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FAMILIAL FACTORS AND DIABETIC NEPHROPATHY IN TYPE 1 DIABETES

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ACADEMIC DISSERTATION

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Contents

List of original publications

This thesis is based on the following original publications, which will be referred to in the text by their Roman numerals:

I. Fagerudd JA, Tarnow L, Jacobsen P, Stenman S, Nielsen FS, Pettersson-Fernholm KJ, Grönhagen-Riska C, Parving HH, Groop PH: Predisposition to essential hypertension and development of diabetic nephropathy in IDDM patients. Diabetes 47: 439–444, 1998.

II. Fagerudd JA, Pettersson-Fernholm KJ, Grönhagen-Riska C, Groop PH: The impact of a family history of Type II (non-insulin-dependent) diabetes mellitus on the risk of diabetic nephropathy in patients with Type I (insulin-dependent) diabetes mellitus. Diabetologia 42: 519–526, 1999.

III. Fagerudd JA, Pettersson-Fernholm KJ, Grönhagen-Riska C, Groop PH: Glucose metabolism in relatives of type 1 diabetic patients with albuminuria. (submitted).

IV. Fagerudd JA, Pettersson-Fernholm KJ, Riska MK, Grönhagen-Riska C, Groop PH: Albuminuria in non-diabetic relatives of IDDM patients with and without diabetic nephropathy. Kidney International 58: 959–965, 2000.

V. Fagerudd JA, Riska MK, Pettersson-Fernholm KJ, Groop PH: No evidence of an exaggerated albuminuric response to physical exercise in non-diabetic siblings of type 1 diabetic patients with diabetic nephropathy. The Scandinavian Journal of Clinical & Laboratory Investigation 60: 449–456, 2000.

Abbreviations

ACE	angiotensin-converting enzyme
AGEs	advanced glycosylation end-products
AUC	area under curve
BMI	body mass index
DN+	diabetic nephropathy (micro- or macroalbuminuria)
DN–	normal urinary albumin excretion rate
ELISA	enzyme-linked immunosorbent assay
ESRD	end-stage renal disease
GBM	glomerular basement membrane
GFR	glomerular filtration rate
HbA_{1c}	glycosylated hemoglobin A_{1c}
IGT	impaired glucose tolerance
ITT	insulin tolerance test
NS	not significant
OGTT	oral glucose tolerance test
OR	odds ratio
PKC	protein kinase C
RIA	radioimmunoassay
SEM	standard error of mean
TGF-β	transforming growth factor-β
UAER	urinary albumin excretion rate
W	watt
WHR	waist/hip ratio
24 h ABPM	24 hour ambulatory blood pressure monitoring
95% CI	95% confidence interval

Introduction

The discovery of insulin by Banting and Best in the early 1920's was one of modern medicine's most important achievements. The patients with diabetes due to an incapability of their pancreatic beta-cells to produce insulin, subsequently termed insulin-dependent or type 1 diabetic patients, could be rescued from otherwise inescapable death. However, although exogenous insulin prevented acute death from diabetic ketoacidosis, it was unable to fully normalize glucose metabolism, thus leading to higher blood glucose levels in insulin-treated diabetic patients than in healthy subjects. Later, long-lasting diabetes was found to be associated with secondary complications involving the heart, blood vessels, eyes, nerves, and the kidneys.

In 1936, Kimmelstiel and Wilson [1] described the specific structural changes in diabetic kidney disease (diabetic nephropathy) in combination with the clinical features of elevated blood pressure, grossly enhanced excretion of protein in the urine, edema, and renal failure. In addition, it became evident that the condition was not solely a disease of the kidneys but was associated also with severe forms of retinal changes leading to visual impairment, with dysfunction of the nervous system, and with a massively increased risk for cardiovascular morbidity and early death.

Epidemiological studies have demonstrated that diabetic nephropathy occurs in approximately one-third to one half of all patients with type 1 diabetes [2] and, today, diabetes is the most important cause of renal failure in the industrialized world [3, 4]. Although elevated blood glucose is of crucial importance [5], it is not the only determinant of diabetic nephropathy. Only a subgroup of patients seems to be susceptible to this long-term complication of diabetes, and, since the condition has been found to cluster in families [6], the development of diabetic nephropathy is likely to be governed also by genetic factors.

In order to elucidate the nature of such genetic determinants, the present studies were undertaken to examine whether any association exists between diabetic nephropathy and the familial clustering of traits such as elevated blood pressure, diabetes, and elevated excretion of protein in the urine.

Review of the literature

History and classification of diabetes

A wonderful but not very frequent affection among men, being a melting down of the flesh and limbs into urine … life is short, offensive and distressing, thirst unquenchable, death inevitable.

This description of the diabetic state by Aretaeus of Cappadocia is approximately 2000 years old [7]. Although he was the first known to use the term 'diabetes' (Greek: *dia* [through] and *bainein* [go]), he had no knowledge of the underlying abnormalities of the disease. However, the typical symptoms and findings of the diabetic patient, with sweet urine, intense thirst, profuse urination, weight loss, vomiting, drowsiness, coma, and death, are described in the old Hindu literature probably originating from the period between 2500 and 600 BC [8]. In European medicine, attention was drawn to the sweet taste of the urine in some patients by Willis in England in the 17th century [8], but it was not until the work by Claude Bernard in the middle of the 19th century that the basic principles of glucose metabolism were understood [9].

Hindu medicine recognized two types of diabetes, one affecting strong and corpulent persons, the other weak and lean ones, and stated that the two subtypes should be treated differently [8]. Investigations in the era of modern medicine confirmed this observation, and in 1936, Himsworth proposed at least two clinical types of diabetes, one insulin-sensitive due to insulin deficiency, the other insulin-insensitive [10]. Later, the terms 'juvenile onset' and 'maturity onset' diabetes were widely used, but the lack of a general consensus regarding their definition caused confusion. Therefore, at the end of the 1970s and beginning of 1980s, two separate expert committees [11, 12] agreed on the classification of diabetes into type 1 or insulin-dependent diabetes mellitus and type 2 or non-insulin-dependent diabetes mellitus in addition to a spectrum of other types of diabetes. The diagnostic criteria for diabetes have recently been revised [13, 14] as the knowledge of the consequences of minor elevations of blood glucose levels has increased.

The natural history of diabetic nephropathy

Although proteinuria had long been recognized as associated with diabetes, observations by Kimmelstiel and Wilson in 1936 became the foundation of the work leading to the current understanding of diabetes-specific lesions of the kidney, also called diabetic nephropathy [1]; they described the specific renal histology of diabetic nephropathy in association with the clinical features of hypertension, albuminuria, edema, and renal failure in an autopsy study on a series of eight patients of whom seven had diabetes.

All patients with type 1 diabetes do not develop nephropathy. According to epidemiological studies performed to describe the natural history of the disease [2, 15], diabetic nephropathy develops in slightly less than half (35–45%) the patients during the 40 years following diagnosis of diabetes. A striking peak in annual incidence rate occurs after 10 to 20 years of diabetes, after which the risk for nephropathy diminishes. Furthermore, male gender increases risk.

The stages in development and progression of diabetic nephropathy in type 1 diabetes are

Table 1. Stages in the development of diabetic nephropathy in type 1 diabetes

Stage	Chronology	UAER	GFR	Kidney structure	Blood pressure
I. Acute hypertrophy-hyperfunction	At diagnosis	Normal[a]	↑↑	Kidney size ↑ glomerular size ↑	Normal[b]
II. Normoalbuminuria	1–5 years	Normal (<20 μg/min)	↑↑	GBM thickness ↑ mesangial expansion ↑	Normal[b]
III. Microalbuminuria (incipient diabetic nephropathy)	6–15 years	↑ (20–200 μg/min)	↑↑	GBM thickness ↑↑ mesangial expansion ↑↑	↑
IV. Proteinuria (overt diabetic nephropathy)	15–25 years	↑↑↑ (>200 μg/min)	↑–↓↓	Pronounced abnormalities	↑↑↑
V. End stage renal disease	Over 25 years	Not measurable	↓↓↓	Advanced abnormalities	↑↑↑

[a]May be transiently elevated. [b]As in the background population.

depicted in Table 1 (modified from Mogensen [16]). The initial stage is characterized by renal hyperfunction that is normalized by the initiation of insulin therapy [17]. The kidney structure is normal at the onset of diabetes, but already during the first, clinically silent phase of normoalbuminuria, renal structural changes become discernible [18, 19]. The first clinical sign of renal microvascular complications is a slight increase in urinary albumin excretion rate (UAER). This condition, referred to as microalbuminuria or incipient diabetic nephropathy, is a powerful predictor of subsequent overt nephropathy [20–22], even though its predictive value seems to be dependent on the duration of the diabetes [23]. Blood pressure rises together with the increase in albuminuria and is clearly elevated early at the stage of overt diabetic nephropathy [24, 25]. At the time proteinuria develops, kidney function is usually normal, but soon, glomerular filtration rate (GFR) relentlessly deteriorates. The rate of decline in GFR averages approximately 10 ml/min/year, with a considerable interindividual variability [26, 27]. The process culminates in a complete loss of kidney function.

Without intervention other than insulin, the prognosis of diabetic nephropathy is poor. Half the patients are dead within seven years after the development of persistent proteinuria [2], predominantly due to renal failure, but also due to a massively increased risk for cardiovascular death [2, 28, 29]. Life-expectancy in type 1 diabetes is highly dependent on the development of nephropathy. In comparison to the background population, there is a two-fold increase in relative mortality even in type 1 diabetic patients who do not develop proteinuria. However, in type 1 diabetic patients with nephropathy, relative mortality is extremely high, increasing to a maximum of 100-fold at age 35 [30]. In addition, quality of life is severely impaired in patients with nephropathy due to the clustering of other micro- and macrovascular complications [28, 31–34].

The changing natural history of diabetic nephropathy

The natural history of diabetic nephropathy has changed dramatically through progress in the care of diabetic patients during recent decades. *Intensified insulin therapy* has a beneficial effect on development of diabetic renal complications. Shortly after onset of diabetes, initiation of insulin pump therapy normalizes the initial glomerular hyperfiltration [35, 36]. Later, as demonstrated in The Diabetes Control and Complications Trial, intensified insulin therapy, achieved either by insulin pump treatment or multiple insulin injections, resulting in a lowering of mean glycosylated hemoglobin A_{1c} (HbA_{1c}) from 9% to 7% over six years, diminishes risk for microalbuminuria by 39% and that for albuminuria by 54% [5]. Furthermore, improve-

ment in glycemic control (change in HbA_{1c} from 10% to 8.5%) by means of continuous subcutaneous insulin infusion retards the progression of morphological renal changes [37]. Whether intensified insulin therapy has a similar beneficial effect on the rate of decline in GFR is unclear [38–40], although such an effect was demonstrated in a study including patients with microalbuminuria at baseline [40]. Thus, intensive glycemic control has great potential, especially in the primary prevention of diabetic nephropathy, and should always be an aim.

Effective *antihypertensive treatment* reduces the rate of decline of GFR [41–43]. Inhibitors of the angiotensin-converting enzyme (ACE) have been proposed to have a specific renal protective effect beyond their blood pressure-lowering ability in patients with overt nephropathy [44, 45], although recent evidence suggests that other agents, for instance calcium-channel blockers, may be equally capable of preserving kidney function [46]. However, in the primary prevention of diabetic nephropathy, ACE inhibitors are indisputably efficient [47–50] and may also prevent progression of diabetic retinopathy [51].

Prior to the 1970´s, *renal replacement therapy* was generally not offered to a patient with uremia which was due to diabetic nephropathy, since the prognosis was considered as too poor [4, 52]. Today, diabetes is the most common reason for actively treated uremia in the Western world [3, 4]. Renal-transplant recipients seem to have a favourable prognosis compared to patients treated with dialysis [53], and promising results have emerged from combined pancreas–kidney transplantation [54].

These improvements in the care of the diabetic patient have indeed had an impact on prognosis. In one cohort with excellent glycemic control, a pronounced decrease has occured in the cumulative incidence of nephropathy [55]. Furthermore, recent evidence suggests that median survival time from onset of proteinuria has doubled to 14 years [2, 56], most likely an effect of intensified antihypertensive treatment (Figure 1). Finally,

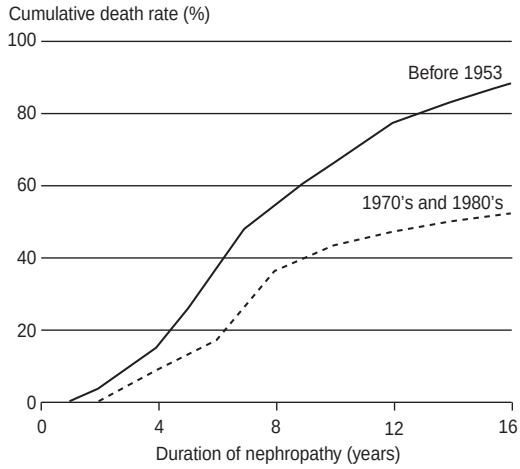

Figure 1. Cumulative death rate after onset of diabetic nephropathy in patients diagnosed with diabetes before 1953 according to Andersen et al [2] and in patients diagnosed with diabetes mainly in 1970s and 1980s according to Rossing et al [56]. The prognosis of diabetic nephropathy has improved (median survival 7 vs 14 years after onset of proteinuria).

according to the Finnish Registry for Kidney Diseases, median survival time in type 1 diabetic patients with diabetic nephropathy who developed end-stage renal disease (ESRD) in the time period 1985–1998, was approximately 5.5 years from onset of uremia [4]. In other words, the prognosis of a patient with ESRD today is quite similar to that of a patient with the first sign of diabetic renal disease – dipstick-positive proteinuria – two or three decades ago. However, despite these recent improvements, diabetic nephropathy is still an important cause of increased morbidity and premature death among patients with diabetes, and constitutes a heavy and growing burden on the health care systems throughout the world.

Renal structural changes in diabetic nephropathy

Kimmelstiel and Wilson, the first investigators to describe the characteristic “intercapillary”, nodular thickening of the mesangium, related these findings to the clinical syndrome

Table 2. Structural lesions in diabetic nephropathy (adapted from Mauer et al [244])

GBM thickening[a]
Mesangial expansion (diffuse glomerulosclerosis)[a]
Intense immunofluorescence staining for albumin in GBM, tubular basement membrane and Bowman's capsule[a]
Kimmelstiel-Wilson nodules (nodular glomerulosclerosis)[b]
Afferent and efferent glomerular arteriolar hyalinization[b]
Tubular basement membrane thickening
Subendothelial hyaline exudative lesions (fibrinoid cap)
Parietal Bowman's capsular surface "capsular drop"[b]

[a]Always present. [b]If present, highly characteristic of diabetic nephropathy.

of diabetic nephropathy [1]. Characteristics of the glomerular and interstitial lesions in advanced diabetic nephropathy are listed in Table 2.

Structural changes in relation to albuminuria

As a group, type 1 diabetic patients with long-duration diabetes and a UAER within the range of normoalbuminuria display mild structural lesions in their kidneys, such as thickening of the glomerular basement membrane (GBM) and mesangial expansion [57]. There is, however, within the group a considerable overlap, with kidney structure ranging from normal to rather advanced lesions [57]. At the stage of microalbuminuria, there is a further thickening of the GBM, together with more pronounced expansion of the mesangium [57–60]. In overt nephropathy, these structural changes become more advanced, although a considerable heterogeneity exists in lesions between patients and even from one glomerulus to another within the same patient [59].

As can be expected, UAER correlates with a variety of glomerular lesions such as GBM thickening and degree of mesangial expansion [58]. However, in one follow-up study with two kidney biopsies performed at a five-year interval in type 1 diabetic patients with UAER varying from normo- to macroalbuminuria, the structural variable most consistently correlating with increase in UAER was the mesangial volume fraction [61].

Structural changes in relation to kidney function

Even the initial glomerular hyperfiltration, early in the course of type 1 diabetes, is associated with structural changes in the kidney such as increase in kidney size [17] and in the size of the glomeruli [62]. The glomerular filtration surface area, i.e., the part of the capillary wall that is directed towards the urinary space and is not in contact with the mesangium, is similarly increased [63]. In overt diabetic nephropathy, decline in GFR correlates strongly with the decreasing glomerular filtration surface area [64, 65]. Late stages of diabetic nephropathy are characterized by a high percentage of occluded glomeruli in combination with compensatory hypertrophy and increased filtration surface area in the non-occluded glomeruli [66].

Pathogenesis of diabetic nephropathy

Hyperglycemia

Hyperglycemia is the key player in the development of diabetic nephropathy. The characteristic structural lesions of diabetic nephropathy are absent at the onset of type 1 diabetes. However, after two years of diabetes, GBM thickening and mesangial expansion are already distinguishable, and at five years, these changes are advanced [18, 19]. Furthermore, when normal kidneys are transplanted into a diabetic milieu, lesions typical of diabetic nephropathy develop [67–71]. In type 1 diabetic patients with microalbuminuria, improved glycemic control by means of intensified insulin therapy retards the progression of morphological changes [37]. Furthermore, as demonstrated in a group of type 1 diabetic patients with mild to advanced glomerular lesions at the time of pancreas transplantation, ten years of normoglycemia has induced reversal of renal structural lesions [72]. Fi-

nally, intervention studies have demonstrated in diabetic patients with intensive glycemic control a decreased risk for progression of UAER [5, 73].

Which are the biochemical mechanisms responsible for the harmful effects of high blood glucose on the kidney? Chronic exposure to hyperglycemia causes formation of *advanced glycosylation end-products (AGEs)* via non-enzymatic glycosylation of extracellular macromolecules [74]. AGEs are formed from early glycosylation products in a chemical reaction that is irreversible. Tissue accumulation of AGEs is associated with cross-linking of long-lived extracellular proteins and induces abnormalities in critical matrix protein functions such as basement membrane self-assembly and the binding of heparan sulfate proteoglycan. AGEs also induce increased formation of extracellular matrix via stimulation of production of growth-promoting cytokines [74]. High levels of AGEs have been found circulating in the serum and are found incorporated into the arterial walls of diabetic patients who have nephropathy [75]. Furthermore, administration of advanced glycosylated albumin to non-diabetic rats induces proteinuria and morphological changes similar to those seen in diabetic nephropathy [76]. The important role of AGEs in development of diabetic nephropathy is highlighted when administration to diabetic rats of aminoguanidine, an inhibitor of formation of AGEs, prevents the expected rise in albuminuria [77].

Through the *polyol pathway*, glucose is transformed into sorbitol, a reaction in which aldose reductase is the rate-limiting enzyme. When sorbitol and other polyols accumulate intracellularly, disturbances in the cellular osmoregulation and a decrease in the intracellular myoinositol follow, with tissue damage as the consequence [78]. After treatment of diabetic rats with an aldose reductase inhibitor, diminished proteinuria was evident [79, 80]. Unfortunately, findings in human beings have been conflicting [81, 82].

The *protein kinase C (PKC)* family includes at least eleven isoenzymes that act as intracellular serine/threonine kinases and are involved in various cellular signal transductions. Glucose-induced activation of PKC is associated with increased permeability, increased production of cytokines and of extracellular matrix, with cell proliferation, and with angiogenesis in vascular cells [83]. A specific inhibitor of the PKC-β isoform has been shown in diabetic rats to normalize glomerular hyperfiltration and decrease UAER [84].

Abnormalities in extracellular matrix

In the healthy glomerulus, the barrier between the capillary and the urinary space can be thought of as a membrane perforated by pores and coated with an inner layer of negatively charged molecules, mainly heparan sulfate proteoglycan and sialic acid [85]. The transglomerular passage is therefore dependent on both the size and the charge of a molecule in addition to hemodynamic forces. The heparan sulfate proteoglycan content is decreased in the capillary wall of type 1 diabetic patients with nephropathy [86]. In addition, the undersulfation of heparan sulfate molecules demonstrated in experimental diabetes [87], results in a further reduction in the anionic sites of the GBM. One consequence is an increased ability of the negatively charged albumin to pass through the glomerular filter. Since this increased permeability is not limited to the glomerulus but is present throughout the vascular bed, a decrease in the anionic content of the lining of the endothelium has been proposed as a common denominator for the deleterious triumvirate of long-term diabetic complications: nephropathy, retinopathy, and macrovascular disease [88]. Interestingly, treatment of patients with type 1 diabetes with low molecular weight heparins, assumed to restore the heparan sulfate proteoglycan content, has been found to reduce albuminuria [89] and to improve retinal hard exudates [90]. Abnormalities in the extracellular matrix may thus be important in the cascade leading in diabetes both to nephropathy and to associated micro- and macrovascular complications.

Hemodynamic factors

Autopsy findings in a patient with long-standing diabetes and unilateral renal artery stenosis revealed only mild ischemic changes on the stenotic side, whereas advanced diabetic nephropathy was evident on the contralateral side exposed to both hyperglycemia and hypertension [91]. Surgical induction of unilateral renal artery stenosis in rats has shown a similar effect [92]. Hemodynamic factors thus seem to influence the development of diabetic nephropathy. As put forward by Hostetter, Rennke, and Brenner [93], intraglomerular hypertension and single-nephron hyperfiltration induced by the diabetic state may lead to increased transglomerular passage of proteins, resulting in their accumulation in the mesangium. This may act as a stimulus for proliferation of mesangial cells, leading to sclerosis of the glomeruli. Compensatory hyperfiltration in the surviving glomeruli then completes the vicious cycle and induces progressive loss of renal function.

This hypothesis gains support from several observations. Glomerular hyperfiltration is frequently present in both type 1 and type 2 diabetes, in particular during poor metabolic control [17, 94]. Patients who later develop microalbuminuria or proteinuria have shown a higher GFR early in the course of their diabetes [95]. In an 8-year follow-up study in patients with type 1 diabetes [96], initial hyperfiltration predicted development of micro- or macroalbuminuria, although another study found the role of early hyperfiltration to be less pronounced [97]. Furthermore, reduction in number of nephrons is associated with a reduced filtration surface area and single nephron glomerular hyperfiltration [98]. Indeed, several conditions associated with a reduced number of nephrons, such as low birth weight [99, 100] and short stature [101] and also loss of one kidney [102, 103], have been linked in diabetes to an increased risk of elevated UAER. Hemodynamic factors thus seem to play an important role in the development and progression of diabetic glomerulopathy.

The issue as to whether the rise in systemic blood pressure usual in diabetic nephropathy precedes or follows the rise in UAER, has been subject to debate [104–106]. However, results from a longitudinal study applying 24 h ambulatory blood pressure monitoring (24 h ABPM) indicate a parallel rise in UAER and in systemic blood pressure in the transition from normo- to microalbuminuria [24]. In that study, base-line systemic blood pressure did not differ between progressors and non-progressors, but the rise in progressors' UAER was closely correlated with their rise in 24 h ambulatory blood pressure during follow-up.

Growth factors

The term 'growth factor' is used for any substance capable of inducing cellular differentiation or proliferation and embraces an increasing number of peptides. In the initiation and progression of diabetic nephropathy, growth factors such as growth hormone, insulin-like growth factor, epidermal growth factor, transforming growth factor, platelet-derived growth factor, tumor necrosis factor-α, and fibroblastic growth factors have all been suggested to play a role [107]. Of these, transforming growth factor-β (TGF-β) is of particular interest due to its wide effects on extracellular matrix [108]. In the normal situation, TGF-β induces deposition of extracellular matrix after tissue injury. However, in the diabetic state, a sustained secretion of TGF-β resulting in an inappropriate production of collagen IV, fibronectin, and proteoglycans among others, may be caused by several stimuli. First, high glucose concentration induces messenger RNA expression of TGF-β in glomerular cells [109] and the increased production of collagen by mesangial cells exposed to high glucose is at least in part mediated by TGF-β [110]. Second, activation of the renin-angiotensin system with enhanced formation of angiotensin II may influence extracellular matrix protein synthesis through TGF-β [111]. Third, AGEs stimulate collagen production by mesangial cells via pathways

that involve TGF-β [112]. The importance of TGF-β is further underlined by the demonstration of a rapid development of glomerulosclerosis after in vivo transfection of the TGF-β1 gene into the kidneys of non-diabetic rats [113].

Genetic factors

Renal disease, regardless of underlying cause, has been found to aggregate in families [114, 115]. In addition, substantial racial differences exist in the occurrence of diabetic nephropathy – for instance, non-Ashkenazi Jewish type 1 diabetic patients are at higher risk than are Ashkenazi patients [116]; moreover, Pima Indian, African-American, and Mexican-American type 2 diabetic patients have a higher risk than do Caucasian patients [117–119]. A genetic predisposition both to diabetic and non-diabetic nephropathy thus seems likely.

Seaquist et al [6] were the first to describe a *familial aggregation of albuminuria* in type 1 diabetes. Elevated UAER was found among 83% of diabetic siblings of patients with nephropathy, but in only 17% of the siblings of patients without nephropathy. Three subsequent studies [120–122] confirmed this finding, although their degree of familial clustering was less pronounced. A recent study of mainly normoalbuminuric type 1 diabetic sibling pairs demonstrated a familial effect not only on UAER, but also on the severity and pattern of glomerular structural lesions [123]. Familial aggregation of nephropathy has also been demonstrated in type 2 diabetes [124–127]. Interestingly, minor abnormalities in UAER seem to be present in individuals with a potential genetic susceptibility to diabetic nephropathy even in the absence of diabetes. This suggestion is based on the finding of an elevated UAER in ordinary urine collections [126, 128–130] as well as of an exaggerated albuminuric response to physical exercise [129] in non-diabetic offspring and siblings of type 2 diabetic patients with micro- or macroalbuminuria. Whether similar abnormalities in UAER are present also in the non-diabetic relatives of type 1 diabetic patients with nephropathy remains unknown.

Susceptibility to diabetic nephropathy may be linked to *familial predisposition to hypertension*, since parents of type 1 diabetic patients with proteinuria have been found to have higher arterial blood pressure than do parents of non-proteinuric patients, a finding originally described by Viberti and co-workers [131]. Although some subsequent studies have confirmed this finding, others have yielded conflicting results [132–137, 201], making the role of familial predisposition to essential hypertension in development of diabetic nephropathy in type 1 diabetes still unclear.

Furthermore, activity of the sodium–lithium countertransporter in red blood cells, a potential indicator of a genetic predisposition to hypertension [139, 140], has been found to be increased in type 1 diabetic patients with nephropathy [132, 141] and in their parents [135]. Abnormalities have also been reported in the closely related sodium–hydrogen countertransporter [142]. However, conflicting results have also appeared [134] and the exact role of these phenotypic markers of essential hypertension in the development of diabetic nephropathy remains to be established.

Earle and co-workers [136] demonstrated that a *parental history of cardiovascular disease* was associated with increased risk for diabetic nephropathy. A Danish case-control study [143] did not, however, find any difference in prevalence of cardiovascular disease between parents of patients with and without nephropathy. The prevalence of cardiovascular disease was much lower in the latter study, probably due to lower age (parental mean age 58 vs 64), which could explain some of the differences between the two studies.

In non-diabetic subjects with a *family history of diabetes*, a spectrum of metabolic derangements such as insulin resistance, abdominal obesity, elevated blood pressure, and lipid abnormalities has been described [144, 145]. Interestingly, similar metabolic and hemodynamic derangements are also found at

the early stages of diabetic nephropathy [26, 146–151]. It could therefore be hypothesized that a family history of diabetes increases the risk for diabetic nephropathy, and indeed, in the Pima Indians, a link was demonstrated between familial diabetes and diabetic nephropathy [152]. Whether a family history of diabetes increases the risk for nephropathy in type 1 diabetes is unknown. However, family members of patients with microalbuminuria have shown metabolic derangements such as hyperinsulinemia, elevated cholesterol and apolipoprotein B levels, and an increased LDL/HDL cholesterol ratio [153].

Genetics

Because the evidence for genetic factors in the development of diabetic nephropathy is convincing, efforts have been made to identify one or several genes increasing susceptibility to diabetic nephropathy. Recent results from segregation analyses in pedigrees of type 2 diabetic patients speak in favor of a *major gene effect* in the development of albuminuria [154, 155]. Similar analyses are very difficult to perform in type 1 diabetes due to the scarcity of large pedigrees with many affected individuals. However, in the study on type 1 diabetic sibling pairs by Quinn et al [121], the cumulative incidence rate for diabetic nephropathy after 25 years of diabetes was much higher in siblings of patients with nephropathy than in siblings of patients without nephropathy, 71% vs 25%, respectively. This finding has been interpreted as indicative of a major gene effect on diabetic nephropathy also in type 1 diabetes. Therefore, in the light of present understanding, diabetic nephropathy seems to be the result of one or several major or minor genes in combination with environmental factors, of which the most important is hyperglycemia [156].

Table 3 presents some of the genes suggested to be involved in the development of diabetic nephropathy. Thus far, the study design most widely used to identify such genes has been the *candidate gene approach* in case-control study settings. As an example, the candidate gene most thoroughly studied has been the insertion(I)/deletion(D) polymorphism in the ACE gene. This polymorphism is especially attractive, since it accounts for half of the variance in serum ACE level [157]. Numerous studies have addressed the issue of a role for the ACE I/D-polymorphism in the development of diabetic nephropathy with diverging results [158]. Some evidence suggests that the D-allele may increase the risk for diabetic nephropathy in Japanese type 2 diabetic patients, while the effect in Caucasian type 1 diabetic patients, if present at all, seems to be only minor [158, 159]. However, it should be noted that case-control studies are especially prone to biases such as selective survival and population stratification. At present, none of the candidate genes in Table 3 qualifies as a gene with a major impact on the development of diabetic nephropathy.

Linkage analysis in type 1 diabetic sibling pairs discordant for diabetic nephropathy has identified a region on chromosome 3 in linkage with diabetic nephropathy [160]. The most obvious candidate gene in this region is the angiotensin II type 1 receptor gene, but subsequent analysis has found no evidence of DNA sequence differences in this gene with any impact on the development of nephropathy. However, this region may harbor a yet-unidentified gene with a major effect on development of diabetic nephropathy.

Other factors

Restriction of *dietary protein intake* retards in animal models the progression of renal disease [161]. Suggested mediators of this beneficial effect include a reduction in glomerular hyperfiltration/hypertension, a preservation of proper glomerular permeability, and actions mediated via lipid metabolism. In humans, dietary protein restriction has been thoroughly studied in non-diabetic kidney diseases, and, according to a meta-analysis [162], a low protein diet has a beneficial effect on the rate of loss of kidney function. In diabetic nephropathy, the studies conducted thus far have been small, and in some cases,

Table 3. Examples of candidate genes for diabetic nephropathy

Mechanism	Candidate gene
1. Renin angiotensin system	Angiotensin converting enzyme Angiotensinogen Angiotensin II type 1 receptor Kallikrein
2. Blood pressure regulation, cardiovascular disease	α-adducin Apolipoprotein E Nitric oxide synthase (eNOS) Glycogen synthase
3. Other	Aldose reductase Transforming growth factor-β Heparan sulphate proteoglycan (Perlecan gene) Methylenetetrahydrofolate reductase

uncontrolled [163–167], and although the combined results indicate that protein restriction slows the progression of diabetic nephropathy [162], larger studies are needed to define the role of protein restriction in diabetic renal disease.

Type 1 diabetic patients with diabetic nephropathy display *abnormalities of lipid metabolism* including slightly elevated levels of total and LDL cholesterol, triglycerides, and apolipoprotein B [148, 168]. Such an atherogenic profile may contribute to the massively increased risk for cardiovascular complications in patients with nephropathy [30]. In type 2 diabetes, high cholesterol levels increase the risk for development of elevated UAER [169]. In type 1 diabetes, multiple abnormalities of lipid metabolism are already evident at the microalbuminuria stage [148, 168], but little information exists on the role of lipid abnormalities in the progression from normal to elevated UAER. In overt nephropathy, hypercholesterolemia has been associated with an accelerated decline in renal function [170–172] and with cardiovascular death [170]. However, although a recent animal study reported positive effects on renal function of lipid-lowering therapy with lovastatin [173], treatment with statins in hypercholesterolemic patients with elevated UAER failed to show any benefit regarding progression of UAER or rate of decline of GFR [174]. However, no data are available from long-term, prospective, randomised trials.

Among other factors, *smoking* has been found to increase the risk for development and progression of diabetic nephropathy [175–177] and thereby constitutes an important modifiable risk factor in diabetic nephropathy.

In addition, some evidence suggests a beneficial effect of the *c-peptide* molecule on kidney function in type 1 diabetes [178], and patients with persisting c-peptide secretion due to only partial destruction of pancreatic β-cells may be protected against diabetic microvascular late complications [179].

Aims of the study

One-third of all patients with type 1 diabetes develop diabetic nephropathy. In addition to being the most important cause of chronic renal failure in the industrialized world, diabetic nephropathy is also associated with a massively increased risk for cardiovascular complications. At present, growing evidence exists of a role for genetic factors in the development of diabetic nephropathy. Identification of such predisposing factors would allow targeting of high-risk individuals and a possibility for intervention even at diagnosis of diabetes. Little is known, however, about the nature of the genetic factors increasing the risk for nephropathy. The main objectives of the present study were therefore to answer the following questions:

1. Is diabetic nephropathy in type 1 diabetes associated with familial predisposition to hypertension? (I)
2. Is diabetic nephropathy in type 1 diabetes associated with familial predisposition to diabetes? (II)
3. If so, which abnormalities in glucose metabolism are responsible for the excess prevalence of diabetes seen in relatives of type 1 diabetic patients with diabetic nephropathy? (III)
4. Are abnormalities in UAER present in non-diabetic first-degree relatives of type 1 diabetic patients with diabetic nephropathy? (IV, V)

Study design and subjects

All studies were *cross-sectional case-control studies*. Characteristics of the type 1 diabetic patients are depicted in Tables 4 and 5. The studies were conducted in accordance with the Declaration of Helsinki [180], and the study protocols were approved by the local ethics committee. All patients and relatives gave their written informed consent prior to their participation.

A total of 318 Caucasian type 1 diabetic patients took part. Type 1 diabetes was defined as onset of diabetes before the age of 35, initiation of insulin therapy within a year after diagnosis, and present treatment with at least two daily insulin injections without the use of any other antidiabetic drug. The subjects were recruited from a random sample of all patients with diabetic nephropathy attending the renal outpatient clinic or the dialysis unit of the Helsinki University Central Hospital between November, 1995, and December, 1997 (n = 137), and from a consecutive sample of all patients attending the diabetic outpatient clinic of the same hospital from September, 1990, to February, 1992 (n = 73), and via an advertisement in the newsletter of the Helsinki Diabetes Association (n = 20). Consequently, a total number of 174 patients with signs of diabetic nephropathy and 56 patients with normal UAER were studied in Helsinki. In addition, 88 Danish patients attending the outpatient clinic at the Steno Diabetes Center, Gentofte, Denmark, participated in Study I. Of these, 44 patients were randomly selected from among all type 1 diabetic patients with diabetic nephropathy who had their GFR measured in 1993, together with 44 patients from an age-, duration- and sex-matched control group with normal UAER.

Familial predisposition to hypertension (I)

Study aim. To evaluate whether diabetic nephropathy is associated with a familial predisposition to hypertension.

Subjects. The parents (n = 109) of 73 patients with overt diabetic nephropathy and those (n = 112) of 73 patients with normal UAER and a duration of diabetes exceeding 15 years (Tables 4 and 5). In each group, 44 of the patients were recruited from the Steno Diabetes Center in Gentofte, Denmark, while the rest came from the outpatient clinics of the Helsinki University Central Hospital.

Study design. Any antihypertensive medication taken by the parents was recorded, and blood pressure was measured auscultatorily in all parents not on antihypertensive medication (n = 162). Furthermore, 131 of the 162 parents taking no antihypertensive medication volunteered for a 24 h ABPM. The prevalence and cumulative incidence of hypertension in the two groups of parents were calculated after exclusion of those parents with clear evidence of hypertension secondary to renal disease. The impact of parental hypertension on the development of hypertension in the diabetic offspring was also assessed.

Familial predisposition to diabetes (II)

Study aim. To elucidate whether diabetic nephropathy is associated with a familial predisposition to type 2 diabetes.

Table 4. Characteristics of the type 1 diabetic patients

Study	Patients with	n	Sex (M/F)	Age (years)	Duration (years)	HbA_{1c} (%)	Serum creatinine (μmol/l)	ESRD (%)	UAER (μg/min)
I	DN+	73	39/34	37±1	25±1	9.4±0.2***	94 (57–1141)***	10	758 (48–8824)[a]
	DN–	73	36/37	37±1	25±1	8.3±0.1	78 (53–111)	–	8 (1–19)
II	DN+	137	87/50*	42±1	30±1	8.9±0.1***	173 (68–1176)***	43	512 (19–8936)[a]
	DN–	54	25/29	42±1	28±1	8.1±0.1	83 (57–111)	–	4 (1–19)
III	DN+	43	22/21	37±1	25±1	9.1±0.2**	92 (62–1176)**	21	124 (9–3386)[a]
	DN–	39	18/21	40±1	26±1	8.2±0.1	85 (65–111)	–	5 (1–19)
IV	DN+	80	52/28**	41±1	30±1	8.8±0.2*	177 (68–1176)***	51	928 (70–8936)[a]
	DN–	25	8/17	41±2	27±2	8.1±0.2	78 (57–101)	–	4 (1–11)
V	DN+	21	16/5	40±2	29±2	9.0±0.3**	152 (58–848)***	38	997 (191–8936)[a]
	DN–	24	10/14	41±2	29±2	8.1±0.1	82 (57–108)	–	4 (1–19)

[a]Includes patients with present UAER less than the cut-off levels for elevated UAER, but with previously documented persistent micro- or macroalbuminuria. *P < 0.05, **P < 0.01 and ***P < 0.001 in comparison of patients with DN+ and DN– within the different studies (significance of differences in prevalence of ESRD and in UAER not assessed).

Subjects. Parents of 137 patients with overt diabetic nephropathy and of 54 patients with normal UAER (Tables 4 and 5) recruited from the outpatient clinics of the Helsinki University Central Hospital and via the Helsinki Diabetes Association.

Study design. Prevalence of known diabetes and hypertension as well as overall and cardiovascular death rate in the parents were assessed in the two groups of patients. Furthermore, an oral glucose tolerance test (OGTT) and anthropometric measurements were carried out in 95 and 55 living, non-diabetic parents of patients with and without diabetic nephropathy, respectively.

Familial abnormalities in glucose metabolism (III)

Study aim. In order to explore the mechanisms behind a possible association between diabetic nephropathy and familial type 2 diabetes, glucose metabolism was assessed in first-degree relatives of type 1 diabetic patients with and without elevated UAER.

Subjects. First-degree relatives (n = 114) of 43 patients with elevated UAER (microalbuminuria, n = 18; overt diabetic nephropathy, n = 25), and 93 relatives of 39 patients with normal UAER. The patients were recruited from the outpatient clinics of the Helsinki University Central Hospital and via the Helsinki Diabetes Association.

Study design. After exclusion of relatives with treatment for diabetes or with fasting hyperglycemia, an OGTT was performed on all relatives, with measurement of plasma glucose and serum insulin responses. On a second occasion, insulin sensitivity was measured by the short insulin tolerance test (ITT) in 106 (89%) and 84 (89%) relatives of patients with and without elevated UAER.

Familial abnormalities in urinary albumin excretion rate (IV–V)

Study aim. Non-diabetic relatives of type 2 diabetic patients with elevated UAER have been found to display abnormalities in UAER. The aim was to discover whether this was also

Table 5. Characteristics of the relatives

Study	Relatives of patients with	n	Parents/ siblings	Sex (M/F)	Age (years)	BMI (kg/m^2)	Smokers (%)
I	DN+	109	109/–	42/67	66±1	27.1±0.5*	47
	DN–	112	112/–	42/70	68±1	25.9±0.3	36
II	DN+	95	95/–	36/59	65±1	28.2±0.4	19
	DN–	55	55/–	22/33	65±1	27.2±0.6	24
III	DN+	114	56/58	49/65	51±1	26.1±0.4*	25
	DN–	93	44/49	43/50	53±2	25.0±0.4	30
IV	DN+	186	77/109*	85/101	52±1	26.4±0.3	27
	DN–	52	30/22	22/30	54±2	26.0±0.6	25
V	DN+	21	–/21	12/9	43±2	25.1±0.6	43
	DN–	24	–/24	14/10	43±2	24.5±0.6	33

*P<0.05 in comparison of relatives of patients with DN+ and DN– within the studies.

the case in non-diabetic relatives of type 1 diabetic patients with diabetic nephropathy.

Subjects. In Study IV, UAER was measured in 186 first-degree relatives of 80 patients with overt diabetic nephropathy and in 52 relatives of 25 patients with normal UAER (Tables 4 and 5). In Study V, basal and exercise-induced UAER was measured in three urine collections from 21 and 24 age-, sex- and body mass index (BMI)-matched siblings of patients with and without diabetic nephropathy, respectively. These patients were recruited from the outpatient clinics of the Helsinki University Central Hospital and via the Helsinki Diabetes Association.

Study design. In Study IV, UAER was measured in one overnight urine collection from relatives, in whom diabetes was excluded by medical history. In Study V, an OGTT was performed to exclude abnormalities in glucose metabolism. UAER was measured from urine collections which the relatives performed overnight, during the OGTT, and during a submaximal bicycle ergometer test.

Methods

Assessment of medical history

A careful medical history regarding the presence of hypertension, diabetes, cardiovascular disease, smoking habits and regular medication was taken from all participating relatives in Studies I to V by use of a standardized questionnaire. *Hypertension* was considered present if the subject was on medication prescribed for elevated blood pressure. *Diabetes* was defined as a diagnosis of diabetes made by a physician. Diabetes in the relatives was classified as type 1 diabetes if age at onset of the disease was less than or equal to 40 years, and since diagnosis it had been treated with no other antidiabetic drug than insulin. All other cases of diabetes were classified as type 2 diabetes. A history of *cardiovascular disease* was defined as a history of acute myocardial infarction or stroke. In cases with incomplete information, medical records were reviewed.

In Studies II and III, the participating type 1 diabetic patients were all interviewed with a standardized questionnaire regarding the health status of their first-degree relatives. The questionnaire assessed whether the relatives were alive, the age of the relatives at the time of the study and presence of diabetes or antihypertensive treatment in the relatives. If a relative was deceased, cause of death and age at death were requested. Parental cardiovascular death was considered to have taken place if the proband reported death from cardiac causes, stroke, or rupture of an aortic aneurysm. In Study II, the reliability of the information obtained from the patients was tested by interviewing living parents and by reviewing medical records and death certificates in those deceased. Confirmation of data was carried out for all parents classified as diabetic by their diabetic offspring (n = 66; 50 parents of patients with and 16 of patients without nephropathy) and furthermore, in a sample of 221 (71%) of all 311 presumed non-diabetic parents (217 parents of patients with and 94 parents of patients without nephropathy). The sample of 221 presumed non-diabetic parents, comprising 138 (64%) of patients with and 83 (88%) of patients without nephropathy, was selected as follows: first, their medical history was obtained from those parents subsequently interviewed at the outpatient clinic when they had their assessment of oral glucose tolerance (n = 95 and 55). Second, in order to test the reliability of data on those presumed non-diabetic parents not attending the outpatient clinic, the medical record and death certificate validation procedure was then performed on a subgroup of 71 parents (n = 43 and 28) of whom 18 were alive (n = 14 and 4) and 53 dead (n = 29 and 24).

Assessment of blood pressure and hypertension

Systolic (Korotkoff I) and diastolic (Korotkoff V) *office blood pressure* was measured auscultatorily on the right arm with an ordinary calibrated mercury sphygmomanometer (Studies I to IV) or a Hawksley random zero sphygmomanometer (Study I) with a properly sized cuff after at least 5 min of rest. The mean value of at least two recordings was used in the analysis. In Study V, an aneroid sphygmomanometer was employed to measure auscultatory blood pressure after the subject had rested 10 min supine.

In Study I, 24 h *ambulatory blood pressure* was measured oscillometrically with a SpaceLabs 90207 device (SpaceLabs Inc., Redmond, WA, USA). The monitor was checked before, after, and once each month

during the study by comparison with a calibrated mercury sphygmomanometer according to the instructions of the manufacturer. Blood pressure was measured every 15 min from 07:00 to 22:00 and every 30 min from 22:00 to 07:00 with a properly sized cuff during 24 h of normal daily activities. Blood-pressure monitoring was accepted if there was at least one successful blood-pressure recording per hour during at least 20 of the 24 h of monitoring. Day- and night-time blood pressures were calculated based on individually recorded awake and sleeping hours.

Hypertension was defined as current use of antihypertensive medication, an office blood pressure exceeding or equal to 140/90 (Studies I, III) or 160/95 mmHg (Study II), or a 24 h ABPM exceeding or equal to 135/85 mmHg [181, 182].

Assessment of diabetic complications

The degree of renal involvement in the diabetic patients was based on at least three urine collections [183]. *Overt diabetic nephropathy* was defined as an UAER exceeding either 200 µg/min (overnight urine collections) or 300 mg/24 h (24 h urine collections), and *microalbuminuria* as UAER 20 to 200 µg/min or 30 to 300 mg/24 h in two out of three consecutive urine collections in the absence of any clinical or laboratory evidence of other renal disease. *Normal UAER* was defined as UAER persistently below 20 µg/min or 30 mg/24 h. In some patients, UAER had decreased, for instance after initiation of antihypertensive therapy. In these cases, classification of microalbuminuria and overt diabetic nephropathy was based on past UAER recordings. *ESRD* was considered present after initiation of renal replacement therapy (dialysis or kidney transplantation). A history of retinal photocoagulation served as an indicator of severe *retinopathy*.

Assessment of glucose metabolism

An *OGTT* was performed in Studies II, III, and V. Plasma glucose and serum insulin were measured in the morning after a 10 to 12 h overnight fast and at 30, 60, and 120 min after ingestion of 75 g of glucose in a volume of 200 ml of water. Diabetes and impaired glucose tolerance (IGT) in the OGTT were defined according to 1985 World Health Organization criteria [184]. Abnormal glucose tolerance was defined as IGT or diabetes. Incremental area under the curve (AUC) was calculated according to the trapezoidal rule.

In order to measure insulin sensitivity, the short *ITT* [185] was applied in Study III. Two intravenous cannulas were inserted, one in a deep cubital vein and a second in retrograde position in a dorsal vein of the contralateral hand. The hand was kept in a heated (+55 °C) box in order to achieve arterialization of venous blood. After a baseline period of 20 to 30 min, fasting plasma glucose was measured twice, and an intravenous bolus of short-acting insulin (0.1 IU/kg body weight) was given. Arterialized venous blood samples for measurement of plasma glucose level were drawn every min from 3 to 15 min after the insulin bolus. After the 15 min test-period, a glucose infusion was initiated, and the subject received a meal. The percentage decline in logarithmically transformed plasma glucose per min during 3 to 15 min after administration of the insulin bolus was calculated by least square analysis and expressed as the K_{ITT}-value (%/min).

Assessment of albuminuria in relatives

In Study IV, UAER was measured in the non-diabetic relatives from a *timed overnight urine collection*. In Study V, UAER was measured from three *timed urine collections*: (I) overnight, (II) during an OGTT, and (III) during a submaximal bicycle ergometer test. In order to maintain sufficient diuresis, the subjects were given 600 ml of water to drink during the OGTT, and approximately 10 ml of water per kilogram body weight before the exercise test was initiated. Prior to the exercise test, each subject lay for 10 min supine. The test began at a level of 40 W in male and 30

W in female subjects, and the work-load was increased by 40 W in male and by 30 W in female subjects every 4 min. The target was 90% of the age-adjusted maximal heart rate (205 minus age in years divided by 2). Blood pressure was measured at rest and during the last min at every work-load level, while heart rate was being recorded with a pulse-sensor device at rest and at 1-min intervals during the test. The test was followed by a 10-min rest in the supine position, after which the subject voided the final urine sample.

Assessment of smoking and anthropometric measurements

Smoking was defined as present smoking of at least one daily cigarette, cigar, or pipe during the year prior to participation in the studies. Body *weight* (to the closest 0.1 kg) and *height* (to the closest cm) were measured in light clothing. *BMI* was calculated as weight (kg) / (height (m)^2). *Waist circumference* was measured midway between the iliac crest and the lowest rib and *hip circumference* at the widest part of the gluteal region. *Waist/hip ratio* (WHR) was calculated as waist (cm)/hip (cm).

Assays

Plasma glucose was measured in duplicate by a glucose oxidase method (Beckman Glucose Analyser II, Beckman, Fullerton, CA, USA) with a coefficient of variation of 1.0%. *Serum insulin* was measured by enzyme-linked immunosorbent assay (ELISA; Dako Diagnostics Ltd, Cambridgeshire, UK) in Study III and by radioimmunoassay (RIA; Insulin-RIA, Pharmacia-Upjohn, Uppsala, Sweden) in Study V with coefficients of variation of 9% and 8%, respectively. In Studies II to V and in the Finnish subjects in Study I, *urinary albumin* was measured by use of RIA (Albumin-RIA, Pharmacia-Upjohn, Uppsala, Sweden) with a coefficient of variation of 4%. In the Danish subjects in Study I, urinary albumin was measured by ELISA [186]. *HbA_{1c}* was measured by high-pressure liquid chromatography with a normal range of 4.0 to 6.0% (Finland) and 4.1 to 6.1% (Denmark). *Serum creatinine* was assayed by a kinetic Jaffé method (normal range in Finland: women 50–110, men 55–115 µmol/l; normal range in Denmark: women 40–110, men 60–130 µmol/l). *Serum cholesterol* (normal range: 3.6–7.0 mmol/l), *HDL-cholesterol* (normal range: women 1.10–2.35, men 0.95–2.00 mmol/l) and *triglycerides* (normal range: 0.4–1.7 mmol/l) were all measured on a Hitachi 917 automated analyzer with enzymatic colorimetric tests.

Statistical analysis

The significance of difference in *categorical variables* between the groups was tested with the Chi squared test. The significance of difference in normally distributed *continuous variables* was tested with Student's t-test, while differences in non-normally distributed variables were assessed with the Mann-Whitney U-test or Student's t-test after logarithmic transformation. Adjustment for confounding factors was performed by analysis of covariance. The cumulative incidence of parental hypertension and overall and cardiovascular parental death rate was calculated with a *life-table method*, which takes into account the variability in length of follow-up. The significance of the difference in cumulative incidence and survival between the two groups was determined with the *logrank test*. In order to evaluate the independent association between familial factors and diabetic nephropathy in Study II, a *multiple forward stepwise logistic regression analysis* was performed and the adjusted odds ratio (OR) and the 95% confidence interval (95% CI) calculated. A two-tailed P-value less than 0.05 was considered statistically significant. Normally distributed continuous variables are presented as mean± standard error of mean (SEM) and non-normally distributed variables as median (range).

Results

Familial predisposition to hypertension (I)

Antihypertensive therapy for essential hypertension was being administered to 37 (34%) of the parents of patients with diabetic nephropathy (DN+) and to 22 (20%) of the parents of patients without diabetic nephropathy (DN–), P < 0.05. Regarding the 162 parents with no antihypertensive treatment, office blood pressure was measured for all and 24 h ABPM for 131. A successful 24 h ABPM was obtained for 128 of the parents (55 of DN+ and 73 of DN–). The 31 parents (14 of DN+ and 17 of DN–) for whom a 24 h ABPM was not performed were slightly older (69 vs 66 years; P = 0.034), but did not differ regarding sex, BMI, or office blood pressure from the rest of the parents.

There was no significant difference in office or 24 h ambulatory blood pressure between the two groups of parents not receiving antihypertensive treatment (Table 6). However, the proportion of parents on antihypertensive medication or with a 24 h ambulatory blood pressure 135/85 mmHg was 57% among parents of DN+ and 41% among parents of DN–, P < 0.05. When office blood pressure was used to identify parents with untreated hypertension, the difference in prevalence of hypertension (antihypertensive medication or office blood pressure 140/90 mmHg) between parents of DN+ patients and DN– patients did not reach statistical significance (64% vs 57%, P = NS).

The cumulative incidence of hypertension was higher in parents of patients with DN+ than in parents of patients with DN– (Figure 2).

Table 6. Office and ambulatory blood pressure (mmHg) in parents without antihypertensive medication

	DN+	DN–
Office blood pressure		
n	72	90
Systolic	141±2	142±2
Diastolic	83±1	82±1
Ambulatory blood pressure		
n	55	73
24 h systolic	126±2	126±1
24 h diastolic	76±1	74±1
Daytime systolic	131±2	131±2
Daytime diastolic	81±1	79±1
Night-time systolic	116±2	115±2
Night-time diastolic	67±1	65±1

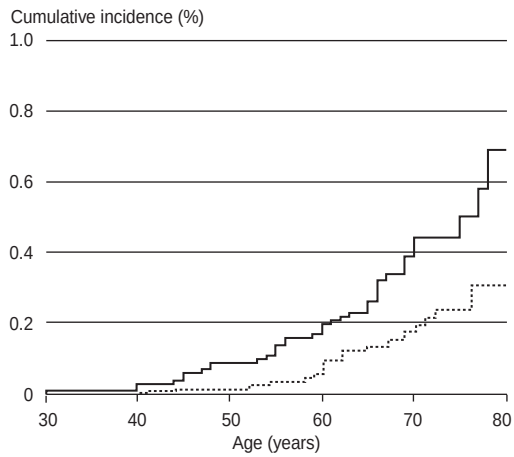

Figure 2. Cumulative incidence of antihypertensive medication in parents of DN+ (solid line; n = 109) and parents of DN – (hatched line; n = 112). Cumulative incidence of antihypertensive medication was higher in parents of DN+ (logrank test: P = 0.002).

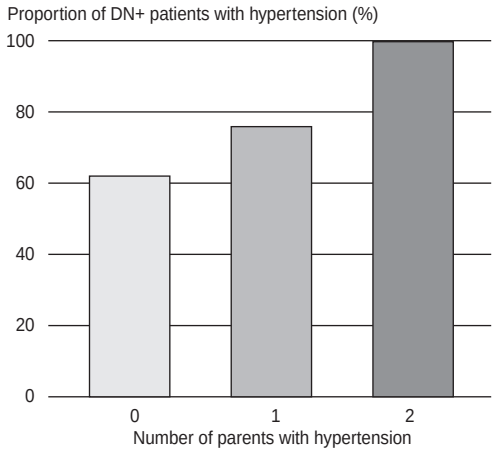

Figure 3. Proportion of patients with DN+ treated for hypertension in relation to antihypertensive treatment in neither, one, or both parents. Hypertension was more common in patients with hypertension in both parents compared to patients without parental hypertension (100% vs 61%, P < 0.05).

After inclusion of data on non-attending parents furnished by their participating spouses, absence or presence of antihypertensive medication could be determined in both parents of 63 (86%) DN+ patients and of 68 (93%) DN– patients. A parental history of hypertension was more common among DN+ patients (56% vs 29%, P < 0.01). In addition, among DN+ patients, parental antihypertensive therapy was associated with increased risk for systemic hypertension in the patients themselves (Figure 3).

No difference existed in prevalence of cardiovascular disease between parents of DN+ and DN– patients (21% vs 18%, P = NS), whereas diabetes was more common in parents of DN+ patients than in those of DN– patients (16% vs 8%, P = 0.034).

Familial predisposition to diabetes (II)

The reliability of the information obtained from diabetic patients was tested by interviewing a subgroup of parents and by reviewing the medical records and death certificates of a subgroup of deceased parents. The patients had identified parental diabetes with a specificity of 93% and a sensitivity of 100%. The corresponding figures for parental hypertension were 89% and 90%, respectively, and for parental death from cardiovascular causes 83% and 97%. Since the main objective was to assess the impact of a family history of diabetes on the development of diabetic nephropathy, only confirmed cases of parental diabetes were included in the analysis, while patient reported data were used regarding parental hypertension and cardiovascular death.

Table 7. Adjusted odds ratios (OR) for variables independently associated with diabetic nephropathy

Variable	OR (95% CI)	P
HbA_{1c}	1.76 (1.29–2.40)	< 0.01
Parental type 2 diabetes	2.95 (1.03–8.40)	< 0.05
Male sex	2.30 (1.13–4.67)	< 0.05
Parental hypertension	2.06 (1.00–4.24)	< 0.05

At the time of the study, 39% and 32% of the parents of patients with DN+ and DN– were deceased (P = NS) with thus no significant difference in proportion of those deceased. However, a survival analysis taking into account the time of follow-up (that is, age at death, or age at the time of the study) revealed impaired survival in parents of DN– patients (logrank test: P < 0.05). No significant difference was found in cardiovascular death rate between the parents in the two groups, although there was a tendency towards higher cardiovascular death rate among mothers of DN+ patients than mothers of DN– patients (P = 0.095) in a sex-stratified analysis.

Diabetes was more prevalent among parents of DN+ patients (16% vs 6%, P < 0.01), mostly due to an excess in type 2 diabetes (14% vs 6%, P < 0.05). In addition, there was an excess of hypertension in parents of DN+ patients (36% vs 19%, P < 0.01). Parental history of type 2 diabetes and hypertension, gender, diabetes duration, smoking, and HbA_{1c} were entered into a forward stepwise multiple logistic regression analysis (Table 7). In addition to glycemic control and male gender, parental history of type 2 diabe-

Table 8. Characteristics of parents with no history of diabetes

Variable	DN+ n=95	DN– n=55	P
Fathers/mothers	36/59	22/33	NS
Age (years)	65±1	65±1	NS
WHR, fathers	0.97±0.01	0.94±0.01	<0.05
WHR, mothers	0.85±0.01	0.83±0.01	NS
BMI, fathers (kg/m^2)	28.0±0.7	26.1±0.9	<0.05
BMI, mothers (kg/m^2)	28.4±0.6	27.9±0.7	NS
Fasting plasma glucose (mmol/l)	5.7±0.1	5.4±0.1	<0.05
Glucose AUC (mmol/l × min)	326±18	300±21	NS
Abnormal glucose tolerance[a] (%)	33	24	NS
Antihypertensive therapy (%)	34	18	<0.05

[a]IGT or diabetes

tes and hypertension were both independently associated with diabetic nephropathy.

An OGTT was performed in parents with no history of diabetes (Tables 5 and 8). Parents of patients with nephropathy had higher fasting plasma glucose levels and had more often been treated for hypertension. Fathers of DN+ patients also had higher WHR than fathers of DN– patients.

Familial abnormalities in glucose metabolism (III)

No differences in insulin sensitivity or in insulin secretion were discernible between relatives of DN+ and DN– patients (Table 9). The relatives of the DN+ showed an excess of hypertension (46% vs 29%, P < 0.05) and more frequently a family history of type 2 diabetes (34% vs 20%, P < 0.05) than did relatives of the DN–. Selection of one relative at random for each diabetic patient did not influence the results (DN+ vs DN–: K_{ITT} 4.0±0.2 vs 3.9±0.1 %/min, P = NS; ratio of insulin AUC/glucose AUC 21.1±4.0 vs 22.1±4.0, P = NS).

Twenty-five relatives of patients with DN+ and 13 relatives of patients with DN– had abnormal glucose tolerance (IGT or diabetes; Table 10). Relatives of DN+ patients were younger. As shown in Table 11, no difference existed in insulin sensitivity between the two groups of glucose-intolerant relatives. However, their insulin response to a rise in plasma glucose (insulin AUC/glucose AUC-ratio) in the OGTT was impaired among relatives of DN+ patients (Table 11). This difference in insulin secretion between relatives of DN+

Table 9. Plasma glucose and serum insulin response to oral glucose load and insulin sensitivity measured in first-degree relatives of DN+ and DN–

Variable	Relatives of DN+ (n=114)	Relatives of DN– (n=93)	P
Fasting plasma glucose (mmol/l)	5.3±0.1	5.3±0.1	NS
Fasting serum insulin (mU/l)	5±1	5±1	NS
Glucose AUC (mmol/l × min)	276±16	225±12	NS
Insulin AUC (mU/l × min)	3349±248	3380±280	NS
Insulin AUC/glucose AUC-ratio	17.9±1.9	17.8±1.9	NS
K_{ITT} (%/min)[a]	4.0±0.1	4.2±0.1	NS

[a]Measured in 101 and 84 relatives of DN+ and DN–, respectively.

Table 10. Characteristics of relatives with abnormal glucose tolerance

Variable	Relatives of DN+ (n=25)	Relatives of DN– (n=13)	P
Sex (M/F)	9/16	6/7	NS
Age (years)	57±3	66±2	0.027
BMI (kg/m^2)	28.1±0.9	28.5±1.3	NS
WHR male subjects	1.00±0.03	0.98±0.02	NS
WHR female subjects	0.84±0.02	0.87±0.02	NS
Hypertension (%)	74	46	0.049
Smoking (%)	16	23	NS
HbA_{1c} (%)	5.6±0.1	5.8±0.1	NS
Proportion IGT/diabetes	18/7	9/4	NS

Table 11. Insulin sensitivity and insulin secretion in relatives with abnormal glucose tolerance

Variable	Relatives of DN+ (n=25)	Relatives of DN– (n=13)	P
K_{ITT} (%/min)[a]	3.3±0.2	3.2±0.3	NS
Fasting plasma glucose (mmol/l)	5.8±0.1	5.7±0.1	NS
Fasting serum insulin (mU/l)	7±1	8±1	NS
Glucose AUC (mmol/l × min)	493±36	387±38	NS
Insulin AUC (mU/l × min)	4089±744	6209±1396	NS
Insulin AUC/glucose AUC-ratio	8.9±1.4	16.8±3.9	0.039

[a]Performed in 23 and 11 relatives of patients with DN+ and DN–, respectively.

and DN– patients was already discernible at the IGT stage (Figure 4).

Familial abnormalities in urinary albumin excretion rate (IV–V)

Study IV

The two groups of relatives participating were comparable regarding sex distribution, age, BMI, and prevalence of smoking (Table 5). Similarly, no difference existed in blood pressure (systolic: 138±2 vs 138±3 mmHg, P = NS; diastolic 84±1 vs 84±2 mmHg, P = NS), prevalence of antihypertensive treatment (17% vs 13%, P = NS), or serum creatinine concentration (80 [44–128] vs 80 [57–112] µmol/l, P = NS) between relatives of patients who were DN+ and DN–, respectively. Overnight UAER did not differ between relatives of DN+ and DN– patients (Figure 5). The proportion of relatives with a UAER 10 µg/min was 12% for the DN+ compared to 8% for the DN– (P = NS). Stratified analyses of males and females as well as of parents and siblings revealed no difference in UAER between the two groups (Table 12).

Table 12. UAER (µg/min) in subgroups of relatives in Study IV

Subgroup	DN+	DN–	P
Male	3.4 (0.1–372)	4.2 (1.1–24.3)	NS
Female	3.5 (0.2–118)	3.5 (0.2–61.5)	NS
Parents	3.5 (0.1–372)	4.0 (0.2–61.5)	NS
Siblings	3.4 (0.2–118)	3.6 (0.4–14.4)	NS

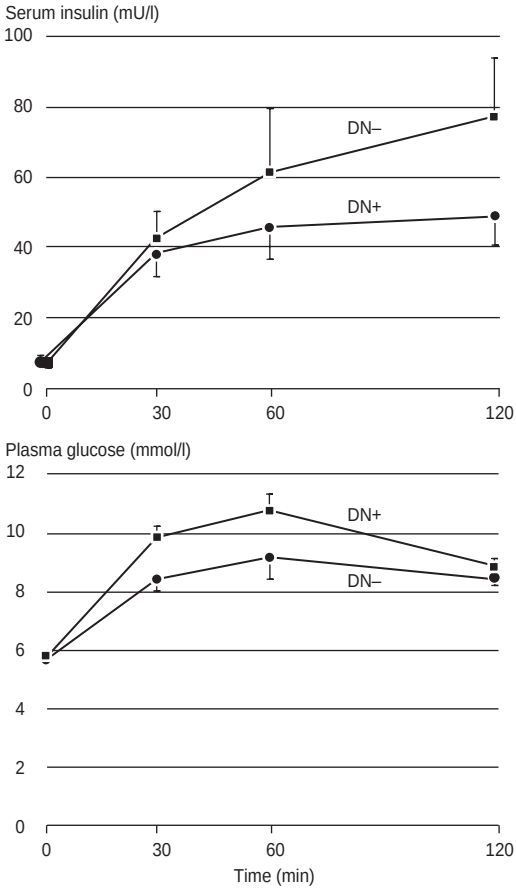

Figure 4. Serum insulin and plasma glucose levels in relatives with impaired glucose tolerance (IGT). The insulin secretion (insulin AUC/glucose AUC-ratio) was impaired in IGT-relatives of DN+ compared to IGT-relatives of DN– (9.3±1.7 vs 16.2±3.4, P=0.058), with no difference observed in insulin sensitivity between these two groups (3.6±0.2 vs 3.6±0.3 %/min, P = NS).

Of relatives of DN+ patients, 32 (17%) were treated for hypertension, and the corresponding number for relatives of the DN– was 7 (13%; P = NS). Among relatives of the DN+, those on antihypertensive treatment had a higher UAER than did those without: 5.0 (0.5–372) vs 3.4 (0.1–26.5) µg/min; P < 0.01. A similar phenomenon was absent from relatives of the DN–, where UAER was comparable in relatives with and without treatment for hypertension: 3.6 (2.1–24.3) vs 4.0 (0.2–61.5) µg/min; P = NS. In order to assess

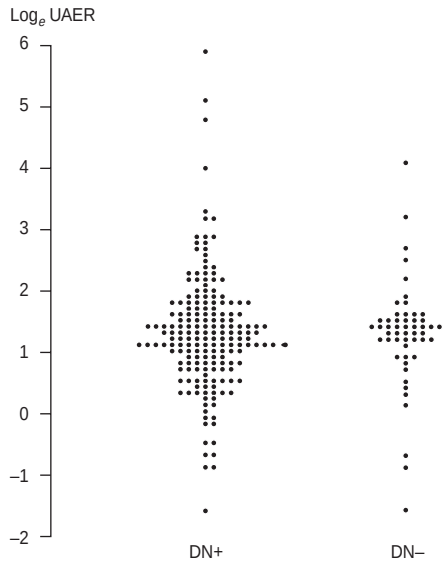

Figure 5. Logarithmically transformed UAER in relatives of patients with DN+ and DN–. There was no difference in UAER between relatives of DN+ and DN– (3.4 [0.1–372] vs 4.0 [0.2–62] µg/min, respectively; P = NS).

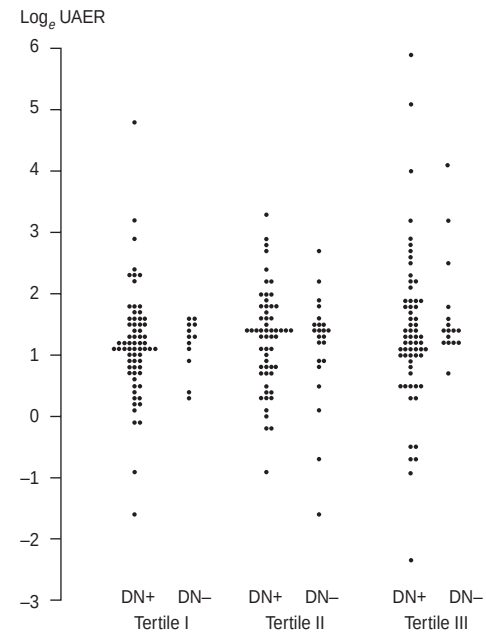

Figure 6. UAER in relatives of patients with DN+ and DN– divided into age tertiles. The tertiles correspond to an age below 43 years (I), between 43 and 62 years (II), and above 62 years (III).

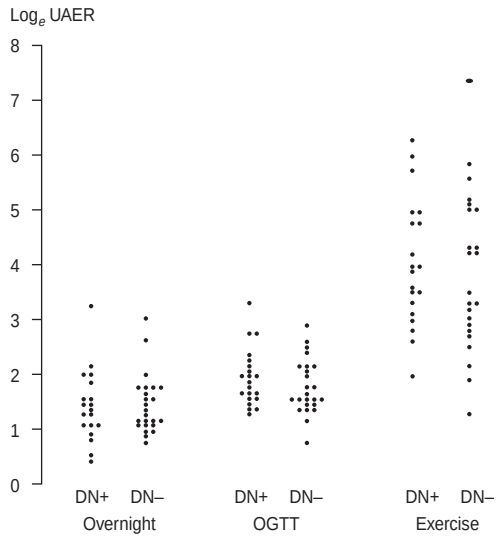

Figure 7. Logarithmically transformed UAER in siblings of patients with DN+ and with DN–. There was no significant difference between siblings of patients with DN+ and DN– in UAER measured overnight: median (range): 3.8 (1.3–24.1) vs 3.5 (2.0–21.0) μg/min; P = NS, during the OGTT 6.3 (3.2–26.0)] vs 4.8 (1.9–15.7) μg/min; P = NS or during the exercise test 44.8 (7.0–535) vs 30.0 (3.4–1614) μg/min; P = NS.

any effect of impaired survival among relatives of patients with nephropathy, the relatives were further divided into tertiles according to age (Figure 6). No difference in UAER was observable in any of the tertiles. In order to control for the range in number of relatives studied per diabetic patient, one relative per diabetic patient was randomly selected. In this analysis, no difference in UAER was observed between the two groups: DN+ (n = 80) vs DN– (n = 25): 3.6 (0.1–168) vs 3.6 (0.2–61.5) μg/min; P = NS.

Study V

As a result of the matching procedure, the two groups of siblings participating in Study V were similar regarding age, gender, BMI, and smoking habits (Table 5). Furthermore, no significant differences were apparent in serum creatinine, blood pressure levels, or plasma glucose or serum insulin levels in the OGTT (Table 13). All siblings had normal glucose tolerance except for one sister of a DN+ patient with IGT. UAER measured from timed urine collections performed overnight, during the OGTT, and during the submaximal exercise test did not differ between the two groups of relatives (Figure 7). Furthermore, the exercise-induced proportional increase in UAER was approximately eight-fold in both groups of siblings; DN+ vs DN–: 8.3 (2.1–193) vs 7.9 (1.7–119)-fold increase; P = NS.

Table 13. Renal function, blood pressure, and glucose metabolism in siblings of patients with DN+ and DN– in Study V

Variable	DN+ (n = 21)	DN– (n = 24)	P
Serum creatinine (μmol/l)	78±2	82±3	NS
Systolic blood pressure (mmHg)	125±2	124±3	NS
Diastolic blood pressure (mmHg)	76±2	76±3	NS
Fasting plasma glucose (mmol/l)	5.1±0.1	5.0±0.1	NS
Glucose AUC (mmol/l × min)	125±27	135±26	NS
Fasting serum insulin (mU/l)	7±1	6±1	NS
Insulin AUC (mU/l × min)	4282±690	3644±801	NS

Discussion

Subjects and methods

The Finnish patients were recruited from the nephrological unit and the diabetic outpatient clinic of the Helsinki University Central Hospital as well as from among members of the Helsinki Diabetes Association, and the Danish patients in Study I from the outpatient clinic of the Steno Diabetes Center in Gentofte, but in both countries the health care systems as well as the epidemiology of type 1 diabetes [187] and its long-term complications [2, 188] are very similar. It is therefore unlikely that the inclusion of patients from two different source populations in Study I would cause problems in terms of validity of the results. Most of the patients with type 1 diabetes residing in the Copenhagen area are treated at the Steno Diabetes Center. Similarly, Helsinki University Central Hospital runs the only nephrological unit in the Helsinki area. Patients from these sources can be considered representative of type 1 diabetic patients with long-duration diabetes with and without diabetic nephropathy. In addition, patients came from the diabetic outpatient clinic of the Helsinki University Central Hospital and via an advertisement in the newsletter of the Helsinki Diabetes Association. These patients could have been affected by some selection bias: first, complicated cases are more easily referred to a university hospital; second, members of a diabetes association can be assumed to be more actively involved in gaining knowledge of their disease than the average diabetic patient. However, as the aim of the present studies was to assess the effect of familial traits on the development of diabetic nephropathy, it is highly unlikely that any selection bias would have had an effect on occurrence of familial traits in patients with and without diabetic nephropathy. In terms of familial factors, the patients included in this study are therefore most likely representative of Caucasian type 1 diabetic patients with and without diabetic nephropathy.

In Study I, *24 h ABPM* was used to measure parental blood pressure in addition to office blood pressure. The SpaceLabs 90207 24 h ABPM device achieved a B-rating [189] when tested according to the British Hypertension Society Protocol [190] and was consequently recommended by the authors for measurement of 24 h ambulatory blood pressure. Day- and night-time blood pressures were calculated by use of individually recorded waking and sleeping hours [191]. Most studies have reported a better correlation with end-organ damage [192–194] and a better predictive value for future cardiovascular events [195] and for treatment-induced regression of left ventricular hypertrophy [196] with 24 h ABPM than with conventional office blood pressure. Thus, 24 h ABPM is considered superior to conventional blood pressure in identifying subjects with clinically relevant hypertension. This superiority is even more pronounced if office blood pressure is measured on a single occasion, since high screening values tend to overestimate and low screening values underestimate true blood pressure, a phenomenon referred to as regression dilution bias [197]. At present, little information is available from prospective studies on the cut-off level for hypertension in 24 h ABPM, but in Study I we applied the operational threshold for hypertension (135/85 mmHg) proposed by Staessen et al [181] and the American Society of Hypertension [182].

In Study III, whole-body insulin sensitivity was measured with the *short ITT* [185]. When glucose disappearance rate is measured

Table 14. Parental hypertension (HT) or blood pressure (BP) and development of diabetic nephropathy (DN) in type 1 diabetes

	No of patients with		Definition of DN+ (UAER)	Parental HT or BP in		Association between parental HT and DN
	DN+	DN–		DN+	DN–	
History of hypertension						
Krolewski 1988[a] [132]	33	56	>70 μg/min	77%	45%	Yes
Barzilay 1992[a] [133]	43	61	>250 μg/min	70%	38%	Yes
Molitch, 1993[a] [201]	73	642	>28 μg/min	39%	40%	No
Rudberg 1998[a] [202]	75	225	>15 μg/min	29%	8%	Yes
Earle 1992[b] [136]	61	61	>30 μg/min	21%	14%	No
Tarnow 1998[b] [200]	163	163	>300 mg/24h	27%	24%	No
Study II[b]	137	54	>200 μg/min	36%	19%	Yes
EURODIAB 1998 [203]	≈1000	≈2250	>20 μg/min	–[c]	–[c]	Yes
Office BP						
Viberti 1987 [131]	17	17	>0.15 g/l	122±17	111±11	Yes
Walker 1990 [135]	20	20	>150 μg/min	99 (77–121)	98 (89-118)	No
Office BP + AHT						
Jensen 1990 [134]	49	49	>300 mg/24h	25%	19%	No
De Cosmo 1997 [205]	31	31	>45 μg/min	42%	14%	Yes
Verhage 1999 [206]	29	28	>20 μg/min	16%	20%	No
24h ABPM + AHT						
Study I	73	73	>200 μg/min	57%	41%	Yes

[a]Proportion of diabetic patients with a history of parental hypertension. [b]Prevalence of hypertension in the parents. [c]No prevalence estimate of parental hypertension available, but a family history of hypertension was associated with an age-adjusted odds ratio for albuminuria of 1.3. Note that regarding the patients included, there is an overlap between studies [132] and [133], [200] and [134], [200] and Study I, and Study I and Study II.

from arterialized venous blood as was the case in Study III, the index of insulin sensitivity in the short ITT (the K_{ITT}-value) has been found to correlate well ($r > 0.8$) with insulin sensitivity measured with the gold standard: the insulin clamp technique [185]. Performed in this way, the short ITT has been found to be reproducible with coefficients of variations of 6 to 13% [185, 198].

Parental mortality

In a family study by Earle and co-workers [136], increased mortality was found among parents of type 1 diabetic patients who showed elevated UAER compared to that of parents of patients with normal UAER. This was mostly due to an excess of parental cardiovascular mortality. This finding was, however, not confirmed by a Danish study [143], but it is worth noting that the number of patients involved in both studies was rather small.

In Study II, we found survival to be impaired among parents of patients with nephropathy. In support of this view, two other recent studies have confirmed increased mortality rates among parents of patients with nephropathy when assessed by survival analysis [199, 200]. Both studies found an excess of cardiovascular mortality in such parents [199, 200], especially an increase in death from stroke in one [199]. In Study II, there was a tendency towards increased cardiovascular mortality rate among mothers of patients with nephropathy compared to mothers of patients without nephropathy. No difference

was evident in cardiovascular death rate in fathers alone or in all parents, and we could confirm no particular increase in deaths from stroke among parents of patients with nephropathy (data not shown). However, Study II did not have the same power as the other recent studies [199, 200]. Thus, diabetic nephropathy in type 1 diabetic patients is associated with impaired survival in the parents, a phenomenon that seems to be due to an excess of cardiovascular disease.

Familial predisposition to hypertension

Viberti and co-workers [131] were the first to report an association between high blood pressure in non-diabetic parents and proteinuria in their offspring with type 1 diabetes. Retrospective analysis of a family study conducted in the 50's revealed a higher arterial blood pressure in 26 parents of 17 patients with a urinary protein concentration exceeding 0.15 g/l than in 26 parents of 17 non-proteinuric patients. This led the authors to put forward the hypothesis that susceptibility to diabetic nephropathy is linked to a familial predisposition to hypertension. However, at the time Study I was initiated, a controversy existed regarding this subject, with some studies supporting the original observation [132, 133], and others unable to find higher blood pressure [134, 135] or an excess of hypertension [136, 201] in parents of patients with signs of diabetic nephropathy.

Table 14 presents an update of studies published thus far dealing with the subject. These exhibit huge variation in sample size, and their definitions of diabetic nephropathy range from the upper level of normoalbuminuria in a Swedish study on pediatric patients [202] to ESRD because of diabetic nephropathy, as in Study II. The methods of determining familial hypertension vary from assessing parental history of hypertension [132, 133, 136, 200–204], to measuring parental office blood pressure [131, 135], to combining antihypertensive medication and office blood pressure [134, 205, 206] or 24 h ABPM [207] in order to calculate total prevalence of parental hypertension. There is an overlap between some of the studies.

At first glance, the results of the studies seem to diverge substantially, but a more detailed analysis enables some conclusions to be drawn. First, although the difference in parental history of hypertension between patients with and without nephropathy is not statistically significant in all studies, there is indeed an evident overall tendency toward an excess of hypertension among parents of patients with nephropathy. The results of the large EURODIAB study [203] speak in favor of such a view. Second, studies relying on office blood pressure may be hampered by obvious methodological shortcomings, as previously discussed. Since no routine treatment for hypertension was available when blood pressure was measured by Viberti et al [131], this may explain why the same group detected no difference in a subsequent study authored by Walker et al [135]. Regarding hypertension defined as office blood pressure or as antihypertensive treatment, only one of four studies [134, 205–207] demonstrates a significant difference between parents of patients with and without nephropathy. The relatively high prevalence of hypertension in Study I can be explained by a lower cut-off level (140/90 mmHg) for untreated hypertension than in two of the other studies (160/95 mmHg) [134, 206] and to the fact that the parents in Study I were older than in the other studies. Study I is the only study having used 24 h ABPM to identify parents with untreated hypertension. In conclusion, an excess of familial hypertension appears in type 1 diabetic patients with diabetic nephropathy, but in order to detect this excess, proper evaluation of parental blood pressure status and sufficient statistical power are required.

Thus far, Study I is also the only study making any attempt to evaluate severity of hypertension by taking into account the age of onset of parental hypertension. Parents of patients with diabetic nephropathy showed an increased cumulative incidence rate of hypertension, and a pronounced excess of hypertension with a rather young age at onset

(Figure 2). Two studies on adolescent [202] and pediatric patients [137] support these findings. In the study by Rudberg et al [202], a parental history of hypertension was associated with a four-fold risk for micro- or macroalbuminuria in the offspring. Likewise, Freire and co-workers [137] found that among normoalbuminuric patients with an UAER in the highest tertile, 79% had a family history of hypertension compared to only 29% of patients with UAER in the lowest tertile. Based on the patients' age, the mean age of the parents in these studies must have been low, and both report strong associations between elevated UAER and parental hypertension. Thus, a familial predisposition to a phenotypically more severe hypertension, manifested in the parents at a relatively young age, may be of particular importance as a determinant of renal outcome in patients with type 1 diabetes. However, before drawing any further conclusions in terms of identifying high-risk patients, our hypothesis needs to be tested in large-scale prospective study settings.

Hypertension was more often present in those patients suffering from nephropathy who had a parental history including antihypertensive therapy. Although this finding must be regarded as preliminary because comparisons were made between relatively small groups, it suggests that for patients with diabetic nephropathy, a familial predisposition to hypertension is associated not only with their increased risk for diabetic nephropathy, but also with their increased risk for systemic hypertension.

Why would a predisposition to hypertension increase risk for nephropathy in type 1 diabetes? The importance of hemodynamic factors in the genesis of diabetic nephropathy has been discussed above. It is of interest that a family history of hypertension has been associated both with elevated systemic blood pressure and with increased glomerular filtration in recent-onset type 1 diabetic patients as well as in non-diabetic subjects [208, 209]. Of these abnormalities, glomerular hyperfiltration may be more important in the initiation of renal damage, since such hyperfiltration [96] is a more powerful predictor of subsequent microalbuminuria or overt diabetic nephropathy than is the level of systemic blood pressure [24, 96, 104, 105]. However, at a later stage, the level of systemic blood pressure is strongly positively correlated with the rate of decline in renal function.

Familial predisposition to diabetes

In Pima Indians, diabetic nephropathy in the parents, more than parental diabetes alone, has been associated with an increased risk for diabetes in the offspring [152], suggesting a link between familial diabetes and development of diabetic nephropathy. Studies in type 1 diabetes evaluating parental hypertension [131, 134, 135, 202] and prevalence [136, 143, 200] or mechanisms [205, 206] of parental cardiovascular disease, have not reported any excess of parental diabetes in type 1 diabetic patients with nephropathy. However, these studies have in general been small. Support of our observation in Study II comes from two recent large-scale studies. An association between parental diabetes and albuminuria in the type 1 diabetic offspring was observed in the EURODIAB Study [203]. In the Pittsburgh Epidemiology of Diabetes Complications Study [210], the prevalence of nephropathy was significantly higher in univariate analysis among type 1 diabetic patients with familial type 2 diabetes than in those without.

In Study II, parental type 2 diabetes was associated with an almost three-fold risk for nephropathy in the offspring, which exceeds the 95% CI of the EURODIAB Study [203]. Additionally, in the EURODIAB Study, the finding was limited to female patients alone, whereas the association in male patients, after adjustment for glycemic control, failed to reach statistical significance. However, the classification of patients into albuminuric and normoalbuminuric in the EURODIAB Study was based on a single urine collection, and the control group had had a relatively short diabetes duration and could therefore have included some proportion of patients at con-

siderable risk for subsequent development of nephropathy. Misclassification of cases and controls in a study with a case-control design will lead to an underestimation of any associated phenomenon. Therefore, the stronger association between diabetic nephropathy and parental diabetes found in our Study II is most likely a result of the more robust classification of cases and controls we used.

In the insulin resistance syndrome, hypertension and abnormalities of glucose metabolism are closely intertwined [14], so that an association of diabetic nephropathy with parental diabetes may be just another reflection of its association with parental hypertension. However, the association of diabetic nephropathy with a family history of diabetes was independent of that of a family history of hypertension in Study II, which was also the case in the EURODIAB Study [203]. Therefore, a family history of diabetes and familial predisposition to hypertension seem to influence the risk of nephropathy via different mechanisms.

In order to identify the mechanisms linking a family history of diabetes to diabetic nephropathy in type 1 diabetes, a further characterization of the mechanisms behind the familial clustering of diabetes is needed. The two key variables involved in the regulation of glucose metabolism are insulin sensitivity and insulin secretion [211]. Study II found a particular excess of parental type 2 diabetes, defined as all cases of diabetes other than those with their onset before age 40 and that had been treated with insulin alone, but it is evident that this definition will include cases of diabetes with a wide range of different etiologic mechanisms [212]; it is thus impossible to further characterize the pathogenetic mechanisms based on this observation.

Impaired insulin sensitivity has been found present in the early stages of diabetic nephropathy in both type 1 [146, 147, 213] and type 2 diabetes [150, 214]. This impairment in insulin sensitivity has in part been attributed to genetic factors, since insulin resistance, either based on fasting hyperinsulinemia [153] or measured with the short ITT [205], has been found present in relatives of type 1 diabetic patients with micro- or macroalbuminuria. Furthermore, assessment of the non-diabetic parents participating in Study II revealed, in parents of patients with diabetic nephropathy, an excess of abdominal obesity, higher fasting plasma glucose, and more hypertension – all factors associated with the insulin resistance syndrome [14]. However, in Study III, we found no significant impairment of insulin sensitivity in non-diabetic relatives of patients with nephropathy compared to that in relatives of patients without nephropathy, despite Study III's being the largest one thus far that has addressed this question.

Differences in study design may explain some of this discrepancy. Study III focused only on relatives with normal fasting blood glucose, and this differs from the strategy applied by De Cosmo and coworkers, who also included diabetic parents [205]. In Study III, there was an excess of diabetes among relatives of patients with nephropathy, and exclusion of these diabetic individuals most likely resulted in an underestimation of the true abnormalities in glucose metabolism in this group. However, chronic hyperglycemia has well-known effects on glucose metabolism, including an impairment in both insulin sensitivity and insulin secretion [215]. If we had included diabetic relatives in Study III, we would have been unable to exclude the fact that any impairment observed in, for instance, insulin sensitivity was merely a consequence of their chronic hyperglycemia. Focusing on those relatives without severe hyperglycemia offered us an opportunity to identify the underlying mechanisms of the excess of diabetes in relatives of patients with nephropathy.

Moreover, De Cosmo's group [205] observed parents alone, not, as in the present study, all first-degree relatives. Indeed, in Study III, insulin sensitivity tended to be impaired, especially in fathers of patients with nephropathy (K_{ITT} 2.9 vs 3.7%/min, respectively). We measured insulin sensitivity with the short ITT, a method found to be both re-

liable and reproducible in estimating whole-body insulin sensitivity [185, 198]. However, one cannot ignore the fact that the use of more sophisticated methods, for instance the insulin clamp technique, could have detected a difference in insulin sensitivity between the two groups. A role for impaired insulin sensitivity cannot, therefore, be excluded in the familial clustering of diabetes in patients with nephropathy, but if impaired insulin sensitivity does play a major role, one would have expected it to be detected with our sample size, despite these various shortcomings. Another recent study found, in parents of patients with nephropathy, no clustering of factors associated with the metabolic syndrome [206].

The new finding in Study III was impaired insulin secretion in relatives of patients with nephropathy, a finding not evident when assessed in all relatives, but isolated in relatives with signs of abnormal glucose tolerance. Since this was the result of a post-hoc analysis, the finding should be interpreted with caution. It is, however, rational to focus on those relatives with evidence of mild abnormalities in glucose metabolism, since only a minority of all the relatives in this study will ever develop diabetes, and the mechanisms responsible for an excess of familial diabetes in patients with nephropathy will most likely be found in that group. In this respect, it is noteworthy that insulin response to a glucose load is related to degree of derangement of glucose metabolism, with a more marked hypoinsulinemia as the degree of hyperglycemia increases [216]. The question arises, whether the impairment of insulin secretion observed is a primary phenomenon, or whether it is just a consequence of subclinical hyperglycemia. Although the latter is possible, it should be emphasized that all the relatives studied had only mild abnormalities in glucose metabolism – in fact, they would all have been classified as non-diabetic based on their fasting plasma glucose level. Furthermore, no differences existed between the groups in the proportions of IGT and diabetes, or in the level of HbA_{1c}. Finally, although based on comparison between very small groups, the impairment in insulin secretion in relatives of patients with nephropathy was already present at the stage of IGT. Therefore, even though our results are preliminary and need to be confirmed, they suggest that the excess of diabetes in the family history for type 1 diabetic patients with nephropathy at least in part may be due to a primary, possibly inherited, impairment in insulin secretion.

By what mechanism could such familial abnormalities increase the risk for diabetic nephropathy in the type 1 diabetic patient? First, patients with an inherited tendency toward impaired insulin sensitivity may be at increased risk for nephropathy since good metabolic control is likely to be more difficult to achieve in such patients. They may also require higher doses of exogenous insulin, and the resultant hyperinsulinemia may increase UAER via increased endothelial permeability [217] or via effects on glomerular hemodynamics [218, 219]. Factors associated with impaired insulin sensitivity, such as hypertension [220] and dyslipidemia [221], may also exert deleterious effects on the glomerulus.

Second, type 1 diabetic patients with residual endogenous insulin secretion are at lower risk of diabetic microvascular complications than are patients with complete loss of beta-cell function [179]. An inherited insulin-secretion defect may increase risk for nephropathy by causing a more complete loss of beta-cell function at the time of onset of diabetes. This could simply result in worse metabolic control, with harmful effects on the kidneys. However, beneficial effects of c-peptide on renal function have also been described [222], and the absence of circulating c-peptide could thereby increase risk for diabetic nephropathy. Furthermore, gestational diabetes develops when the beta cells are unable to respond to the increased need for insulin due to the insulin resistance induced by pregnancy [223]. The recent observation in Pima Indians that offspring of mothers who have diabetes during their pregnancy are at increased risk for elevated UAER [224] may provide an additional mechanism for an increased risk

for nephropathy in patients with familial defects in insulin secretion, because intrauterine hyperglycemia exerts detrimental effects on fetal kidney function [225].

In the light of our findings, several questions need to be answered in large-scale study settings for type 1 diabetic patients. First, is it possible to confirm that preserved beta-cell function protects against development of diabetic nephropathy? If so, is this just an effect of enhanced metabolic control, or is there perhaps some protective effect from the c-peptide molecule? Second, what is the relation between familial diabetes and residual beta-cell function in the type 1 diabetic patient? Third, is there any association between maternal gestational diabetes and development of diabetic nephropathy in the offspring? The answers to these questions will determine the importance of our finding of impaired insulin secretion in glucose-intolerant relatives of patients with elevated UAER.

Familial abnormalities in urinary albumin excretion rate

Studies in both type 1 [6, 120, 121, 123] and type 2 diabetes [124, 126, 127] have reported familial clustering of microalbuminuria and/or proteinuria in diabetic siblings. Thus, in the presence of diabetes, genetic factors seem to influence UAER. Several family studies have reported an elevated UAER also in non-diabetic relatives of those type 2 diabetic patients showing micro- or macroalbuminuria when compared with that in relatives of normoalbuminuric patients [126, 128–130]. Therefore, at least for relatives of type 2 diabetic patients, abnormalities leading to an elevated UAER seem to be present even in the absence of diabetes in subjects with a potential genetic susceptibility to diabetic nephropathy. Studies IV and V thus far offer the only such data available in type 1 diabetes and indicate that similar abnormalities in UAER are absent from non-diabetic relatives of patients with type 1 diabetes and nephropathy.

Since there was no difference in UAER between relatives of patients with and without nephropathy, the power to detect such a difference is of crucial importance. Assuming that the distributions of UAER in both groups were similar to those observed, Study IV would have detected a relatively small difference in median value between the two groups (5.1 µg/min in relatives of DN+ vs 4.0 µg/min in relatives of DN– patients). Studies performed in type 2 diabetes [126, 128, 129] have reported clearly elevated (approximately 2-fold) UAER in non-diabetic relatives of patients with nephropathy compared to that in relatives of patients without nephropathy. A recent study of relatives of Finnish type 2 diabetic patients found this difference to be somewhat smaller [130]. Although our study cannot totally exclude a minimal elevation in UAER in non-diabetic relatives of type 1 diabetic patients with nephropathy, it seems justified to question the relevance of an elevation in UAER in such relatives not detected in a sample of our size.

Features associated with elevated albuminuria such as cardiovascular morbidity and mortality [226, 227], diabetes [228], insulin resistance [229], and hypertension [230] have all been found to cluster in family members of type 1 diabetic patients with diabetic nephropathy [131, 136, 153, 203, 204, 207]. Therefore, since we studied UAER in surviving non-diabetic relatives in Study IV and in non-diabetic siblings with no antihypertensive therapy in Study V, an underestimation of the "true" UAER in relatives of type 1 diabetic patients with nephropathy may have taken place. However, also in type 2 diabetes, relatives of patients with nephropathy are at increased risk for hypertension [231] and diabetes [152] compared to relatives of patients without nephropathy. Most importantly, the original observation by Gruden et al [128] was made in a case-control study with 20 offspring in each group, all of whom were normotensive and had normal glucose tolerance. The confirming observation by Strojek et al [129] was also based on offspring selected to be normotensive and to have normal oral glucose tolerance. The studies on siblings of type 2 diabetic patients [126, 130] may readily

have been affected by selective mortality. In other words, a similar selection bias is likely to affect all the studies in type 2 diabetes. Therefore, the most likely explanation for the discrepancy between ours and the previous findings is that a true difference exists between mechanisms regulating UAER in non-diabetic relatives of type 1 and type 2 diabetic patients.

In Study V, the relatives selected were those not receiving antihypertensive therapy, nor was any difference observed in the prevalence of hypertension between the two groups of relatives in Study IV. The latter fact was somewhat surprising, since there was an excess of familial hypertension in the whole study population, as demonstrated in Study II. A recent study has concluded that common genetic determinants of UAER and blood pressure exist in families with type 2 diabetes [232]. Therefore, the absence of a difference in blood pressure level in Studies IV and V may have contributed to the lack of difference in UAER. However, it should be noted that all [126, 128, 130] but one [129] of the four studies in type 2 diabetes found no difference in blood pressure between relatives of patients with and without nephropathy despite the difference observed in UAER.

Exercise increases albuminuria in both diabetic and non-diabetic subjects [233, 234], but more pronouncedly in diabetic patients with early signs of diabetic nephropathy [233]. Furthermore, a prominent rise in exercise-induced UAER has been predictive of subsequent microalbuminuria [235]. The study by Strojek et al [129] found an exaggerated albuminuric response to physical exercise in relatives of albuminuric compared to normoalbuminuric type 2 diabetic patients, a 16-fold vs a 6-fold increase, respectively [129]. No such tendency was evident in Study V, which further supports the theory of differences in mode of inheritance of UAER between type 1 and type 2 diabetes.

How can such a difference between type 1 and type 2 diabetes be explained? One possibility may be that a common familial susceptibility to elevated UAER exists in both types of diabetes, but that an additional trigger, such as diabetes, dyslipidemia, or hypertension, is needed for the susceptibility to manifest itself as increased UAER. Non-diabetic individuals with a family history of type 2 diabetes display various metabolic and hemodynamic alterations [144, 145] not present in non-diabetic subjects with a family history of type 1 diabetes [236]. It may be hypothesized that such an unmasking trigger is present in the non-diabetic relatives of type 2 diabetic patients, but not in those of type 1 diabetic patients. It is therefore of interest that in Study IV, hypertensive relatives had higher UAER than normotensive relatives when it was specifically assessed in the relatives of patients with nephropathy. No such effect appeared among relatives of patients without nephropathy. The comparison was, however, made between small groups and does not allow any further conclusions to be drawn, but if the albuminuric response to hypertension indeed differs between relatives of patients with and without nephropathy, it would favor this hypothesis. Moreover, the finding that persistently elevated UAER is often present at diagnosis [237] and even precedes development of type 2 diabetes [238] but is usually absent at diagnosis of type 1 diabetes [239] is compatible with this hypothesis.

On the other hand, the discrepancy may also reflect differences in susceptibility to elevated UAER between type 1 and type 2 diabetes. In support of this, only 30% of type 2 diabetic patients with microalbuminuria (comparable to the cases included in the studies in type 2 diabetes [126, 128–130]) have been found to have structural lesions typical of diabetic nephropathy [240]. Type 1 diabetic patients with overt proteinuria (comparable to our cases) have the morphologically rather monotonous pattern typical of diabetic nephropathy [59]. Microalbuminuria is, furthermore, a strong predictor of overt diabetic nephropathy in type 1 diabetes [20–22], while in type 2 diabetes, microalbuminuria is, instead, prognostic of cardiovascular events [241]. Therefore, differences regarding

mechanisms behind elevated UAER, and perhaps also in genetic susceptibility to albuminuria, may exist between type 1 and type 2 diabetes.

Familial clustering – genes or environment?

The basis for genetic susceptibility to diabetic nephropathy is mostly based upon evidence of familial clustering, not only of diabetic nephropathy [6, 120–123], but also of traits clustering in the family members of patients with nephropathy, as in the present studies. However, familial clustering can be demonstrated for almost any disease [242], and it is difficult to differentiate to what extent this is due to shared genes on the one hand and to similar known and unknown environmental factors on the other. A simulation study has provided some insight into this problem [243]. By assuming no genetic susceptibility at all, it found that familial clustering of environmental factors with a relative risk for disease of less than 10 lead to only a minor increase in familial clustering of disease, even in the case of complete correlation of exposure. Therefore, genetic factors, acting either on their own or in concert with the environment, ought to be important in the familial clustering of a disease. This view is supported by recent segregation analyses in type 2 diabetes [154, 155].

During the last decade, many attempts have been made to identify one or several genes that increase susceptibility to diabetic nephropathy, but thus far, no gene has been identified with any major impact on the development of nephropathy [156]. What are the implications of the present studies in the search for diabetic nephropathy susceptibility genes? First, the association of diabetic nephropathy with familial hypertension is confirmed (Study I). If identified, genes associated with hypertension of early onset are of particular interest. Second, genes related to diabetes, to the metabolic syndrome, or especially to impaired insulin secretion qualify as candidate genes for diabetic nephropathy (Studies II and III). Finally, and most importantly, differences may exist between susceptibility to elevated UAER in type 1 and type 2 diabetes (Studies IV and V). Distinct subphenotyping of the type of diabetes in the patients studied may therefore prove worthwhile, and results from studies with mixed type 1 and type 2 diabetic patients should be interpreted with caution.

Summary and conclusions

1. Diabetic nephropathy was associated with a familial predisposition to hypertension. Genetic or environmental factors related to hypertension, especially to a more severe form of hypertension manifested relatively early in life, may be important in the development of diabetic nephropathy in type 1 diabetes.
2. Diabetic nephropathy was associated with a familial predisposition to diabetes. Factors related to familial clustering of diabetes may predispose to diabetic nephropathy in type 1 diabetes.
3. In relatives of patients with diabetic nephropathy, diminished insulin secretion rather than impaired insulin sensitivity, characterized early abnormalities in glucose metabolism. This may explain the excess of diabetes found in relatives of patients with nephropathy. In the development of diabetic nephropathy, factors related to impaired insulin secretion need to be further evaluated.
4. No abnormalities of UAER were present in non-diabetic first-degree relatives of type 1 diabetic patients with diabetic nephropathy. This differs from what has been found in type 2 diabetes and may suggest differences in susceptibility to albuminuria between type 1 and type 2 diabetes.

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