Candidate Genes for Late Diabetic Complications

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2007

Candidate Genes for Late Diabetic Complications

Academic dissertation

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LUNDS
UNIVERSITET
Medicinska fakulteten

With the permission of the Medical Faculty of Lund, to be presented for public examination at the Clinical Research Centre, Malmö University Hospital, December 4, 2007 at 9.15 a.m.

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ISSN 1652-8220

ISBN 978-91-85897-25-4

Printed by Media-Tryck, Lund University, Lund, Sweden

Cover picture: © HSB Turning Torso, Fotograf: Pierre Mens

Wer sie nicht kennte
Die Elemente,
Ihre Kraft
Und Eigenschaft,
Wäre kein Meister
Über die Geister.

Johann Wolfgang von Goethe
Faust

(Den som ej känt
vart element,
ej vet att märka,
huru de verka,
kan ej befalla
andarne alla)

(översättning Viktor Rydberg)

Organization	Document name
LUND UNIVERSITY Faculty of Medicine Department of Clinical Sciences Malmö University Hospital	DOCTORAL DISSERTATION
	Date of issue December 4th 2007
	Sponsoring organization
Author(s) Eero Lindholm	

Title and subtitle

Candidate Genes for Late Diabetic Complications

Abstract

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The new WHO criteria for classification of diabetes takes into account also clinical stages dividing the diabetic patients into not insulin requiring (NIR), insulin requiring for control (IRC) and insulin requiring for survival (IRS) subgroups. Diabetic complications are the result of chronically elevated blood glucose. Genetic factors are believed to play role in pathogenesis of diabetic complications.
The aim of this study was
1) To test the usefulness of the new WHO criteria for clinical staging of diabetes in the characterization of diabetic patients.
2) To test a putative association between late diabetic complications and candidate gene polymorphisms.
In study I we could show that the WHO clinical staging of diabetes could discriminate between clinically meaningful subgroups. The IRC patients represented a group with more severe diabetes than acknowledged in the etiological classification with high frequency of diabetic complications. In study II we demonstrated that polymorphisms in the UCP1-3 genes did not play a major role in the development of micro- or macroalbuminuria in Scandinavian diabetic patients. In study III we showed that a polymorphism in the MHC class II transactivator gene (MHC2TA) was associated with cardiovascular mortality and predictors of cardiovascular mortality, microalbuminuria and metabolic syndrome. In study IV and V we showed that polymorphisms in the LTA, TNF and AGER genes were associated with diabetic complications. The association was complex and dependent on the HLA-DQB1 genotypes, with partly different alleles conferring susceptibility in type 1 and type 2 diabetic patients. We cannot exclude that these genes are a part of a large haplotype block that also includes HLA-DQB1 risk genotypes.
Although this study revealed several associations with putative candidate gene polymorphisms and diabetic complications, the studied polymorphisms can only explain part of the genetic risk factors for diabetic complications. More studies are needed to enable mapping of the susceptibility genes for diabetic complications. Revealing the genetic risk factors could help us to identify the patients at risk and understand the pathogenesis of diabetic complications and making it possible to find novel treatments for diabetic complications.

Key words: diabetes mellitus, nephropathy, retinopathy, neuropathy, genetic, UCP, HLA-DQB1, MHC2TA, AGER, TNF, LTA

Classification system and/or index termes (if any):

Supplementary bibliographical information:	Language English
ISSN and key title: ISSN 1652-8220	ISBN 978-91-85897-25-4
Recipient's notes	Number of pages 117 / Price
	Security classification

Distribution by (name and address)

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Contents

List of original publications

I. **Lindholm E**, Agardh E, Tuomi T, Groop L, Agardh C-D. Classifying diabetes according to the new WHO clinical stages. *Eur J Epidemiol* 17: 983-989, 2001

II. **Lindholm E**, Klannemark M, Agardh E, Groop L, Agardh C-D. Putative role of polymorfisms in *UCP1-3* genes for diabetic nephropathy. *J Diabetes Complications* 18:103-107, 2004

III. **Lindholm E**, Melander O, Almgren P, Berglund G, Agardh C-D, Groop L, Orho-Melander M. Polymorphism in the *MHC2TA* gene is associated with features of the metabolic syndrome and cardiovascular mortality. *PLoS ONE* Dec 20;1:e64, 2006.

IV. **Lindholm E**, Bakhatadze E, Sjögren M, Cilio CM, Agardh E, Groop L, Agardh C-D. *RAGE* –374 T/A polymorphism is associated with type 1 *HLA-DQB1* risk genotypes and late diabetic complications. *Diabetologia.* Nov;49(11):2745-55, 2006.

V. **Lindholm E**, Bakhatadze E, Cilio CM, Agardh E, Groop L, Agardh C-D. Linkage disequilibrium between *LTA*, *TNF* and *AGER* polymorphisms and their association to late diabetic complications. (*Submitted*), 2007.

Abbreviations

ACE	angiotensin-I converting enzyme
AER	Urinary albumin excretion rate
AGER	Gene encoding the receptor for advanced glycation end-products
BMI	Body mass index
CHD	Coronary heart disease
DAG	1,2-diacylglycerol
DCCT	Diabetes Control and Complications Trial
DME	Diabetic macular edema
DNA	Deoxyribonucleid acid
DNs	Diabetic neuropathies
DPN	Diabetic peripheral neuropathy
ESRD	End stage renal disease
GADA	Glutamic acid decarboxylase antibody
HLA	Human leukocyte antigene
ICAM-1	Intracellular adhesion molecule-1
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IL	Interleukin
IRC	Insulin requiring for control
IRS	Insulin requiring for survival
LOD	Logarithm of the odds
LTA	Lymphotoxin alpha
MDC	Malmö Diet and Cancer Study
MHC2TA	Major histocompatibility complex class II transactivator gene
MI	Myocardial infarction
NAPDH	Nicotinamide adenine dinucleotide
NIR	Not insulin requiring
OGTT	Oral glucose tolerance test
PCR	Polymerase chain reaction
PDR	Proliferative diabetic retinopathy
PKC	Protein kinase C
RAAS	Renin angiotensin aldosterone system
RBX	Ruboxistaurin
ROS	Reactive oxygen species
T1D	Type 1 diabetes mellitus
T2D	Type 2 diabetes mellitus
TNF	Gene encoding for tumor necrosis factor alpha
UCP	Uncoupling protein
UKPDS	United Kingdom Prospective Diabetes Study
VEGF	Vascular endothelial growth factor
WHO	World health organisation

1. Introduction

The earliest known record of diabetes mentioned comes from the 3rd dynasty Egyptian papyrus by the physician Hesy-Ra [1]. The word diabetes means “going through” and was used by Aretaeus of Cappadocia in the 2nd century AD. The word mellitus is latin and refers to the sweetness of the urine from a diabetic subject [1]. The British physician George Harley commented in 1866 that “there are at least two distinct forms of the disease” and the French physician Etienne Lancereaux made a distinction between fat and thin diabetes: “Le diabete gras et le diabete maigre” [2]. This was indeed an important distinction as most of the children and young adults with diabetes died within a few months whereas the older persons who were only treated with diet could survive for years.

Until the early twentieth century the chances of a young diabetic surviving long enough to develop diabetic complications were poor, and the chances of an older patient to survive the attendant vascular complications long enough to develop diabetic nephropathy or retinopathy was equally poor.

Despite the obvious difficulties, the discovery of diabetic complications streches back to the eighteenth and nineteenth centuries. Domenico Cotugno (1736-1822) wrote in 1770 propably the first proper description of proteinuria, noticing that he had seen diabetics with coagulable urine. The first one to suggest that the albuminuria seen in patients was caused by diabetes (and not the opposite) was the German physician Wilhelm Griesinger (1817-1868) [3].

Diabetic retinopathy was first documented by Eduard Jaeger in 1855 and proliferative retinopathy was described in 1876 by Wilhelm Manz [4]. The first clinical description of diabetic neuropathy was done by John Rollo of London in 1798, who described the pain and paresthesia in the legs of diabetic patients [5].

The discovery of insulin by Banting and Best in 1921 did indeed revolutionize the treatment of diabetes, but it also transformed the diabetes from an acute fatal illness to a chronic disease with serious long-term complications. Dr. Elliot P. Joslin wrote in 1931, only ten years after the discovery of insulin: “With the advent of insulin, we moved from the era of diabetic coma to the era of diabetic complications" [6].

Paul Kimmelstiel and Clifford Wilson published in 1935 a paper describing details of nodular renal lesions in eight maturity-onset (48-68 year old) diabetic patients. It was however Arthur Allen that in 1941 established these lesions to be specific for diabetes and by no means rare [3]. Similarily, although diabetic retinopathy was described earlier, it was first in 1943 that Arthur James Ballantyne suggested that diabetic retinopathy is a unique vasculopathy and not only a product of hypertension or atherosclerosis [4]. The final acceptance for the concept that diabetic microangiopathy was specific for diabetes came after work of Knud Lundbæk [7].

The risk of developing diabetic nephropathy in type 1 diabetic patients before 1950 was nearly 50% and seven years after the onset of proteinuria 49% of the patients had died [8]. The prevalence of proliferative diabetic retinopathy (PDR) in the Wisconsin epidemiologic study of diabetic retinopathy (1980-1982) was 60% after 35 years of diabetes. The risk of severe visual loss from PDR is approximately 40% six years after onset of PDR if not treated with laser photocoagulation The prevalence of legal blindess in the Wisconsin study was 3% in patients with a diabetes duration over 15-19 years and increased to 12% in those with a duration $\geq$ 30 years [9].

Several factors such as self monitoring of blood glucose, screening program and laser treatment of proliferative diabetic retinopathy and macular edema, more effective management of blood pressure treatment with ACE inhibitors have all contributed to better management of diabetes and decline in both nephropathy [10-14] and retinopathy [11, 14] in type 1 diabetic (T1D) patients as well as in type 2 diabetic (T2D) patients. Discovery of

glycated hemoglobin (HbA_{1c}) finally made it possible to show in large studies such as the Diabetes Control and Complications Trial (DCCT) and the UK Prospective Diabetic Study (UKPDS) that better metabolic control could prevent diabetic complications in both T1D and T2D patients, respectively [15, 16].

The last decades have also meant new challenges as the prevalence of T2D is increasing (www.who.int) and better management of cardiovascular complications in T2D patients means longer survival and sufficient time to develop severe microvascular complications such as diabetic nephropathy and proliferative retinopathy. Diabetic nephropathy is today the most common cause of new cases of end stage renal disease (ERDS) in need for dialysis or renal replacement therapy in Sweden and the number of T2D patients with ERDS is increasing [17]. Diabetic retinopathy remains a major cause of severe visual impairment in the Western world [18].

2. Diabetes mellitus- diagnosis and classification

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Table 1 summerizes changes in the WHO diagnostic criteria for diabetes mellitus and intermediate hyperglycemia over time. The first WHO guidelines for the diagnosis and classification of diabetes was published in 1965 [19]. The first widely accepted classicification system was published by WHO in 1980 [20] and modified in 1985 [21]. This classification included two major types of diabetes; insulin dependent (IDDM) and non-insulin dependent (NIDDM) diabetes mellitus. It was a compromise between clinical and etiological classifications and allowed classification of patients even when the specific cause or etiology was unknown. As more data on etiology and the importance of intermediate non-diagnostic glucose values emerged, a new diagnostic criteria and classification system was introduced by WHO in 1999 [22].

Table 1. Changes in WHO diagnostic criteria for diabetes and intermediate hyperglycemia over time.

Year	**1965**	**1980**	**1985**	**1999**
Normal		Not defined	Not defined	
Fasting P-Glucose	Not specified			<6.1 mmol/l
2-h P-glucose	<6.1 mmol/l			Not specified but <7.8 mmol/l implied
IFG	Not defined	Not defined	Not defined	
Fasting P-Glucose				≥6.1 and <7.0 mmol/l and
2-h P-glucose				<7.8 mmol/l (if measured)
IGT	Referred to as borderline state			
Fasting P-Glucose		<8.0 mmol/l and	<7.8 mmol/l and	<7.0 mmol/l and
2-h P-glucose	6.1–7.1 mmol/l	≥8.0 and <11.0 mmol/l	≥7.8 and <11.1 mmol/l	≥ 7.8 and <11.1 mmol/l
Diabetes				
Fasting P-Glucose	Not specified	≥8.0 mmol/l and / or	≥7.8 mmol/l or	≥7.0 mmol/l or
2-h P-glucose	≥7.2 mmol/l	≥11.0 mmol/l	≥11.1 mmol/l	≥11.1 mmol/l

From Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation. World Health Organization 2006 [23].

Diagnostic criteria were based on either a fasting plasma glucose concentration above 7.0 mmol/l on two different days or a 2-hour 75 g oral glucose tolerance test (OGTT) value above

11.0 mmol/l [22]. A new category of impaired fasting glucose (IFG) was introduced and defined as fasting plasma glucose values below the diagnostic cut-off for diabetes (<7.0 but ≥6.1 mmol/l). Impaired glucose tolerance (IGT) was now defined as a 2-h glucose ≥7.8 but <11.1 mmol/l and fasting plasma glucose <7.0 mmol/l.
The diagnostic fasting plasma glucose concentration was lowered because several studies showed increased risk of microvascular [24] and macrovascular complications [25] in patients with normal 2-h OGTT values but fasting plasma glucose values ≥7.0 mmol/l.
Fasting plasma glucose of 7.0 mmol/l will also in most subjects represent a diagnostic 2-h post-load concentration [24, 25] and seems as an optimal cut off level to separate the bimodal frequency distribution of fasting plasma glucose levels [24].
In 2003 the American Diabetes Association (ADA) reviewed its diagnostic criteria and recommended a plasma glucose level of 5.6 mmol/l as a new treshold for IFG. The latest WHO report recommends that the current diagnostic criteria for diabetes should be maintained [23]. Because lack of evidence for benefits of lowering the cut off the study group recommended that the current cut off for IFG should be left unchanged. They also recommended the use of OGTT as a diagnostic test because it is the only way to identify IGT and because fasting plasma glucose alone fails to diagnose 30% of the patients with diabetes [23].

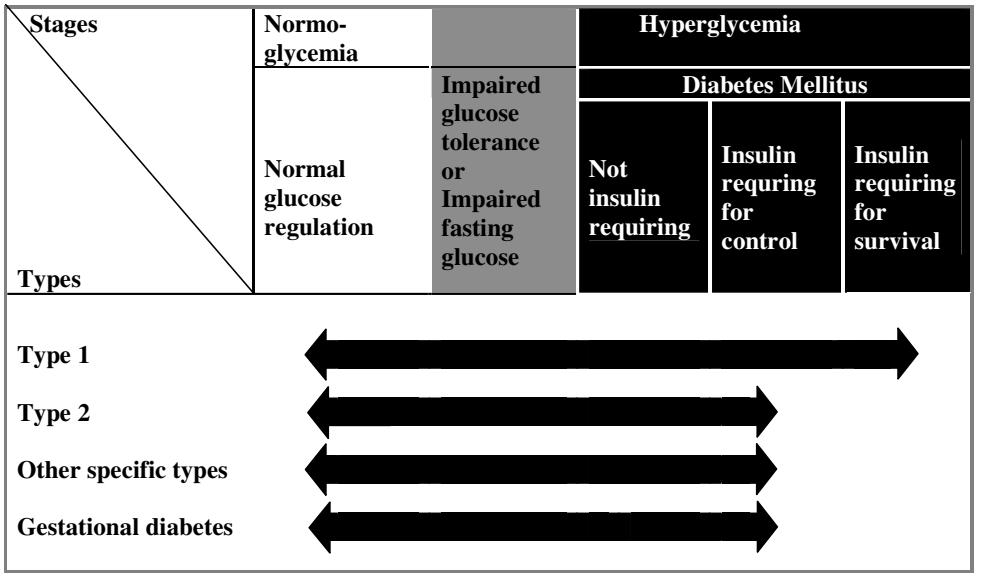

Figure 1. Disorders of glycemia, etiological types and clinical stages. From [22].

The new classification system of diabetes encompasses both etiological types and clinical stages of diabetes and different categories of hyperglycemia [22] (Figure 1). The etiological types are: 1) T1D, usually autoimmune, sometimes idiopathic, 2) T2D, which may range from predominantly insulin resistant with a relative insulin defiency to a predominantly secretory defect with or without insulin resistance, 3) other specific types of diabetes, including monogenic forms, diseases in endocrine pancreas and 4) gestational diabetes.
Besides etiological processes, the new WHO criteria for classification of diabetes takes into account also clinical stages based upon the degree of glycemia and mode of treatment. The pre-diabetic stages are IFG and/or IGT. Diabetes mellitus is subdivided into three clinical

stages: not insulin requiring (NIR), insulin requiring for control (IRC) and insulin requiring for survival (IRS). All patients with diabetes mellitus can be categorized according to clinical stages and the stage of glycemia may change over time so that individual patients can move from one stage to another in either direction. The clinical classification is therefore a complement to the etiological classification, even though the WHO classification does not clearly define the difference between IRC and IRS stages [22].

3. Diabetic complications

3.1 Epidemiology

3.1.1 Natural history and epidemiology of diabetic nephropathy

The renal involvement in diabetes mellitus is a gradual process and rather well defined in T1D. Mogensen [26] suggested that the development of renal changes may be divided into five different stages (Table 2). Stage 1 is present at diagnosis of diabetes and includes hyperfunction/ hypertrophy of the kidneys. Urinary albumin excretion rate (UAE) may be increased, however not permanently. Most of the abnormalities seen at this stage may be reversed with improvement of metabolic control by initiation of insulin treatment. Stage 2 usually lasts at least five years from diagnosis. The glomerular filtration rate (GFR) is increased and exercise-induced microalbuminuria may be present. Blood pressure is however normal. Stage 3 is present typically after 6-15 years of diabetes and UAE is 20-200 µg/min. Overt nephropathy (stage 4) occurs usually 15-25 years from the onset of T1D. GFR declines 10 ml/min per year and if not treated, the blood pressure is high. Stage 5 represents the final outcome of diabetic kidney disease with ESRD and usually occurs 25-30 years or more after diagnosis of diabetes. GFR is now <10 ml/min and blood pressure is always high if untreated.

Table 2. Stages of diabetic nephropathy.

	Stages	Chronology	GFR	Baseline UAE	Exercise induced UAE	Main structural changes	BP
1	Hyperfunction / hypertrophy	At diagnosis	↑↑	Normal or ↑	↑	Kidney hypertrophy, increased glomerular size	Normal
2	Normo-albuminuria	First five years	↑↑	Normal	Normal or ↑	Increasing basement membrane thickness and mesangium expansion	Normal, Increase by 1 mmHg/ year
3	Incipient diabetic nephropathy	After 6-15 years from diagnosis (in ≈ 35% of patients)	↑	20-200 µg/min	↑		Increase by 3 mmHg/ year
4	Proteinuria	After 15-25 years from diagnosis.	↓↓	>200 µg/min, increasing	↑↑↑	Increasing glomerular occlusion	High
5	End-stage renal failure	After 25-30 years from diagnosis	↓↓↓	Decline	Not studied	and severe mesangial expansion	High

Adapted from [26]. BM=basement membrane, UAE=urinary albumin excretion rate, BP= blood pressure

The course of kidney disease seems to be similar in T2D [26] with some important exceptions. High blood pressure is normally present in T2D patients even before the onset of

diabetic nephropathy. Because T2D can remain undiagnosed several years, microvascular complications like nephropathy may be present already at the time of diagnosis.
The peak incidence of nephropathy in T1D is 15 years from the onset of diabetes. Patients who do not develop nephropathy during the first 20-25 years of diabetes have a very low risk to develop nephropathy later on (about 1 % per year) [27]. It has been estimated that diabetic nephropathy will ultimately develop in 35 % of the patients with T1D [8]. The incidence of diabetic nephropathy in T1D patients is however declining as shown in several populations [10-14].
The prevalence of diabetic nephropathy has previously been reported to be lower in T2D than in T1D patients. The prevalence of diabetic nephropathy seems to differ between different ethnic groups. Because proteinuria is a risk factor for cardiovascular disease it is possible that previous studies underestimate the prevalence of diabetic nephropathy. It seems that the risk for proteinuria at any given duration of diabetes is similar in both T1D and T2D. As 90% of the diabetic patients have T2D and the diabetes prevalence is increasing, there has also been a rise in the prevalence of diabetic nephropathy and diabetes is now the most common single cause of ESRD in Europe [28].

3.1.2 Natural history and epidemiology of diabetic retinopathy

The early stage of diabetic retinopathy (DR) is characterized by loss of retinal pericytes. This is followed by development of weakness in the capillary wall that leads to formation of microaneurysm and leakage from capillaries as their walls become more permeable. Impaired vascular function gradually develops leading to areas of ischemia and infarction. In response to these changes local growth factors are secreted that contribute to new vessel formation.[29]. Macular edema (ME), and proliferative retinopathy are the two major sight threatening manifestations of diabetic retinopathy and they represent the end manifestations of increased vascular permeability and vascular occlusion.
Several clinical classifications have been proposed and in 2002 the Global Diabetic Retinopathy Project Group agreed upon a retinopathy scale, which consists of five different stages (Table 3).

Table 3. Diabetic Retinopathy Disease Severity Scale*

Proposed disease severity level	Findings observable on dilated ophtalmoscopy
No apparent	No abnormalities
Mild nonproliferative diabetic retinopathy	Microaneurysms only
Moderate nonproliferative diabetic retinopathy	More than just microaneurysms but less than severe nonproliferative diabetic retinopathy
Severe nonproliferative diabetic retinopathy	Any of the following: more than 20 intraretinal hemorrhages in each of 4 quadrants; definite venous beading in 2+ quadrants; Prominent intraretinal microvascular abnormalities in 1+ quadrant and no signs of proliferative retinopathy
Proliferative diabetic retinopathy	One or more of the following: neovascularization, vitreous/preretinal hemorrhage

*From [30].

Macular edema (if present) is diveded in three cathegories: 1) Mild with some retinal thickening or hard exudates in the posterior pole but distant from the center of the macula, 2) Moderate with retinal thickening or hard exudates approaching the center of the macula but

not involving the center, and 3) Severe with retinal thickening or hard exudates involving the center of the macula [30].
DR is still the leading cause of blindness in older adults (45-74 years) accounting for more than one third of the cases and the fourth common cause of blindness in younger adults (15-44 years) in the Western world [18]. The prevalence of diabetic retinopathy is correlated to diabetes duration but unlike nephropathy, retinopathy shows no decline in incidence after 15-20 years of diabetes duration. The Wisconsin study reported a 70% overall prevalence of diabetic retinopathy in T1D patients (onset of diabetes before 30 years) and 39% in insulin treated T2D patients (age at onset ≥30 years). Prevalence of proliferative diabetic retinopathy (PDR) was 23% and 14%, respectively, and clinically significant ME 14% and 11% in T1D and insulin treated T2D patients, respectively [31]. With increased duration retinopathy prevalence reaches almost 100% in T1D patients and 85% in patients with insulin treated T2D [32]. The incidence of severe retinopathy seems to be declining, perhaps due to better metabolic control [11, 14]. In a 14-year follow-up to the Wisconsin Epidemiologic Study of Diabetic Retinopathy, the cumulative incidence of PDR over a period of 15 years in persons with T1D was still 37% but there appeared to be a decline in the estimated annual rates of progression to proliferative retinopathy and the incidence of ME in the last 4-year period of the study compared to earlier periods of the study [33]. A Swedish study from Linköping showed also that the cumulative proportion of severe retinopathy in T1D patients diagnosed in childhood is declining [11].

3.1.3 Natural history and epidemiology of diabetic neuropathy

Diabetic neuropathy encompasses a wide range of nerve abnormalities and is common, with prevalence rates reported between 5–100% depending on the diagnostic criteria [34-36]. Due to the variaty of clinical manifestation there is no universally accepted classification of diabetic neuropathy. Neuropathy is often divided into sensorimotor and autonomic neuropathy (Table 4).

Table 4. Classification of diabetic neuropathy

Sensorimotor neuropathy
Distal symmetric polyneuropathy
Focal neuropathy
Diabetic mononeuropathy (cranial, truncal, peripheral nerves)
Mononeuropathy multiplex
Diabetic amyotrophy
Autonomic neuropathy
Hypoglycemic unawareness
Abnormal pupillary function
Cardiovascular autonomic neuropathy
Vasomotor neuropathy
Sudomotor neuropathy (sweat glands)
Gastrointestinal autonomic neuropathy
Gastric atony
Diabetic diarrhea or constipation
Fecal incontinence
Genitourinary autonomic neuropathy
Bladder dysfunction
Sexual dysfunction

From [37].

In this study we have only considered distal symmetric polyneuropathy also known as diabetic peripheral neuropathy (DPN). DPN is a causal factor in most foot ulcerations and the incidence of foot ulcers increases three-fold in patients with DPN [38]. Several studies have suggested that improved glycemic control will be beneficial in preventing diabetic nephropathy [15, 39, 40]. There are however no population based studies on trends in the incidence of neuropathy. Treatment strategies of diabetic neuropathy based on pathogenetic mechanisms like aldose reductase inhibitors or protein kinase C inhibitor (ruboxistaurin) have been disoppointing so far [41, 42].

3.1.4 Natural history and epidemiology of macrovascular complications

Macrovascular disease is the leading cause of mortality in subjects with diabetes and also a major cause of morbidity [43]. The major manifastations of macrovascular disease include heart disease, cerebrovascular disease and peripheral vascular disease. The prevalence and incidence of macrovascular disease in diabetes mellitus is still not very well studied [43] and there are many sources for potential biases. Both diabetes mellitus and macrovascular disease are common conditions and can therefore occur together by chance. Both diseases can also be subclinical for years and silent coronary heart disease is more common in diabetic than non-diabetic subjects [44] which might underestimate the prevalence. T2D patients tend to have a more severe diabetes with longer duration and studies based on subjects with already known diabetes are likely to give falsely high prevalence of macrovascular disease in T2D patients [43]. The risk for coronary heart disease is almost 10-fold in T1D men and even greater in women compared to non-diabetic subjects [43]. T2D male patients have a 2-3 fold risk of coronary heart disease, and the risk is even greater in women. The risk for stroke is approximately 2-fold increased in persons with known or newly diagnosed diabetes [45]. Diabetes is also a well known risk factor for peripheral vascular disease. The prevalence of intermittent claudication was 3 times higher in diabetic men and almost 6 times higher in diabetic women than in non-diabetic subjects of the same sex [46]. The prevalence of peripheral vascular disease in the UKPDS was 1.2% at diabetes diagnosis and increased to 12.5% by 18 years [47] with hyperglycemia, dyslipidemia, blood pressure and smoking as associated risk markers.

3.2 Pathogenesis of diabetic complications

3.2.1 Environmental factors

The occurence of hyperglycemia is an absolute condition for development of diabetic nephropathy, retinopathy and neuropathy and several large studies have shown the importance of glycemic control in preventing microvascular complications [15, 16]. In contrast, the role of glycemic control in macrovascular complications is not as obvious. It seems though as diabetes mellitus itself is a risk factor for macrovascular disease and some studies have also shown association between glycemic control and the risk for coronary heart disease [48-50], whereas more aggressive treatment of glycemia in the UKPDS study did not show any benefits in terms of lower prevalence of MI [39]. However, the DCCT study group could recently show that T1D patients in the intensive treatment group had significantly less atherosclerosis than the conventionally treated group suggesting that better glycemic control might prevent atherosclerosis in T1D [51].
Diabetes duration [52] and smoking [53] are both risk factors for micro- and macrovascular complications. Male gender has been a risk factor for diabetic nephropathy [54] but not for diabetic retinopathy in most of studies [55].
Elevated blood pressure is a risk factor for diabetic nephropathy, retinopathy and macrovascular disease [52]. Elevated blood pressure is usually present at the time of

diagnosis of T2D, whereas in T1D blood pressure usually rises at the onset of microalbuminuria [56]. In the UKPDS tight blood pressure control was shown to cause a 34% reduction in progression of retinopathy and a 47% reduced risk of deterioration in visual acuity of three lines (ETRDS scale) in association with a 10/5 mm Hg reduction in blood pressure [57]. The same study showed after six years a 29% reduction in risk of having urinary albumin concentration >50 mg/l.

There are also complex interrelationships between different diabetic complications so that presence of one diabetic microvascular complication influences the risk of developing a second complication. Proliferative retinopathy is associated with microalbuminuria [58] although 35% of T1D patients with proliferative retinopathy did not show any signs of nephropathy [59] which suggests that at least partially different patophysiological mechanisms might be operative behind retinopathy and nephropathy.

Severity of diabetic nephropathy is associated with the severity of retinopathy and prevalence of diabetic neuropathy. Microalbuminuria is also known to be a risk marker for macrovascular disease and presence of retinopathy in T2D is associated with increased risk for coronary heart disease, independent of other known risk factors [60]. Presence of autonomic neuropathy has been associated with both diabetic nephropathy and retinopathy [61].

Ethnic background has been suggested to play role for development of diabetic nephropathy. The incidence and severity of diabetic nephropathy are increased in blacks, Mexican-Americans, and Pima Indians with T2D compared to the white population. Although some of the variation is due to differences in prevalence of hypertension and socio-economic background it seems that even after correction for hypertension and socio-economic status there is still an increased risk of ESRD caused by diabetic nephropathy in blacks [62].

Pregnancy is known to be a risk factor both for development and progression of diabetic retinopathy and sudden improvement in poorly controlled diabetes may cause a rapid worsening of retinopathy [63].

Lipid abnormalities are already present in the microalbuminuric stage and increased serum triglyceride levels are an independent risk factor for diabetic nephropathy in T1D [64] and T2D [65].

3.2.2 Major pathogenic pathways

Protein kinase C

Protein kinase C (PKC) constitutes a superfamily of serine-threonine kinase isoenzymes, many of which are activated by cofactors such as diacylglycerol (DAG) and phosphatidylserine [66]. The DAG-PKC pathway is activated in diabetes mellitus because of an increased de novo synthesis of DAG. High glucose can through PKC dependent mechanisms induce formation of reactive oxygen species (ROS) in the cell [66]. Activation of PKC leads to phosphorylation of a variety of target proteins and PKC appears to be important in the pathogenesis of diabetic complications. The first clinical studies on the selective PKC-β inhibitor ruboxistaurin mesylate (RBX) showed some beneficial effects on diabetic complications; urinary albumin excretion was reduced and GFR maintained during one year treatment with RBX [67]. RBX had no effect on progression of diabetic retinopathy even if it seemed to reduce the risk of visual loss [68]. RBX did not have any effect on vibration threshold or neurological symptoms, although patients with less severe neuropathy showed some improvement in symptoms and nerve function [42].

Polyol Pathway

Reduction of glucose by aldose reductase (AR) leads to the formation of sorbitol, which, in some tissues is further oxidized to fructose upon sorbitol dehydrogenase-catalyzed oxidation. Conversion of glucose to fructose results in utilization of NAPDH and NAD^+ which might lead to a state of pseudohypoxia with depletion of NAPDH and accumulation of reduced NAD. Increase in NADH due to increased polyol pathway activity could lead to synthesis of DAG and activation of the protein kinase C pathway. Accumulation of sorbitol in the cell also leads to osmotic stress and membrane damage [69]. Inhibition of the polyol pathway with specific aldose reductase inhibitors seemed a promising pharmacological approach to prevent microvascular diabetic complications, but the outcomes of clinical trials have been disappointing so far. Most studies showed only modest improvement with multiple side effects [69].

Glycoxidation

Advanced glycation end products (AGEs) are formed as a result of a complicated series of reactions between glucose, fructose, or glycolytic intermediates and amino groups of proteins, lipids, or nucleic acids [70].

The reaction begins with glucose attachment to amino groups, thus forming a reversible Schiff base adduct. This reaction occurs over a period of hours, and the Schiff base in turn undergoes a slow intramolecular rearrangement to form Amadori products. Amadori products were previously thought to be practically irreversible [70], but a new mechanism involving enzymatic deglycation has recently been discovered [71]. Glycated proteins can undergo further reactions to form AGEs. Formation of AGEs is non-enzymatic and therefore dependent only on concentration and temperature. Macrophages and other cellular systems can endocytose and degrade AGEs via receptor or non-receptor pathways, resulting in low molecular weight AGE peptides which can be catabolised and excreted through the kidneys [72] and AGEs will therefore accumulate in kidney failure.

AGEs constitute a heterogenous group of molecules and they can cause tissue damage by cross-links that disrupt the structure and function of proteins and lipids [72]. AGE cross-links kidney matrix proteins lead to changes in their structure and function. These changes can be inhibited in diabetic animals by administration of the cross-link breaker ALT-711 [73]. AGEs accumulate in the retina of patients with diabetic retinopathy and AGE accumulation is also found in different nervous tisssues and vascular wall where AGEs form cross-links [72].

AGEs can also cause tissue damage by interacting with cell surface receptors which leads to altered intracellular events that induce oxidative stress and inflammation [72]. AGE receptors have been found in renal mesangial cells and binding of AGE to its receptors leads to increased extracellular matrix production, induction of oxidative stress and activation of PKC-β [72]. RAGE overexpression in diabetic mice resulted in increased albuminuria, renal hypertrophy, elevated serum creatinine, mesangial expansion and glomerulosclerosis [74]. The galectin-3 (AGE-R3) knockout mice showed accelerated AGE-induced glomerular injury [75] suggesting protective effect of the galectin-3 receptor against AGE induced renal injury.

Retinal endothelial cells exposed to AGE overproduce vascular endothelial growth factor (VEGF) through oxidative stress induction, PKC activation and abnormal endothelial nitric oxide synthase (eNOS) expression. In a recent study systemic administration of sRAGE significantly inhibited blood-retinal barrier breakdown, leukostasis, and expression of ICAM-1 in the retina in the diabetic C57/BJ6 and RAGE-transgenic mice [76].

Several ways of reducing AGE-induced damage in diabetes have been proposed. Better glycemic controll will reduce formation of AGEs and this can be futher reduced by smoking cessation and dietary measures [72]. Antioxidants can potentially protect against glycation

derived free radicals and some of them like pyridoxamine and benfotiamine can inhibit AGE formation; the latter is now in phase II trials [77]. Some of the drugs already used in treatment of diabetes mellitus including metformin, pioglitazone, ACE inhibitors and angiotensin II receptor inhibitors, have shown to also have AGE inhibiting activity [77]. Acetylsalicylic acid has shown to reduce glycation in vitro and in animal experiments and salicylate based anti-inflammatory drugs can inhibit the early lesions of diabetic retinopoathy in rats [77]. The best studied specific AGE blocker is aminoguanidine. It reacts with with carbonyl groups from reducing sugars and 3-deoxyglucosones and in diabetic animals aminoguanidine can prevent/reduce formation of diabetic nephropathy [78], retinopathy [79] and neuropathy [80].

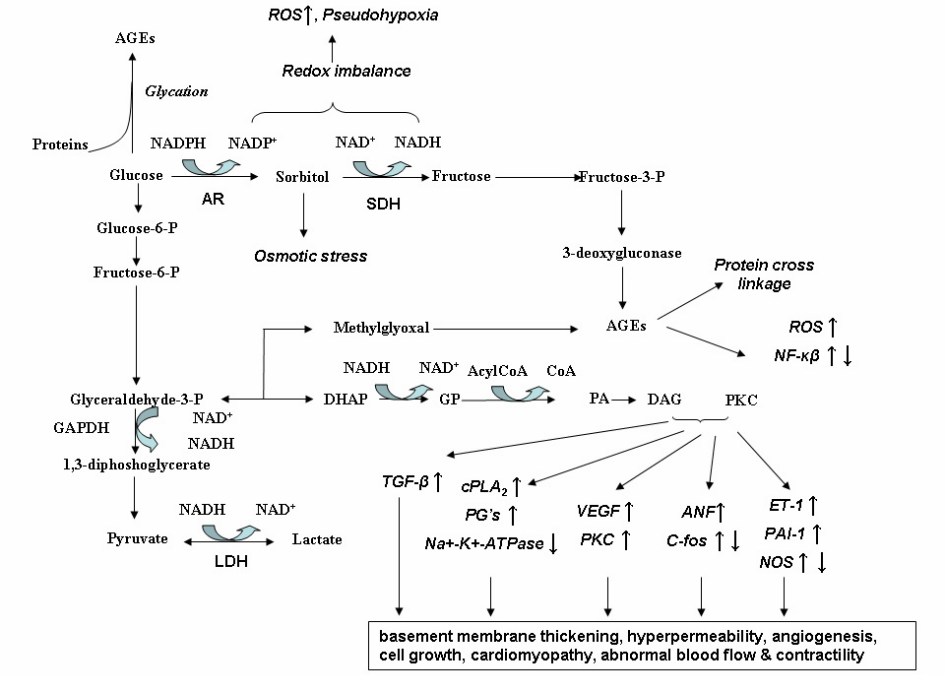

Figure 2. Interplay between glycolysis, aldose reductase pathway, glycoxidation and PKC pathways. ABBREVIATIONS: AR, aldose reductase; SDH, sorbitol dehydrogenase; LDH, lactate dehydrogenase; DHAP, dihydroxyacetone phosphate; GP, glycerol-3-phosphate; PA, phosphatidic acid; DAG, diacylglycerol; PKC, protein kinase C; TGF-β, transforming growth factor beta; $cPLA_2$, cytosolic enzyme phospholipase A_2; PG, prostaglandins; VEGF, vascular endothelial growth factor; ANF, atrial natriuretic factor; ET-1, endothelin-1; PAI-1, plasminogen activator inhibitor-1; NOS, Nitric oxide synthase; ROS reactive oxygen species; AGEs, advanced glycation end-products. Modified from [81, 82].

In a clinical trial aminoguanidine has been shown to have protective effects against diabetic retinopathy and nephropathy [83]. A similar trial on T2D patients was, however, prematurely stopped due to serious side effects [70].

Oxidative stress - a common mediator?

Nishikawa et al. have shown that inhibition of mitochondrial superoxide production could block all three major pathways of hyperglycemic damage [84]. They blocked the formation of reactive oxygen species (ROS) by an inhibitor of electron transport chain complex II, by an uncoupler of oxidative phosphorylation and by uncoupling protein-1 and by manganese superoxide dismutase (MnSOD). In each case they could prevent glucose induced activation of PKC, formation of AGEs and sorbitol accumulation in the cell. They suggest that formation of superoxide in the mitochondria is a causal link between elevated glucose and each of the three main pathways responsible for hyperglycemic damage. Recently Vecchione et al. showed that some of the beneficial effects of statins are mediated through reduction of oxidative stress in diabetic vasculature [85].

Inflammation

Inflammation may play a role in the pathogenesis of obesity, insulin resistance and T2D [86]. In diabetic nephropathy, several pro-inflammatory cytokines are secreted by blood-borne cells mainly as monocytes and macrophages, as well as intrinsic renal cells [87]. Upregulation of monocyte chemoattractant protein-1 is a feature of diabetic renal injury and associated with macrophage recruitment and progression of diabetic nephropathy [88, 89]. Intracellular adhesion molecule-1 (ICAM-1) is involved in the activation of leukocytes and macrophagess to sites of inflammation and patients with nephropathy have elevated concentrations of ICAM-1 [90, 91]. The proinflammatory cytokines IL-1β, IL-6, IL-18 and TNF-α have also been associated with diabetic nephropathy both in experimental models of diabetes and in clinical studies [87]. IL-1β stimulates mesangial cell proliferation and extracellular matrix synthesis, which would lead to expansion of the mesangium and thickening of the glomerular basement membranes. IL-1β has also been involved in the development of intraglomerular microcirculatory abnormalities related to the stimulation of prostaglandin synthesis by mesangial cells and it increases endothelial procoagulant activity and endothelial permeability [87]. IL-6 has been related to increased glomerular basement membrane thickening and it enhanced fibronectin expression. It also affects extracellular matrix dynamics at both mesangial and podocyte level, stimulates mesangial cell proliferation, and increases endothelial permeability [87]. IL-18 has been independently associated with urinary albumin excretion rate in T2D subjects [92]. TNF-α on the other hand, plays a critical role in the development of microvascular diabetic complications, including nephropathy [93]. It does not only mediate the inflammatory response but also reduces glomerular blood flow and filtration rate, induces damage to the glomerular permeability barrier which, in turn, will lead to albuminuria, apoptosis, recruitment of inflammatory cells to the kidney and increased coagulant activity [93].

Diabetic retinopathy shows many features of chronic inflammation, i.e., increased nitric oxide production, ICAM-1 upregulation, leukostasis and increased vascular permeability. Chronic hyperglycemia can cause a low-grade chronic inflammation in the retina which in turn leads to increased production of cytokines, tissue damage and cell death [94].

The role of inflammation in diabetic neuropathy is not as well studied as in retinopathy and nephropathy but a recent study by González-Clemente et al. showed that the activity of the TNF-α system is increased in subjects with T1D and diabetic neuropathy suggesting that inflammation may play a pathogenic role also in the development of diabetic neuropathy [95].

Inflammation plays also a crucial role in diabetic macrovascular disease by promoting and destabilizing atherosclerotic plaques [96]. Several inflammatory markers have been linked to cardiovascular disease progression, but their clinical usefullness is still unclear [96].

3.2.3 Genetic factors

Familiar clustering and heritability

The challenge of family studies on diabetic complications is that there must be an aggregation of both diabetes and the specific complication in the family why studies on familial aggregation of diabetic complications are rare. Evidence that genetic susceptibility plays an important role in diabetic nephropathy in T1D was first presented by Seaquist et al. [97] who could show that 83% of siblings of probands with diabetic nephropathy also suffered from renal disease, compared with 17% of those without nephropathy. Borch-Johnsen et al. [98] also showed that there was a familiar clustering of diabetic nephropathy and concluded that this could be either due to shared genetic or enviromental factors as the HbA_{1c} correlated within sib-pairs. The heritability of urinary albumin excretion rate in Finnish nuclear families was 30% being strongest from mothers to sons [99]. Both albumin-creatinine ratio and GFR showed strong familiality, having a heritability of 75% and 46% in Caucasians with T2D, respectively [100]. The cumulative risk of diabetic nephropathy in T1D patients after 30 years duration was 71.5% in the siblings of index cases with nephropathy and 25.4% in siblings of index cases without nephropathy implicating familial aggregation of diabetic nephropathy in T1D [101]. Retinopathy, on the other hand seems to affect all patients to some extent. The DCCT found familiar clustering of severe diabetic retinopathy and nephropathy in T1D patients [102]. Familial aggregation of severe retinopathy was also demonstrated in Mexican American T2D patients [103]. A recent study of 322 families with at least two T2D siblings from Southern India reported a 3-fold increased risk for retinopathy in siblings of probands with retinopathy compared to siblings of those without [104].

Previous studies suggest an ethnic predisposition to diabetic neuropathy. T1D Algerians develop diabetic neuropathy earlier and more often than French T1D patients [105]. However, no studies on heritability of diabetic neuropathy has been performed so far and our knowledge of genetic risk factors for diabetic neuropathy is mainly derived from different association studies on candidate genes.

In contrast to neuropathy, the heritability of cardiovascular disease is well studied. The Framingham Heart Study demonstrated that a positive family history of a parent [106] or a sibling [107] is a risk factor for coronary artery disease (CAD) and the familial risk is greater the lower the age at first manifastation of the disease in affected family members [108]. A family history of stroke and MI was associated with stroke at a younger age [109]. A recent study suggested that heritability of ischemic stroke was greater in women than in men, with an excess of affected mothers and sisters in female probands independently of traditional vascular risk factors [110]. A family history of peripheral vascular disease [111] and premature cardiovascular disease [112] are risk factors for peripheral vascular disease in young adults.

Genome-wide scans

A genome-wide linkage analysis requires no previous knowledge of the putative gene. In a genome-wide scan, markers randomly spread over the entire genome cover all chromosomes. If one marker is inherited from parents more often to affected than to unaffected individuals than predicted, the region is considered linked to the disease and may harbor a susceptibility gene. Linkage is reported as a logarithm of the odds (LOD) score and a genome wide linkage requires a LOD score >3.6 and suggestive evidence for linkage a LOD score of 2.2 [113].

The first genome scan search for nephropathy and retinopathy loci was performed in 98 Pima Indian sibling pairs concordant for diabetic nephropathy. The strongest evidence for linkage

to nephropathy was observed on chromosome 7q (LOD score 2.7) [114]. Vardarli et al. reported a strong linkage (LOD score 6.6) to nephropathy on chromosome 18q22.3-23 in Turkish T2D patients [115]. A genome scan for diabetic nephropathy in 206 African American T2D sibling pairs concordant for severe diabetic kidney failure (ESRD or advanced diabetic nephropathy) from 166 families showed no LOD scores above 2.0. In an ordered subset analysis there was nominal support for susceptibility locus on chromosome 18q (LOD score 3.72) [116].
A linkage analysis in 63 families with multiple members with T2D found support for linkage to albumin-creatinine ratio on chromosome 22q (LOD score 3.7) and chromosome 7q (LOD score 3.1). When the analysis was restricted to 59 Caucasian families, support for linkage in all relatives increased and became significant for 5q (LOD score 3.4) [117].
Genome-wide scan for T1D nephropathy in Finns did not show any significant linkage. However, one locus on 3q reached suggestive linkage (LOD score 2.7) [118]. In a a genome-wide search for linkage to renal function in West Africans with T2D the strongest linkage was observed to creatinine clearance on chromosome 16 (LOD score 3.6) [119]. Recently, the first results from a large genome-wide scan from the Family Investigation of Nephropathy and Diabetes (FIND) study were published [120]. This study found nominal evidence for linkage to diabetic nephropathy on chromosomes 7q21.3, 10p15.3, 14q23.1, and 18q22.3, and to albuminuria on 2q14.1, 7q21.1 and 15q26.3. Results regarding 7q, 10p and 18q were replications from linkage to diabetic nephropathy in other populations [120].
The first genom-wide scan on diabetic retinopathy in Pima Indians suggested nominal linkage between regions on chromosomes 3 and 9 and the occurrence of retinopathy in 136 affected siblings (103 affected pairs) with T2D, with a maximum multipoint LOD score of 1.46 for the region on chromosome 9 [114]. Recently, a larger scan for diabetic retinopathy susceptibility genes in 393 Mexican American families showed suggestive linkage for any retinopathy on chromosomes 3 (LOD score 2.4) and 12 (LOD score 2.5). In an ordered subset analysis ranking families by average age of diabetes diagnosis LOD scores > 2.0 for any retinopathy occurred on chromosomes 12 (LOD score 4.47), 15 (LOD score 3.65) and 20 (LOD score 2.67) while LOD scores >2.0 was seen for either moderate-to-severe nonproliferative or proliferative retinopathy on chromosomes 5 (LOD score 2.53), 6 (LOD score 2.2), and 19 (LOD score 2.21) [121]. A genome wide linkage analysis that was conducted in 211 Pima Indian sibships showed suggestive linkage for diabetic retinopathy on chromosome 1p (LOD score 3.1) [122].
Several genome-wide scans have tried to find linkage to cardiovascular disease but none of them has been specfically conducted in diabetic patients. Genome scans suggested loci linked to coronary artery disease on chromosome 2q21-22 (LOD score 3.2), Xq23-26 (LOD score 3.5) in a Finnish population [123] and on 16p13 (LOD score 3.0) in North-Eastern Indian populations [124]. Other scans performed in German [125] and US populations [126] suggested linkage between MI and regions on chromosome 14q (LOD score 3.9) and 1p34-36 (LOD score 11.68), resepctively, whereas stroke was linked to to a region on hromosome 5q (LOD score 4.4) in an Icelandic population [127].

Candidate genes for diabetic nephropathy

A large number of genes have been associated with diabetic nephropathy, but most of the studied gene variants has not been convincingly replicated in different populations. The candidate genes in the majority of the studies have been chosen on the basis of complex pathways that are believed to be involved in the pathogenesis of diabetic nephropathy including increased activity of a variety of growth factors and cytokines (i.e., transforming growth factor beta (TGF-β), growth hormone (GH), insulin-like growth factor 1 (IGF-1), vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF)); activation

of PKC isoforms; increased release of renin, angiotensin, endothelin, and bradykinin; formation of ROS; increased formation of AGEs; increased activity of the aldose reductase pathway; and abnormalities in glucose transport mechanisms [128]. Best studied are genes that are involved in the renin-angiotensin-aldosterone system (RAAS), especially angiotensin-I converting enzyme insertion/deletion (*ACE* I/D) polymorphism. Despite a pletora of studies, the role of *ACE* I/D polymorphism in the pathogenesis of diabetic complications is still unclear. A recent meta-analysis of 47 studies published between 1994-2004 including 14,727 subjects, suggested that the *ACE* II polymorphism is protective against diabetic nephropathy in Asian T2D patients but not in Caucasian T1D or T2D patients [129]. The *ACE* I/D polymorphism seems, however, to affect the response to treatment with ACE-inhibitors, and the renoprotective effect is mainly seen in patients with the II genotype [130, 131]. Table 5 summarizes some of the candidate genes for diabetic nephropathy. All of the genes presented have been shown to be associated with diabetic nephropathy in at least two different populations.

Candidate genes for diabetic retinopathy

Candidate genes for diabetic retinopathy have also been selected from the proposed pathogenic pathways that include the polyol patway, AGEs, the renin-angiotensin system, growth factors (VEGF, GH and IGF-1), oxidative damage and ROS, PKC, GLUT1, PPARγ, extracellular matrix homeostasis and tissue matrix metalloproteinases, inflammation, trombogenesis, apolipoprotein (a) and vitamin D [132].

VEGF is a central regulator of both physiological and pathological angiogenesis and plays an important role in neovascularisation of proliferative retinopathy and increased vascular permeability of diabetic macular edema [133]. Several studies have shown associations between polymorphisms in the *VEGF* gene and diabetic retinopathy [134-137] and treatment with anti-VEGF aptamer (Pegaptanib) has in a phase II study shown to have beneficial effects in treatment of diabetic macular edema [138].

A $(A\text{-}C)_n$ repeat polymorphism in the aldose reductase gene (*AKR1B1*) has been associated with diabetic retinopathy in different ethnic populations [132]. Clinical trials with aldose reductase inhibitors have, however, been a disappointment [139].

The *ACE* I/D polymorphism has been intensively studied also in diabetic retinopathy, mostly with negative results [132]. Table 6 lists candidate genes and studies, that have shown association with diabetic retinopathy in more than one population.

Candidate genes for diabetic neuropathy

There are few studies on candidate genes for diabetic neuropathy and most of the positive associations lack confirmation in other populations. Perhaps the best studied gene is the aldose reductase gene [145-149], but low sample size and different ways of characterizing diabetic neuropathy have given conflicting results. The D allele of the *ACE* I/D polymorphism has been associated with increased risk for diabetic neuropathy in Turkish T2D patients [140] and in female but not male British T2D patients [141]. Also the genes for apolipoprotein E (*APOE*) [142], uncoupling protein 2 (*UCP2*) [143], genes encoding the enzymes Mn-SOD, the extracellular superoxide dismutase (EC-SOD) [144], catalase [145], Na/K ATPase (*ATP1-A1*) [146], tumor necrosis factor receptor 2 (*TNFRSF1B*) [147] and human leukocyte antigene (*HLA*) [148] have been associated with diabetic neuropathy.

Table 7 summarizes some of the candidate genes for diabetic nephropathy all of which have been associated with diabetic neuropathy in at least one population.

Candidate genes for diabetic macrovascular disease

Atherosclerosis is a very complex disease or condition with probably hundreds of susceptibility genes [149]. In a recent review on suceptibility genes for MI the authors could find almost 5000 studies on candidate genes for CAD and MI [150]. Positive and reproducible findings were shown for 192 polymorphisms from 102 genes in at least two independent populations. Most studies have investigated the renin-angiotensin system, lipid metabolism, inflammation and the clotting cascade [150]. The situation is even more complicated by the fact that many of the cardiovascular risk factors like blood pressure, diabetes, and lipid levels have themselves a significant genetic component [149] and candidate genes for these traits might therefore affect suceptibility to cardiovascular disease.

Several genome-wide scans for CAD and stroke have identified loci of interest [151]. However, only the locus on 2p11 has been replicated in two independent studies, one American [126] and one British [152]. Three genes responsible for MI and/ or stroke have been identified in genome-wide scans: a four marker SNP haplotype of *ALOX5AP*- 5 lipooxygenase activating protein (*FLAP*) [153], a five to seven SNP marker haplotype of leukotrien A4 hydroxylase (*LTA4H*) [154] and a gene encoding phosphodiesterase 4D (*PDE4D*) [127]. Two genome-wide association studies have been conducted. The first one by Ozaki et al. assessed 92,788 SNPs in 13,738 genes. They mapped a susceptibility locus to the lymphotoxin-α gene with an odds ratio of 1.8 for MI. In a follow up study they showed that variation in the gene encoding for galectin-2 (*LGALS2*) was associated with MI. Shiffman et al. [155] used 11,053 SNPs in 6891 genes in a genome-wide association study to identify four gene variants associated with MI: *Palladin* (a cytoskeletal protein), *ROS1* (a tyrosine kinase) and two G-protein coupled receptors *TAS2R50* and *OR13G1*. In a follow up study they tested 11,647 SNPs in three case and control cohorts [156]: two variants in a gene modulating platelet degranulation (*VAMP8*) and a gene encoding for ribonuclear protein (*HNRPUL-1*) were associated with MI. The *palladin* and *ROS1* SNPs were tested also in the follow up study but were not associated with MI. A recent genome-wide association study could identify three suceptibility locus that were found associated with coronary artery disease in the Wellcome trust case control consortium and replicated in a German MI family study [157]. The strongest association was found on chromosome 9p21.3 (SNP, rs1333049) ($p=1.80\times10^{-14}$ and $p=6.12\times10^{-5}$, respectively) and two other loci were on chromosome 6q25.1 (rs6922269, $p=6.33x10^{-6}$ and p=0.009, respectively) and on chromosome 2q36.3 (rs2943634, $p=1.19x10^{-5}$ and p=0.03, respectively).

Table 5. Candidate genes for diabetic nephropathy.*

Gene	OMIM name	Role	Reference
AGE receptor	*AGER*	Glycation	[158-160]
Aldose reductase	*AKR1B1*	Polyol pathway	[161-163]
Angiotensin I	*AGT*	RAAS	[164, 165]
Angiotensin I converting enzyme	*ACE*	RAAS	[166, 167]
Angiotensin II receptor type 1	*AGTR1*	RAAS	[165, 168]
Apolipoprotein E	*APOE*	Lipid metabolism	[169-171]
Atrial natriuretic peptide (ANP)	*NPPA*	Blood pressure, glomerular pressure	[172-174]
Bradykinin receptor	*BDKRB2*	Inflammation	[175, 176]
Carnosinase gene	*CNDP1*	Free radical scavenger ? Cleavage of AGE ?	[177, 178]
Chemotactic cytokine receptor 5 (RANTES receptor gene)	*CCR5*	Inflammation	[179-181]
Cytochrome b-245	*CYBA*	Generation of superoxide	[182-184]
Ectonucleotide pyrophosphatase/phosphodiesterase	*ENPP1/PC-1*	Insulin resistance	[185, 186]
Glucose transporter 1	*SLC2A1*	Glucose transport in glomeruli	[187, 188]
Hemochromatosis	*HFE*	Diabetes, iron overload in kidney	[189, 190]
Heparan sulphate proteoglycan of basement membrane (Perlecan)	*HSPG2*	Component of the glomerular basement membrane	[191, 192]
Interleukin 1 receptor antagonist	*IL1RN*	Inflammation	[193-195]
Interleukin 1 -Beta	*IL1B*	Inflammation	[193, 195, 196]
Lipoprotein Lipase	*LPL*	Lipid metabolism	[171, 197-199]
Manganese superoxide dismutase (Mn-SOD) (Mitochondrial superoxide dismutase)	*SOD2*	free radical scavenging enzyme	[200-202]
Matrix metalloproteinase-9	*MMP9*	Inflammation	[183, 203, 204]
Methylenetetrahydrofolate reductase	*MTHFR*	Hyperhomocysteinemia, endothelial dysfunction	[205-207]
Nitric oxide synthase 3 (eNOS)	*NOS3*	Blood pressure	[208-210]
Paraoxonase 1	*PON1*	Lipid oxidation	[211, 212]
Paraoxonase 2	*PON2*	LDL oxidation, associated with CAD.	[213, 214]
Peroxisome proliferator-activated receptor-gamma	*PPARG*	Inflammation, insulin resistance, type 2 diabetes	[215, 216]
Plasminogen activator inhibitor 1	*PAI1*	Fibrinolysis, extracellular matrix turnover	[217, 218]
Protein kinase C beta	*PRKCB1*	PKC pathway	[219, 220]
Solute carrier family 12 (sodium/chloride transporters),member 3	*SLC12A3*	Blood pressure	[221-223]
Transforming growth factor beta 1	*TGFB1*	extracellular matrix turnover	[224, 225]
Tumor necrosis factor alpha	*TNF*	Inflammation	[226, 227]

*The candidate genes are derived from a medline search using key words [diabetes or diabetic], [gene or genetic] and [nephropathy].

Table 6. Candidate genes for diabetic retinopathy.*

Gene	OMIM name	Role	Reference
AGE receptor	*AGER*	Glycation	[158, 228, 229]
Aldose reductase	*AKR1B1*	Polyol pathway	[230-233]
Angiotensin I converting enzyme	*ACE*	RAAS	[234, 235]
Nitric oxide synthase 2 (iNOS)	*NOS2A*	Blood pressure	[236, 237]
Nitric oxide synthase 3 (eNOS)	*NOS3*	Blood pressure	[238-240]
integrin alpha-2/beta-1 (or platelet glycoprotein Ia/IIa)	*ITGA2*	Cell surface glycoprotein	[241, 242]
Intercellular adhesion molecule-1	*ICAM1*	Inflammation	[243, 244]
Methylenetetrahydrofolate reductase	*MTHFR*	Hyperhomocysteinemia, endothelial dysfunction	[245-247]
Neuropeptide Y	*NPY*	Angiogenesis	[248, 249]
Plasminogen activator inhibitor 1	*PAI1*	Fibrinolysis, extracellular matrix turnover	[250, 251]
Paraoxonase 1	*PON1*	Lipid oxidation	[211, 213]
Tumor necrosis factor alpha	*TNF*	Inflammation	[252, 253]
Vascular endothelial growth factor	*VEGF*	Neovascularitation	[134-137]

*The candidate genes are derived from a medline search using key words [diabetes or diabetic], [gene or genetic] and [retinopathy] and from reference [132].

Table 7. Candidate genes for diabetic Neuropathy.*

Gene	OMIM name	Role	Reference
Aldose reductase	*AKR1B1*	Polyol pathway	[254-256]
Aldehyde dehydrogenase 2	*ALDH2*	Vulnerability to oxidative stress	[257]
Angiotensin I	*AGT*	RAAS	[140, 141]
Apolipoprotein E	*APOE*	Lipid metabolism	[142]
catalase gene	*CAT*	antioxidant enzyme	[145]
extracellular superoxide dismutase (EC-SOD)	*SOD3*	free radical scavenging enzyme	[144]
Human leukocyte antigene	*HLA*	Inflammation	[148]
Manganese superoxide dismutase (Mn-SOD) (Mitochondrial superoxide dismutase)	*SOD2*	free radical scavenging enzyme	[144]
Na/K ATPase gene (ATP1-A1)	*ATP1A1*	Reactive oxygen species	[146]
Tumor necrosis factor receptor 2	*TNFRSF1B*	Inflammation	[147]
Uncoupling protein 2	*UCP2*	Reactive oxygen species	[143, 258]

*The candidate genes are derived from a medline search using key words [diabetes or diabetic], [gene or genetic] and [neuropathy].

4. Aims of the present study

The aims of this study were:

1. To test the usefulness of the new WHO criteria for clinical staging of diabetes in the characterization of diabetic patients.

2. To study whether there is an association between polymorphisms in the *UCP1-3* genes and diabetic nephropathy.

3. To study whether there is an association between *MHC2TA* -168 A→G polymorphism and cardiovascular morbidity and mortality, microalbuminuria and the metabolic syndrome.

4. To study whether *AGER* -374 T/A polymorphism is associated with diabetic nephropathy, retinopathy, neuropathy or macrovascular disease.

5. To study whether polymorphisms in *LTA*, *TNF* and *AGER* genes alone or together (as haplotypes) increase the risk for diabetic nephropathy, sight-threatening retinopathy and macrovascular disease.

5. Subjects and methods

5.1 Diabetes registry

A diabetes registry in Southern Sweden (Diabetes 2000) was initiated in 1996 and hitherto 7,461 patients have been registered. The majority of the patients (4,981) have been registered at the Department of Endocrinology, University hospital MAS, Malmö, the remaining were registred at the Trelleborg hospital or heath care centers in the Malmö and Trelleborg regions.
The registry includes information on onset of diabetes, and mode of treatment. At registry inclusion and at least once a year thereafter the following measurements are performed: body weight, height, blood pressure, fasting concentrations of plasma glucose, HbA_{1c}, serum total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides in addition to the urinary albumin excretion rate (AER) and P-creatinine. Plasma glucose, C-peptide and GAD antibodies (GADA) are measured at the registry inclusion. At annual follow-ups, signs of retinopathy, nephropathy, neuropathy and macrovascular disease are recorded. All patients gave their informed consent and the registry was approved by the Swedish Data Inspection Board and the Ethics Committee of Lund University.

5.2 The Botnia population and the myocardial infarction case-control population from the Malmö diet and cancer study

The Botnia Study was initiated in 1990 and represents a large population-based T2D family study in Finland and Sweden, aiming at identification of genes increasing susceptibility to T2D, metabolic syndrome and associated complications [259, 260].
The Malmö Diet and Cancer study population (MDC) [261] includes 28,098 randomly selected men (born 1923–1945) and women (born 1923–1950) living in the city of Malmö (population 250,000) in Sweden. A baseline examination was carried out between 1991 and 1996 encompassing a comprehensive assessment of lifestyle factors, heredity, medication as well as previous and current diseases.

5.3 Assessment of complications

5.3.1 Classification of nephropathy

The urinary albumin concentration was determined by immunonephelometry (Beckman Instruments, Fullerton, CA, USA) up to 1998 and thereafter by an immunoturbimetric method (Beckman Coulter, Beckman Instruments). Albuminuria was reported either as µg/min (AER), mg/24 h or as a urinary albumin:creatinine ratio (g/mol). Microalbuminuria was defined as 20–200 µg/min, 30–300 mg/24 h or 2.0–25 g/mol in men and 2.8–25 g/mol in women. For the definition of macroalbuminuria we also considered older values given as the urinary albumin concentration measured in a first morning specimen. Values of 30–300 mg/l were considered as microalbuminuria. Values above the upper limit were indicative of macroalbuminuria.
Diabetic nephropathy was defined as the presence of macroalbuminuria. Macroalbuminuria was considered present when at least two values above the cut-off limit for macroalbuminuria were recorded. One positive measurement only was considered as macroalbuminuria if the patient thereafter was treated with ACE inhibitors or angiotensin II receptor blockers or if the patient previously had had persistent microalbuminuria. Patients with known other kidney diseases were excluded from the analysis. Normoalbuminuria required that all urinary albumin measurements were in the normal range, otherwise the albuminuria status was

considered as unknown. Duration of albuminuria was calculated from the onset of microalbuminuria when known, or from the latest measurement with no albuminuria. If not known, duration was calculated from the first registered value with micro- or macroalbuminuria. When calculating the genotype frequencies in patients with normoalbuminuria, only patients with a diabetes duration ≥10 years were included.

5.3.2 Classification of retinopathy

Fundus photography (2-4 fields per eye) or fundus examination by biomicroscopy revealed the degree of diabetic retinopathy. In study I, retinopathy was defined as any type of retinopathy affecting at least one eye. In study IV and V patients were divided into two groups; subjects with no or non-proliferative retinopathy without macular edema requiring photocoagulation and subjects with sight-threatening retinopathy, which included patients with proliferative retinopathy and/or photocoagulation treatment (panretinal and/or focal/grid for macular edema). The duration of sight-threatening retinopathy was defined from the first information of diagnosis or laser treatment. When calculating the genotype frequencies in patients without sight-threatening retinopathy only those with a diabetes duration ≥10 years were included.

5.3.3 Classification of neuropathy

Peripheral sensory neuropathy was assessed by measuring vibration sensation thresholds by a biothesiometer on the medial malleoli (Bio-Thesiometer; Bio-Medical Instruments, Newbury, OH, USA) and defined as a threshold ≥25 V. The duration was calculated from the first value ≥25 V. When calculating the genotype frequencies for patients with vibration threshold <25 V, only patients with a diabetes duration ≥10 years were included.

5.3.4 Classification of macrovascular disease

Diabetes registry. Information on macrovascular disease was obtained from medical records. In study I, III and IV macrovascular disease was defined as previous MI and/or stroke. In paper V macrovascular disease was defined as previous MI, angina pectoris, transitory ischemic attack (TIA), stroke and/or peripheral vascular disease.

Botnia study. In the Botnia study (study III) a structured questionnaire was completed by specially trained nurses, covering information about diseases other than T2D (particularly hypertension, coronary heart disease, MI and stroke) and data on smoking habits at the baseline examination. Diagnosis of MI was always established in the hospital.

Total and cardiovascular mortality were assessed with a median follow up time of 7.9 years and the mortality data was obtained from the central death-certificate registry in Finland. Cardiovascular mortality was classified using the 9th revision of the International Classification of Diseases (cardiovascular diagnosis codes 390–459) before 1997 and the 10th revision (codes 100–199) thereafter. Causes of death were classified as 1) cardiovascular death (coronary heart disease), cerebrovascular disease (including both thrombotic and hemorrhagic stroke) or other cardiovascular (including pulmonary embolism, abdominal aortic aneurysm, hypertensive complications, general atherosclerosis and peripheral artery disease with gangrene), or 2) other causes of death (neoplasm, violent or other).

The MI case- control population from the MalmöDiet and Cancer Study (MDC). On December 31st, 2000 the study population was matched against the Swedish National Board of Health and Welfare's National Patient Registry and Cause of Death Registry. MI cases (first MI) were identified in the Swedish Patient Registry or in the Swedish Cause of Death Registry; using ICD 9–10 codes 410 and I21 in the Swedish Patient Registry and 410–414 and I21–I25 in the Swedish Cause of Death Registry.

Two age- (±1 year) and gender-matched controls without MI from MDC were assigned to each MI patient, resulting in a case-control material consisting of 1,244 MI patients and 2,488 control subjects.

5.4 Genotyping

Study II

Genotyping of the *UCP1* -3286 A→G and *UCP3* -55 C→T polymorphisms was performed using PCR and thereafter cleavage with appropriate restriction enzymes and separation of the alleles on agarose gel. Genotyping of the *UCP2* I/D polymorphism was performed with PCR and following separation of the alleles on agarose gel. A total of 434 diabetic patients and 106 non diabetic control subjects were genotyped for the polymorphisms in the *UCP1-3* genes. The genotyping success rate was 99.0% for UCP1 and UCP2 and 98.9% for UCP3.

Study III

The -168 A/G polymorphism (rs3087456) was genotyped using an allelic discrimination method on the ABI 7900 instrument (Applied Biosystems, Foster City, CA, USA). Totally 11,064 individuals were successfully genotyped. The genotyping success rate was 97.9, 98.0 and 99.0% in Botnia, MDC and Diabetes registry, respectively.

Study IV

AGER –374 T/A polymorphism (rs1800624) was genotyped using an allelic discrimination method on the ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). A total of 867 T1D, 2 453 T2D patients and 205 non-diabetic control subjects were genotyped for the *AGER* –374 T/A polymorphism. The genotypic success rate was 98.4% and regenotyping was performed in 115 samples with 100% genotyping concordance rate.

The *AGER* 63 bp insertion/deletion polymorphism was genotyped in a randomly selected subset of T1D (n=390) and T2D (n=410) diabetic patients with *AGER* –374 T/T or A/A genotypes with PCR amplification resolving the PCR products on a 2% agarose gel.

The *HLA-DQB1* genotyping was performed using a primer-pair with a biotinylated 3' primer, the 158 bp second exon of the *HLA-DQB1* gene was amplified by PCR. The amplification product was bound to streptavidin-coated microtitration plates and denatured with NaOH. After washing, bound DNA was assessed using two different hybridisation mixtures (A and B) with lanthanide (III) chelate-labelled DNA probes specific for the *HLA-DQB1* alleles. Mixture A contained a europium (Eu)-labelled internal reporter probe for *DQB1* *0602 and *0603 alleles (*0602–*0603), samarium (Sm)-labelled probe for *0603 and *0604 (*0603–*0604) alleles, and terbium (Tb)-labelled consensus sequence specific probe (Tb-*DQB1* control) as a control for PCR amplification. Mixture B contained Tb-, Sm- and Eu-labelled probes specific for *DQB1**02, *0301, *0302 alleles. When the sample is positive for both the Eu-labelled probe (*0602–*0603) and the Sm-labelled probe (*0603–*0604) and the second *HLA-DQB1* allele is any DQB1 allele except *02, *0302 and *0301 (we used the symbol X to mark an unknown *DQB1* allele), then the whole genotype is denoted as 0602-03-04/X. Thus, the *HLA-DQB1* genotype is *HLA-DQB1**0603/X, if it is homozygous for the *0603 allele, or *HLA-DQB1**0602/*0603 or *HLA-DQB1**0602/*0604 or *HLA-DQB1**0603/*0604.

The *HLA-DQB1* genotyping was done in 825 T1D, 1 179 T2D diabetic patients and 205 non diabetic control subjects.

Study V

The *AGER* -374 T/A polymorphism and HLA-DQB1 polymorphism were genotyped as previously described [158]. In addition *LTA* T60N C→A (rs1041981) and *TNF* -308 G→A (rs1800629) polymorphisms were genotyped using the allelic discrimination method on the ABI 7900 instrument (Applied Biosystems, Foster City, CA, USA). A total of 726 T1D, 2,920 T2D patients and 200 non-diabetic control subjects were successfully genotyped for *LTA* polymorphism and 729 T1D, 2,927 T2D patients and 205 non-diabetic control subjects were genotyped for *TNF* polymorphism. The genotyping success rates were 99.0% for the *LTA* and 98.7% for the *TNF* polymorphisms. Regenotyping was done in 133 (*LTA*) and 136 (*TNF*) samples with a 100 % genotyping concordance rate.

5.5 Statistical methods

Descriptive data, unless otherwise stated, are expressed as mean ± standard deviation, or median value [25^{th}-75^{th} percentile]. Categorical variables were compared by chi-square test. Normally distributed continuous variables were subjected to Student's t-test, while non-normally distributed were analyzed by the Mann-Whitney U-test. In order to assess factors associated with diabetic complications, a multiple logistic regression analysis with forward selection was performed. All data were analyzed with a NCSS (NCSS statistical software, Kaysville, UT, USA). A p-value <0.05 was considered statistically significant. To evaluate putative haplotype blocks in study V, linkage disequilibrium (LD) between the SNPs was analyzed using Haploview 3.32 and D' values were calculated with 95% confidence intervals (CI) when the genotype frequencies were in Hardy-Weinberg equilibrium [262]. A corrected p-value was obtained after 100,000 permutations of individual SNPs and haplotype blocks including the *TNF*, *LTA* and *AGER* polymorphisms. Power analysis was made using Genetic Power calculator [263]. HW-QuickCheck software [264] was used for testing of putative excess of heterozygous/ homozygous patients.

Genetic Power

Study II: A post-hoc power calculation shows that the genetic power to detect differences in *UCP1* allele frequencies between cases and controls assuming dominant model and genotype relative risk of 1.2 was 42.4% for *UCP1*, 40.6% for *UCP2* and 40.9% for *UCP3*.
Study III: The statistical power to detect differences in risk of MI according to genotype assuming dominant model and a genotype relative risk of 1.2 was 32.0% in Botnia, 95.1% in the MDC cohort and 36.5% for Swedish T2D patients (from Diabetes 2000).
Study IV&V: Power assuming $\alpha=0.05$ and a relative risk of 1.3 was 11%, 31% and 32% for T1D patients and 62%, 81% and 80% for T2D patients with or without diabetic nephropathy for the *LTA*, *TNF* and *AGER* polymorphisms. The power for retinopathy was 68%, 86% and 85 % in T1D and 58%, 73% and 73% in T2D and for macrovascular disease 20%, 28% and 28% in T1D and 97%, 99% and 99% in T2D.

6. Results

6.1. WHO clinical stages (I)

This study evaluated whether the clinical stages proposed by WHO in 1999 [22], especially if they can discriminate between clinically meaningful diabetic subgroups. We defined the clinical stages as following: patients still on diet and/or oral treatment were considered as not insulin requiring (NIR), patients who required insulin therapy after one year from diagnosis were considered to be insulin requiring for control (IRC) and patients who because of deteriorating hyperglycemia within one year required insulin were considered as insulin requiring for survival (IRS).

Table 8. Clinical characteristics in different clinical stages.

	NIR	IRC	IRS
Number of patients	711	543	743
Males/Females (n)	427/284	295/248	404/339
Age (years)	61.1±12.0*	62.4±11.6	43.6±14.7***
Age at diabetes diagnosis (years)	52.1±12.0***	48.7±12.9	22.7±15.3***
Diabetes duration (years)	7.0 (4.0-13.0)***	13.0 (8.0-18.8)	19.0 (10.0-29.0)***
BMI (Kg/m^2)	29.3±5.2**	28.0±4.8	24.2±3.4***
f-P-Glucose (mmol/L)	12.3±4.0**	12.9±4.6	13.4±5.9
HbA_{1c} (%)	7.21±1.65***	7.62±1.61	7.64±1.50
P-C-Peptide (nmol/L)	0.98 (0.72-1.31)***	0.56 (0.28-0.94)	0.05 (0.05-0.05)*
GADA positivity (n ;%)	38 (5.5)***	87(16.8)	318(44.5)*
S-Cholesterol (mmol/L)	5.39±1.08	5.44±1.14	5.09±1.03
S-HDL Cholesterol (mmol/L)	1.18±0.34*	1.27±0.40	1.58±0.46***
S-Triglycerides (mmol/L)	1.96 (1.36-2.92)	1.69 (1.12-2.53)	0.98 (0.74-1.37)***
S-LDL Cholesterol (mmol/L)	3.25±0.93	3.27±0.95	2.97±0.86
U-Albumin (µg/min)	14 (8-47)*	18 (9-74)	10 (6-23)*
P-Creatinine (µmol/L)	83.0 (74.0-94.0)***	90.0 (77.0-109.0)	86.0 (77.0-96.0)
Systolic BP (mmHg)	146.2±21.2	147.0±20.5	134.8±19.9
Diastolic BP (mmHg)	80.8±11.8	78.9±9.6	75.6±8.9

Figures are given as mean±SD or median (interquartile range). *p<0.05, **p<0.01, ***p<0.001 Compared with IRC. P-values adjusted for age, duration sex and BMI. NIR = Not insulin requiring, IRC = Insulin requiring for control, IRS = Insulin requiring for survival.

The three clinical stages showed clearly different features (Table 8). NIR patients were older at the time of diabetes diagnosis (p<0.05), had a higher BMI (p<0.01, adjusted for age, sex and duration), higher C-peptide concentrations (p<0.001) and lower frequency of GADA (p<0.001) than the IRC patients. They also had lower HbA_{1c} concentrations (p<0.001), lower HDL-cholesterol concentrations (p<0.05), lower AER (p<0.05) and lower P-creatinine than IRC patients. Data were adjusted for age, sex, BMI and duration, where appropriate.

Table 9. Presence of complications in patients belonging to the clinical stages.

	NIR	IRC	IRS
Microalbuminuria	211(36.6)*	179 (43.4)	158 (25.0)***
Macroalbuminuria	78 (17.6)***	107 (31.5)	90 (16.0)***
Retinopathy	60 (14.5)***	179 (43.9)	417 (62.1)***
Neuropathy	186 (59.0)*	211 (68.1)	238 (42.7)***
Macrovascular disease	77 (20.5)*	116 (28.2)	66 (10.4)***

Data are given as number of patients (n) and frequency (%). *p<0.05, **p<0.01, ***p<0.001, compared with IRC. NIR = Not insulin requiring, IRC = Insulin requiring for control, IRS = Insulin requiring for survival.

The IRC patients had a higher prevalence of micro- or macroalbuminuria (p<0.05 and p<0.001), retinopathy (p<0.001), neuropathy (p<0.05), and macrovascular disease (p<0.05) than the NIR (Table 9). In a subset of patients (n=426) matched for diabetes duration, age and sex, the IRC patients had still higher frequency of macoalbuminuria (29.7 vs. 17.2%, p<0.001) and retinopathy (40.0 vs. 17.2%, p<0.001) than IRS patients.
The IRC patients were older at the time of diabetes diagnosis (p<0.001), had higher BMI (p<0.001, adjusted for age, sex, BMI and duration) and higher C-peptide concentrations (p<0.001) and lower frequency of GADA (16.8 vs. 44.5%; p<0.001) than the IRS patients. The IRC patients had a higher frequency of micro- and macroalbuminuria (p<0.001), neuropathy (p<0.001) and macrovascular disease (p<0.001) but lower prevalence of retinopathy (62.1 vs. 43.9%) than the IRS patients (p<0.001).
In conclusions this study suggest that the WHO clinical staging of diabetes can discriminate clinically meaningful subgroups and the IRC patients represent more severe from of diabetes than acknowledged in the etiological classification.

6.2. The role of UCP1-3 genes in diabetic nephropathy (II)

Increased production of reactive oxygen species (ROS) has been suggested as a cause of diabetic complications and blocking the mitochondrial superoxide production in vivo can block all three main pathways behind diabetic complications [86]. The uncoupling proteins (UCPs) represent a family of proteins that are able to dissipate the proton gradient in the inner mitochondrial membrane thereby uncoupling the oxidative phosphorylation and reducing formation of ROS. The aim of this study was therefore to study whether variation in the *UCP1*, *UCP2* and *UCP3* genes was associated with increased risk of diabetic nephropathy.
No differences in allele and genotype frequencies of the *UCP1–3* polymorphisms were seen between healthy control subjects, diabetic subjects with normal AER, and diabetic subjects with micro- or macroalbuminuria (Table 10). The *UCP3* C/T polymorphism was, however, associated with BMI.
We concluded that the the studied polymorphisms in *UCP1–3* genes do not play a major role in the development of diabetic nephropathy in Scandinavian patients.

Table 10. *UCP1-3* genotype and frequencies.

	UCP1		***UCP2***		***UCP3***	
	G/G or A/G	A/A	I/I or I/D	D/D	T/T or C/T	C/C
Control subjects	38	68	59	47	55	51
	(35.8%)	(64.2%)	(55.7%)	(44.3%)	(51.9%)	(48.1%)
Diabetic subjects						
Normo-albuminuria	97	121	115	103	104	114
	(44.5%)	(55.5%)	(52.8%)	(47.2%)	(45.7%)	(52.3%)
Micro- or macro-albuminuria	84	132	105	111	110	106
	(38.9%)	(61.1%)	(48.6%)	(51.4%)	(50.9%)	(49.1%)

Data are given as number of patients (n) and frequency (%). The *UCP2* I/D and *UCP3* C → T (-55) polymorphisms were in linkage disequilibrium (93 % of the subjects homozygous for the D/D genotype were homozygous for the *UCP3* C allele $p < 0.0005$).

6.3. MHC2TA gene polymorphism, the metabolic syndrome and cardiovascular mortality (III)

Variation in the MHC class II transactivator gene (*MHC2TA*) has recently been shown to be associated with increased susceptibility to MI [265]. The aim of this study was therefore to try to confirm this association in three different populations and also to study the role of the *MHC2TA* polymorphism in microalbuminuria, the metabolic syndrome and cardiovascular mortality. Patients were selected from three large populations in Finland and Sweden; the Botnia study, the Malmö Diet and Cancer Study (MDC) and the Diabetes Registry in Southern Sweden (Diabetes 2000) (DR).

The genotype and allele frequencies of the *MHC2TA* -168 A/G polymorphism were similar in patients with or without MI in all three study populations. The *MHC2TA* polymorphism was not associated with cardiovascular mortality in the whole population, but in a subgroup of patients with previous history of MI the *MHC2TA* AG/GG genotypes were associated with cardiovascular death (Figure 3).

The *MHC2TA* AG/GG genotypes were more frequently found among patients with the metabolic syndrome (40.1 vs. 36.9%, p=0.030) as well as among non-diabetic individuals with microalbuminuria in the Botnia cohort (50.0% vs. 36.0%, p=0.003, Table 11). In contrast, the AG/GG genotypes were not associated with microalbuminuria among T2D patients, neither in the Botnia, nor in the DR cohort (Table 11).

Table 11. The genotype frequencies of the *MHC2TA* – 168 A/G polymorphism in different study populations according to history of previous MI and microalbuminuria status.

	MI+	MI-	p	Micro-albuminuria	Normo-albuminuria	p
Botnia						
Non-diabetic subjects	112 (63.4/33.0/3.6)	2686 (62.8/33.3/3.9)	0.90	99 (49.5/45.5/5.0)	1940 (64.0/32.4/3.6)	0.003
T2D subjects	184 (59.2/38.0/2.7)	1326 (59.7/36.0/4.2)	0.90	99 (58.9/38.8/2.3)	756 (63.6/32.4/4.0)	0.33
MDC						
Non-diabetic subjects	1071 (58.0/35.7/6.3)	2312 (55.7/37.4/6.9)	0.21	-	-	-
T2D subjects	151 (45.7/43.7/ 10.6)	123 (52.8/42.3/4.9)	0.24	-	-	-
Diabetes registry						
T2D subjects	316 (57.0/37.3/5.7)	1974 (54.4/39.1/6.4)	0.44	827 (53.8/39.4/6.8)	1311 (56.4/37.1/6.4)	0.23

Data are given as number of patients and allele frequencies. P-values are for the frequency of risk alleles (AG or GG) with or without MI or albuminuria respectively.

Taken together, we could not confirm an association between *MHC2TA* and MI, however we showed that the AG/GG genotypes of the *MHC2TA* -168 A→G polymorphism were associated with microalbuminuria and features of the metabolic syndrome and increased risk of cardiovascular mortality in patients with previous MI.

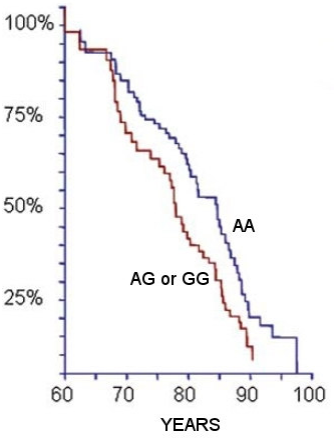

Figure 3. Kaplan-Meir curve of cardiovascular mortality in the Botnia cohort in patients with previous MI. The data has been treated as left truncated and right censored.

6.4. Genetic risk factors for diabetic complications- the role of the HLA region on chromosome 6p (IV and V)

The HLA locus located on the short arm of chromosome 6 is among the most polymorphic regions in the human genome. Many of the genes are involved in inflammatory responses and may therefore be considered as candidate genes for late diabetic complications. Previous association studies on this chromosomal region have given conflicting results concerning risk for late diabetic complications [159, 228, 229, 253, 266, 267]. The aim of the first study (IV) was to investigate a putative association between *AGER* -374 T/A polymorphism and diabetic nephropathy, retinopathy, neuropathy and macrovascular complications. We could show that T1D patients had higher frequency of the *AGER* -374 A/A or T/A genotypes than T2D patients (51.1% vs. 44.9%, p=0.002) and control subjects (51.1% vs. 47.6%, p=0.0006). The RAGE -374 T→A polymorphism was associated with *HLA-DQB1* genotypes; patients with HLA-risk genotypes had higher frequency of the A/A or T/A genotypes than patients with other *HLA-DQB1* genotypes (60.3% vs. 40.3%, p<0.000001). In T1D patients, the frequency of the A/A or T/A genotypes was higher in patients with than without diabetic nephropathy (61.1% vs. 46.8%, p=0.006) and with than without sight-threatening retinopathy (56.1% vs. 47.6%, p=0.03). In T2D patients with HbA_{1c} below the median, the T/T genotype was more frequent in patients with than without diabetic nephropathy (54.3% vs. 38.2%, p=0.02). We could not demonstrate any association between *AGER* polymorphism and diabetic neuropathy or macrovascular complications.

We concluded that the *AGER* -374 T→A was associated with diabetic nephropathy and possibly retinopathy in T1D patients but not in T2D patients. We could however not exclude that other genes in the region like *TNF* or *LTA* could influence susceptibility to diabetic nephropathy or retinopathy.

In the follow up study we therefore included *LTA* T60N C→A and *TNF* -308 G→A polymorphisms. The study population was partly the same as in the previous study, however, because of the previously shown association of *AGER* polymorphism with *HLA-DQB1* high risk genotypes, a special attention was made to exclude possible LADA patients. We therefore excluded all GADA positive T2D patients as well as T1D patients with an age at diagnosis over 35 years. Our results showed that the *AGER* -374 A allele was more common in T1D patients with than without diabetic nephropathy (31.2 vs. 28.4%, p=0.007) independently of *LTA* or *TNF* genotypes (Table 12). In a logistic regression analysis however, the *LTA* polymorphism (and not the *AGER*) was associated with increased risk of diabetic nephropathy in T1D patients. The *AGER* -374 A allele was associated with increased risk for macrovascular disease in T1D patients (OR 2.05 [1.19-3.54], p=0.01), but with decreased risk in T2D patients (OR 0.66 [0.49-0.90], p=0.009). The *AGER* A allele was independently associated with sight-threatening retinopathy in T2D (OR 1.65[1.11-2.45], p=0.01). The *TNF* A allele was associated with increased risk for macrovascular complications in T2D (OR 1.53 [1.04-2.25], p=0.03), but not in T1D patients (Figure 4).

In addition the AA haplotype of *TNF* and *LTA* was more common in T2D patients with than without macrovascular disease (21.5% vs. 18.1%, p=0.003).

Taken together this study showed that the *LTA*, *TNF*, *AGER* genes increased the risk of diabetic micro- and macroangiopathy either alone or in concert. The association was very complex and the genes might be part of a large haplotype block that also includes *HLA-DQB1* risk genotypes.

Table 12. Allelic association of *LTA* T60N (C→A), *TNF* -308G→A and *AGER* -374 T→A polymorphism with diabetic nephropathy, retinopathy and macrovascular complications.

		T1D				T2D			
Nephropathy		N		p	p*	N		p	p*
LTA T60N C→A	Controls	340	27.9/53.2/18.9	0.317	0.918	442	33.3/47.3/19.5	0.205	0.592
	Cases	113	18.6/63.7/17.7			314	37.2/45.9/16.9		
TNF -308 G→A	Controls	342	47.7/46.5/5.8	0.503	0.983	438	63.9/30.4/5.7	0.559	0.970
	Cases	113	44.2/48.7/7.1			314	65.0/30.9/4.1		
AGER -374 T→A	Controls	345	52.2/41.2/6.7	0.007	0.023	439	54.2/37.6/8.2	0.119	0.509
	Cases	114	34.2/57.9/7.9			315	58.1/37.1/4.8		
Retinopathy									
LTA T60N C→A	Controls	310	29.4/53.5/17.1	0.154	0.461	584	34.1/49.8/16.1	0.608	0.986
	Cases	310	21.6/60.6/17.7			296	37.5/45.6/16.9		
TNF -308 G→A	Controls	307	50.2/43.6/6.2	0.573	0.982	583	65.0/30.7/4.3	0.368	0.878
	Cases	315	46.0/48.9/5.1			295	67.8/28.8/3.4		
AGER -374 T→A	Controls	313	48.9/45.3/5.8	0.295	0.774	583	54.9/36.5/8.6	0.734	0.998
	Cases	315	44.8/47.9/7.3			298	50.3/44.0/5.7		
Macrovascular complications									
LTA T60N C→A	Controls	609	27.6/55.2/17.2	0.034	0.122	1802	38.1/47.5/14.4	0.102	0.456
	Cases	112	15.2/64.3/20.5			885	35.0/48.6/16.4		
TNF -308 G→A	Controls	611	49.3/44.0/6.7	0.426	0.916	1804	67.3/29.2/3.5	0.003	0.017
	Cases	113	42.5/52.2/5.3			886	61.4/34.1/4.5		
AGER -374 T→A	Controls	617	48.8/44.6/6.6	0.299	0.797	1809	54.4/38.1/7.5	0.010	0.052
	Cases	111	41.4/52.3/6.3			888	58.8/35.9/5.3		

p*= Corrected p-values after 100 000 permutations (Haploview).

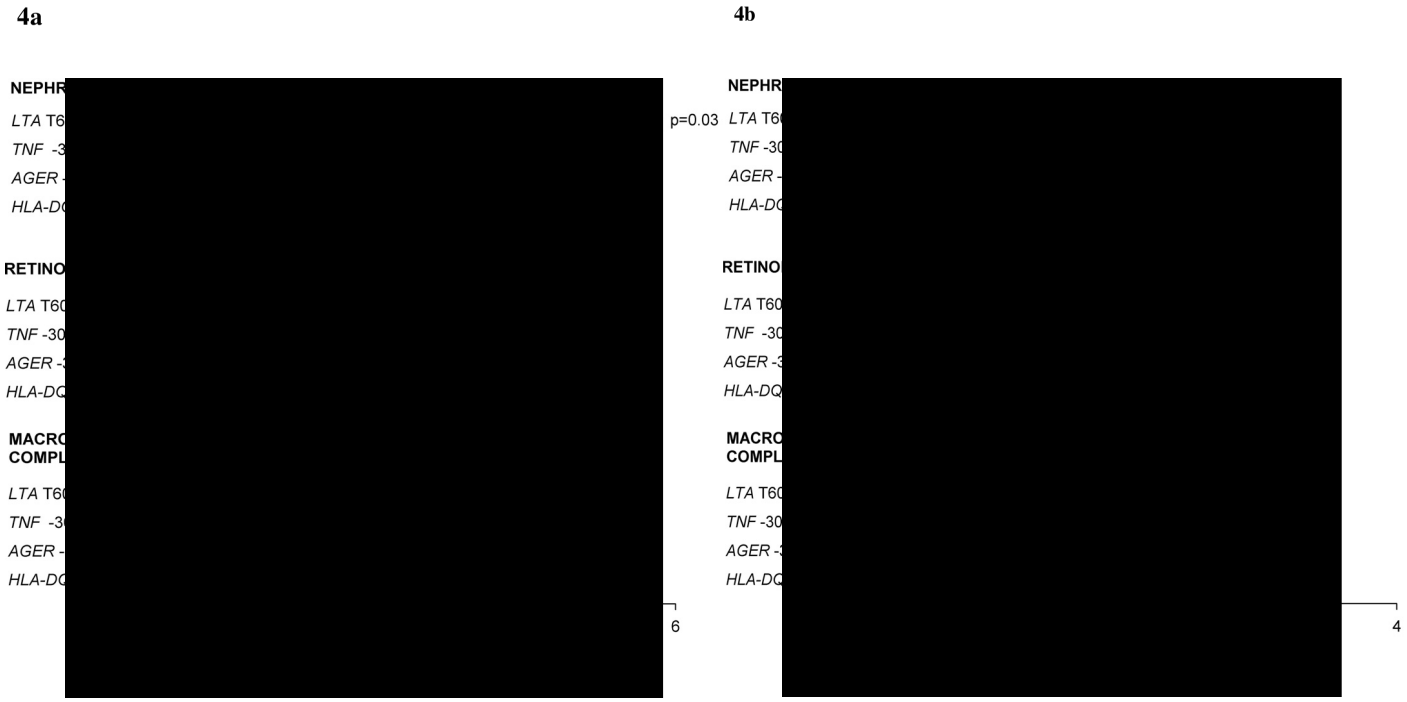

Figure 4. Logistic regression analysis in T1D (4a) and T2D (4b) patients with *LTA* T60N (C→A), *TNF* -308 G→A, *AGER* -374 T→A polymorphisms and HLA-DQB1 risk genotypes as independent and diabetic complications as dependent variable. Age, systolic and diastolic blood pressure, sex, previous/current smoking were included in all models. BMI was included in the models for nephropathy and macrovascular disease, duration in the models for nephropathy and retinopathy and age at diagnosis in the model for macrovascular disease.

7. Discussion

7.1 Study Population

The patients in our studies were mainly recruited from the local diabetes registry (Diabetes 2000). In the first study the population included 1,997 patients and in the last study a total of 5,474 patients. The majority of the patients were recruited in Malmö and 22% of the patients were other than Scandinavian origin and therefore not included in the genetic studies. The majority of the patients were recruited at the Department of Endocrinology in Malmö and at the Hospital in Trelleborg. As the majority of the T1D patients are treated at the hospital outpatient clinics, the diabetes registry covered most of the T1D patients in the region. On the other hand the T2D patients are usually referred to the hospital clinic due to problems with metabolic control or diabetic complications and consequently, one could expect an enrichment of complications in this material.

According to the Swedish national guidelines for diabetes (1999) all patients with hypertension or microalbuminuria should be treated with ACE inhibitors or angiotensin receptor II (AT2) blockers. Because a majority of the T2D patients have hypertension a wast majority was treated with ACE inhibitors/AT2 blockers. We can therefore not exclude that some of the patients in the control group with normal AER might have developed micro- or macroalbuminuria if not treated. Treatment with RAAS-blockade could also have beneficial effects in preventing diabetic retinopathy [268] and therefore influence studies on diabetic retinopathy.

Similarly, wide spread use of statins in treatment of hyperlipidemia in diabetic patients is a confounding factor in studies regarding macrovascular complications. Previous data would also suggest that approximately 30% of the T2D patients without diabetic retinopathy and macroalbuminuria have other kidney diseases than diabetic nephropathy [269]. Since all patients with nephropathy due to diabetes also have retinopathy it would be possible to separate between diabetic nephropathy from other causes excluding T2D patients without diabetic retinopathy. This procedure, however did not change the results, possible because we had already excluded all patients with previously known other kidney diseases.

In studies II-V we applied the candidate gene approach. The *UCP1-3* genes have been previously studied for association with obesity but not with diabetic complications. All other genes i.e. *AGER*, *TNF*, *LTA*, *HLA* and *MHC2TA* had previously been associated with diabetic complications in other populations. Previous experience has demonstrated the difficulty in reproducing results in other populations. Several reasons have been suggested, e.g small sample size and ethnic differences etc. One additional problem is publication biases, i.e. positive association are more likely to be published than negative ones. A good example of this so called “winners curse” is the ACE I/D polymorphism, which has been extensively studied for a role in diabetic complications. A recent meta-analysis of 47 studies suggested that the II genotype is protective for diabetic nephropathy especially in Asian T2D patients [129]. The majority of the studies are were, however, small and how this polymorphism modifies the effect of ACE inhibitors is not clear and the effect may be different between Asian and Caucasian populations [270].

A special concern in studies of diabetic complications is a possible survival bias. Genes that may influence susceptibility to e.g diabetic nephropathy may be the same that influence the risk for macrovascular complications as seen in studies III and V. This could, in turn lead to survival biases.

7.2 Study I

This is the first and to our knowledge the only study on the clinical staging proposed by WHO 1999. The advantages of the clinical staging are obvious: it offers a way to classify diabetes without any knowledge of the etiological type of diabetes and does not require any measurement of beta-cell function, auto-antibodies or DNA testing and can therefore be applied worldwide. In this study the clinical stages were defined as not insulin requiring (NIR) if the patient was currently treated with diet/oral agents only. The definition between insulin requiring for control (IRC) and insulin requiring for survival (IRS) was based on the timepoint for insulin treatment. If the insulin treatment was started within one year of diabetes, the patient was considered to require insulin for survival, otherwise only for control.
This kind of definition of the clinical stages creates an obvious problem because the patients that are considered as IRC cannot later be defined as IRS. This is especially true for the GADA positive patients in the IRC group, who in most cases would represent latent autoimmune diabetes in adults (LADA) and who after several years will develop β-cell failure [271]. Still, the classification seemed to discriminate between three clinically meaningful subgroups. The NIR group represented a classical T2D population with high BMI, high c-peptide levels and high lipid levels. IRS patients were mainly T1D patients with low c-peptide levels and high frequency of GADA. The IRC group turned out to be a high risk group for diabetic complications, with a high frequency of both micro– and macrovascular complications. We conclude that the IRC patients clearly represent a more severe form of diabetes than acknowledged in the etiological classification.

7.3 Study II

Studies on *UCP2* and *UCP3* knock out mice suggest that *UCP2* and 3 are more important for the regulation of ROS than for energy metabolism as previously thought [272, 273]. This would make them obvious candidate genes for diabetic complications. In this study we tested the hypothisis that polymorphisms in the *UCP1-3* genes could be associated with diabetic nephropathy. We concluded that it was not the case at least not in this population. The study population was well matched for metabolic control, sex and duration of diabetes. The power was not enough to detect small differences in allele frequencies and we can therefore not exclude that *UCP2* or *UCP3* genes could have some effect on the risk for diabetic nephropathy. Rudofsky et al. found an association between diabetic neuropathy and polymorphisms in the *UCP2* (-866 G→A) and *UCP3* (-55 C→T) genes [154]. No association was however found with diabetic nephropathy or retinopathy. The study population was even smaller, 227 patients, than in our study and larger studies would be needed to be able to definitely solve this issue. Recently Rudofsky et al. could show that *UCP2* and *UCP3* polymorphisms were associated with diabetic neuropathy in T1D [143] but not in T2D [274]. In contrast, they could not see any association with between diabetic nephropathy or retinopathy and *UCP2-3* polymorphisms [274].

7.4 Study III

One previous study suggested that a polymorphism in the gene (*MHC2TA*) is associated with MI [265]. The investigators also suggested that the same polymorphism was associated with susceptibility of rheumatoid arthritis and multiple sclerosis.
Our study included 11,064 individuals from three different populations with a large number of MI cases (651 with T2D and 1,183 without diabetes mellitus) and was thus well powered. Nevertheless, we could not confirm any association between this gene and MI. The *MHC2TA* polymorphism was however associated with mortality in individuals with a previous MI and

also with features of the metabolic syndrome. The lack of association with MI in our study could be due to a differences in the ascertianment of MI as the information on MI was collected retrospectively in our study. The *MHC2TA* polymorphism was associated with microalbuminuria only in non-diabetic subjects. This could reflect the fact that in diabetic subjects other factors including hyperglycemia may influence the day-to-day variation in albumin excretion.
Our results also suggest that the G-allele of the *MHC2TA* polymorphism (and in particular the AG genotype) could be a risk factor for cardiovascular mortality after MI, although the mechanism remains unclear.

7.5 Study IV

The gene for the receptor for advanced glycation end-product (*AGER*) has previously been associated with diabetic nephropathy, retinopathy and also with MI. We could show that the genotype frequencies of the *AGER* -374 T→A polymorphism differed between different ethnic groups and between T1D and T2D patients. A natural explanation for this is that the *AGER* is located in the HLA region and also associated with *HLA-DQB1* risk genotypes, the frequencies of which are known to differ between populations. Our finding that the A allele is a risk factor for diabetic nephropathy is in conflict with previous studies where the A allele has been protective in T1D patients with high HbA_{1c}. This could be explained by other genes in the HLA-region that are in linkage disequilibrium with *AGER* -374 T→A polymorphism.

7.6 Study V

Variants in the lymphotoxin alpha (*LTA*) and TNF-alpha (*TNF*) genes have previously been associated with diabetic complications. As they are located in the region that harbors genes of the MHC class III and also the *AGER* gene, association between variants in any of these genes might depend on variation in other genes. In this study we confirmed that all three genes (*LTA*, *TNF* and *AGER*) are associated with T1D *HLA-DQB1* risk genotypes. Because of excess of heterozygosity in T1D patients, we could not calculate LD between the three loci. In T2D patients the *LTA* and *TNF* were in tight LD, whereas the *AGER* was not. We conclude that the gene polymorphisms studied were associated with diabetic complications in a very complex way. They are propably part of a larger haplotype block that includes the *HLA-DQB1* risk genotypes. Although the short arm of chromosome 6 still remains as a very intresting region in the search for candidate genes for diabetic complications, there are many issues to be solved and future studies must therefore be larger and include more extensive genotyping than hitherto.

Conclusions

The WHO clinical staging of diabetes can discriminate between clinically meaningful subgroups. The insulin requiring for control patients represent a group with more severe diabetes than acknowledged in the etiological classification with high frequency of diabetic complications.

The *UCP1* -3862 A→G , *UCP2* insertion/deletion (I/D) polymorphism in exon 8 and the *UCP3* -55 C→T polymorphisms do not play a major role in the development of micro- or macroalbuminuria in Scandinavian diabetic patients.

The -168A→G polymorphism in the MHC class II transactivator gene (*MHC2TA*) is associated with cardiovascular mortality, microalbuminuria and the metabolic syndrome.

Polymorphisms in the *LTA*, TNF and *AGER* genes are associated with diabetic complications. The association is complex and dependent upon the *HLA-DQB1* genotypes, with partly different alleles conferring susceptibility in type 1 and type 2 diabetic patients. We cannot exclude the possibility that the genes are part of a large haplotype block that also includes *HLA-DQB1* risk genotypes

Populärvetenskaplig sammanfattning

Diabetes karakteriseras av kroniskt förhöjt blodsocker som beror på bristande insulininsöndring och/eller bristfällig känslighet för insulin i kroppens vävnader. Diabetes kan indelas i olika typer beroende på orsak (etiologiskt typ). Förutom tidigare kända typ 1 och typ 2 diabetes finns nu även väl kända mindre vanliga former av diabetes där den bakomliggande genförändringen som orsaker sjukdomen är känd (t.ex. MODY).
Den nya WHO-klassificeringen innehåller förutom etiologisk klassificering även en ny klinisk stadieindelning. Den kliniska stadieindelningen innefattar även fördiabetiska stadier av nedsatt glukostolerans (IGT) och icke-diabetisk fastehyperglykemi (IFG). Diabetes i sin tur indelas i tre stadier, dvs. icke-insulinberoende diabetes (NIR), diabetes som är insulinberoende för kontroll (IRC) och diabetes som är insulinberoende för överlevnad (IRS).
Kroniskt förhöjt blodsocker har skadliga effekter i olika organ som t.ex. i njurar (nefropati), ögon (retinopati), nerver (neuropati) och i blodkärlen (makrovaskulära komplikationer).
Orsakerna till diabeteskomplikationer är delvis okända, man vet dock att nivån av blodsocker och blodtryck och blodfetter spelar en viktig roll i uppkomsten av olika komplikationer. Det finns dock patienter som har haft diabetes flera decennier utan att drabbas av allvarliga diabeteskomplikationer. Tidigare studier har också kunnat fastställa att det finns en anhopning av nefropati och retinopati i vissa familjer vilket tyder på att det kan finnas genetiska orsaker till diabeteskomplikationer. Det samma gäller risken för hjärt- och kärlsjukdom som är ökad vid diabetes och även den verkar ha ärftliga orsaker.
Målsättning med denna studie var att undersöka:

1) Hur användbar är den av WHO föreslagna kliniska stadieindelningen för att identifiera kliniskt meningsfulla diabetiska undergrupper?
2) Hur påverkar variationen i olika kända gener risken att drabbas av diabeteskomplikationer?

Studie I visade den föreslagna kliniska stadieindelningen på ett meningsfullt sätt karakteriserar patienter med kliniska undergrupper oberoende av etiologisk typ. IRC visade sig ha den högsta risken att utveckla diabeteskomplikationer och hade dålig sockerkontroll, högt blodtryck och höga blodfetter.
Studie II visade att variationen i *UCP1*, *UCP2* och *UCP3* generna inte är associerade med diabetesnefropati hos svenska diabetespatienter.
Studie III visade att variation i *MHC2TA* genen är associerad med utsöndring av äggvita hos finska kontrollpersoner utan diabetes. Samma genvariation var också associerad med metabolt syndrom och med ökad mortalitet hos patienter som tidigare haft en hjärtinfarkt.
Studie IV och V visade att variationer i *LTA*, *TNF* och *AGER* generna var associerade med diabetes nefropati, retinopati och makrovaskulära komplikationer på ett komplicerat sätt och att samband i vissa fall var olika vid typ 1 och typ 2 diabetes och dessutom beroende på vilken s.k. vävnadstyp (HLA-typ) patienten hade.
Trots att dessa studier visar en del positiva samband mellan diabetes komplikationer och variation i de studerade generna, kan de förklara endast en liten del av den ärftliga risken. Det krävs flera studier för att kunna kartlägga de ärftliga faktorerna bakom diabeteskomplikationer och för att kunna dra några definitiva slutsatser angående orsak och verkan.

Yleistieteellinen yhteenveto

Diabetes on sairaus, jota luonnehtii plasman kroonisesti kohonnut glukoosipitoisuus, joka johtuu insuliinin puutteesta tai insuliinin heikentyneestä vaikutuksesta tai molemmista. Diabetes jaetaan totunnaisesti oletetun etiologian (syyn) mukaan tyypin 1 ja tyypin 2 muotoon. Sen lisäksi tiedetään jo useita diabetesmuotoja, joissa diabeteksen aiheuttava muutos perinnöllisyystekijöissä (geeneissä) on tiedossa (esim. MODY).

WHO:n uusi diabetesluokitus sisältää paitsi ennestään tunnetun etiologisen luokittelun myös luokituksen kliinisen kehitysasteen mukaan. Kliiniset luokat sisältävät diabeteksen esiasteet heikentynyt glukoosinsieto (IGT) ja poikkeava paastoglukoosi (IFG) sekä varsinaisen diabeteksen joka jaetaan kolmeen kliiniseen luokkaan: insuliinia tarvitsematon diabetes (NIR), insuliinia glukoositasapainon takia tarvitseva diabetes (IRC) ja insuliinia elämän välittömään ylläpitoon tarvitseva diabetes (IRS).

Kroonisesti kohonneet sokeriarvot johtavat usein diabeteksen kroonisiin lisäsairauksiin eli komplikaatioihin kuten munuaisvaurioihin (nefropatia), silmänpohjan verkkokalvon muutoksiin (retinopatia), hermomuutoksiin (neuropatia) sekä sydän- ja verisuonitauteihin (makroangiopatia). Perimmäiset syyt diabeteksen komplikaatioihin ovat ainoastaan osittain tiedossa. Tiedämme esim että veren sokeri ja rasva-arvoilla sekä verenpaineella on suuri merkitys potilaan riskiin sairastua diabeteksen komplikaatioihin. Toisaalta kaikki potilaat eivät välttämättä sairastu komplikaatioihin ja tutkimustulokset osoittavat myös että komplikaatioiden riski on suurempi perheessä jossa on jo henkilö jolla on joku diabeteksen komplikaatio. Sama koskee myös sydän- ja verisuonitauteja jotka ovat myös osaksi perinnöllisiä.

Tämän tutkimuksen tarkoituksena oli vastata seuraaviin kysymyksiin:

1) Kuinka käyttökelpoinen WHO:n ehdottama kliininen luokittelu on ts. voiko sen avulla jakaa diabeteksen mielekkäisiin luokkiin?
2) Miten tietyt muutokset tunnetuissa perintötekijöissä vaikuttavat alttiuteen sairastua diabeteksen komplikaatioihin?

Tutkimus I osoitti että WHO:n ehdottamat kliiniset luokat ovat käyttökelpoinen tapa jakaa diabetes eri luokkiin riippumatta siitä mikä potilaan etiologinen tyyppi on. IRC potilaiden glukoositasapaino osoittatui erityisen huonoksi ja heillä oli myös erityisen korkeat veren rasva-arvot sekä korkea verenpaine. Heillä oli myös erityisen paljon diabeteksen komplikaatioita.

Tutkimus II osoitti että muutokset *UCP1-3* geeneissä eivät lisänneet potilaiden alttiutta nefropatialle.

Tutkimus III osoitti että muutos *MHC2TA* geenissä oli erityinen riskitekijä kohonneelle virtsan albumiinin eritykselle suomalaisissa potilaissa ilman diabetestä. Sama geenimuutos lisäsi myös metabolisen oireyhtymän alttiutta sekä kuolleisuutta sydäninfarktin jälkeen.

Tutkimus IV ja V osoittivat että muutokset *LTA*, *TNF* ja *AGER* geeneissä lisäsivät diabeteksen komplikaatioiden alttiutta. Tulokset osoittivat että geenimuutosten vaikutus oli osittain erilainen tyypin 1 ja tyypin 2 diabetikoissa. Vaikutus oli myös riippuvainen potilaan kudostyypistä (eli ns. HLA-tekijöistä).

Vaikka tämä tutkimus osoittikin, että tietyt geenimutokset voivat lisätä alttiutta komplikaatioihin, voi niiden avulla ainoastaan selittää pienen osan alttiutta lisäävistä perintötekijöistä. Lisätutkimukset ovat tarpeen jotta diabeteksen komplikaatioihin liittyvät perinnölliset tekijät sekä syy-seuraussuhteet voidaan kartoittaa.

Acknowledgements

I would like to thank my supervisor Professor Carl-David Agardh and my co-supervisor Leif Groop for their support, enthusiasm, constructive criticism and source of inspiration.

I am also grateful to Professor Elisabet Agardh for her advice, ideas and comments as well as to associate professor Marju Orho-Melander for her support, critical comments and advice with paper III.

I would like to thank all my other co-workers Ekaterine Bakhtadze, Göran Berglund, Corrado Cilio, Mia Klannemark, Olle Melander, Marketa Sjögren and Tiina-Maija Tuomi for their ideas and critical comments.

Peter Almgren (also a co-author) and Timo Kanninen for their help in statistical, database and programming issues. Aki Suomalainen for help with programming and Johan Hultman for his help with computers.

I am also grateful to people in the lab especially Margareta Svensson and Esa Laurila for their skillful assistance and for Britt Bruveris-Svenburg and Anita Svensson for GADA analysis and Lena Rosberg who did the genotyping in paper III.

Bodil Israelsson and Lovisa Johansson for all help with practical issues with my experiments.

Henrik Jansson, Bengt Hallengren, Camilla Cervin, Barbro Holm, Targ Elgzyri, Devjith Tripathy, Åsa-Linda Lethagen, Harvest Feng Gu and Hayan Li for co-operation in studies that are not included in this work.

Maj-Lis Smith for interesting scientific discussions.

Professor Åke Lernmark for his efforts to explain to me the secrets of the HLA.

Leif Persson at the department of clinical chemistry for his help with Diabetes 2000 registry.

All people from the old floor 3 at Wallenberg laboratory and department of endocrinology during these years:
Avinash Abhyankar, Gertrud Ahlqvist, Kristina Bengtsson, Anna Berglund, Kerstin Berntorp, Philippe Burri, Maria Carlsén, Emma Carlsson, Anders Dahlin, Mozhgan Dorkhan, Malin Eliasson, Karl-Fredrik Eriksson, Jenny Fredriksson, Charlotte Granhall, Barbro Gustavsson, Per Hagert, Forouzan Haghanitar, Barbro Holm, Johan Holmkvist, Xudong Huang, Lennart Hulthén, Martins Kalis, Markku Lehto, Charlotte Ling, June Ljungberg, Holger Luthman, Valeriya Lyssenko, Marie Nilsson, Hemang Parikh, Martin Ridderstråle, Nael Shaat, Malin Svensson, Mona Svärdh, Jianping (Jesse) Weng, Ylva Wessman, Fredrik von Wowern.

I also want to specially mention the late Göran Sundkvist, Professor, who always took time to answer questions whether they yielded practical clinical management or scientific issues.

Last but not least thanks to my wife Beata and my son André – Your are always in my heart!

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