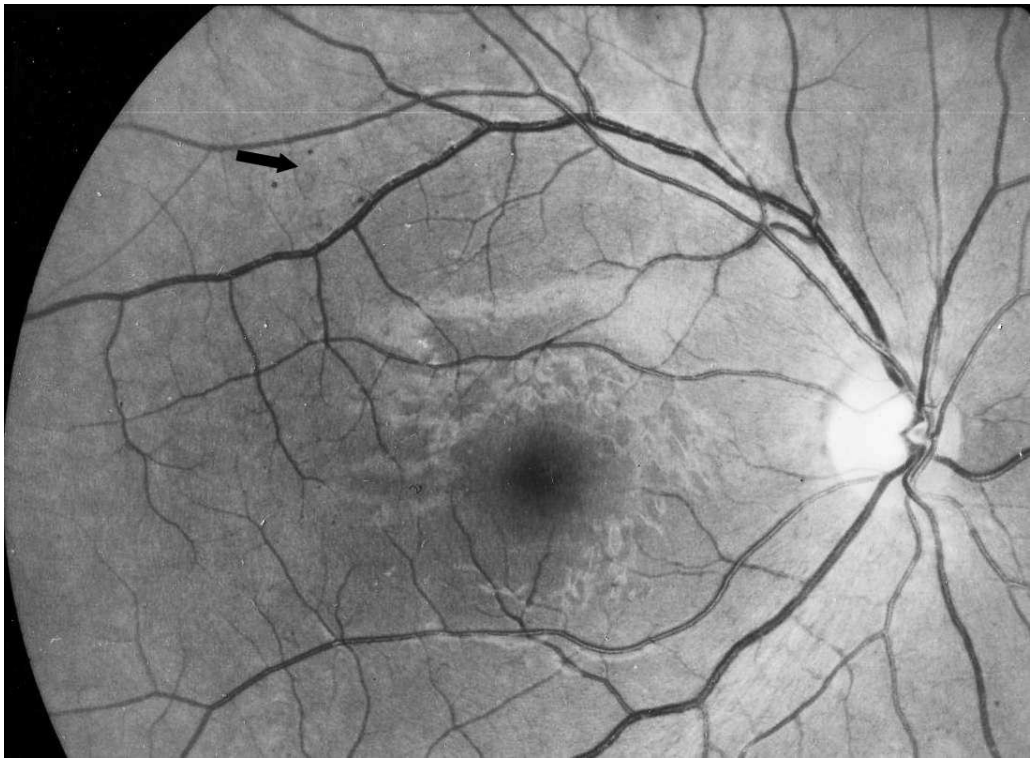


Figure 3 Venous irregularity (beading), microaneurysms, and mild IRMA (intraretinal microvascular abnormality) in the upper temporal quadrant (arrow).

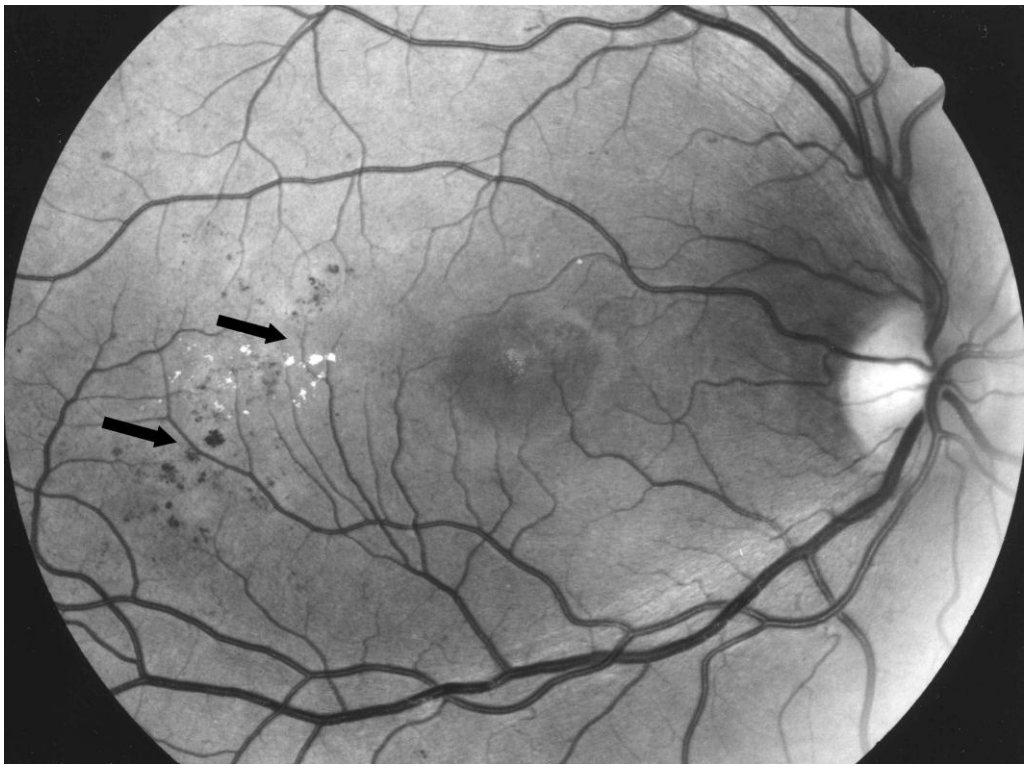


Figure 4 Macular edema with lipid exudates (upper arrow), intraretinal haemorrhages (lower arrow), and microaneurysms.

The hallmark of DRP is the damage to the vascular endothelial cells and pericytes secondary to abnormalities in these cells themselves or in the nearby retinal cells. Retinal edema occurs mainly as a result of the disruption of the blood-retinal barrier (BRB) which leads to increased accumulation of fluid within or under the neuroretinal layers (108). The vascular endothelium is an important component of the inner BRB, and endothelial cell dysfunction and death are important in the development of retinopathy. The occlusion of the vascular lumen by white blood cells or platelets may also lead to the obliteration of the small capillaries (107). As the area of retina with acellular capillaries increases and coalesces, the terminal arterioles that supply these areas also become occluded (Fig. 5). Adjacent to the nonperfused retina, tortuous hypercellular vessels may develop, and these vessels are called intraretinal microvascular abnormalities (IRMA) (Fig.3). IRMA changes could represent both intraretinal neovascularisations, and dilated capillaries and this uncertainty is indicated in the term IRMA (109).

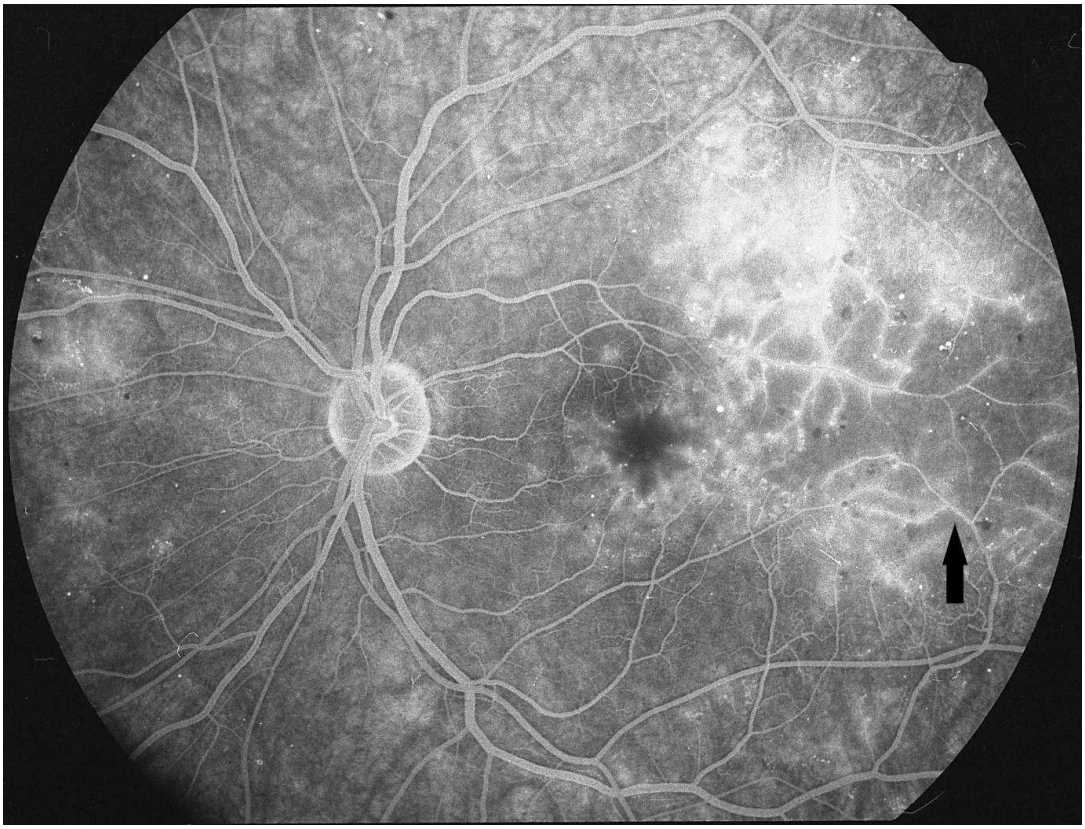


Figure 5 Fluorescein angiogram of extensive capillary closure (arrow), enlarged foveal avascular zone, and macular edema.

As the retinal ischaemia becomes extensive other, changes can also be seen, such as dilated, irregular segments in retinal veins that are called venous beading (VB) (Fig. 3 and 5) and ischaemic intraretinal haemorrhages.

A commonly seen change in the retinal arteries is the loss of elasticity and increase in the thickness of the vascular wall by the replacement of the vascular wall smooth muscle fibers with fibrous tissue. The changes in the retinal vessels may lead to branch retinal vein occlusion and even to the occlusion of the retinal arteries (Fig. 6). Eventually, neovascularisation ensues to compensate for the widespread impaired circulation and ischemia in the retina due to the damaged and occluded vessels (109). The definition of PDR (proliferative diabetic retinopathy) requires the presence of newly formed blood vessels or fibrous tissue resulting from the regression of new vessels, or both arising from the retina or optic disc and extending along the inner surface of the retina or optic disc or into the vitreous cavity (110). When neovascularisation occurs on the surface of the retina, but not at the optic disc, it is called NVE (neovascularisation elsewhere) (Fig. 6). When it occurs on the optic disc, it is termed NVD (neovascularisation at the disc) (Fig. 7). Severe NPDR is usually predictive of neovascularisation. However, sometimes the fundus may appear quiet or featureless since haemorrhages, microinfarctions, and IRMAs tend to disappear after an extensive capillary closure. The characteristic features of severe NPDR are, thus, not always present when neovascularisations are first recognised (110). Furthermore VBs and IRMAs may not always be easily distinguished from other than red-free images, which enhance the detection of haemoglobin containing structures (111-114)

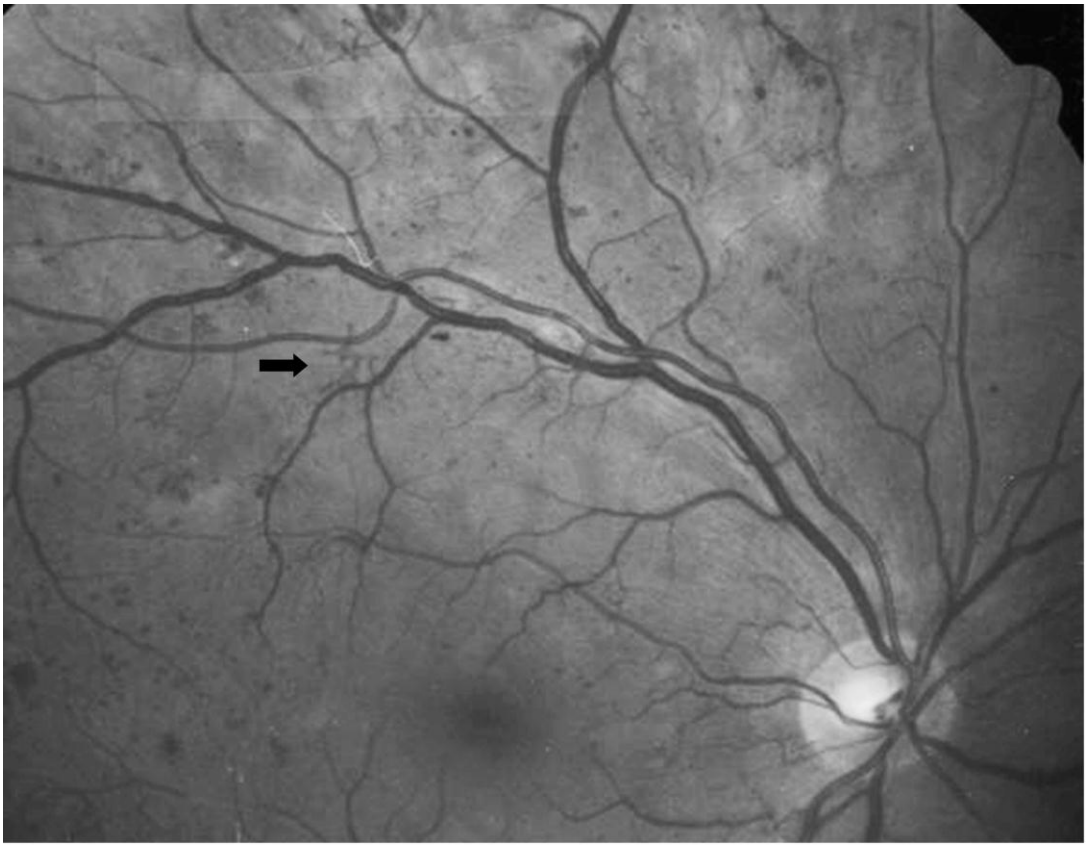


Figure 6 Venous irregularity (beading) and an early stage of neovascularisation (arrow).

As the extent of the midperipheral capillary closure increases, so does the severity of the neovascularisation process, which increases in the following order: from one or more local NVEs to NVD and, finally, the anterior chamber angle with neovascular glaucoma (NVG) (115). Neovascularisations may become fibrotic and rarely regress even without any treatments. However, the proliferative process, especially if posterior vitreous detachment has not taken place, leads to retinal traction (Fig. 8), repeated haemorrhages, and, finally, TRD (tractional retinal detachment).

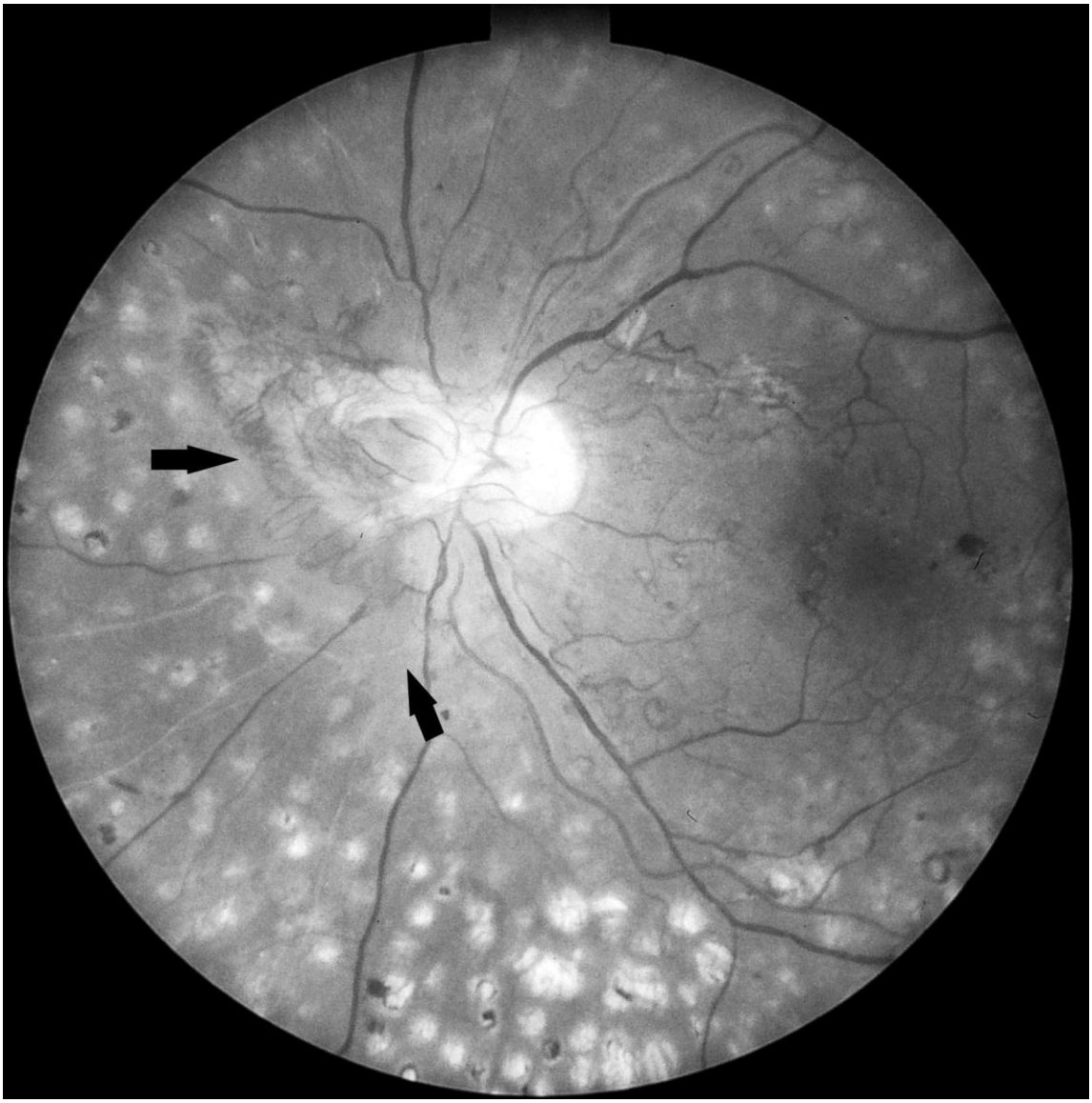


Figure 7 Neovascularisation at the disc (NVD) as an extensive fibrovascular membrane (upper arrow). Occluded artery (lower arrow) in the inferior nasal quadrant and scatter laser scars.

Without proper treatment of PDR, the patient is at a high risk of becoming blind (116, 117). If vitreous detachment has taken place, the proliferative process is confined to the surface of the retina or the optic disc without the risk of TRD (110). NVEs and the remaining damaged capillaries have increased permeability which may lead to the accumulation of fluid in the macula and decreased visual acuity (118, 119). If vitreous detachment has occurred, it may promote the spontaneous resolution of DME (diabetic macular edema) and, consequently, improve visual acuity (120).

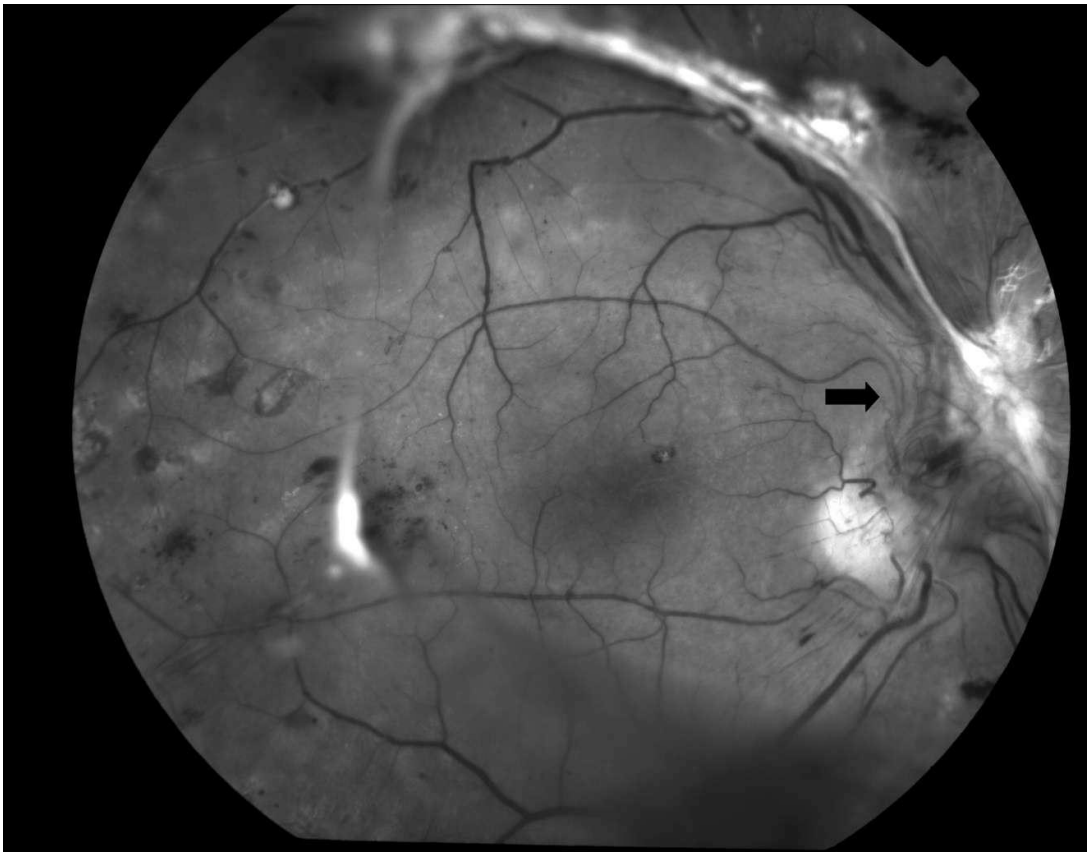


Figure 8 Fibrovascular traction extending from the optic disc to the upper nasal area and along the upper temporal archide (arrow). A smaller traction is present temporal to the macular area.

5.3.6.2 Epidemiology of diabetic retinopathy

Mild retinopathy changes without diabetes, indistinguishable from DRP, are quite common (5-10)% in generally healthy adults that are ≥ 43 years old (121-124). The retinopathy without diabetes is usually associated with aging, hypertension, and impaired glucose metabolism, although it can occur even without these risk factors (121-124). The five and ten year incidences of retinopathy in people without diabetes have been reported to vary from 6 to 19% in the Beaver Dam and Blue mountains eye studies (125-127). Hyperglycaemia significantly increases the prevalence of any retinopathy changes (121, 124, 128). When retinopathy occurs in the presence of diabetes it is considered DRP. However, similar retinopathy changes may be found in many systemic diseases such as inflammation (vasculitis), blood dyscrasias, and any retinal vascular dysfunction, the most common being retinal vein occlusion and age-related macular degeneration.

There are many studies published on the natural history and risk factors of DRP (129). However, many of the studies give inconsistent results

regarding the prevalence and incidence of different DRP phenotypes. This is probably related to variable study methods used (129). The most consistent epidemiologic evidence comes from population-based studies. One such study is the WESDR which began in 1979 and included 2990 type 1 and type 2 patients across 11 counties in southern Wisconsin, USA. The patients in WESDR were more likely to have evidence of nonproliferative or PDR the longer the duration of their diabetes (130). After 20 years of diabetes almost all patients with onset before the age of 30 years showed some signs of retinopathy (131). The WESDR also showed that insulin treatment was associated with an increased frequency of nonproliferative retinopathy and PDR in type 2 diabetes patients (130). Essentially the same results were shown a few years later in two separate studies from Sweden (132, 133). The incidence of PDR rises almost linearly after 10 years of diabetes duration (132, 134), and the prevalence of PDR varies between 13 and 50 % after 15-25 years of diabetes in patients that use insulin (131, 135). The risk of developing high-risk characteristics PDR in the ETDRS after a 1-year follow-up ranged from 1% (95%CI 0.6-1.1) for the patients with mild DRP at baseline, to 3 % (95%CI 2-5%) – 9 % (95%CI 7-11) for moderate, 15 % (95%CI 12-17) for severe, and 45 % (95%CI 34-55) for very severe NPDR. The incidence of high risk characteristics PDR rose to 16 % (95%CI 13-18), 27 % (95%CI 24-30) – 39 % (95%CI 36-43), 56 % (95%CI 52-60), and 71 % (95%CI 62-80) after 5 years, respectively (136).

In the WESDR, the patients whose age at the diagnosis of diabetes was less than 30 years and who were taking insulin, the prevalence rates of DME (diabetic macular edema) varied from 0% in those who had had diabetes for less than 5 years to 29% in those whose duration of diabetes was 20 years or more. For those whose age at the diagnosis was 30 years or older, the prevalence rates of DME varied from 3% in those who had diabetes less than 5 years to 28% in those whose duration of diabetes was 20 or more (137). Similar prevalences have been reported also in other studies in which the prevalences of DME ranges between 12 to 29 % after 18-25 years of diabetes. DME has usually been found to be more common with the diabetes onset after 30 years of age and with insulin use (130, 132, 138). The natural course of DME is significantly different from PDR, since DME may resolve by its own, without any treatments and also without producing severe visual disturbances (139). This is not the case with PDR which is almost certain to produce severe visual disturbances that are eventually noticed by the patient (116, 117). DME also differs from PDR in that it causes a more severe visual loss in the elderly patients which may be related to the longer natural duration of edema in these patients (108). The natural course of DME may also be reflected upon the incidence rates of DME. The incidence of DME is likely to be higher in studies in which annual examinations are performed as compared to studies with less frequent examinations. This is because resolved DME may not be accounted for in studies with less frequent examinations. For example the annual incidence of DME in type 1 diabetes

patients examined yearly with diabetes duration from 10 to 20 years is 6.7% which is approximately three times the rate calculated from WESDR data from a similar era (140). A third feature of DME that makes it different from PDR is the requirement of stereoscopic fundus examination for the classification of treatable lesions (141). The requirement for stereoscopic fundus examination may also result in a larger variation in the prevalence estimates.

There have been differences in the prevalences of DRP between various ethnic groups (142, 143). In the third National Health and Nutrition Examination Survey in the USA, the prevalence of DRP was 46% higher in blacks and 84% higher in Mexican Americans than in whites with type 2 diabetes (143). Studies in South Africa and the UK report a similar prevalence of DRP between people of African, European, and Indian origin with type 2 diabetes (144), between Asians and Europeans (145), and between black West-Indians, Jamaicans, and Caucasians (146) The differences between the ethnic groups are probably related to differences in predisposing conventional risk factors because when adjusted for baseline variables, ethnicity has not been associated with the prevalence of sight-threatening retinopathy (142, 144-146).

The effect of sex has shown inconsistent associations with DRP. In the 25-year analysis of the WESDR, male sex was associated with retinopathy progression, and males also had less improvement in DRP than females. However, PDR was not associated with the male sex in the WESDR (131). The effect of sex may be modified by the age at onset of type 1 diabetes. The higher the age at onset, the higher the risk of microvascular complications in males as compared to females (147).

The medical and ophthalmic care of diabetes patients has improved over time. It has been shown in many studies that the DRP incidence and associated severe visual loss has been greatly reduced (148, 149). In a study of elderly Medicare beneficiaries diagnosed with diabetes, those diagnosed with diabetes in 1999 and in 2003 showed lower rates of NPDR and PDR within 1 year after diagnosis and during 6 years of follow-up as compared to a cohort diagnosed in 1994. Similarly, six-year rates of surgical procedures for DRP were lower among beneficiaries in the 1999 cohort than in the 1994 cohort (150). In a meta-analysis of temporal trends of DRP progression, the overall incidence of PDR and severe visual loss in studies after 1985 (e.g., 2.6% for PDR and 3.2% for severe visual loss at 4 years) were substantially lower than the rates observed before 1985 (19.5% for PDR and 9.7% for severe visual loss at 4 years). The substantially lower progression rates may reflect improvements in the overall care and the management of diabetes and associated risk factors (151), together with earlier identification of type 2 diabetes (149). A decline in severe DRP in type 1 diabetes has also been observed in the FinnDiane study population from the 70's to the end of the century (152). The incidence of severe visual loss due to DRP has been reduced by half during the same time in Finland (153).

5.3.6.3 Risk factors for diabetic retinopathy

The risk factors for DRP can be categorized roughly into those that can and those that cannot be treated (Table 1). Although epidemiological studies are able to show associations between different retinopathy phenotypes and potential risk factors, these associations may be obscured by various known and unknown confounding factors. For example dyslipidaemia may be associated with hyperglycaemia and, thus, produce erroneous results in the statistical analysis. Although known confounding factors can be taken into account in multivariate models, randomised controlled trials are ultimately needed to establish a causal relationship between a risk factor and the outcome of interest. In DRP, such studies are available regarding glycaemic control, BP and, triglycerides.

DRP seems to have an inherent momentum of progression once severe enough. The more severe the NPDR, the more likely it is to progress even further (94, 95, 154-157). To prevent the progression of retinopathy, the most important treatable risk factor is considered to be the glycaemic control. The threshold for the HbA_{1c} in making the diagnosis of diabetes was chosen to be 6.5 % in part because of the sharp increase in the prevalence of retinopathy in patients whose HbA_{1c} rises above this level (17). It has also been noted in many randomised controlled trials, that a poor glycaemic control increases the incidence and the progression of DRP in both type 1 and type 2 diabetes (158-160). In the DCCT primary prevention cohort, the risk of any DRP was reduced by 27% over a mean follow-up of 6.5 years in the conventional vs. intensive blood glucose control groups (90 vs. 70%)(161). For the primary prevention cohort, the cumulative 8.5 years rates of 3-step or more DRP progression were 54.1% and 11.5% in intensive vs. conventional groups respectively. For the secondary prevention cohort, the cumulative 3-step progression was 49.2% and 17.1%, respectively (157). In the ADVANCE trial with type 2 diabetes patients, the intensive glycaemic control did not decrease the DRP progression, although it decreased the progression of nephropathy (162).

Table 1. *Risk factors for retinopathy progression. Level of evidence from selected key studies.*

Treatable risk factors	**Effect**	**Type of study**	**Studies**
Poor glycaemic control	Increases risk	Randomised controlled	DCCT*, UKPDS
High blood pressure	Increases risk	Randomised controlled	UKPDS
High triglycerides	Increases risk	Randomised controlled	FIELD, ACCORD
High cholesterol	Effect not certain	Prospective observational	ETDRS*
Diabetic nephropathy	Increases risk	Population-based prospective	WESDR*
Anaemia	Effect not certain	Prospective observational	ETDRS*
Large waist-to-hip ratio	Increases risk	Prospective observational	EURODIAB*
Pregnancy	Increases risk	Prospective observational	DIEP*, DCCT*, EURODIAB*
Cataract surgery	Effect not certain	Observational	Several cohort studies*
Smoking	Effect not certain	Prospective observational	WESDR*, EURODIAB*
Risk factors not treatable	**Effect**	**Type of study**	**Studies**
Diabetes duration	Increases risk	Population-based prospective	WESDR*
Age at onset of diabetes	Modifies risk	Observational	Several cohort studies
Familial susceptibility	Increases risk	Prospective observational	DCCT*
Candidate genes	Effect not certain	Observational	Several cohort studies*
Myopia	Effect not certain	Population-based prospective	WESDR*
High intraocular pressure	Effect not certain	Population-based prospective	WESDR*
Large venular diameter	Increases risk	Population-based prospective	WESDR*
Smaller retinal arteriole/venule ratio, A/V nicking,, focal arteriolar narrowing	Increases risk	Cross-sectional	ARIC

**The study included patients with type 1 diabetes*

Evidence for a beneficial effect of lowering of the BP in type 2 diabetes patients comes from the UKPDS study (163) where a 2-step or more deterioration on the ETDRS scale was significantly different at 4.5 years of follow-up with 25% less patients in the tight BP control group progressing 2 steps or more as compared to the less-tight BP control group. Patients allocated to tight BP control had a 35% reduced risk of laser treatment, and 42% reduced risk of laser-treated DME (163). However, the positive effect of BP lowering was not statistically significant in the subsequent ACCORD and ADVANCE–trials (160, 164). The lowering of triglycerides with fenofibrates in type 2 diabetes patients has been shown to reduce the progression of DRP in two randomised controlled trials. In the ACCORD study, the odds of DRP progression were reduced by 40% in patients with fenofibrate along with statin treatment, and in the FIELD study, the risk of the first laser treatment was reduced by 31% in the fenofibrate group (160, 165). However, there are no large-scale randomised controlled trials investigating the effects of statins alone on the progression of DRP, despite the fact that total cholesterol and HbA_{1c} were the only factors that predicted the development and persistence of severe visual loss due to PDR and DME in the ETDRS (166). The improvement of glycaemic control is usually associated with a less rapid progression of DRP over extended follow-up periods. However, if the glycaemic control is greatly improved over a short period of time, there can be a paradoxical worsening of DRP. This has been shown in many studies (157, 167-171). The analyses of the DCCT data indicate that the magnitude of the change in HbA_{1c} is more important than the rate of change. The most important risk factors for early worsening were higher HbA_{1c} level at screening and the magnitude of the reduction of this level during the first 6 months after randomisation. DCCT found no evidence to suggest that more gradual reduction of hyperglycaemia might be associated with less risk of early worsening (172). Despite identical mean HbA_{1c} values, patients may show a large variation in their long-term glycaemic profile. Further analyses of the DCCT also indicated that variability in the long-term glycaemia, defined as intrapersonal standard deviations (SDs) of a quarterly measured HbA_{1c}, is a risk factor for a 3-step progression of DRP on the ETDRS 12-step severity scale (173).

Evidence for other risk factors that can be treated comes from observational studies. Microalbuminuria and diabetic nephropathy have been associated with DRP and DME especially in the younger onset patients (134, 174). The association between nephropathy and retinopathy has been inconsistent, and in some studies no association has been found (175, 176). The reduction of systemic fluid overload by haemodialysis may improve or even resolve DME (177). A similar improvement and stabilization of DRP has been noted after renal transplantation (178, 179).

There are case reports suggesting that anaemia can exacerbate the development of PDR and DME (180, 181). Erythropoietin treatment has been

associated with the improvement of DME and visual acuity (182). In the ETDRS, low haematocrit increased the risk for severe PDR during a five year follow-up (95). Likewise, in a study from northern Finland, patients with normocytic anaemia tended to have an increased risk of DRP, especially of the severe form (183). However, in population-based studies, haematocrit has not always been associated with the prevalence or severity of DRP (184).

In the EURODIAB prospective complications study, WHR (waist-to-hip ratio) was an independent risk factor for the development of PDR in patients with type 1 diabetes (185). Higher WHR increased the incidence of DRP in type 2 diabetes patients in the prospective Hoorn study where it was a more important risk factor than BMI (body mass index) (186).

Pregnancy in type 1 diabetes induces a transient increase in the risk of DRP. The Diabetes in Early Pregnancy Study (DIEP) showed that elevated HbA_{1c} at baseline and the magnitude of improvement of glucose control through week 14 were associated with a higher risk of progression of DRP (187). Fortunately, the long-term risk of progression of DRP seems to remain unaffected by pregnancy. Although individual patients showed a transient worsening of DRP during pregnancy in DCCT, even to the proliferative levels, their mean levels of DRP were comparable to those in patients who had not become pregnant within each treatment group at the end of the DCCT (188). Compared with nonpregnant women, pregnant women in the intensive treatment group had a 1.63-fold greater risk of any worsening of their retinopathy during pregnancy. The risk was 2.48-fold greater for pregnant vs. non-pregnant women in the conventional group. The transient nature of DRP worsening was also shown in EURODIAB study where having a first or subsequent pregnancy did not seem to be a risk factor for a long-term progression of any microvascular complications (189).

It has been suspected for a long time that cataract surgery can exacerbate DRP, but the evidence has been inconsistent which may be due to significant advances in the surgical techniques. Since the advent of the modern phacoemulsification technique, the cataract surgery does not seem to cause clinically important DRP progression when the preoperative DRP is less advanced than PDR (190-193). There is, however, evidence that uncomplicated phacosurgery can be associated with the onset or worsening of DME (diabetic macular edema) (190, 192, 194).

The relationship between smoking and DRP appears to be complex and probably mediated through some confounding factors such as worse glycaemic control in patients who smoke. The independent significance of smoking on the progression of DRP is, therefore, difficult to assess (195-197).

The above mentioned risk factors are to a certain extent treatable, but in addition to these factors, there are several factors that are not treatable. The most important factor is the length of the hyperglycaemic exposure itself. The longer the diabetes duration, the higher the cumulative incidence and risk of DRP (130). Interestingly, the effect of diabetes duration on the risk of DRP may be nonuniform. The onset of type 1 diabetes before puberty appears to

prolong the time to DRP with an increasing delay in the onset of complications in those with a longer prepubertal diabetes duration (198). The effect of prepubertal duration on the risk of DRP is, thus, smaller than the postpubertal diabetes duration (199, 200). In particular, the diabetes duration without DRP is significantly longer (2-4 years) for those diagnosed before the age of 5 years compared with those diagnosed after the age of 5 (198). This difference in diabetes duration without DRP may be related to the observation that diabetes onset at puberty is associated with an increased risk of DRP (200-202). In addition to modifying the survival time free of any DRP, the age at onset may also modify the DRP phenotype, as was shown in the EURODIAB study, where the onset before puberty was associated with an increased risk of PDR (185). The finding of the age at onset as a risk factor for DRP is not limited to type 1 diabetes. In type 2 diabetes the higher age at onset has been shown to be associated with a reduced risk of DRP (203). The risk difference may be related to residual insulin secretion. The DCCT data indicated that patients with any residual C-peptide secretion, but especially those with the highest stimulated concentrations, had a reduced incidence of DRP and nephropathy (204). The patients with any residual C-peptide secretion were also slightly older than the patients with no C-peptide secretion (204). However, the role of C-peptide has been somewhat controversial since it has not been linked to DRP in other studies (131).

Genetic factors are likely to play a role in the development of DRP. Previous studies have been able to show familial clustering of severe NPDR in families with type 2 diabetes, (205, 206) and in families with a mixture of both type 1 and type 2 patients (207). The heritability has been reported to be 27 % for DRP and 25 % for PDR in a mixed sample of type 1 and type 2 diabetic siblings (208). Three genome wide linkage scans have offered suggestive evidence of linkage, but on a number of different chromosomes in patients with type 2 diabetes (209-211). The first genome wide association analysis was published in 2011 in type 2 diabetes and suggested an association to loci in chromosome 5 (212). The associations with various biologically relevant candidate genes have been extraordinarily difficult to replicate (213, 214). This is probably because the heritability estimates are typical of multifactorial inheritance that would require a very large sample size to show significant associations.

There has been a suspicion for a long time that some ocular factors could be involved in the development of DRP. Myopia has been associated with less severe DRP in some studies, but the association has been inconsistent (215-221). A higher intraocular pressure or bilateral glaucoma do not appear to affect the progression of DRP (215, 216). In the ARIC study (Atherosclerosis Risk in Communities study), smaller retinal arteriole/venule ratio, presence of retinal arteriole/venule nicking, and focal arteriolar narrowing were independently associated with the severity of DRP after controlling for systemic variables (184). Independently of the DRP severity level and the systemic factors, the widening of the retinal venular but not arteriolar

diameter was associated with subsequent incidence and progression of DRP (222). One ocular factor that encouraged the use of light photocoagulation in the treatment of DRP was the observation that the eyes with retinochoroidal scarring from trauma or inflammation had a markedly reduced prevalence and severity of diabetic DRP (223, 224).

5.3.6.4 Treatment of diabetic retinopathy

Severe visual loss is mostly due to either PDR or DME (166). The most severe form of DRP is PDR, and without any treatments most of the patients will become blind after 5-10 years (116, 117). DME does not usually lead to blindness, but it may cause low vision and, thus, affect the quality of life. Laser treatment is considered the cornerstone of the treatment of both PDR and DME. The beneficial effect of laser treatment in severe PDR was clearly shown by the DRS. The laser treatment in DRS was specified as widespread scatter (panretinal) photocoagulation with 800 to 1600 500 μm laser burns of 0.1 seconds duration (225). Severe visual loss was defined in the DRS as visual acuity <5/200 in two consecutive visits at 4 months apart. DRS demonstrated that the risk of severe visual loss was reduced to half by photocoagulation treatment (225). As a continuation to the DRS, the ETDRS recruited 3 711 patients of whom 1 444 patients had type 1 diabetes and 2 267 patients had type 2 diabetes (226). These patients were followed regularly for 3-9 years with 4-month intervals. Those patients with either a poor prognosis for 5-year survival or only less than a mild background DRP in either eye were not considered eligible for the study. The ETDRS study showed that laser photocoagulation had a beneficial effect of reducing the so called CSME (clinically significant macular edema). The ETDRS demonstrated that a focal or scatter laser treatment of DME reduced the risk of moderate visual loss by half in eyes fulfilling the CSME definition, whereas the effect was less obvious in the group with DME without CSME characteristics (139). The moderate visual loss was defined in the ETDRS as loss of 15 letters or more between the baseline and follow-up visits, equivalent to doubling of the visual angle (136). There were numerous studies even before the ETDRS which demonstrated the beneficial effect of laser treatment on DME (227-234). However, these studies were difficult to compare and to apply as treatment guidelines. The ETDRS provided such a guideline with proven efficiency for treating CSME with focal or grid laser treatments. In the focal treatment, microaneurysms and other focal leakage sites received 50-100 μm laser burns of 0.1 s duration or less. The treatment of lesion closer to 500 μm to the fovea was not required initially. However, if the vision was less than 20/40 and the retinal edema and leakage persisted, treatment of lesions up to 300 μm from the center was recommended, unless there was a perifoveal capillary dropout which might have been worsened by the laser treatment. In the grid treatment, areas of diffuse leakage or nonperfusion within 2 disc diameters

of the center of the macula were treated in a grid pattern. The goal was to produce a light/moderate intensity burn not more than 200 μm in diameter. A spot size of 50-200 μm was used to achieve this. A space one burn wide was left between each burns and a minimum distance of 500 μm was kept to fovea. The ETDRS protocol did not specify the number of burns used in the treatment of CSME (136, 139). Later on, the ETDRS also showed the positive effect of laser treatment in severe NPDR especially in type 2 diabetes patients (235). Today, the number of burns needed and the extent of the area treated varies according to the severity and location of PDR and location of severe NPDR as well as the technical qualities of laser equipment

The laser treatment may have adverse side-effects, the most notable of which are the constriction of visual fields and a slight reduction of visual acuity following dense panretinal photocoagulation (225). In ETDRS, these adverse effects were most evident in the months immediately following the treatment and were less frequent in eyes assigned to less extensive scatter photocoagulation (136). If a patient has less than severe NPDR or does not have CSME, the patient may be followed and treated if progression to the aforementioned severity levels takes place (235). In more advanced stages of PDR, the patient will have VHs and/or TRDs. The beneficial effect of vitrectomy at this stage was reported first by Robert Machemer (236). In the DRVS type 1 diabetes patients had a three times higher risk of attaining good visual acuity (≥10/20) due to early vitrectomy as compared to conventionally managed patients (35.6% vs. 11.7%) (237).

When DRP is less severe, the treatment focuses on systemic factors such as BP, glycaemic control and lipids (238, 239). The treatment of DME and DRP is changing at the moment. Emerging new treatments, such as anti-VEGF injections and cortisone injections and implants, are promising alternatives that are already widely used. However, research into the every-day-clinical protocols and the long-term effects of these treatment modalities is scarce, and further research is still needed (239-242).

6 THE AIMS OF THE STUDY

A large proportion of the risk of sight-threatening, severe DRP remains unexplained. There are well-established risk factors for DRP such as diabetes duration, the level of hyperglycaemia, and BP. However, these risk factors explain much less than half of the risk (12, 13). Therefore, the aim of the present study was to elucidate other risk factors for the development of sight-threatening DRP like PDR and CSME in type 1 diabetes.

6.1 STUDY I

Familiality may affect the progression of DRP. Whether PDR also clusters in families is not known. The aim of the study was to assess the familial clustering of PDR in patients with a longstanding type 1 diabetes and to estimate the degree of familiality by calculating the h^2 heritability of PDR.

6.2 STUDY II

Age at the onset of diabetes modifies the risk of DRP. However, it is not known how a late age at onset of type 1 diabetes affects the risk of PDR as compared to a very early onset of type 1 diabetes, whether young age at onset is a protective factor even in the long run, or whether it only delays the onset of PDR. Therefore, the aim of this study was to elucidate how the age at onset of type 1 diabetes influences the long-term risk of PDR in patients with type 1 diabetes.

6.3 STUDY III

Despite identical mean HbA_{1c} values, patients may have a large variation in their long-term glycaemic profile which has shown to affect the progression of NPDR (173). Whether HbA_{1c} variability is also associated with a higher risk of laser treatment and PDR is not known. Therefore, the aim of this study was to investigate the effect of HbA_{1c} variability on the long-term risk of laser treatment and PDR.

6.4 STUDY IV

Several risk factors for DME have been identified. However, the long-term incidence of CSME has not yet been studied with specific focus on the age at

onset of type 1 diabetes. Therefore, the aim of this study was to assess the impact of age at onset of type 1 diabetes on the long-term incidence and risk of CSME and to see what the differences are in risk factors between CSME and PDR.

6.5 STUDY V

In addition to a genetic component, the familial clustering of DRP is likely to include shared, non-genetic factors that are largely unknown. The aim of this study was to elucidate whether diabetes onset within sibships, as adjusted for conventional risk factors, age at onset of type 1 diabetes and sibship size influences the long-term risk of PDR.

7 PATIENTS AND STUDY DESIGN

7.1 THE FINNDIANE STUDY POPULATION

The FinnDiane study was initiated in 1997 primarily to find out why one third of type 1 diabetes patients develop nephropathy. This aim was soon extended to include all late complications of type 1 diabetes. A longitudinal, observational patient database, consisting of clinical, genetic, and environmental variables was collected from a large number of participating study centres (Table 2). The participating study centres comprise diabetes and renal outpatient clinics at all five university central hospitals, all 16 central hospitals, the majority (n= 27) of all regional hospitals, and 31 major primary health care centres in Finland. All adult patients with type 1 diabetes at these centres were invited to participate and 78% responded positively (243). The FinnDiane database now comprises over 4 800 patients with type 1 diabetes and the geographic distribution of the patients closely follows the distribution of the general population of Finland (Fig. 9). The collection of the prospective follow-up data started in 2004. To date, nearly 4 000 FinnDiane-patients have either been re-examined or, alternatively, their medical files reviewed by the FinnDiane investigators at the participating study centres. As the number of type 1 diabetes patients is approximately 30 000 in Finland, the FinnDiane study therefore represents 16% of all type 1 diabetes patients in Finland. The study protocol is in accordance with the Declaration of Helsinki, and was approved by the ethics committee of the Helsinki University Central Hospital. The DRP study presented here was undertaken as one of the many substudies of the FinnDiane Study.

Table 2. *Clinical characteristics of the FinnDiane patients (n=4 895), male/female 2 585 / 2 310.*

Duration of diabetes (years)	21.8±12.2
Age (years)	38.8±12.4
Age at onset (years)	17.0±11.1
HbA_{1c} (%)	8.4±1.5
MSBP (mmHg)	135±18
MSDP (mmHg)	80±10
MAP(mmHg)	98±11
AHT (%)	38%
ASA treatment (%)	13%
Diabetic Nephropathy (%)	22%
Triglycerides (mmol/l)	1.0 (IQR 0.7-1.4)
Total Cholesterol (mmol/l) males	5.0±1.0
HDL Cholesterol (mmol/l) males	1.5±0.4
HDL Cholesterol (mmol/l) females	1.7±0.5
Statin treatment (%)	11%
WHR males	0.91±0.07
WHR females	0.82±0.07
BMI	25.1±3.6
History of smoking	23%
Mortality until 24.3.2009 (%)	10%

The values are expressed as means ± standard deviations, or medians (IQR=interquartile range). MAP= Mean Arterial Pressure, BMI= Body Mass Index, CRP =C-reactive protein, WHR= Waist-to-Hip Ratio, ASA=Aspirin (Acetylsalicylic Acid), AHT= Antihypertensive Treatment, MSBP =Mean systolic Blood Pressure, MDBP =Mean Diastolic Blood Pressure

Figure 9 Distribution of the FinnDiane-patients. Each dot represents the home address of a FinnDiane study patient.

7.2 PATIENTS IN STUDIES I AND V

The heritability of PDR was calculated in study I in siblings with type 1 diabetes. As a part of the baseline visit, the patients answered a question whether any of their close relatives had type 1 diabetes, as defined by having an age at onset of 40 years or less and insulin treatment initiated within one year of the diagnosis. These criteria identified 188 families with at least two siblings with type 1 diabetes. All siblings were contacted and those siblings, who agreed to take part, signed a consent form and were studied at a

FinnDiane center. The same patient data was used in study V which assessed the impact of diabetes onset within siblings on the long-term risk of PDR.

Records of fundus examinations by dilated ophthalmoscopy and/or fundus photographs were obtained for 369/396 (93%) of the patients. Both were available for 217/369 (59%) patients. The fundus photographs taken for screening or documentary purposes were available for 251/369 of the patients (68%). These patients had been photographed on a median of 3 (IQR 1-5) times. A diabetologist's evaluation of the fundi was the only source of information for 3/369 of the patients, all of whom had a mild diabetic retinopathy. All the other patients had had dilated ophthalmoscopies carried out by an ophthalmologist.

7.3 PATIENTS IN STUDY II

In the study of the effects of age at onset of type 1 diabetes on the long-term risk of PDR, a sample of 1 117 was drawn from the FinnDiane database. The inclusion of the patients into this study was based on the ascending order of the FinnDiane patient identification number. Thus, the consecutively recruited patients from the very beginning of the FinnDiane study were the first to be included in the study. This approach has two advantages. First, there should be no significant biases with regards to the treatment of diabetes and its complications. Secondly, these patients had the longest duration of type 1 diabetes as they were the first ones to participate in the FinnDiane study.

Only in 19/367 (5.2%) patients, PDR was discovered at their first fundus examination by an ophthalmologist. Thus, there were no available reference points for these patients before they had developed PDR. All the other patients (n=348) had had at least one ophthalmic examination on a median of 0.7 (0.3-1.8) years prior to the diagnosis. Additionally, records of treatment and follow-up were available for nearly all (364/367) patients with PDR.

7.4 PATIENTS IN STUDY III

The effect of HbA_{1c} variability was assessed in 2 019 FinnDiane Study patients. The patients were studied in two partially overlapping subcohorts with either verified first laser treatment (n=1459) or DRP severity and progression graded from ophthalmic records with the ETDRS scale (n=1 346) (Fig 9). There were 786 overlapping patients between the subcohorts. The laser treatment subcohort consisted of 1 459 patients that were followed up for the occurrence of the first laser treatment. In January 2012, the

FinnDiane database had 2 019 patients with follow-up data on whether the patient had been laser-treated (yes/no) as verified from medical files by the attending physician, data on the year of the first laser treatment, and data on at least two HbA_{1c} measurements one year apart. Of the 2 019 patients, 1 459 did not have any prior laser treatment episodes before the first FinnDiane visit. The patients that were not included in the subcohort of 1 459 patients had either been laser-treated before the first FinnDiane visit, or the ophthalmic data and/or serial HbA_{1c} measurements were insufficient in order to construct a follow-up period after the first FinnDiane visit.

The specific risk factors for PDR were further analysed in a subcohort of 1 346 patients. Fundus photographs taken for screening or documentation purposes and/or records of dilated slit-lamp fundus examinations performed by a specialist in ophthalmology were obtained for 1 346 consecutively recruited patients. These patients were the earliest to have been recruited in the FinnDiane study and had, thus, the longest duration of diabetes. The records of fundus examinations by ophthalmologists were available for 1 076/1 346 (79.9%) patients and fundus photographs were available for 1 052/1 346 (78.2%) of the patients. The patients had been photographed on a median of 3 (IQR 1-5) times per patient. All the patients who were not examined by ophthalmologists had had serial fundus photographs. Both ophthalmic records and photographs were available for 782/1 346 (58.1%) patients.

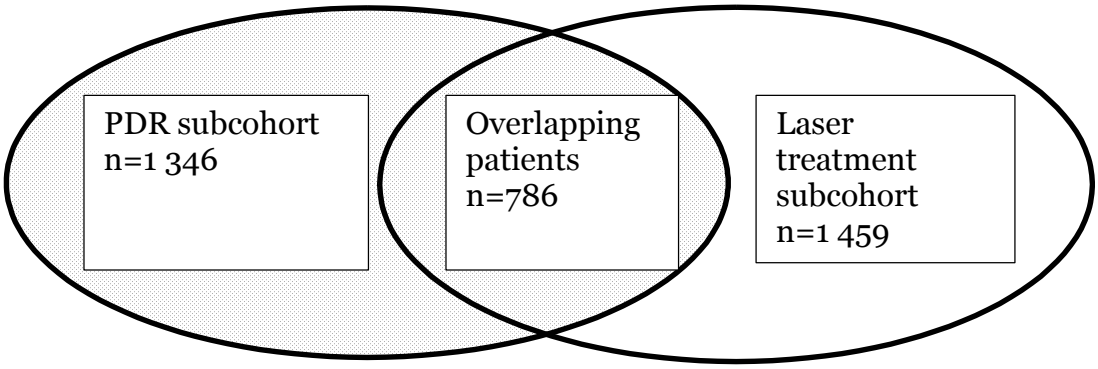


Figure 10 The PDR and laser treatment subcohorts (n=2 019). There were 786 overlapping patients between proliferative retinopathy (PDR) (n=1 346) and laser treatment (n =1 459) subcohorts.

7.5 PATIENTS IN STUDY IV

As for the study II, the inclusion of patients for this study was based on an ascending order of the FinnDiane patient identification number. However, in this study, the fundus photographs were required for every patient which made the cohort slightly different from that used in study II. The patients had been photographed on a median of 3 (IQR 1-5) times per patient. Roughly one third of the patients in study II were, therefore, not included in

this study. The data also included patients that were recruited later for study I, but this did not significantly shorten the mean duration of diabetes as compared to study II.

The DRP and maculopathy status was determined in 1 354 patients. The indications for each individual laser treatment and for each laser-treated eye were verified from ophthalmic files for all the 516 patients that had received any laser treatment. The patients had received a median of 5 (IQR 3-7) laser treatments for the right eye and 5 (IQR3-8) for left the eye, with a total of 11 (IQR 7-16) laser treatments. Those patients, whose laser treatment was performed with the specific intent to reduce macular edema and who had fundus photographs consistent with the CSME definition (i.e. hard exudates, microaneurysms/haemorrhages, or photocoagulation burns in macular area), were considered to have CSME. Laser-treatment alone was not taken as evidence of CSME or PDR since severe NDRP is also an indication for laser photocoagulation. In 29/242 (12%) patients, CSME was discovered at their first fundus examination by an ophthalmologist. Thus, there were no available reference points for these patients before they had developed CSME. All the other patients (N=213) had had at least one ophthalmic examination on a median of 1.9 ±1.8 years prior to the CSME.

8 METHODS

8.1 MEDICAL HISTORY

The data were collected at the regular visits to the patients' attending physician. Patients filled in a questionnaire which was then verified from the medical files by the attending physician. The questionnaire included a standardised check list regarding medication, the year of the onset of diabetes and the year of initiation of permanent insulin treatment. The check list also included questions about microvascular and macrovascular events, such as the year of the first laser treatment, and renal and cardiovascular status. The data on all-cause mortality were obtained until 24.3.2009 from the Population Register Centre of Finland.

8.2 ANTHROPOMETRIC MEASUREMENTS

The patients' weight was measured using a standardized scale and registered to closest 0.1 kg. Height was registered to the closest 1 cm. Waist circumference was measured midway between the lowest rib and the iliac crest and hip circumference at the widest part of the gluteal region. BP was measured twice in the sitting position using a mercury sphygmomanometer after a rest of at least 10 min, or an automated BP measurement device in accordance with the clinical practice at the local study centres. MAP (mean arterial blood pressure) was calculated according to the formula: MAP = diastolic BP + 1/3(systolic BP - diastolic BP). BMI was calculated as weight divided by height squared (kg/m2). WHR was calculated by dividing waist with hip circumference.

8.3 LABORATORY MEASUREMENTS AND ASSAYS

Blood was drawn for laboratory measurements. These measurements included HbA_{1c} using standardized assays at the participating study centres. Serum lipoproteins were measured centrally at the research laboratory of the Helsinki University Central Hospital with automated methods that use the Cobas Mira analyser (Hoffman La Roche, Basel, Switzerland). An equation for the eGDR modified for use with HbA_{1C} instead of HbA_1 (eGDR = 24.4-12.97•WHR - 3.39•AHT- 0.6•HbA_{1C}), was used as a measure of insulin sensitivity. In this equation WHR stands for waist to-hip ratio and AHT for antihypertensive treatment and/or BP ≥140/90 mmHg (yes=1, no=0) (244).

AHT was defined as the current use of at least one antihypertensive drug. C-peptide was measured with radioimmunoassay (until 2004 LKB Wallac, Mt. Waverley VIC, Australia, and thereafter Riagamma Delfia, PerkinElmer, Waltham, MA, USA). CRP was measured with radioimmunoassay (Orion Diagnostica, Espoo, Finland) (245). The renal status was based on the UAER in two out of three overnight or 24-hour urine collections. The UAER was centrally determined by radioimmunoassay until November 2002 (Pharmacia, Uppsala, Sweden) and, thereafter, by immunoturbidimetry with a correlation coefficient of 0.96 between the two methods. The patients were divided by renal status into four categories: Those with normal UAER (<20 μg/min or <30 mg/24h), microalbuminuria (UAER ≥ 20 and <200 μg/min or ≥ 30 and < 300 mg/24h), and macroalbuminuria (UAER ≥200 μg/min or ≥300 mg/24h). The patients were considered to have ESRD if they had received a kidney transplant or if they were undergoing dialysis treatment. Diabetic nephropathy (yes/no) was defined as persistent macroalbuminuria or ESRD

8.4 RETINOPATHY DATA

8.4.1 LASER TREATMENT

In Finland, the national guidelines for the screening and treatment of DRP were published already in 1992 (246), and updated in 2006 (247). Consequently, the main indications for laser treatment such as CSME and PDR have been well-recognised by ophthalmogists as an important indication for laser treatment. Since laser treatment is regarded a surgical procedure, its indications are invariably stated in the ophthalmic records. The indications for each individual laser treatment session and for each laser-treated eye were verified from the ophthalmic files for all patients that had received any laser treatment. The patients with laser treatment had received a median of 5 (IQR 3-7) laser treatments for the right eye and 5 (IQR 3-8) for left the eye, with a total of 11 (IQR 7-16) laser treatment sessions. Importantly, laser treatment alone was not taken as evidence of DME or PDR since severe NDRP (i.e. preproliferative DRP) is also an indication for laser photocoagulation

8.4.2 FUNDUS PHOTOGRAPHS

The fundus photographs taken for screening or documentation purposes as well as fluorescein angiographies were obtained from the participating study centers and scanned and stored in a digital archive (Table 3). The fundus photographs were available for 1 441/1 866 (78%) of the patients. The fundus

photographs were graded with the ETDRS-scale (94, 95). Approximately 3% of the photographs were judged ungradable due to poor image quality. The patients with photographs had been photographed on a median of 3 (IQR 1-5) times per patient. The high percentage of fundus photographs increases the sensitivity of the study since retinal photography has been reported to be the most sensitive screening method for any diabetic retinopathy. The sensitivity is in the excess of 80% in detecting severe DRP. Ophthalmoscopy has less sensitivity but conversely a higher specificity (248).

Table 3. *Fundus photographs. The patients with photographs available (n=1 441) had been photographed on a median of 3 (IQR 1-5) times per patient.*

Photographic fields and their coverage	**Number**
1 x 45	2 024
2 x 45	445
1 x 60 or 1 x 50	1 296
2 x 60 or 2 x 50	712
30 degree (number of fields not specified)	373
Types of photographs	**Number**
Polaroid	748
Colour photo	2 843
Colour slide	73
Grayscale	556
Red-free	330
FAG	219
Digital photos	81
Total	**4 850**

8.4.3 OPHTHALMIC RECORDS

Ophthalmic records were obtained and scanned into a digital archive. The combination of the fundus photography with clinical examination gives a good sensitivity and specificity for the detection of severe DRP. The majority of the patients in this study had attended several screening examinations and 1 441/1 866 (77%) had fundus photographs available. Practically all of the patients without fundus photographs had been examined by ophthalmologists, and only 9 patients had been examined by a diabetologist. The clinical fundus examination by an ophthalmologist is important because it has a good specificity as compared to the screening photographs (248) and because the stereoscopic fundus examination is a requirement for the detection of DME.

8.4.4 GRADING OF DIABETIC RETINOPATHY SEVERITY

The retinopathy severity scale produced in the ETDRS was used in this study to assess the severity of DRP in the fundus photographs (Table 4) (95). The ETDRS scale is not commonly used in clinical practice, or in the retinopathy screening, where the descriptions of the fundus changes resemble more the proposed international DRP severity scale (249). The proposed international severity scale is a derivative of the ETDRS scale and can, thus, be roughly compared to the ETDRS scale as shown in Figure 10. Some of the patients in this study did not have fundus photographs available, but instead they had verbal descriptions of clinical fundus examinations that could thus be approximated to numerical values in the ETDRS scale.

Table 4. *The ETDRS retinopathy severity scale (95)*

Level	Severity	Definition
10	DRP absent	Microaneurysms and other characteristics absent
14/15	DRP questionable	Microinfarcts, IRMA, haemorrhages, but no microaneurysms
20	Very mild NPDR	Only microaneurysms
35	Mild NPDR	One of the following: • Microaneurysms and lipid exudates, microinfarcts, venous loops, questionable IRMA and venous beading (VB) • Haemorrhages and microaneurysms as in standard photograph 1 in 1-4/4 quadrants
43	Moderate NPDR	One of the following (not both) • Haemorrhages and microaneurysms > standard photograph1 in 4/4 quadrants or ≥ standard photograph 2a ¼ quadrants • Mild IRMA in 1-3/4 quadrants
47	Moderately severe NPDR	One of the following: • Both level 43 characteristics, or one of the following: o Haemorrhages and microaneurysms ≥ standard photograph 2a in 2-3/4 quadrants o Venous beading as in standard photograph 6a in ¼ quadrants o Mild IRMA in 4/4 quadrants i.e. 2-1-4- rule with one characteristic true
53 (53A-D)	Severe NPDR	One of the following: • Two or more level 47-characteristics (i.e. 2-1-4- rule with 2 characteristics true) • Haemorrhages and microaneurysms ≥ standard photograph 2a in 4/4 quadrants • Venous beading as in standard photograph 6a in 2-4/4 quadrants • Severe IRMA ≥ standard photograph 8b in 1-4/4 quadrants i.e. 4-2-1-rule with 1 characteristics true
55 (53E)	Very severe NPDR	Two of the following : • Haemorrhages and microaneurysms ≥ standard photograph 2a in 4/4 quadrants • Venous beading as in standard photograph 6A in 2-4/4 quadrants • Severe IRMA ≥ standard photograph 8B in 1-4/4 quadrants i.e. 4-2-1-rule with 2 characteristics true
61	Mild PDR	NVE (Neovascularisation elsewhere) <0,5 Disk Area (DA) in 1-4/4 quadrants
65	Moderate PDR	One of the following: • NVE (Neovascularisation elsewhere, more than 1 disc diameter distance from optic disc) < 0,5 DA in 1-4/4 quadrants and vitreous haemorrhage (VH) or preretinal haemorrhage (PRH) < 1 disc area • NVD (Neovascularisation at Disc) <standard photograph 10A (<0.25-0.33 DA) • NVE ≥0.5 DA in 1-4/4 quadrants
71/75	High-risk PDR	One of the following: • NVD ≥standard photograph10a • NVE ≥0.5 DA or NVD<standard photograph 10A and vitreous haemorrhage (VH) or preretinal haemorrhage (PRH) • Vitreous haemorrhage (VH) or preretinal haemorrhage (PRH) ≥1 DA (thus obscuring possible neovascularisation)
81/85	Advanced PDR	Fundus partially obscured by vitreous haemorrhage and either new vessels ungradable or retina detached at the center of the macula
90	Cannot grade	Cannot grade even sufficiently for levels 81/85

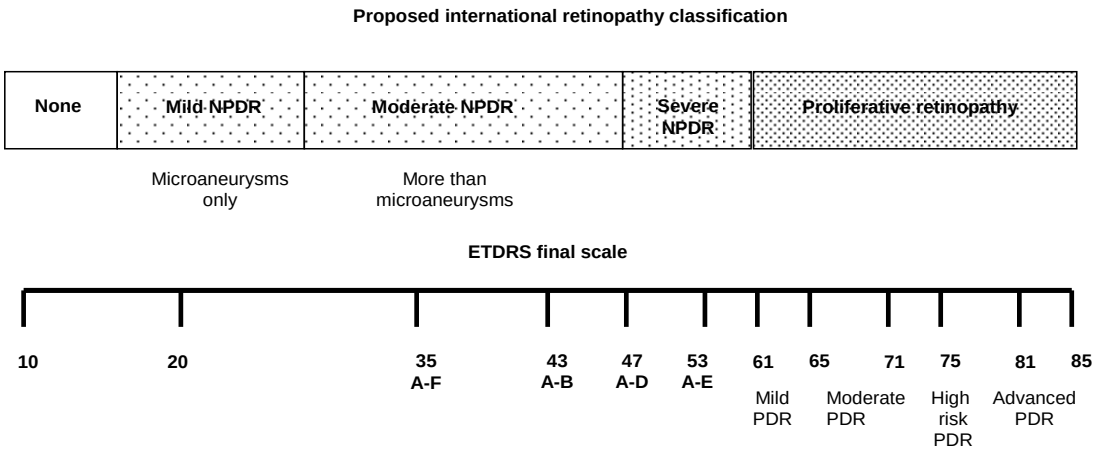


Figure 11 The ETDRS and the proposed international retinopathy classifications with the terminology commonly used in clinical practise for different stages of retinopathy severity.

The DME was assessed based on the fundus photographs and the descriptions in the ophthalmic files. The ETDRS study had two definitions for DME. The division was based on the distance of retinal thickening from the fovea. In the ETDRS, any retinal thickening within 1 disc diameter from the fovea was taken as evidence of DME. If the retinal thickening involved or threatened the center of the macula (even if visual acuity was not yet reduced), the retinal thickening would then be defined as CSME. CSME was defined 1) as the presence of retinal thickening at or within 500 μm of the center of the macula, or 2) hard exudates at or within 500 μm of the center of the macula if associated with thickening of the adjacent retina or zones of retinal thickening at least 1 disc area in size, at least a part of which is within 1 disc diameter of the center of macula. The assessment of the CSME in the ETDRS was made by stereo contact lens biomicroscopy or stereo photography (250). Because of the complex nature of the CSME definition, an easier definition has been suggested which is also closer to the terminology and descriptions used by clinical ophthalmologists (249). The proposed international maculopathy severity scale can be approximated to the ETDRS definitions as shown in table 5 (249).

Table 5. *Classifications of diabetic macular edema (DME)*

Proposed international maculopathy classification				
None	DME apparently present	Mild DME	Moderate DME	Severe DME
No apparent retinal thickening or hard exudates in the posterior pole	Some apparent retinal thickening or hard exudates in the posterior pole	Some retinal thickening or hard exudates in the posterior pole, but distant from the center of macula	Retinal thickening or hard exudates approaching, but not involving the center of the macula	Retinal thickening or hard exudates involving the center of the macula
ETDRS maculopathy classification				
No DME		DME but not clinically significant	Clinically significant DME (CSME)	
Fluorescein leakage without retinal thickening		Retinal thickening or hard exudates within 1 disc diameter of the fovea	The presence of retinal thickening at or within 500 μm of the center of the macula or hard exudates at or within 500 μm of the center of the macula if associated with thickening of the adjacent retina or zones of retinal thickening at least 1 disc area in size, at least part of which is within 1 disc diameter of the center of macula	

8.4.5 STATISTICAL METHODS

The data are presented as means and SDs for continuous, normally distributed variables and CIs for estimates of cumulative risk, HRs and ORs. Medians and IQRs are given for non-normally distributed variables. SEs are given for mean differences and heritability estimates. Differences between two groups were tested with the t-test, or the Mann Whitney test. The differences between multiple groups were compared with one-way ANOVA

adjusted for multiple comparisons and the differences between proportions with either Kruskal-Wallis or Chi-square test. The trends in normally distributed variables were analysed with linear polynomial contrasts (ANOVA) and Jonckheere-Terpstra test in non-normally distributed variables. Spearman's rho (r_s) was used as a measure of correlation. The overall significance of a categorical risk factor in a regression model was tested with the Wald test. The Nelson-Aalen estimator was used to generate cumulative hazard curves shown in this thesis, and the associated stratified hazard-rate ratios and significance tests, controlling for time, were calculated by using a Mantel-Haenszel method. The regression models were compared with BIC (Bayesian information criteria) in studies III, IV and V. The main advantage of BIC is that the factors included in the regression have a high probability of being associated with the outcome variable. However, BIC is so stringent that some factors could be left out even if they actually are important. Most of the statistical calculations were performed with SPSS 15.0 (SPSS, Chicago, IL, USA), Stata 11.2 (Statacorp, Texas, USA), or R open source software (www.r-project.org). In addition, special software as noted below was used for familial associations in study I and for competing risks assessment in study II.

8.4.5.1 Study I

The unadjusted intrafamilial associations were estimated by calculating ICC (intra-class correlations) for sibpairs. The FCOR-program of the SAGE-software package (Case Western Reserve University, Cleveland, OH) was used with a uniform weighting scheme giving equal weights for each sibship regardless of the number of sib pairs within the sibships (251). Similarly, the correlations between ordinal ETDRS scores and DRP status were calculated with the FCOR-program using the same weighting scheme. The mean differences in current age and duration of diabetes between probands and siblings were calculated using a linear mixed model. In order to study the familial aggregation of PDR or any DRP three complementary analyses were used. First, the presence or absence of PDR in the proband was estimated as a risk factor for the corresponding condition in the other siblings. The familial risks were estimated with logistic regression models, adjusted for conventional risk factors, and fitted with generalized estimating equations using an exchangeable correlation structure to account for correlations within sibships (252). Second, to measure the degree of concordance within sibships, the intraclass correlation of durations of diabetes to the diagnosis of PDR was calculated in the 29 sibships in which two siblings had PDR. Third, the h^2 of PDR was estimated by a liability threshold model as implemented in the SOLAR-software (SOLAR, Version 4.0.7. Southwest Foundation for Biomedical Research, San Antonio, TX) with HbA_{1c}, mean arterial pressure, gender, and the duration of diabetes as covariates. The liability threshold

model is an extension of the variance components model to dichotomous traits, such as PDR (253). In the variance components model, the overall phenotypic variation is partitioned into individual variance components due to polygenic effects (multiple unmeasured genes under an additive variance), covariates (e.g. duration, gender, HbA_{1c}, BP), and random environmental effects. The estimated h^2 is defined as the ratio of the genetic variance component to the residual phenotypic variance, and it is an estimate of the familiality of the trait. The significance of the genetic component was determined by a likelihood ratio test.

8.4.5.2 Study II

Kaplan-Meier survival analysis was used to estimate the time without PDR, and Mantel-Cox logrank test to compare the survival distributions among different age at onset groups. The risk of PDR within the age at onset groups was estimated with a Cox proportional hazards model, controlling for clinically significant covariates. A previously published macro for SAS statistical software (SAS, Cary, NC, USA) was used to account for the competing risk of death (254).

8.4.5.3 Study III

As a normalised measure of variability, the coefficient of variation (CV) for the serially measured HbA_{1C} was calculated as the ratio of intrapersonal SD and the mean to correct for larger SD because of higher absolute values of HbA_{1c} (255). The patients were then ranked into quartiles of HbA1c, variability (CV) in both subcohorts. Cumulative incidences for the laser treatment were calculated with a cumulative incidence function which accounts for the competing risk of death. The differences in cumulative incidences were tested with Gray's test (256). As the cumulative incidences of death (n=32) were borderline significantly different (P=0.09, Gray's test) in the various HbA_{1c} variability quartiles, a recently published modification of Fine & Gray competing risks regression for clustered data (participating study centers) was used to calculate the risk of laser treatment during the follow-up (257). The Fine & Gray model takes into account the follow-up time (failure time) until the event of interest occurs, or the patients are censored without event, and the model also controls for clinically-significant covariates. The effect modification (i.e., whether the effect of a certain variable on the risk of PDR or laser treatment varied according to the level of another covariate) was tested for by adding corresponding interaction terms to the regression models. Pairwise first and second order interactions between the mean HbA_{1c}, the CV (coefficient of variation), and the duration

of diabetes were studied. None of the interaction terms were significant. In addition, Schoenfeld residuals were plotted to confirm that the proportional hazards assumption was met. The model selection was then based on BIC in Fine & Gray regression.

8.4.5.4 Study IV

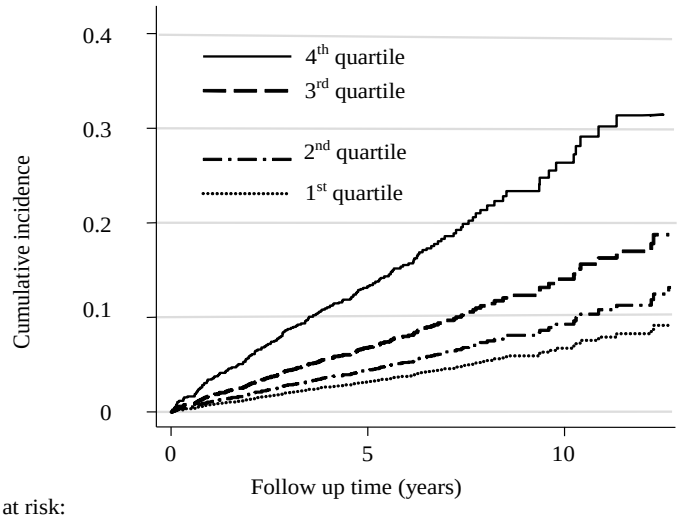
The cumulative incidence function was used in order to account for the competing risk of death, and the differences in cumulative incidences were tested with Gray's test (256). As both cumulative incidences of CSME (P=0.0010, Gray's test) and death (P=0.010, Gray's test) differed significantly between various age at onset groups, the adjusted hazard ratios were calculated with Fine & Gray competing risks regression model. This model takes the duration of diabetes into account until the event of interest has occurred (258). In order to avoid overfitting the Gray & Fine regression model, the covariates were selected with BIC from a very large set of candidate models (258).

8.4.5.5 Study V

The failure times (survival) for the cumulative incidence function were calculated from onset of diabetes until death, PDR, or for the patients without events (censored) to the end of the DRP follow-up. The differences in the distributions of cumulative incidences were tested with Gray's test (256). Secondly, the Cox regression model with a shared gamma frailty was used for calculating hazard ratios. A frailty is an unobservable random effect shared by subjects within a subgroup. Families with a large value of frailty will experience the event of interest at earlier times than families with small values of the random effect. Thus, the most "frail" individuals will get PDR early, and late survivors will tend to come from more robust families (259). The Cox regression model with a shared frailty was adjusted for certain a priori-selected potential risk factors such as HbA_{1c}, sex, MAP, and the size of the sibship. The model selection was based on BIC. The Schoenfeld residuals were plotted to confirm that the proportional hazards assumption was met.

9 RESULTS

The table 6 depicts the clinical characteristics of the study patients according to the retinopathy status. There was a significant overall effect of hyperglycaemia and BP on the risk of severe DRP and laser treatment throughout all studies. This is most clearly shown in the subset of patients from study III who were followed prospectively for the occurrence of the first laser treatment (n=1 459). These patients had a mean diabetes duration of 16.9 (SD 10.4) years before the start of the 5.2 (SD 2.2) years follow-up period. The patients had their HbA_{1c} values measured on a median of 10 (IQR 3-17) times. The mean HbA_{1c} was then ranked into quartiles. The mean HbA_{1c} was 10.0 (SD 0.9) %, 8.6 (SD 0.2) %, 7.9 (SD 0.2)%, and 6.9 (SD 0.5)% from the highest to the lowest quartiles respectively. The cumulative incidences of laser treatment, accounting for the competing risk of death, were significantly higher when the mean HbA_{1c} was high (Figure 11). The highest mean HbA_{1c} quartile had HR 4.3 (95% CI 2.6-7.2, p<0.001) as compared to the lowest mean HbA_{1c} quartile when adjusted for the baseline mean arterial BP (p<0.001) in a Fine & Gray competing risks regression accounting for the competing risk of death. Because of the constant association of BP and hyperglycaemia with the risk of severe DRP, they were used as explanatory covariates in all subsequent regression models.



Number at risk:

4th quartile	368	174	7
3rd quartile	365	192	8
2nd quartile	365	180	9
1st quartile	361	175	7

Figure 12 The cumulative incidence of laser treatment in different quartiles of the mean HbA_{1c} (P<0.001, Gray's test).

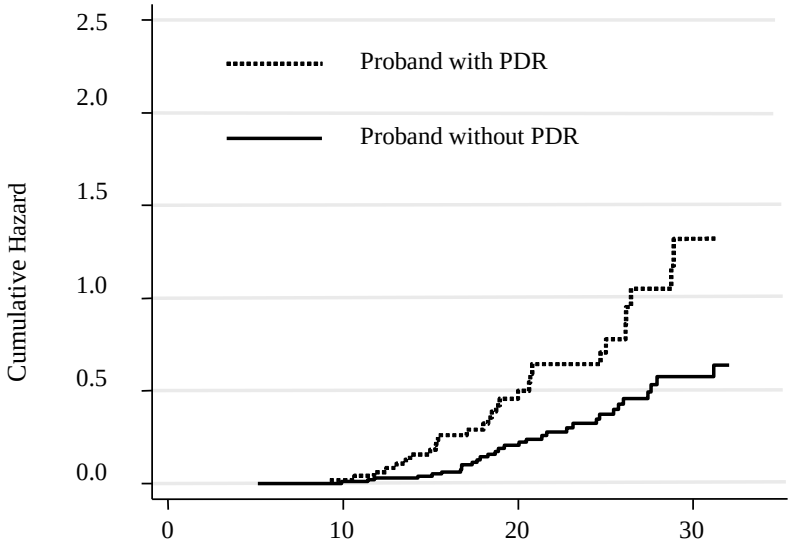
Table 6. *The clinical characteristics of patients according to retinopathy status, male/female 992/874. Of the patients with laser-treated diabetic macular edema (DME) 250/346 (72%) had also had proliferative retinopathy (PDR). Of the patients in PDR group, none had been treated for DME.*

Retinopathy	No retinopathy (n=445)	NPDR (n=690)	Laser-treated DME (n=346)	PDR (n=385)	All Patients (n=1866)
Duration of diabetes (years) *	13.5 ±8.5	24.6 ±9.6	29.8 ±8.5	34.4 ±9.1	24.9 ±11.6
Age (years) *	32.8 ±11.4	38.7 ±11.4	44.9 ±10.4	44.4 ±10.4	39.6 ±11.9
Age at onset (years) *	19.1 ±10.4	14.1 ±9.3	15.1 ±9.4	10.1 ±6.6	14.7 ±9.6
HbA_{1c} (%)*	8.1 ±1.4	8.5 ±1.4	8.9 ±1.5	8.7 ±1.5	8.5 ±1.5
MSBP (mmHg) *	128 ±15	131 ±17	141 ±21	143 ±20	134 ±19
MSDP (mmHg) *	77 ±9	79 ±9	82 ±11	83 ±10	80 ±10
MAP (mmHg)*	94 ±10	97 ±11	102 ±13	103 ±12	97 ±12
AHT (%)*	10.8	28.1	70.3	74.3	41.4
ASA treatment (%)*	3	8	18	27	12
Diabetic nephropathy (%)*	1	9.0	56	63	27
Triglycerides (mmol/l)*	0.9 (IQR 0.7-1.2)	1.1 (IQR .7-1.3)	1.2 (IQR 0.9-1.8)	1.2 (IQR 0.9-1.7)	1.0 (IQR 0.7-1.5)
Total Cholesterol* (mmol/l) males	4.7 ±0.9	4.8 ±0.9	5.3 ±1.1	5.2 ±1.1	5.0 ±1.0
HDL Cholesterol* (mmol/l) males	1.5 ±0.4	1.5 ±0.4	1.4 ±0.4	1.4 ±0.4	1.4 ±0.4
HDL Cholesterol* (mmol/l) females	1.8 ±0.4	1.8 ±0.5	1.7 ±0.6	1.6 ±0.4	1.7 ±0.5
Statin treatment (%)*	3	8	18	17	11
WHR males*	0.89 ±0.7	0.90 ±0.7	0.95 ±0.8	0.94 ±0.7	0.92 ±0.8
WHR females*	0.80 ±0.06	0.81 ±0.06	0.83 ±0.07	0.84 ±0.07	0.82 ±0.07
BMI*	24.5 ±3.4	25.2 ±3.4	25.7 ±4.0	25.6 ±4.1	25.2 ±3.7
hsCRP(mmol/l) *	1.5 (IQR 0.9-3.1)	2.0 (IQR 1.2-4.3)	2.4 (IQR 1.4-5.3)	2.5 (IQR 1.6-5.4)	2.1 (IQR 1.2-4-7)
Mortality (%)* (until 24.3.2009)	2	5	21	25	11

The values are expressed as means ± standard deviations, or medians (IQR=interquartile range). P-value for trend, P <0.05. MAP= Mean Arterial Pressure, BMI= Body Mass Index, hsCRP = high sensitivity C-reactive protein, WHR= Waist-to-Hip Ratio, ASA=Aspirin (Acetylsalicylic Acid), AHT= Antihypertensive Treatment, MSBP =Mean systolic Blood Pressure, MDBP =Mean Diastolic Blood Pressure*

9.1 STUDY I: HERITABILITY OF PROLIFERATIVE DIABETIC RETINOPATHY IN TYPE 1 DIABETES

There were a total of 396 patients in the FinnDiane study, who came from sibships with at least two siblings with type 1 diabetes. The records of treatment and follow-up could be obtained for 369/396 (93%) of these patients. The male/female ratio was 202/167 and the mean diabetes duration 25.9±11.8 years. The mean duration from the onset of diabetes to PDR was 20.9±7.5 years, and the mean age at onset of diabetes was 14.3±10.2 years. PDR was found in 115/369 patients (31%). In 8/115 (7.0%) patient's PDR was discovered at their first examination by an ophthalmologist, and there were no previous fundus examinations for these patients without PDR. The other patients had all had at least one ophthalmic examination on a median of 1.0 (IQR -2.2 – (-) 0.4) years prior to the diagnosis. There were a total of 48/168 (29%) probands (the oldest sibling) with PDR and 61/182 (34%) of the younger siblings who also had PDR. The siblings of probands with PDR had a higher unadjusted cumulative risk of PDR HR 2.2 (95% CI 1.3-3.6, P = 0.002, Mantel Haenszel Chi-square test) when compared to siblings of probands without PDR (Fig. 13).



Number at risk:				
Proband without PDR	131	110	57	17
Proband with PDR	51	48	22	5

Figure 13 The cumulative hazard estimates (Nelson-Aalen) of proliferative diabetic retinopathy (PDR) in 182 siblings stratified according to whether the proband has PDR or not.

The sibships with PDR in the probands had a significantly higher mean arterial BP, 104 mmHg (±13) vs. 99 mmHg (±11) (P<0.001, t-test), as compared to the sibships without PDR in the proband (Fig. 14)

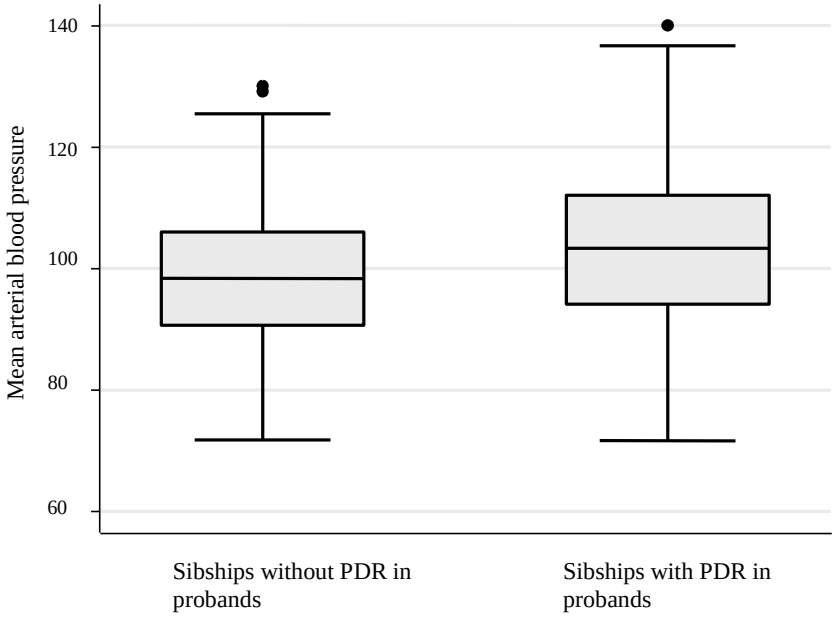


Figure 14 Mean arterial blood pressure (MAP) in sibships (P<0.001, t-test)

The sibships with PDR in the proband also had a higher HbA_{1c}, 8.8±1.5 % vs. 8.4±1.6 % (P<0.03, t-test) than sibships without PDR in the proband (Fig. 15).

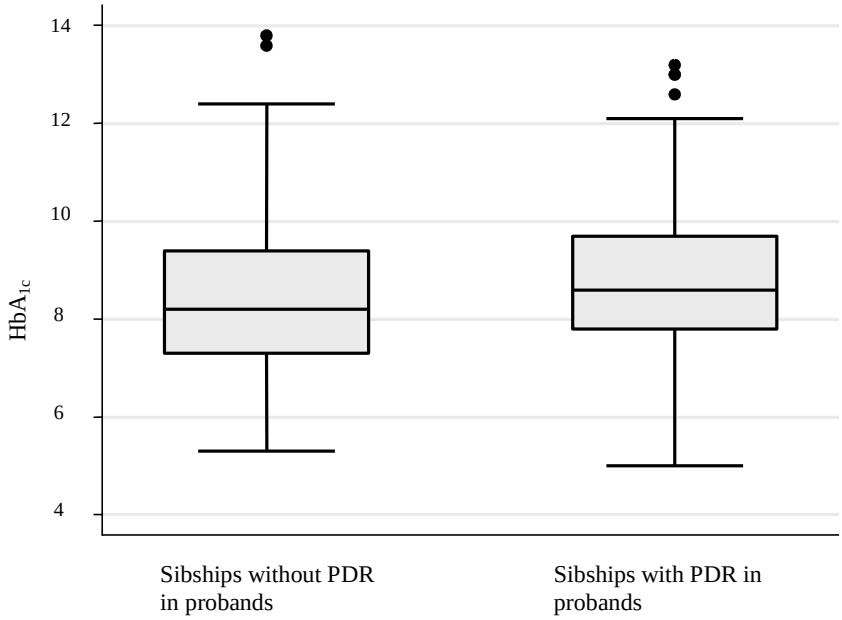


Figure 15 HbA_{1c} in sibships with or without proliferative retinopathy (PDR) (P=0.03, t-test)

Because of these similarities in clinically significant risk factors, the risk estimate was calculated cross-sectionally in a logistic regression model with the duration of diabetes, HbA_{1c}, and the mean arterial BP as covariates. The heritability was estimated in a variance component model in which the proportion of variance attributable to all covariates (HbA_{1c}, duration, and BP) was 0.23 (Kullback-Leibler R^2). PDR in the probands (48/168) was a significant risk factor for PDR in later-born siblings 61/182 (34%) after the adjustment for the similarities in the above mentioned risk factors OR 2.76 (1.25 - 6.11, P=0.01). The 29 proband-sibling pairs, in which both members had PDR were concordant for the survival time without PDR (intra class correlation = 0.47 [0.14-0.71], P=0.004). The heritability of PDR was h^2=0.52 (±0.31, P<0.05) as adjusted for mean arterial BP, HbA_{1c}, and diabetes duration. The sex was non-significant (P>0.1).

9.2 STUDY II: AGE AT ONSET AND THE RISK OF PROLIFERATIVE RETINOPATHY IN TYPE 1 DIABETES

The effect of age at onset of type 1 diabetes on the long-term risk of PDR was studied in a cohort of 1 117 patients, male/female 596/521. The mean age at onset of type 1 diabetes was 13.7±8.5 years, and the mean duration of diabetes 25.0±11.4 years. The longest median duration to development of PDR was 22.2 (IQR 19.1-26.9) years in the 0-4 age at onset group (n= 79), whereas the shortest was 18.2 (IQR 15.0-24.3) years in the 5-14 age at onset group (n= 212). In the 15-40 age at onset group the median duration to PDR was 20.0 (IQR 16.0-27.2) years (n= 76) (Fig. 16).

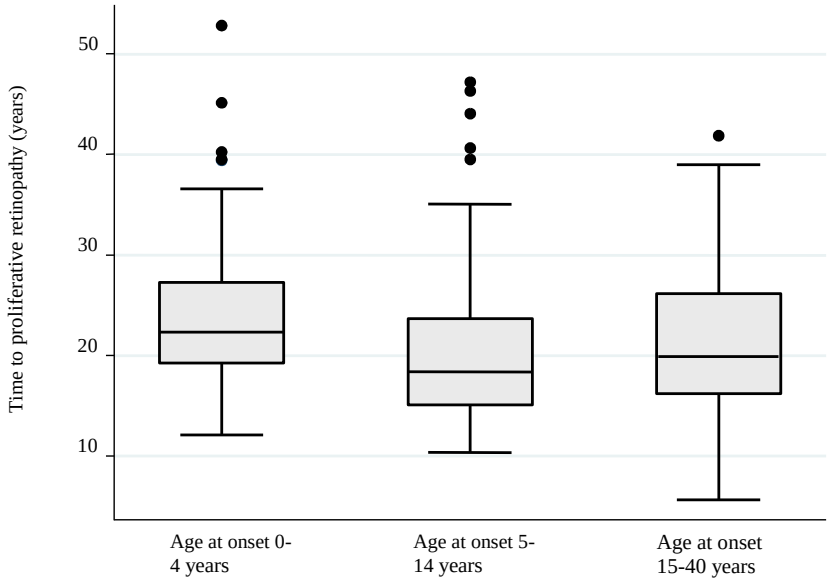
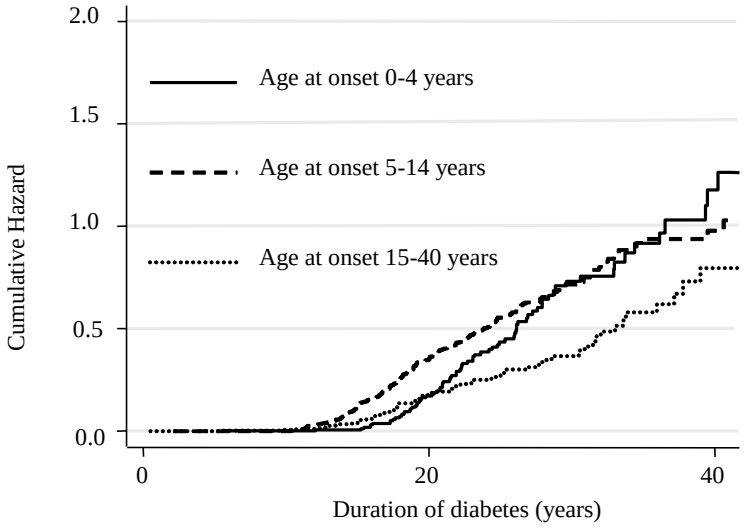


Figure 16 The time to diagnosis of proliferative retinopathy (years) in different age at onset groups (P< 0.001, Anova)

The highest proportion of patients with PDR 47% was observed in the age at onset group of 0-4 years, the second highest in the age at onset group of 5-14 years 39%, and the lowest in the age at onset group 15-40 years 19%. Altogether, PDR was found in 367/1117 (33 %) patients. The differences in the incidence as well as the median survival times resulted in the lowest cumulative risk of PDR for the age at onset group of 15-40 years (Fig. 17)



Number at risk:			
0-4 years	168	120	11
5-14 years	546	263	23
15-40 years	403	170	10

Figure 17 The cumulative hazard estimates (Nelson-Aalen) of proliferative diabetic retinopathy (PDR) in 1117 patients stratified according to the age at onset. (P=0.007, Mantel-Haenszel Chi-square test).

Despite the initially lower risk for the youngest age at onset group, the long-term cumulative risk was similar in the age at onset group 0-4 years and the 5-14 years (Fig. 17). Patients with PDR had the highest mortality 20.4%, the highest HbA_{1c} 8.7 ±1.5%, and the longest duration of diabetes 33.1±9.1 years. The patients in the age at onset group of 15-40 years had the highest proportion (21%) of patients with C-peptide concentrations above the detection limit of 0.033 nmol/l. A total of 99 of the 1 117 patients (8.9%) had died during the follow-up. Of these, 75/99 (75.8%) had been diagnosed with PDR. With such a long follow-up time, a potentially important issue is the competing risk of death. The outcomes from the Kaplan-Meier or the Cox-regression analysis did not change if the cumulative risk of death was taken into account using a previously published SAS macro (254). The risk of PDR, adjusted for HbA_{1c}, BP, sex, and BMI, was the highest in the age at onset groups of 5-14 years, HR 1.90 (95% CI 1.45–2.48, p<0.001) as contrasted to the age at onset group of 15-40 years who had the lowest risk. Similarly,

patients with the age at onset between 0-4 years had a higher adjusted risk of PDR with a HR of 1.6 (95% CI 1.16-2.23, p=0.002) as compared to the age at onset group 15-40 years. BMI and sex were nonsignificant in this model. As for the other covariates, the risk of PDR increased 15% with every unit increase in HbA_{1c} percentage with a HR of 1.15 (95% CI 1.07-1.23, p<0.001), and 3% with every 1 mmHg increase of the mean arterial BP with a HR of 1.03 (95%1.02-1.04, p <0.001).

There may also be a confounding cohort-effect in the present study, since the oldest patients may have a worse prognosis than the younger ones (149). The data were tested for such a cohort effect in two ways. The first was to use the year of the onset of diabetes as a continuous variable in the Cox-regression model. The other was to use the decade of the onset of diabetes as a categorical variable. Both of these variables were statistically non-significant in this model (P=0.74 for year of onset and P=0.72 for decade of onset, Wald test), and they did not alter the overall significance for the categorical variable age at onset.

9.3 STUDY III: HbA_{1C} VARIABILITY INCREASES THE RISK OF RETINOPATHY REQUIRING LASER TREATMENT IN TYPE 1 DIABETES

The effect of HbA_{1c} variability on DRP progression was studied in a total of 2 019 patients, male/female 995/1 024. The patients were divided into two partially overlapping subcohorts with either verified first laser treatment (n=1459) or DRP severity and progression graded from ophthalmic records with the ETDRS scale (n=1 346) (Fig. 10). The mean duration of diabetes was 22.9±11.9 years with a mean age at onset 15.3±9.2 years. A median of 10 (IQR 3-18) HbA_{1c} measurements were recorded per patient. The intrapersonal mean of serially measured HbA_{1c} was 8.4±1.2 %. There was a clear correlation between the HbA_{1c} measured at the baseline and the mean of serial HbA_{1c} measurements (r_s =0.77, P< 0.001). The mean HbA_{1c} also correlated positively with the intrapersonal SD of the HbA_{1c} values (r_s=0.39, P<0.001). Thus, the patients with the highest mean HbA_{1c} values had the highest variation of their HbA_{1c} values. Also the number of HbA_{1c} measurements correlated positively with the intrapersonal SD of the mean HbA_{1c} (r_s=0.32) which could have resulted from the repeated attempts by the attending clinicians to improve the glycaemic control of the patients with high HbA_{1c} values. The regression models were, therefore, adjusted for both the number of HbA_{1c} measurements and the mean HbA_{1c}. There were progressively higher HbA_{1c} values from the 1st to the 4th quartile as shown in Figure 18 for the laser treatment subcohort. Total cholesterol, BP and BMI were no different in the higher quartiles of variability.

There were a total of 1 459 patients with no previous laser treatment and with prospective follow-up data after the FinnDiane baseline visit for a period of 5.2±2.2 years. Of these patients, 74 had their first laser treatment during the follow-up period. The estimated 5-year cumulative incidences of laser treatment, accounting for the competing risk of death, were 19.1 % (95%CI 14.6-23.9) in the 4th quartile of HbA_{1c} variability, 11.8 % (95%CI 8.5-15.7) in the 3rd quartile, 8.8 % (95%CI 5.9-12.4) in the 2nd quartile, and 9.5 % (95%CI 6.5-13.3) in the 1st quartile (P< 0.001, Gray's test). The patients with CV above the median had a higher unadjusted risk of laser treatment HR 1.4 (95%CI 1.03-1.9, P<0.03, Mantel-Haenszel Chi-square test) as compared to the patients with CV below the median (Fig. 19).

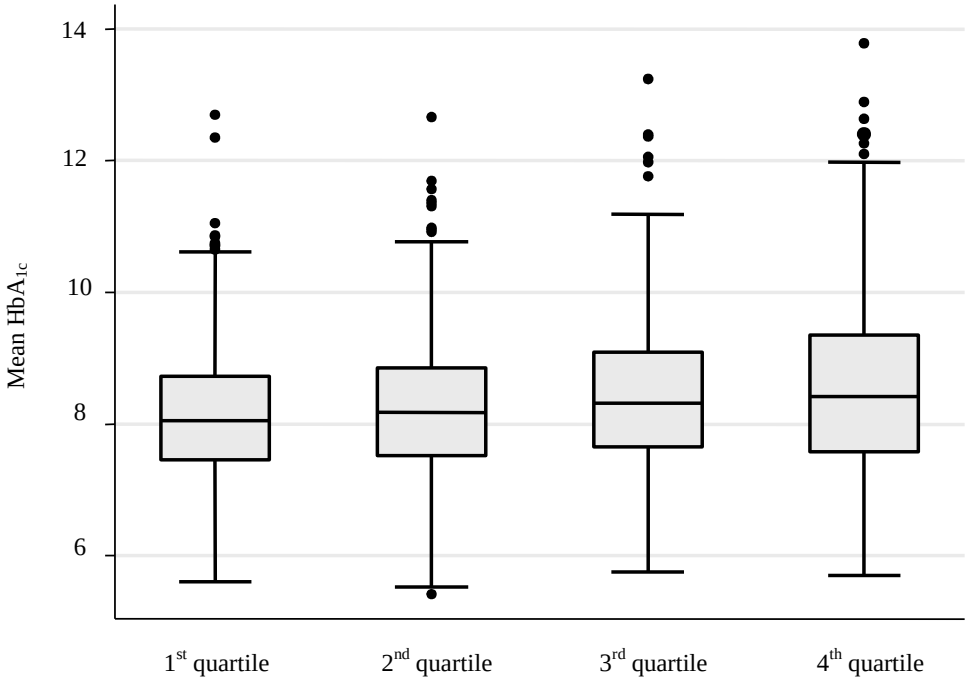
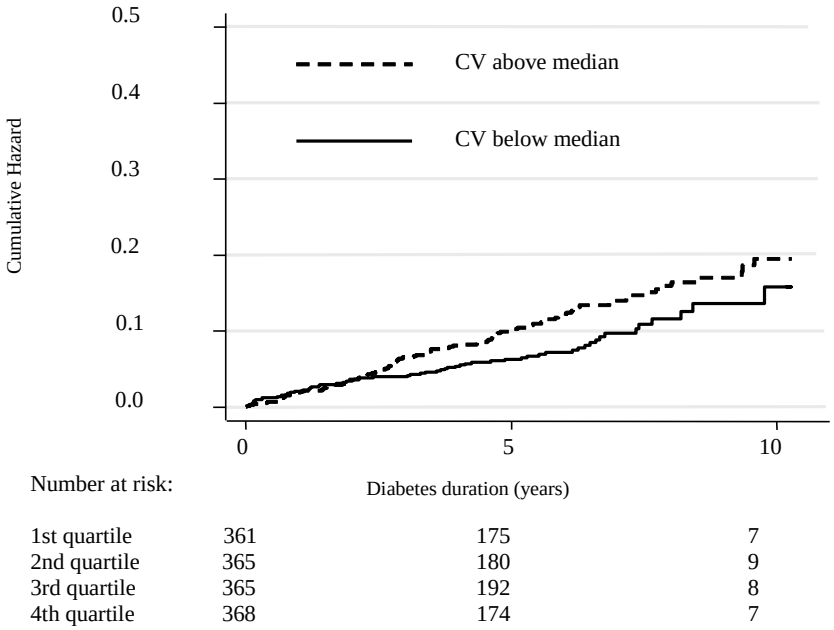


Figure 18 Mean HbA_{1c} in different quartiles of CV (coefficient of variation) in the subcohort of laser-treated patients (P<0.001, Anova)



Number at risk:

1st quartile	361	175	7
2nd quartile	365	180	9
3rd quartile	365	192	8
4th quartile	368	174	7

Figure 19 The cumulative hazard estimates (Nelson-Aalen) of laser treatment in 1 459 patients stratified according to coefficient of variation (CV) below or above the median (P < 0.03, Mantel-Haenszel Chi-square test).

In a Fine & Gray competing risks regression model, adjusted for the baseline duration of diabetes, renal status, mean HbA_{1c}, MAP, sex, and the number of HbA_{1c} measurements, and also accounting for possible correlations within the study centers, the CV quartile was a significant overall risk factor for laser treatment (P=0.04, Wald test). The highest adjusted risk of laser treatment HR 1.6 (95%CI 1.1-2.5, P=0.02) was seen in the 4th quartile as compared to the 1st quartile of CV.

The specific indications for each individual laser treatment were verified from ophthalmic records and fundus photographs for a subcohort of 1 346 patients. The associations between the conventional risk factors, HbA_{1c} variability, and PDR were further analysed in this subcohort (Fig.10). Of these patients, 434/1 346 (32 %) had been diagnosed with PDR. Patients with PDR had a lower than average age at onset of type 1 diabetes (11.0±7.2 years, P<0.001, t-test), higher than average mean HbA_{1c} (8.7±1.3 %, P<0.001, t-test), higher mean arterial BP (102.2±11.9 mmHg, P<0.001, t-test), higher BMI (25.6±3.9 kg/m^2, P<0.001, t-test), and longer duration of diabetes (33.3±9.0 years, P<0.001, t-test). Furthermore, patients with PDR had higher than average values of CRP (2.4 IQR 1.4-4.9 mmol/l, P<0.001, Mann-Whitney test), and triglycerides (1.2 IQR 0.9-1.6 mmol/l, P<0.001, Mann-Whitney test). In a Fine & Gray regression model, the HbA_{1c} variability

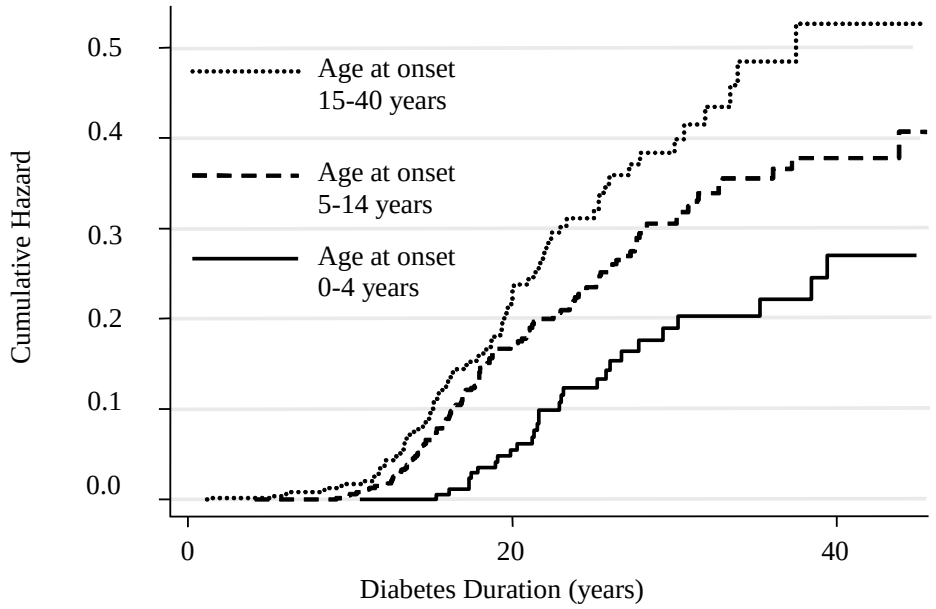
quartile (CV) was significantly associated with PDR (P=0.003, Wald test). The 4th quartile of HbA_{1c} variability (CV) had the highest HR 1.7 (95% CI 1.3, 2.2, P<0.001), as contrasted to the 1st quartile. For the other clinically relevant covariates, the mean HbA_{1c} HR 1.2 (95% CI 1.1, 13, P<0.001) and BP with a HR of 1.02 (95% CI 1.01, 1.03, P<0.001), were significantly associated with PDR. However, male sex was a nonsignificant factor with a HR of 1.2 (95% CI 0.9, 1.3, P=0.5). The patients' highest attained ETDRS score and the renal status showed a significant correlation (r_s = 0.64, P<0.001). Because of this, the renal status was not included in the Fine & Gray regression model for PDR. However, if only patients with no nephropathy (normal UAER, or microalbuminuria, n=969) were included, the HbA_{1c} variability above the median was still associated with PDR with a HR of 1.4 (95% CI 1.01, 2.0, P= 0.04).

9.4 STUDY IV: HIGHER AGE AT ONSET OF TYPE 1 DIABETES INCREASES RISK OF MACULAR EDEMA

The effect of age at onset of type 1 diabetes was studied in a cohort of 1 354 patients (male/female 727/627). The mean age at onset of type 1 diabetes was 14.1±8.7 years and mean duration of diabetes 24.6±11.6 years. CSME was diagnosed in 242 patients (17.9 %) and PDR in 425 patients (31.4 %). PDR and CSME showed a significant overlap as the majority (73 %) of the patients with CSME had also been diagnosed with PDR at some point. The longest mean duration of diabetes to CSME was 23.6 ±6.4 years in the 0-4 age at onset group, whereas the shortest was 17.6 ±6.5 years in the 15-40 age at onset group. The oldest age at onset group had also the highest cumulative incidence of CSME. At 30 years of diabetes duration, the estimated cumulative incidences of CSME were 17.0% (95% CI 10.7–24.5) in the age at onset group 0-4 years, 27.4% (95% CI 22.7-32.4) in the age at onset group 5-14 years, and 34.1% (95%CI 27.3-41.0) in the age at onset group 15-40 years (P=0.001, Gray's test). The highest unadjusted cumulative risk of CSME was seen in the oldest age at onset group (Fig. 20).

Patients with CSME had higher HbA_{1c} (8.8±1.5 %, P<0.001, t-test), higher triglycerides (1.2 IQR 0.9-1.7 mmol/l, P<0.001, Mann-Whitney test) and total cholesterol (5.3±1.1 mmol/l, P<0.001, t-test) (Fig. 21 and 22), higher proportion of patients with nephropathy 47% (P<0.001, Chi-square test), and lower eGDR 4.1±1.5 $mg \bullet kg^{-1} \bullet min^{-1}$ (P<0.001, t-test) than patients without CSME. The adjusted risk of CSME was calculated in a competing risks regression model (Fine&Gray), for which the covariates were selected with BIC. The covariates included were the age at onset group, ETDRS-score, HbA_{1c}, and total cholesterol. The onset of diabetes after 15 years of age increased the risk of CSME the most, HR being 3.72 (95% CI 2.35–5.89, p<0.0001) as contrasted to the age at onset group of 0-4 years. Also, the patients with age at onset between 5-14 years had a higher risk of CSME with

a HR of 1.89 (95% CI 1.22-2.91, P=0.004) as compared to the youngest age at onset group. Other significant risk factors were the ETDRS score with a HR of 1.04 (95% CI 1.03-1.05, P<0.001), HbA_{1c} HR 1.12 (95% CI 1.02-1.23, P=0.016), and total cholesterol HR 1.19 (95% 1.04-1.37, P=0.013). The patients' highest attained ETDRS-score and the renal status showed a strong correlation (r_s = 0.64, P<0.001). However, the nephropathy status and/or BP reduced the goodness of fit (BIC) of the regression model and were, thus, not included in the regression model.



Number at risk:

15-40 (years)	508	179	11
5-14 (years)	662	334	45
0-4 (years)	184	145	30

Figure 20 The cumulative hazard estimates (Nelson-Aalen) of clinically significant macular edema (CSME) in 1354 patients stratified according to the age at onset group (P< 0.001, Mantel-Haenszel Chi-square test).

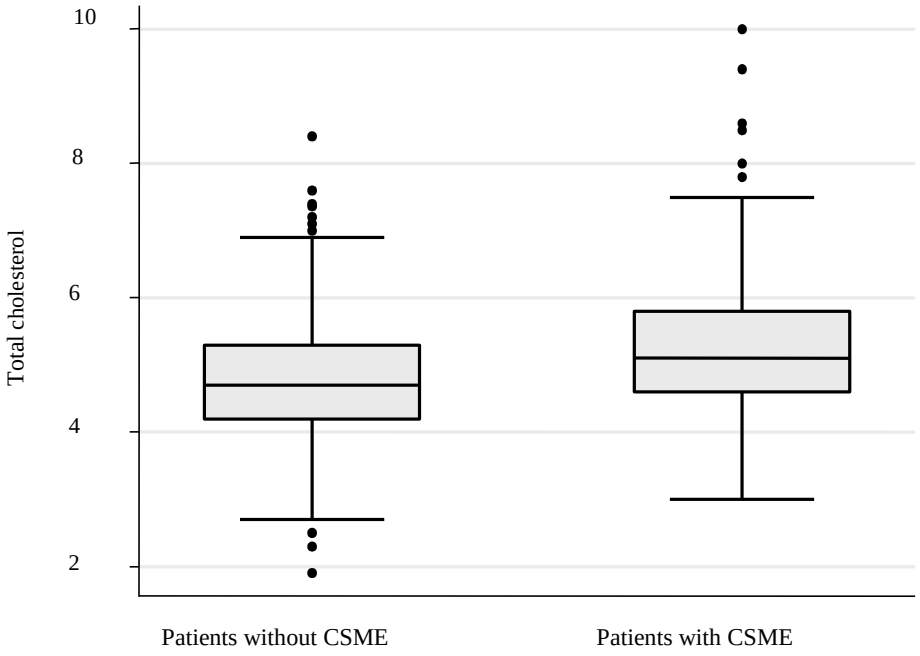


Figure 21 Total cholesterol in patients with or without clinically significant macular edema (CSME) (P < 0.001, t-test).

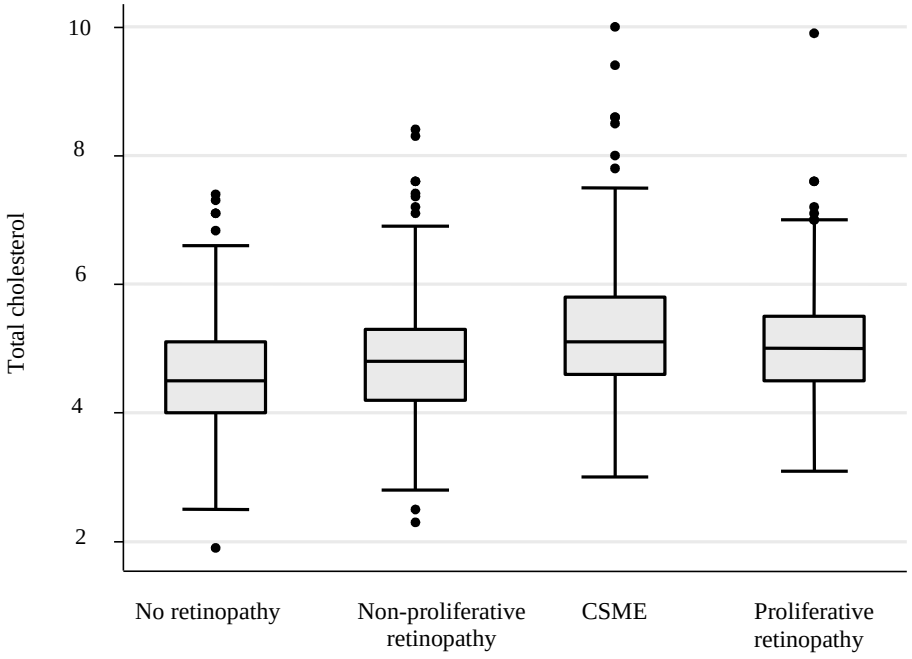


Figure 22 Total cholesterol in different retinopathy groups (P = 0.001, Anova).

the competing risk of death increased with the duration of diabetes and was the highest in the oldest age at onset group. A total of 130/1 354 patients (9.6%) had died. Of these 45/130 (34.6%) had been diagnosed with CSME. The cumulative incidence estimates and regression models, therefore, accounted for the competing risk of death. An additional and potentially important confounding effect may be the improvement in medical and ophthalmic care that is gradually improving the long-term prognosis of DRP (152). The data were tested for such a time dependent confounding effect in two ways. The year of the onset of diabetes was used either as a continuous variable in a competing risks regression model, or the decade of onset of diabetes as a categorical variable. Neither of these changed the overall significance or magnitude of the effect of age at the onset as a risk factor for CSME.

9.5 STUDY V: THE RISK OF PROLIFERATIVE RETINOPATHY IN SIBLINGS WITH TYPE 1 DIABETES

The effect of the age at onset of type 1 diabetes within sibships was studied in the same patient cohort as the effect of heritability in study I. As a difference to study I, where the oldest sibling was the proband, the sibling first diagnosed with type 1 diabetes was designated as the proband in study V. Overall, PDR was diagnosed in 115/369 (31%) of the patients. The number of patients with PDR was higher in the probands as compared to the later affected siblings (36% vs. 27%, P=0.05, Chi Square), and the probands also had a significantly longer duration of diabetes as compared to their later affected siblings (30.3 [IQR 21.8-29.2] vs. 19.6 [IQR 13.2-29.4] years, P<0.001, Mann-Whitney). The median age at onset of type 1 diabetes was 8.4 (IQR 4.2-13.3) years in probands, whereas the median age at onset of type 1 diabetes was 16.9 (IQR 10.2-27.8) years in the later affected siblings (P<0.001 Mann-Whitney). If the cut-off value for puberty was arbitrarily chosen to be at age 12 years for males and at age 11 for females, the difference in the age at onset resulted in a significantly longer median prepubertal diabetes duration for the probands as compared to the later affected siblings (P<0.001, Mann-Whitney) (Fig 23).

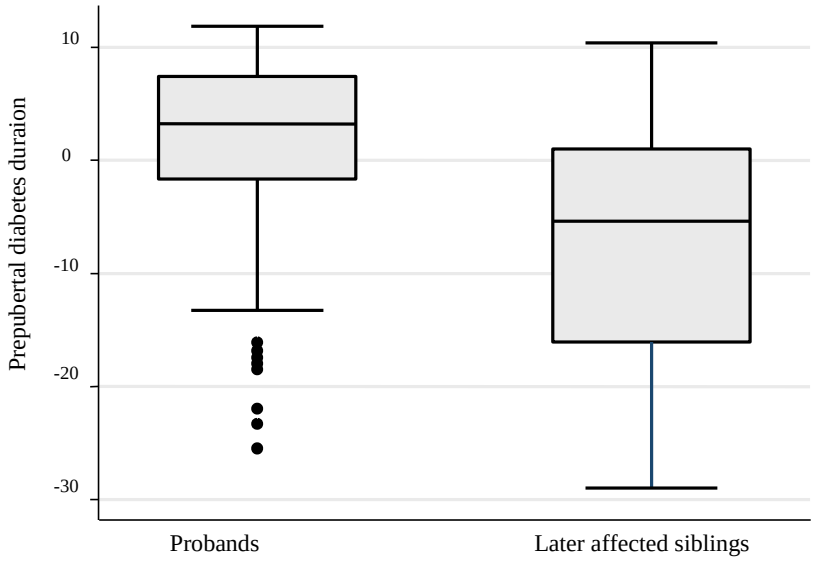
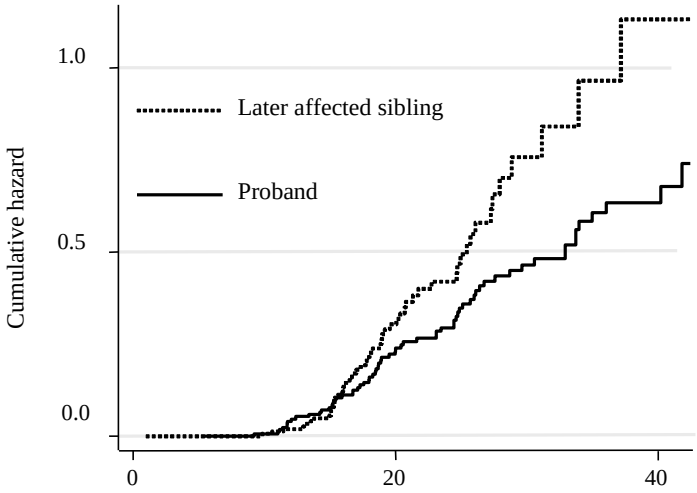


Figure 23 The probands had a longer prepubertal diabetes duration than the later affected siblings (P < 0.001, t-test)

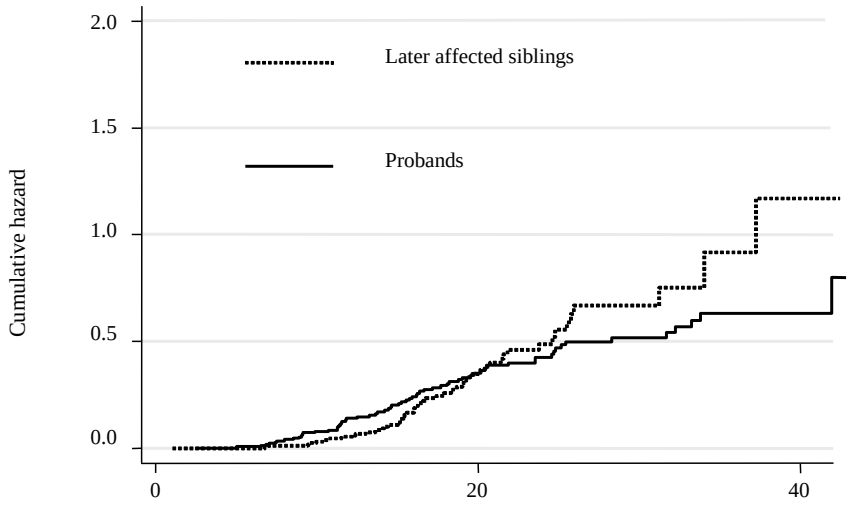
The cumulative incidence estimates for PDR, accounting for the competing risk of death, were 21% (95% CI 15-27) in probands and 26% (95% CI 19-35) in later affected siblings at 20 years of diabetes duration, and the 30 years cumulative incidences were 37% (95% CI 29-45) and 53% (95% CI 40-64) (P=0.05, Gray's test) respectively. The overall cumulative incidence of death was no different between probands (0%) and later affected siblings 2% (95% CI 0-7) after 30 years of diabetes duration (P=0.3, Gray's test). The unadjusted cumulative risk was higher in the probands with a HR of 1.5 (95% CI 1.03-2.1, P=0.04, Mantel-Haenszel Chi-square test) (Fig. 24). When adjusted for the duration of diabetes, HbA_{1c}, MAP, male gender, age at onset of type 1 diabetes, and the size of the sibship, the later affected siblings had a moderately higher risk of PDR as compared to the probands with a HR of 1.76 (95%CI 1.13-2.75), P=0.01).



Number at risk:			
Proband	183	117	23
Later affected sibling	186	67	3

Figure 24 The cumulative hazard estimates (Nelson-Aalen) of proliferative diabetic retinopathy (PDR) in siblings stratified according to the onset of type 1 diabetes (P = 0.04, Mantel-Haenszel Chi-square test).

If prepubertal years were omitted, the cumulative incidences of PDR were no different in probands and later affected siblings (P=0.6, Mantel-Haenszel Chi-square test) (Fig 25).



Number at risk:			
Proband	183	117	23
Later affected sibling	186	67	3

Figure 25 The cumulative hazard estimates (Nelson-Aalen) of proliferative diabetic retinopathy (PDR) in siblings stratified according to the onset of type 1 diabetes without the prepubertal diabetes duration (P = 0.6, Mantel-Haenszel Chi-square test).

Similarly, in the regression models in which the prepubertal diabetes duration was omitted, the effect of proband became nonsignificant. However, in all the regression models in which the prepubertal diabetes duration was taken into account, the effect of the proband was significant. The inclusion of prepubertal diabetes duration also clearly improved the fit of the regression model as assessed with the BIC.

Many of the patients in this study were diagnosed with diabetes already in the 1970's. As a confounding effect, the patients diagnosed with diabetes in the 1970's may have a worse prognosis than the patients diagnosed at the end of the 1980's due to the improvement of medical care of diabetes and DRP (152). The data were tested for this confounding effect by including the birth year as a continuous variable in the regression models. The birth year was nonsignificant (P=0.6, Wald test) and it did not change the overall significance or magnitude of the effect for the probands.

10 DISCUSSION

10.1 OVERALL STUDY DESIGN, STRENGTHS AND WEAKNESSES

The FinnDiane study is an observational study which has recruited a large number of type 1 diabetes patients from different levels of health care, including primary health care centers and university hospitals. The patient sampling frame can be considered geographically representative of type 1 diabetes in Finland (Fig. 9). The enrolled patients are well characterized regarding their medical history and the presence or absence of any diabetic complications. There are, however, a few potential concerns in the study design that need to be taken into account.

The FinnDiane study has many independent study centers which could cluster the data. The statistical models were, therefore, calculated with or without adjusting for correlations within study centers in study III. All these models yielded practically identical results and, thus, indicated no significant clustering within the data. Another potential problem in the patient data is that the focus in the FinnDiane has been in the study of diabetic nephropathy which could have resulted in overrepresentation of microvascular complications in the FinnDiane patients. The FinnDiane patients may also overrepresent central and university hospitals since all of them are included in the FinnDiane and only 11% of the primary health care centers are involved, although most of the diabetes patients are treated in the primary health care system. However, the proportion of patients with PDR in this study was comparable to a previous population-based assessment of PDR in a sample of 1 067 patients in Finland (260). There is also a comprehensive screening system for DRP in the primary health care system which covers practically all of the diabetes patients and provides an easy access to the fundus photographs. This has balanced the patient sampling frame by providing a large number of patients without DRP and/or with milder degrees of DRP. The fundus photography has been used as the primary screening method for a more than 20 years, and a high percentage of patients with diabetes in Finland are undergoing regular fundus photography since the national guidelines for the screening of diabetic DRP were published already in 1992 and updated in 2006 (246, 247). These guidelines emphasized fundus photography as the preferable screening method (261). The majority of the patients in this study had attended several screening examinations. Many were examined and treated by ophthalmologists and eventually 1 441/1866 (77%) had fundus photographs available. Practically all of the patients without fundus photographs had been examined by ophthalmologists, and only 9 patients had been examined by a diabetologist. Retinal photography has been reported to be the most sensitive screening

method for DRP. The sensitivity is in excess of 80% in detecting PDR (248). Ophthalmoscopy has less sensitivity, but conversely a higher specificity. It provides good results in the hands of trained professionals such as ophthalmologists and diabetologists (248). Fundus photography together with clinical fundus examination is, thus, a good combination of sensitivity and specificity. One possible limitation of the study may be the lack of a standardised fundus photography protocol. The majority of the fundus photographs were taken for screening purposes with quite heterogeneous methods. However, if severe NPDR or PDR was suspected based on the screening photographs, the patients were referred to an ophthalmologist for a clinical fundus examination. Furthermore, the 12-step ETDRS scale was used for a significant outcome only in study number IV where the risk of macular edema was adjusted for the ETDRS grade. The outcome of interest was PDR in all the other studies.

The diagnosis of CSME was based on the ophthalmologist's clinical decision to laser treat the patient in order to reduce DME. The detection of DME can be done clinically with biomicroscopy, or the retinal thickening can be detected from stereoscopic fundus photographs. Both of these methods have been shown to be reliable in the assessment of CSME (250). However, the subsequent clinical decision as to which areas need laser treatment and how much may show variation (262). It has been shown that the DME photocoagulation treatment threshold and dosage of laser spots differ depending on whether thickness assessments are based on stereoscopic slit-lamp biomicroscopy or OCT. In addition, retinal specialists differ in the number and placement of planned laser spots even when given identical information concerning CSME and treatable lesions. This variability in the photocoagulation treatment could have led to differences in the clinical course of CSME (262).

10.2 FAMILIAL FACTORS IN THE DEVELOPMENT OF DIABETIC RETINOPATHY

10.2.1 FAMILIAL CLUSTERING OF PDR IN TYPE 1 DIABETES

There was familial clustering of PDR in patients with type 1 diabetes, which could not be accounted for by conventional risk factors. Previous studies have been able to show familial clustering of severe NPDR in families with type 2 diabetes, (205, 206) and in families with a mixture of both type 1 and type 2 patients (207). However, no studies have so far given estimates for the familial risk of PDR. The estimated heritability of PDR was $h^2 = 0.52$ (SE 0.31, $P<0.05$), and the intraclass correlation of survival time without PDR 0.47 (0.14-0.71, $P=0.004$). If the oldest sibling had PDR, the adjusted risk of PDR was OR 2.76 (1.25–6.11, $P=0.01$) for the sibling later affected with type 1

diabetes. A heritability estimate of 25% for PDR in type 2 diabetes was reported only a month after the publication of study I (208). The estimated 25% heritability fits within the fairly wide SE of the present study and is, thus, in line with the results obtained here. The 25% heritability was calculated in patients with type 2 diabetes, who may have a different genetic background and set of risk factors for DRP progression as compared to type 1 diabetes patients. Another fact supportive of the findings of the present study is the comparable familial clustering of diabetic nephropathy (263). Diabetic nephropathy is a microvascular complication that is closely related to DRP (264). In a previous study regarding diabetic nephropathy, roughly 50% of the risk could not be attributed to the familial clustering of conventional risk factors (265). As there is a close correlation between severe DRP and nephropathy it is conceivable that the two could have a common genetic background.

10.2.2 GENETIC SUSCEPTIBILITY TO PDR

It has been observed that severe DRP appears to have an inherent momentum of progression that, by time, leads to an almost linear increase in the incidence of PDR (130). The worsening of DRP continues even after an improvement of glycaemic control (159, 266), and the more severe the DRP is, the longer the delay before a beneficial effect of improved glycaemic control is observed (159). It is possible that this inherent momentum may at least partially be determined by genetic factors. The heritability estimates obtained from this study and from other studies are typical of a polygenic genetic component. It is of note, that some risk factors that are considered environmental, such as BP and HbA_{1c}, also appear to be determined at least in part by genetic factors. In fact, it has been noted that a single HbA_{1c}-measurement offers a fair estimate of the glycaemic control during the previous ten years in type 1 diabetes patients (267). This could be a reflection of the predictive value of biological, between-individual variations in HbA_{1c}, which is distinct from the mean blood glucose, and which is significantly determined by a genetic component (268, 269). In this study, both single measured HbA_{1c} and BP were significant risk factors in the logistic regression and heritability calculations. Increased BP is considered to be a multifactorial trait with an estimated genetic contribution in the range of 30-50 % (270). Although both HbA_{1c} and BP may have a significant familial component they are not likely to be the main factors behind the familial clustering of PDR. It has been shown in a simulation study that familial clustering of two additive environmental risk factors only leads to a slight excess in the clustering of a disease among the siblings (271). Therefore, it is unlikely that the degree of familiality observed in this study is the result of familial clustering of glycaemic control and BP alone.

Despite the apparent genetic contribution to DRP, attempts to find evidence for an involvement of any major loci in DRP have so far turned out inconclusive. Four genome wide-scans have offered suggestive evidence of a linkage, but on a number of different chromosomes in patients with type 2 diabetes (209-212). The associations with various biologically relevant candidate genes have also been extraordinarily difficult to replicate (213, 214). This is not surprising because the heritability estimates imply a multifactorial inheritance with probably a modest risk increase or decrease from any single gene.

10.2.3 THE SIBLINGS FIRST AFFECTED WITH TYPE 1 DIABETES HAVE A YOUNGER AGE AT ONSET

The siblings first affected with type 1 diabetes were significantly younger at the onset of diabetes as compared to their later affected siblings, which is in line with previous studies (26-28). A possible biological explanation for this could be a simultaneous environmental trigger within sibships. The onset of diabetes has been associated, for example, with certain viral infections caused by enteroviruses. Thus, the older age at onset for the later affected siblings within the sibships could be related to a trigger that simultaneously initiates an autoimmune response in both the younger and the older siblings (272). Those siblings that are genetically or environmentally most susceptible to diabetes will then manifest type 1 diabetes at an earlier age. In addition to viral infections, there are likely to be other environmental risk factors that may, over time, interact with the susceptibility genes and modify their penetrance. It has been noted that the risk of diabetes decreases with higher birth order of the children in larger sibships, but the studies have also suggested that this risk reduction is modified by a complex interplay between birth order and maternal age at delivery (23, 24). There has also been an overall trend of diabetes onset at a younger age in the general population and this could have some importance within the sibships as well (273), although such a cohort effect was not observed in this study.

10.2.4 AGE AT ONSET OF TYPE 1 DIABETES AS A RISK FACTOR WITHIN SIBSHIPS

The siblings first affected by type 1 diabetes had a better long-term prognosis with regards to the development of PDR as compared to their later affected siblings. The cumulative incidence estimates for PDR, accounting for the competing risk of death, were 21% (95% CI 15-27) in the probands and 26% (95% CI 19-35)% in the later affected siblings at 20 years of diabetes duration and the respective 30 years incidences were 37% (95% CI 29-45) and 53%

(95% CI 40-64), (P=0.05, Gray's test). The risk of PDR adjusted for conventional risk factors, the age at onset, and sibship size, was higher in the later affected siblings with a HR of 1.76 (95% CI 1.13-2.75, P=0.01) as compared to their probands.

The probands had mainly a prepubertal onset of diabetes whereas the later affected siblings had a postpubertal onset. If only postpubertal duration of diabetes was taken into account, the effect of the proband in the regression model was nonsignificant (Fig. 24 and 25). This suggests that the longer prepubertal duration of diabetes for the probands may explain the better long-term prognosis. It has previously been suggested that the prepubertal duration of diabetes contributes only minimally to the long-term risk of DRP (200). However, the exclusion of the prepubertal duration produced a clearly worse fit for the model (BIC) indicating that the prepubertal years indeed contribute significantly to the risk of PDR. The present study, thus, indicates that the prepubertal years are significant for the long-term prognosis and may modify the long-term prognosis even within sibships. The risk difference of diabetes onset before and after 15 years of age may also be related to the fact that the onset of type 1 diabetes close to the puberty, as observed in the later affected siblings, may increase the risk of severe DRP (201).

10.2.5 OTHER POSSIBLE FACTORS INFLUENCING THE LONG-TERM PROGNOSIS WITHIN SIBSHIPS

There are also other possible explanations for how early childhood could modify late diabetic complications. Very few chronic medical disorders of the childhood affect the young patients, their families, and their social networks as profoundly as type 1 diabetes. The treatment regimen often becomes a major source of stress for the family members and a potential focus of family conflicts (274). Parents that manage their child's type 1 diabetes rate themselves as having stress, and those able to maintain their child's glycaemic control indicate even higher levels of perceived stress (275). A high perceived stress could contribute to a less than optimal medical care of the siblings later affected with diabetes. A stressful family setting, such as a chronic physical problem in a close family member, has indeed been identified as a risk factor for poor glycaemic control (274, 276). This may be important in the long-term, since key social influences early in development may permanently change physiological stress reactivity patterns (277), and severe stress experienced in early life may have long-term consequences on adult physiological functions (278). Moreover, a poor glycaemic control during the first 5 years of diabetes has been observed to accelerate time to the occurrence of DRP (279). This may be related to the carry-over effect of prior glucose exposure, i.e. metabolic memory, which may be explained in

part by early glycation of proteins and AGE formation (280), or possibly by epigenetic phenomena (281, 282).

10.3 AGE AT ONSET OF TYPE 1 DIABETES AS A MODIFIER OF RETINOPATHY PHENOTYPE

10.3.1 EARLY AGE AT ONSET INCREASES RISK OF PDR IN A NONUNIFORM FASHION

The risk of PDR was higher, if the age at onset of type 1 diabetes was less than 15 years. However, this risk increase was not uniform. There was an initially lower risk of PDR for the patients with the age at onset of type 1 diabetes less than 5 years of age. In the long run, this initial advantage was lost (Fig. 17). The longest median duration to PDR was 22.2 (IQR 19.1-26.9) years in the 0-4 age at onset group (n= 79), whereas the shortest was 18.2 (IQR 15.0-24.3) years in the 5-14 age at onset group (n= 212). In the 15-40 group, the median duration to PDR was 20.0 (IQR 16.0-27.2) years (n= 76). The observation of longer duration to PDR in patients with the age at onset less than 5 years, is in line with earlier findings regarding DRP and nephropathy (198, 200, 283). The present study adds to this by showing that the long-term risk of PDR was no different between the age at onset groups of 0-4 years and 5-14 years despite the initial advantage for those with earlier onset of diabetes. Ultimately, the cumulative hazard curves for these two groups cross each other when the duration of diabetes approaches 30 years (Fig. 17).

There are several reasons why the youngest patients may have a lower initial risk of PDR. The advantage for the youngest patients may be related to better self-care skills. It has been shown that a good self-care of diabetes correlates with good metabolic control (284). It may be easier to learn good self-care skills at a very young age as compared to the prepubertal period (283). Learning good self-care skills and adjusting to diabetes may be more difficult for prepubertal children as compared to the younger children which would explain the delayed onset of PDR in the youngest patients. The benefit of better glycaemic control may be compounded by a less steep decline in the endogenous insulin production. This is because a more stringent management of type 1 diabetes in children has been shown to reduce the decline in insulin production (285). In addition to factors related to learning and adjusting, hormonal changes in the puberty may contribute to an initially worse metabolic control in prepubertal children (286) and, at least, partially explain the initial difference in the risk of PDR.

10.3.2 LOWER RISK OF PDR IN PATIENTS WITH AGE AT ONSET AFTER 15 YEARS

In contrast to the younger onset patients, the cumulative risk for PDR in those patients with the age at onset of type 1 diabetes between 15-40 years appears to be consistently lower. The onset of diabetes after the age of 12 years has been associated with a lower risk of PDR in EURODIAB study as well (185). The lower risk may be due to the fact that the diabetes onset after puberty has been linked to a less aggressive form of type 1 diabetes (287), and it has also been observed that β-cells are better preserved when type 1 diabetes begins in adulthood (288). The better prognosis in older onset patients could, thus, be explained by the preservation of β-cells as the patients with later diabetes onset also had the highest C-peptide concentrations. The DCCT data has previously indicated that patients with any residual C-peptide secretion, but especially those with the highest stimulated concentrations, had a reduced incidence of DRP and nephropathy (204). However, the role of C-peptide has been somewhat controversial since it has not been linked to DRP in other studies (131). The association of age at onset with the risk of DRP may not only be limited to type 1 diabetes. It has been noted that a higher age at onset of diabetes reduces the risk of DRP in type 2 diabetes patients as well (203).

10.3.3 LATER AGE AT ONSET OF TYPE 1 DIABETES INCREASES THE RISK OF MACULAR EDEMA

In contrast to PDR, those patients with the age at onset of type 1 diabetes between ages 15-40 years may have a consistently higher risk of CSME than any patient group with the age at onset below 15 (Fig. 20). The patients with the highest age at onset had the highest cumulative incidence and risk of CSME, even when potential risk factors were taken into account. After 30 years of diabetes, the cumulative incidences for CSME were 34% for the age at onset group of 15-40 years, 27% for the age onset group 5-14 years, and 17% in those diagnosed between 0-4 years of age. The corresponding HRs were 3.72 and 1.89 as compared to the youngest (0-4 years) age at onset group.

Because CSME is more common in older patients of whom many have type 2 diabetes, the goal of this study was initially to search for such features in patients with type 1 diabetes that could potentially explain why certain patients are more susceptible to CSME than others. It has indeed been shown in the FinnDiane study that it is possible for patients with type 1 diabetes to have features of type 2 diabetes as well (19, 289). An extensive statistical testing was, thus performed with typical features of type 2 diabetes as the primary suspects for CSME. About a hundred different statistical models

were tested with different combinations of various risk factors. These included eGDR (mg• kg-1•min-1), and triglycerides as surrogate markers for insulin resistance. The age of the patients was also included as well as the BP, BMI, and WHR. However the only associated risk factors, according to the strict BIC were the age at onset of type 1 diabetes, ETDRS-score, HbA_{1c}, and total cholesterol. Prospective studies have shown an increased risk of DME if the baseline cholesterol is high (290). In the ETDRS study HbA_{1c} and cholesterol were the only factors that predicted the development and persistence of severe visual loss due to PDR and DME (166). In this study, these two factors also produced the best fitting model which, thus, provides further support for the role of cholesterol as a risk factor for CSME. Other typical features of type 2 diabetes were less important as they were consistently more associated with a higher ETDRS score than CSME. This meant that the inclusion of these factors together with the ETDRS score deteriorated the fit of the regression equation and rendered them nonsignificant.

10.3.4 NEPHROPATHY AND MACULAR EDEMA IN TYPE 1 DIABETES

End-stage renal disease correlated strongly with the ETDRS score (r_s = 0.64, P<0.001) and, probably because of this, it was not a significant risk factor for CSME if included in the model together with the ETDRS score (P=0.150, Wald test). The ETDRS score even overshadowed the BP in the model selection. The impact of diabetic nephropathy has not always been evident in previous studies either (108), although it has been noted that albuminuria is associated with DME (291), and that renal replacement therapy may stabilize the DRP status and reduce DME as a result of correction of overhydration (177). It is also known that microalbuminuria predicts the development of PDR in patients with type 1 diabetes (292). However, there is still some controversy whether this association is more due to hyperglycaemia or whether nephropathy is truly an independent predictor for DRP. It has been observed, though, that after renal transplantation or initiation of dialysis visual function stabilizes (178). The stabilization could also be due to other factors, such as lower BP during renal replacement therapy (177).

10.3.5 AGING ITSELF MAY BE A MODIFIER OF PROLIFERATIVE RESPONSES

Higher age of the patient could at least partially explain the difference in the responses to hyperglycaemia-induced cellular injury. Hyperglycaemia exerts its detrimental effects only after a certain period of hyperglycaemic exposure. The patients who have had an older age at onset of type 1 diabetes are older

when they have had a long enough hyperglycaemic exposure to cause significant cellular injury. Recent findings in research on ageing suggest that cellular senescence contributes to the development and progression of many disease states (293). The predominant ageing mechanism of mitotic tissues is thought to be due to the gradual accumulation of senescent cells. Senescent cells have undergone an irreversible cell cycle arrest as opposed to most mitotic cells that are found in a reversible growth arrested state known as quiescence. How often the quiescent cells proliferate is dependent upon how frequently cells become damaged or lost. The proliferative response to cellular injury is, thus, attenuated as a result of ageing (293). The immunological responses are also altered by the natural ageing process which leads to a gradual deterioration of the immune system (294). An attenuated proliferative response to intraocular cellular injury can be seen after cataract surgery where old age significantly decreases epithelial cell proliferation and posterior capsule opacification as compared to the younger patients (295). In a similar fashion, the age at onset of type 1 diabetes may determine the specific biological response to the hyperglycaemic cellular injury. The distinct significance of the age at onset as a risk factor for CSME is in line with previous studies showing a higher incidence of CSME in older patients with type 1 and, especially, with type 2 diabetes (130, 132, 140). In addition to cellular senescence, ageing and hypertension may also cause structural changes in the retinal vasculature (296), although the inclusion of BP into the regression model did not improve the fit of the model.

10.4 HbA_{1C} VARIABILITY AS A RISK FACTOR FOR RETINOPATHY

10.4.1 HbA_{1C} VARIABILITY INCREASES RISK FOR RETINOPATHY REQUIRING LASER TREATMENT

The need for laser treatment was higher in those patients who had the widest HbA_{1c} variability. PDR is the most common indication for laser treatment in type 1 diabetes and a large HbA_{1c} variability was associated with an increased risk of PDR. The five-year cumulative incidence estimates of laser treatment were 19.1% in the highest quartile of HbA_{1c} variability as compared to 9.5% in the lowest quartile with 1.6 times higher adjusted risk of laser treatment. Analysis of the DCCT data has previously shown that HbA_{1c} variability was associated with a three-step progression of DRP on the 12 step ETDRS scale (173). This study adds to that knowledge by showing that there may be clinically significant consequences from HbA_{1c} variability, such as laser treatment and PDR. This is true even if the renal status was taken into account as a confounding factor in the statistical analyses. Unlike in the

DCCT, the results obtained in this study reflect a normal clinical setting without any confounding interventions on the HbA_{1c}.

10.4.2 HbA_{1C} VARIABILITY AND POSSIBLE CONFOUNDING FACTORS

Although there was an association between laser treatment, PDR, and HbA_{1c} variability, it still remains to be seen whether HbA_{1c} variability truly causes the DRP to progress. There may be confounding factors that explain the association between HbA_{1c} variability and DRP progression. One such factor may be insulin resistance which is associated with HbA_{1c} variability and has been implicated in the pathogenesis of diabetic complications (297). Higher triglycerides were noted in those patients who were in the highest quartile of HbA_{1c} variability. This supports the possible association with insulin resistance although the inclusion of triglycerides in the models did not change the overall significance of HbA_{1c} variability in either subcohort. Another possible mechanism may be the exponential fashion by which both higher mean HbA_{1c} and higher HbA_{1c} variability increases the risk of microvascular complications (173). Thus, even short periods of higher HbA_{1c} could significantly increase the risk of PDR, even in the presence of comparable mean HbA_{1c} levels. However, the inclusion of first and second order interaction terms in the regression models to account for the exponential risk that increased with higher mean HbA_{1c} and higher HbA_{1c} variability did not change the results in Fine & Gray regression models. Furthermore, the ETDRS score and the mean HbA_{1c} did not show any exponential relationship which suggests that other mechanisms are also likely to play a role.

The present study may be biased by the fact that the serial HbA_{1c} values were collected from available laboratory records as part of the patients' routine clinical follow-ups. Thus, there were no prespecified intervals between the HbA_{1c} measurements, and also the number of measurements per individual patient varied. Another potential limitation is the use of HbA_{1c} measured at the local study centres, not in a central laboratory. However, the individual patient's measurements were performed at the same centre and the Fine & Gray regression models accounted for the possible correlations within the participating study centres. The regression models were adjusted for both the number of HbA_{1c} measurements and the mean HbA_{1c}. Moreover, the standardisation of the HbA_{1c} measurement intervals to one year by resampling of the original measurements retained the significance for variability quartiles as risk factor for both PDR and laser treatment.

10.4.3 HbA_{1C} VARIABILITY AND PATHOGENESIS OF DIABETIC RETINOPATHY

A possible impact of HbA_{1c} fluctuation itself is supported by the fact that a short term DRP worsening has been observed in patients with improved glycaemic control. This has been shown in many studies (157, 167-171). Interestingly, the short term glucose fluctuations do not appear to increase the risk of DRP progression (298). The difference could be related to "metabolic memory" in which periods of even short hyperglycaemia place patients at higher risk (159).

Another potential explanation for this discrepancy is the intermittent, more intensive use of insulin. Insulin induces the expression of VEGF and together with stabilized HIF-1α may explain the initial worsening of DRP which is observed in improved glycaemic control (103, 104). Repeated cycles of intensive insulin use could, therefore, result in a sustained DRP progression. There are, however, many other possible cellular mechanisms that may be involved, such as the induction of growth factor IGF-1 (299, 300) and impaired endothelial function (301). Other detrimental in vitro cellular effects due to oscillating glucose have been noted as well (302-304).

10.4.4 AGE AT ONSET AND LESS VARIABLE HbA_{1C}

A possible factor behind a less variable HbA_{1c} may be the age at onset. The patients with a higher age at onset had less variable HbA_{1c} measurements. As noted before, the β-cells are better preserved when type 1 diabetes begins in adulthood (288). The DCCT data indicated that patients with any residual C-peptide secretion had a reduced incidence of DRP and nephropathy (204). Thus, the preservation of β-cells could explain the connection between a lower risk of PDR and less variable HbA_{1c} measurements. The patients with PDR in the present study had the lowest C-peptides which supports this hypothesis.

11 SUMMARY AND CONCLUSIONS

I. PDR in type 1 diabetes showed familial clustering which could not be accounted for by conventional risk factors. If the oldest sibling in the sibship had PDR, the risk for all the subsequent siblings increased. The heritability estimate suggests the presence of a multifactorial familial component in the development of PDR.

II. The age at onset of type 1 diabetes modified the risk of PDR. The patients diagnosed with type 1 diabetes before 15 years of age had a higher risk of PDR than patients diagnosed after 15 years of age.

III. A wide HbA_{1c} variability increased the risk of severe retinopathy requiring laser treatment. The patients in the highest quartile of HbA_{1c} variability had a 1.6 times higher adjusted risk as compared to patients in the lowest quartile.

IV. There was a significant association between the age at onset of type 1 diabetes and the risk of CSME. The higher the age at onset of type 1 diabetes, the higher the risk of CSME. This observation could not be explained by risk factors associated with old age or features typical of type 2 diabetes. This indicates a potential role for the biological changes related to ageing itself in determining the DRP phenotype.

V. The siblings first affected by type 1 diabetes were younger than the later affected siblings. The first affected siblings had a lower risk of PDR which was most likely related to their longer prepubertal diabetes duration.

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14 INDEX

APPENDIX

THE FINNISH DIABETIC NEPHROPATHY STUDY CENTERS

Anjalankoski Health Center	S.Koivula, T.Uggeldahl
Central Hospital of Central Finland, 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, 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 Hospital of Ostrobothnia, Kokkola	S.Anderson, B.Asplund, U.Byskata, P.Liedes, M.Kuusela, T.Virkkala
City of Espoo Health Centers:	
Espoonlahti	A.Nikkola, E.Ritola
Tapiola	M.Niska, H.Saarinen
Samaria	E.Oukko-Ruponen, T.Virtanen
Viherlaakso	A.Lyytinen
City of Helsinki Health Centers:	
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 Centers:	
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 Central Hospital,	

Department of Medicine, Division of Nephrology	A.Ahola, M.Feodoroff, D.Gordin, O.Heikkilä, K.Hietala, J.Kytö, S.Lindh, K.Pettersson-Fernholm, A.Sandelin, L.Thorn, J.Tuomikangas, T.Vesisenaho, J.Wadén
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, Espoo	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, 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, 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	E.Korpi-Hyövälti, T.Latvala, E.Leijala
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	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