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# Familial Aggregation of Type 1 Diabetes and Diabetic Nephropathy in Finland

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National Public Health Institute Helsinki, Finland
*and*
Department of Public Health, University of Helsinki, Finland

Helsinki 2007

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# FAMILIAL AGGREGATION OF TYPE 1 DIABETES AND DIABETIC NEPHROPATHY IN FINLAND

A C A D E M I C  D I S S E R T A T I O N

*To be presented with the permission of the Faculty of Medicine, University of Helsinki, for public examination in Auditorium XIV, University Main Building, on February 10, 2007 at 10 o'clock.*

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*and*

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# ABSTRACT

Type 1 diabetes (T1D) is a common, multifactorial disease with strong familial clustering. In Finland, the incidence of T1D among children aged 14 years or under is the highest in the world. The increase in incidence has been approximately 2.4% per year. Although most new T1D cases are sporadic the first-degree relatives are at an increased risk of developing the same disease. This study was designed to examine the familial aggregation of T1D and one of its serious complications, diabetic nephropathy (DN). More specifically the study aimed (1) to determine the concordance rates of T1D in monozygotic (MZ) and dizygotic (DZ) twins and to estimate the relative contributions of genetic and environmental factors to the variability in liability to T1D as well as to study the age at onset of diabetes in twins; (2) to obtain long-term empirical estimates of the risk of T1D among siblings of T1D patients and the factors related to this risk, especially the effect of age at onset of diabetes in the proband and the birth cohort effect; (3) to establish if DN is aggregating in a Finnish population-based cohort of families with multiple cases of T1D, and to assess its magnitude and particularly to find out whether the risk of DN in siblings is varying according to the severity of DN in the proband and/or the age at onset of T1D: (4) to assess the recurrence risk of T1D in the offspring of a Finnish population-based cohort of patients with childhood onset T1D, and to investigate potential sex-related effects in the transmission of T1D from the diabetic parents to their offspring as well as to study whether there is a temporal trend in the incidence.

The study population comprised of the Finnish Young Twin Cohort (22,650 twin pairs), a population-based cohort of patients with T1D diagnosed at the age of 17 years or earlier between 1965 and 1979 (n=5,144) and all their siblings (n=10,168) and offspring (n=5,291). A polygenic, multifactorial liability model was fitted to the twin data. Kaplan-Meier analyses were used to provide the cumulative incidence for the development of T1D and DN. Cox's proportional hazards models were fitted to the data. Poisson regression analysis was used to evaluate temporal trends in

incidence. Standardized incidence ratios (SIRs) between the first-degree relatives of T1D patients and background population were determined.

The twin study showed that the vast majority of affected MZ twin pairs remained discordant. Pairwise concordance for T1D was 27.3% in MZ and 3.8% in DZ twins. The probandwise concordance estimates were 42.9% and 7.4%, respectively. The model with additive genetic and individual environmental effects was the best-fitting liability model to T1D, with 88% of the phenotypic variance due to genetic factors. The second paper showed that the 50-year cumulative incidence of T1D in the siblings of diabetic probands was 6.9%. A young age at diagnosis in the probands considerably increased the risk. If the proband was diagnosed at the age of 0-4, 5-9, 10-14, 15 or more, the corresponding 40-year cumulative risks were 13.2%, 7.8%, 4.7% and 3.4%. The cumulative incidence increased with increasing birth year. However, SIR among children aged 14 years or under was approximately 12 throughout the follow-up. The third paper showed that diabetic siblings of the probands with nephropathy had a 2.3 times higher risk of DN compared with siblings of probands free of nephropathy. The presence of end stage renal disease (ESRD) in the proband increases the risk three-fold for diabetic siblings. Being diagnosed with diabetes during puberty (10-14) or a few years before (5-9) increased the susceptibility for DN in the siblings. The fourth paper revealed that of the offspring of male probands, 7.8% were affected by the age of 20 compared with 5.3% of the offspring of female probands. Offspring of fathers with T1D have 1.7 times greater risk to be affected with T1D than the offspring of mothers with T1D. The excess risk in the offspring of male fathers manifested itself through the higher risk the younger the father was when diagnosed with T1D. Young age at onset of diabetes in fathers increased the risk of T1D greatly in the offspring, but no such pattern was seen in the offspring of diabetic mothers. The SIR among offspring aged 14 years or under remained fairly constant throughout the follow-up, approximately 10.

The present study has provided new knowledge on T1D recurrence risk in the first-degree relatives and the risk factors modifying the risk. Twin data demonstrated high genetic liability for T1D and increased heritability. The vast majority of affected MZ twin pairs, however, remain discordant for T1D. This study confirmed the drastic impact of the young age at onset of diabetes in the probands on the increased risk of T1D in the first-degree relatives. The only exception was the absence of this pattern in the offspring of T1D mothers. Both the sibling and the offspring recurrence risk studies revealed dynamic changes in the cumulative incidence of T1D in the first-degree relatives. SIRs among the first-degree relatives

of T1D patients seems to remain fairly constant. The study demonstrates that the penetrance of the susceptibility genes for T1D may be low, although strongly influenced by the environmental factors. Presence of familial aggregation of DN was confirmed for the first time in a population-based study. Although the majority of the sibling pairs with T1D were discordant for DN, its presence in one sibling doubles and presence of ESRD triples the risk of DN in the other diabetic sibling. An encouraging observation was that although the proportion of children to be diagnosed with T1D at the age of 4 or under is increasing, they seem to have a decreased risk of DN or at least delayed onset.

Keywords: type 1 diabetes, twins, co-twin, concordance, heritability, familial aggregation, cumulative incidence, sibling, population-based, sibling recurrence risk, diabetic nephropathy, end stage renal disease, offspring, temporal trend, sex difference

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# TIIVISTELMÄ

Väitöstyön tarkoituksena oli tutkia tyypin 1 diabeteksen (T1D) sekä diabeettisen nefropatian (DN) kasautumista perheissä. Ensimmäisen osatyön tavoitteena oli tutkia monotsygoottisten että ditsygoottisten kaksosten konkordanssia T1D:n suhteen sekä määrittää geneettisten tekijöiden ja ympäristötekijöiden suhteellista osuutta T1D:n synnyssä. Toisen osatyön tarkoitus oli määrittää T1D:sta sairastavien sisarusten kumulatiivinen sairastumisriski T1D:een pitkän seuranta-ajan kuluessa. Tavoitteena oli myös selvittää riskiin vaikuttavia tekijöitä, erityisesti indeksipotilaan (probandin) sairastumisiän vaikutusta sekä eri syntymäkohorttien välisiä eroja. Kolmannen osatyön päämääränä oli tutkia, onko perheen toisella diabeetikkosisaruksella kohonnut riski sairastua diabeettiseen nefropatiaan, jos aiemmin sairastuneella sisaruksella on jo todettu tämä tauti. Tarkoituksena oli myös selvittää, vaikuttaako diabeettisen nefropatian vaikeusaste (dialyysi ja munuaisensiirto) sisarusriskiin sekä sitä, onko diabetekseen sairastumisiällä vaikutusta nefropatiariskiin. Viimeisessä osatyön tarkoitus oli tutkia T1D:ta sairastavien henkilöiden lasten diabetesriskiä longitudinaalisesti ja sitä, eroaako diabeetikkomiesten ja -naisten lasten diabetesriski. Lisäksi tutkittiin, vaikuttaako diabeetikkovanhemman sairastumisikä lapsen riskiin ja erityisesti, onko sairastumisiän vaikutus samanlainen sekä diabeetikkomiesten että diabeetikkonaisten lapsilla. Myös lasten syntymäkohortin vaikutusta tutkittiin.

Kolme laajaa, väestöpohjaista aineistoa oli käytössä. Ensimmäinen aineisto sisälsi kaikki Suomessa vuosina 1958–86 syntyneet kaksosparit (nuorten kaksosten kohortti, n=22,646 paria)). Toinen aineisto koostui vuosina 1965–79 T1D:een alle 18-vuotiaina sairastuneista diabeetikoista (n=5,144) ja heidän sisaruksistaan (n=10,168) ja kolmas aineisto koostui em. diabeetikoista ja heidän lapsistaan (n=5,291).

Tärkeimmät tilastolliset tutkimusmenetelmät olivat Kaplan-Meier-analyysi, Coxin regressioanalyysi, Poissonin regressioanalyysi ja rakenneyhtälömallitus. Monotsygoottisista kaksospareista 27.3 % oli konkordantteja T1D:n suhteen,

kun taas ditsygoottisista kaksospareista vain 3.8 %. Rakenneyhtälömallituksessa parhaiten sopiva malli selitti sairastumiseen liittyvää vaihtelua sekä geneettisin että yksilöllisin ympäristötekijöin ja heritabiliteetin estimaatiksi saatiin 88 %. Identtiset kaksoset sairastuivat hyvin samanikäisinä. Suurin diskordanssiaika konkordanteilla pareilla oli 6.9 vuotta. Identtisten kaksosparien sairastumisiän korrelaatio oli 0.95, kun se epäidenttisillä kaksospareilla oli 0.43.

Diabeetikkojen sisarusten pitkä seuranta osoitti, että T1D:n kumulatiivinen riski 50 ikävuoteen mennessä oli 6.9 %. Riskiin vaikutti kuitenkin hyvin voimakkaasti probandin sairastumisikä. Jos probandi oli sairastunut 0-4, 5-9, 10-14 tai yli 15-vuotiaana, 40 ikävuoden kumulatiivinen riski sisaruksilla oli vastaavasti 13.2, 7.8, 4.7 ja 3.4 %. Mitä myöhäisempään syntymäkohorttiin sisarus kuului, sitä suurempi oli kumulatiivinen riski. Kuitenkin, sisarusten ja taustaväestön ilmaantuvuuksien välinen standardisoitu ilmaantuvuussuhde (SIR) oli noin 12 koko seurantajakson ajan.

Perheessä myöhemmin T1D:een sairastuneella sisaruksella oli 2.3-kertainen riski sairastua DN:aan, jos ensiksi T1D:een sairastuneella oli DN. Jos probandilla oli loppuvaiheen munuaistauti (dialyysi, munuaissiirre), riski kasvoi kolminkertaiseksi. Diabetekseen sairastumisikä vaikutti riskiin siten, että suurin riski oli henkilöillä, jotka olivat sairastuneet murrosiässä tai joitakin vuosia ennen murrosikää.

T1D:ta sairastavien miesten lasten kumulatiivinen riski sairastua T1D:een oli 20 ikävuoteen mennessä 7.8 %, kun taas diabeetikkonaisten lasten riski oli 5.3 %. Kaiken kaikkiaan diabeetikkomiesten lasten sairastumisriski oli 1.7-kertainen diabeetikkonaisten lasten riskiin verrattuna. Kun miesdiabeetikkojen lasten riski kasvoi sitä suuremmaksi, mitä nuorempana miesdiabeetikko oli diagnosoitu, naisdiabeetikkojen sairastumisiällä ei ollut vaikutusta lasten sairastumisriskiin. Lasten ja taustaväestön ilmaantuvuuksien välinen SIR oli noin 10 koko seurantajakson ajan, mutta kumulatiivinen riski oli sitä suurempi, mitä nuorempi syntymäkohortti oli kyseessä.

Avainsanat: Tyypin 1 diabetes, kaksoset, kaksospari, konkordanssi, heritabiliteetti, perheaggregaatio, kumulatiivinen ilmaantuvuus, sisarus, väestöpohjainen, sisarusriski, diabeettinen nefropatia, loppuvaiheen munuaistauti, jälkeläinen, ajallinen trendi, sukupuoliero

# CONTENTS

# ABBREVIATIONS

| | |
|---|---|
| ACE | Angiotensin-converting enzyme |
| BCG | Bacillus Calmette-Guérin –vaccine |
| DCCT | Diabetes Control and Complications Trial |
| CDR | Central Drug Register |
| CI | Confidence Interval |
| CPR | Central Population Register |
| DERI | Diabetes Epidemiology Research International |
| DN | Diabetic nephropathy |
| DN+ | Diabetic nephropathy present |
| DN- | Diabetic nephropathy absent |
| DZ | Dizygotic |
| ESRD | End-stage renal disease |
| $HbA_{1c}$ | Glycosylated haemoglobin $A_{1c}$ |
| HDR | Hospital Discharge Register |
| HLA | Human Leukocyte Antigen |
| ID | Personal identifier |
| ICA | Islet cell antibody |
| $\lambda_r$ | Relative recurrence risk ratio |
| $\lambda_s$ | Sibling recurrence risk ratio |
| MHC | Major histocompatibility complex |
| MMR | Measles, mumps, and rubella vaccine |
| MZ | Monozygotic |
| RR | Relative Risk |
| SIR | Standardized incidence ratio |
| T1D | Type 1 diabetes |
| T2D | Type 2 diabetes |
| UAER | Urinary albumin excretion rate |

# LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles referred to in the text by their Roman numerals:

**I** Hyttinen V, Kaprio J, Kinnunen L, Koskenvuo M, Tuomilehto J. Genetic liability of type 1 diabetes and the onset age among 22,650 young Finnish twin pairs: a nationwide follow-up study. *Diabetes* 52:1052-1055, 2003

**II** Harjutsalo V, Podar T, Tuomilehto J. Cumulative incidence of type 1 diabetes in 10,168 siblings of Finnish young-onset type 1 diabetic patients. *Diabetes* 54:563-569, 2005

**III** Harjutsalo V, Katoh S, Sarti C, Tajima N, Tuomilehto J. Population-based assessment of familial clustering of diabetic nephropathy in type 1 diabetes. *Diabetes* 53:2449-2454, 2004

**IV** Harjutsalo V, Reunanen A, Tuomilehto J. Differential transmission of type 1 diabetes from diabetic fathers and diabetic mothers to their offspring. *Diabetes* 55(5):1517-1524, 2006

# 1 INTRODUCTION

Type 1 diabetes (T1D) is a disease primarily affecting young people although it can occur at any age. Most new T1D cases in any population are sporadic. Importantly, the first-degree relatives are at an increased risk of developing the same disease. Most studies on familial aggregation of T1D have been cross-sectional point-estimations of the prevalence in the first-degree relatives at the time of diagnosis of the proband. The eventual phenotype of unaffected family members cannot be determined with certainty, however, without a long time of follow-up. Therefore, though rare, the natural way to study the topic is a longitudinal follow-up.

Finland is one of the few countries with nationwide and population-based twin registers that comprise the Old Twin Cohort and the Young Twin Cohort. In different populations the observed pairwise concordance for T1D is 13-52% in monozygotic twins but substantially less in dizygotic twins. Notably, the pairwise concordance was estimated to be 13% and probandwise concordance 23% in monozygotic twins in the Finnish Old Twin Cohort. Heritability was estimated to be 74%. Methodological problems, however, may arise in twin studies and may also lead to false conclusions. In particular, in clinic-based opportunistic studies ascertainment biases tend to increase the number of concordant pairs.

Finland has the world's highest incidence of T1D in and it is increasing steadily. The lifetime cumulative risk is supposed to be less varying than the incidence between populations. The European ACE study hypothesizes that the T1D risk amongst first-degree relatives varies across populations in such a manner that it mirrors the pattern of disease incidence in the background population. Little information is, however, available on the birth cohort effects on the recurrence risk of T1D in the first-degree relatives in different populations.

A number of studies has detected preferential sex-specific transmission of T1D. The offspring of fathers with T1D have an increased risk for T1D compared to the offspring of affected mothers. The nature of the factors responsible for this preferential transmission is unclear. One suggested explanation is that the observed preferential transmission might be due to fewer progeny to mothers than fathers with T1D. Few population-based studies with an optimal study design, in which the ascertainment of T1D of the offspring is confirmed through parents with T1D, however exist.

Increasing evidence shows that the age at onset of diabetes in the proband has an impact on the risk of diabetes in the first-degree relatives. Generally, the younger the proband when diagnosed the greater the risk in the close relatives. Only a few studies with rather inconclusive results, however, have assessed this risk of T1D in the offspring according to the age at onset of diabetes and according to the gender of the parent with T1D.

Diabetic nephropathy (DN) with a multifactorial pathogenesis is one of the most severe late complications of diabetes affecting about one third of patients with T1D. Despite a large number of studies, the etiology of DN is still poorly understood. Although some studies have reported familial clustering of DN, these studies have mainly been clinic-based, cross-sectional, and with relatively small sample sizes. Studies providing truly population-based empirical estimates of the sibling recurrence risk of DN do not exist.

In this thesis the first study evaluates the concordance rate of T1D in twins and further estimates the heritability for T1D in the Finnish Young Twin Cohort comprising 22,650 twin pairs. The second study provides empirical long-term estimates of the risk of T1D among 10,168 siblings of the Finnish population-based cohort of patients with T1D (probands) diagnosed at the age of 17 or under during the years 1965-79 (n=5,144). This study also investigated the cumulative risk for different birth cohorts, as well as a number of risk factors predicting the risk. The third study addressed the question of familial aggregation of DN and its magnitude in multiplex T1D families. Finally in the fourth study offspring recurrence risk for T1D was evaluated and the relation to gender and a number of other risk factors. The relation to the incidence in the background population was further explored. All studies investigate the effect of age at onset in the proband on the risk of T1D in co-twins, siblings, and offspring as well as the risk of DN. A common feature of all these studies is that they utilize large, population-based cohorts of study subjects and follow the subjects longitudinally.

# 2 REVIEW OF THE LITERATURE

## 2.1 Fundamentals of genetic epidemiology

### 2.1.1 Genetic epidemiology and familial aggregation

Genetic epidemiology is a comparatively new discipline combining aspects of statistics, genetics and classical epidemiology. The goal of genetic epidemiology is to elucidate the joint action of genetic and environmental factors in the distribution and determinants of diseases within human populations (1). Genetic epidemiology could be defined as a science, which deals with etiology, distribution, and control of disease in groups of relatives and with inherited causes of disease in population (2). The study of familial aggregation is a central theme in genetic epidemiology. Familial aggregation means the occurrence of a disorder at a higher frequency in the first-degree relatives of an affected person compared to the general population. Though, familial aggregation of disease is generally taken as evidence of a genetic etiological mechanism, environmental factors common to the family members cannot be excluded. Putative disease genes are not readily observed, but are hypothesized based on evidence from observed patterns of familial aggregation (3).

The measure most commonly used to measure the degree of family aggregation in genetic epidemiology is called the relative recurrence risk ratio. The ratio between the recurrence risk of the disease in relatives divided by the population prevalence, usually denoted by $\lambda_r$ (4). The $\lambda_r$ parameter in itself does not directly express the genetic transmission, however, only the degree of family aggregation. One widely used measure of familial aggregation is the sibling recurrence risk ratio ($\lambda_s$), which is defined as the ratio of risk disease manifestation, given that one's sibling is affected, as compared with the disease prevalence in the general population.

### 2.1.2 Heritability

Heritability ($h^2$), answers the question of how much of the variation in the risk of disease can be attributed to genetic differences among individuals. Heritability is

defined as the ratio of genetic variance to the total phenotypic variance in the population (1). Heritability, and what it measures, is often misunderstood and misinterpreted as a measure of genetic control. Genetic control determines if the genes are biologically involved in a trait. Heritability does not indicate how important genes are in influencing a trait. Instead, it defines the extent to which genetic individual differences contribute to the observed phenotypic individual differences. It measures how the gentic control can vary. An extreme example of this is when a given phenotypic trait is under direct Mendelian control and all the individuals have the same genotype, no genetic variation and heritability could be 0 (1). On the other hand in the Finnish Old Twin Cohort the heritability was estimated to be 74% and denotes considerable genetic variation. The heritability is strictly relative and a population specific measure (1). If the distribution of exposure to environmental factors changes, the heritability will change even within a population, even with the same set of genotypes.

### 2.1.3. Mendelian patterns of inheritance

Inheritance is the transmission of genetic information across generations. Mendelian type inheritance is the most common form of genetic inheritance. It is based on the transmission of a single gene in a dominant, recessive, or X-linked pattern. In autosomal dominant inheritance, only one copy of a gene causing a specific trait must be present in order for a person to display the trait. Homozygous and heterozygous individuals will be affected equally by the mutation and both will express identical forms of the trait. The second copy of a mutated gene in the homozygous individual does not cause a more severe disease. If a particular trait is autosomal recessive, two copies of the mutated gene causing this trait must be present in order for a person to possess the trait. Therefore, only homozygous individuals will be affected with the trait. Heterozygous individuals will not exhibit characteristics of the trait. These heterozygous individuals are called carriers, because they carry the trait and can pass it onto their children. Sex-linked traits are carried on the X and Y, or sex, chromosomes and may be either dominant or recessive. Most diseases have, however, a complex genetic and environmental basis (5).

### 2.1.4 Penetrance

The impact of a specific genetic factor on disease occurrence is usually measured in terms of penetrance. It is defined as the probability of the disease or phenotype of interest to develop among individuals who carry the specific genotype. It means that a given genotype does not always produce the same phenotype. A true dominant trait will have a penetrance of 100%. Many traits that are said to be dominant do not, however, have complete penetrance. For chronic diseases, the penetrance is equivalent to the lifetime risk of disease in carriers of the genotype (1). The expression of a given genotype may be modified by several factors, for example other genes or environmental factors (6). If the environmental exposure varies, the same genes at a given trait locus may have different effects on the phenotype, due to the genotype-environment interaction. If all individuals with a disease genotype show the disease phenotype, then the disease is said to be completely penetrant. Incomplete penetrance is a statistical concept to reflect that not everyone having the genotype of interest will manifest the phenotype of interest (1).

### 2.1.5 Classical twin method

The importance of twin studies has long been recognised in addressing the question of heritability, because twins provide a natural control for experiments that estimate the contribution of genetic factors to the phenotypic variability in human traits. The usefulness of twins in studying the genetic contribution to the trait was first suggested by Galton in 1875 (7). The systematic analyses of similarity between monozygotic twins (MZ) and dizygotic twins (DZ) was introduced by Siemens, who formulated the rule of pathology: any heritable disease will be more concordant in MZ twins than in DZ twins (8). The rationale is that genetic variance between MZs is zero, but DZs are genetically as variable as full siblings. MZ twin concordance rates of less than 100% emphasise the importance of environmental factors. The basic idea behind the classical twin study approach is to make a crucial assumption that both MZ and DZ twins share the intra-pair environment to the same degree (equal environment assumption). Therefore, the greater phenotypic similarity among MZ twins is consistent with a greater genetic similarity. MZ twins, however, are also often environmentally more similar than DZ twins and even in the complete absence of any genetic component a higher concordance can be detected in MZ twins than in DZ twins (9).

Resemblance between twins is measured as concordance if the trait is dichotomous and as correlation if the trait is continuous. There are two kinds of concordance; pairwise and probandwise concordance. The probandwise concordance is defined as the probability that one twin is affected with disease, given that the co-twin is affected (10). It is estimated by the ratio of the concordant to the sum of the concordant and discordant pairs (11). The pairwise concordance is defined as the probability that both twins are affected, given that at least one is affected, and is estimated by the ratio of the affected concordant individuals to the sum of the affected concordant and discordant individuals (11). Probandwise concordance can be interpreted as the recurrence risk in a co-twin of an affected twin and is more preferable of the two measures.

Many diseases, like T1D, are familial but do not segregate in a mendelian way, but show multifactorial inheritance where both multiple genes and environmental factors play a role in the development of the disease. These can be modeled as threshold traits. According to the liability/threshold model proposed by Falconer (12), all of the factors, which influence the development of a multifactorial disorder, whether genetic or environmental, can be considered as a single entity known as liability. The liabilities of all individuals in a population form a continuous variable, which is assumed to have normal distribution and individuals whose liability exceeds a certain threshold develop the disease (Figure 1). Liability is presumed to be determined, in part, by numerous genes with low penetrance and environmental factors, each with small effects. The sharing of genes and environmental factors by relatives therefore results in a correlation of liability (12).

Normally distributed curves are specified by only two parameters, the mean and the variance. Variances have the property of being additive when they are due to independent causes. Thus the overall variance of the phenotype ($V_P$) is the sum of the variances due to the individual causes of variation, i.e. the environmental variance ($V_E$) and the genetic variance ($V_G$). $V_G$ can be further decomposed into additive ($V_A$) and dominance genetic variation ($V_D$), which is variation due to gene interactions like dominance (between alleles at the same locus) and epistasis (between alleles at different gene loci). Also $V_E$ decomposes into common and unique environmental effects. Narrow sense heritability of the trait can be estimated as the fraction of the variance due to additive genetic factors $V_A/V_P$, whereas broad sense heritability is due to all additive and non-additive genetic factors $(V_A + V_D)/V_P$ (13).

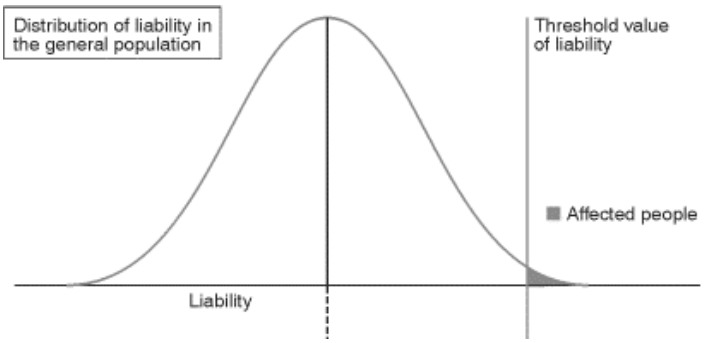


**Figure 1.** Falconer's polygenic threshold model for dichotomous nonmendelian characters. Liability to the condition is assumed to be polygenic and normally distributed. People whose liability is above a certain threshold value are affected

## 2.2 Type 1 diabetes

T1D is a common, multifactorial, and genetically heterogeneous autoimmune disease affecting about 0.3% of the world's population and accounting for about 10% of all diabetes (14). T1D was previously defined as insulin-dependent or juvenile diabetes. The new classification of diabetes, according to The American Diabetes Association and the World Health Organization, is now primarily based on the pathogenesis rather than on the requirement for insulin therapy (15; 16). T1D is now classified as type 1A (autoimmune) and type 1B (not immune-mediated). These phenotypes are differentiated by the presence or absence of autoreactive antibodies in the serum of affected individuals, stressing the involvement of immune-mediated mechanisms in type 1A diabetes. Type 1A is the most common form of diabetes among children and adolescents of European origin, and is usually characterized by an acute onset and dependence on exogenous insulin for survival. In adults, the disease is nearly as frequent as in children, but often with a less dramatic onset that may lead to misclassification as T2D and a delayed insulin treatment. About 60% of individuals with T1D are diagnosed in their adult life.

In most cases, a preclinical period marked by the presence of autoantibodies to pancreatic β-cell antigens precedes the onset of overt T1D. At least one of these autoantibodies is present in 85–98% of newly diagnosed children (17). Everyone who develops T1D seems to be genetically predisposed to it. On the other hand, a

genetic predisposition does not guarantee that the disease will occur. T1D clusters in families, but does not segregate with a known mode of inheritance.

### 2.2.1 Genetics of Type 1 diabetes

The onset of T1D is attributed to both genetic risk factors and external triggers. The genetic component cannot be classified according to a classical model of inheritance but T1D is probably due to an interaction between different genes and environmental factors and thus a typical multifactorial, complex disease. The etiology of this disorder remains, however, unclear.

The most studied and major genetic predisposition to T1D is conferred by genes in the HLA region on the short arm of chromosome 6 (6p21.3) (IDDM1). The association between HLA alleles and T1D was first documented in the 1970s (18-20). HLA genes encode molecules that are crucial to the immune system. The HLA region of chromosome 6 contains genes that encode class I (HLA-A, B, C), class II (HLA-DR, DQ, DP), and class III antigens, as well as numerous other genes that control immune response (Figure 2) (21; 22).

The identification of the primary susceptibility determinants, within the MHC region, is confounded by strong linkage disequilibrium between the genes. Particular alleles of the HLA-DQA1, -DQB1 and -DRB1 loci all are primarily involved in the genetic predisposition to T1D, but due to strong linkage disequilibrium it is difficult to study the effect of individual HLA-DQ or –DR genes separately.

Table 1 shows HLA Class II DR-DQ linkage patterns and T1D susceptibility in Caucasians (23; 24). The highest susceptibility to T1D is associated with two combinations of DQA1 and DQB1 alleles: DQA1*0501.DQB1*0201 and DQA1*0301.DQB1*0302, which encode the HLA-DQ2 and HLA-DQ8 molecules. Two DRB1 alleles, DRB1*03 and DRB1*04 (which encode the DR3 and DR4 molecules), are also associated with an increased risk of disease. About 90% of patients with T1D carry one or both of these haplotypes and the highest genetic risk of the disease is conferred by the DR3.DQ2/DR4.DQ8 heterozygous genotype.

Although DQ8 is the principal disease determinant on this haplotype, its influence on disease risk may be modified by the DRB1 subtype. The DRB1*0401, DRB1*0402, and DRB1*0405 subtypes have been reported to increase the risk of diabetes independent of DQ8, whereas DRB1*0403 is neutral. “Protection” against

T1D is conferred by the DQA1*0102.DQB1*0602 haplotype, which encodes the HLA-DQ6.2 molecule. This molecule occurs in approximately 20% of the healthy caucasian population, but is rarely found among patients with diabetes (24).

The inheritance of particular HLA alleles account for over half of the genetic risk of T1D (24). Comparison of disease concordance between HLA-identical siblings (15-20%) and MZ-twins (35-45%) indicates that other loci have to be involved in the genetic transmission of T1D. Also, the fact that the risk of a sibling sharing none of the HLA-haplotypes with a proband is greater than the population prevalence suggests that additional factors are involved (25; 26).

**Table 1.** HLA Class II DR-DQ linkage patterns and T1D susceptibility in Caucasians (23; 24)

| HLA-DR | DQA1 | DQB1 | DRB1 | SUSCEPTIBILITY |
|---|---|---|---|---|
| DR2 | 0102 | 0602 | 1501 | ”Protective” |
| DR2 | 0102 | 0502 | 1601 | Predisposing |
| DR2 | 0103 | 0601 | 1502 | Neutral |
| DR3 | 0501 | 0201 | 0301 | High Risk |
| DR4 | 0301 | 0302 | 0401 | High Risk |
| DR4 | 0301 | 0302 | 0402 | Predisposing |
| DR4 | 0301 | 0302 | 0403 | Neutral |
| DR4 | 0301 | 0302 | 0404 | Predisposing |
| DR4 | 0301 | 0302 | 0405 | High Risk |
| DR4 | 0301 | 0301 | 0401 | Neutral |
| DR4 | 0301 | 0303 | 0401 | Neutral |
| DR6 | 0101 | 0503 | 1401 | ”Protective” |
| DR7 | 0201 | 0303 | 0701 | ”Protective” |

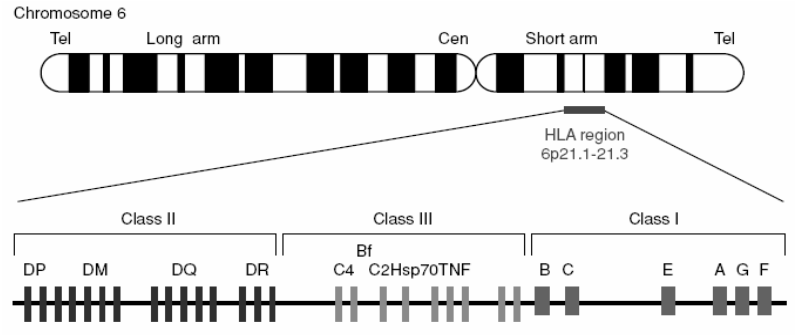


**Figure 2.** Gene map of the human leukocyte antigen (HLA) region

Genome-wide linkage scans have enabled the identification of additional chromosomal regions that might contain susceptible genes for T1D (27-31). These scans have confirmed that the HLA gene region is the major genetic determinant of T1D risk, but they have further provided evidence that approximately twenty regions of the genome are also linked to susceptibility (Table 2) (23). The symbols of these loci are labeled from IDDM1 to IDDM18 (www.gene.ucl.ac.uk/nomenclature).

Polymorphisms in the regulatory region of the insulin gene *INS* (IDDM2) have been shown to be the second most important and plausible candidate of the T1D susceptibility and contributes to about 10% of the genetic susceptibility (31; 32). Also the gene *CTLA4* (IDDM12) is a leading candidate gene outside the HLA region (24; 33). The contribution of most of the candidate genes outside the HLA region to the T1D susceptibility, however, has turned out to be difficult to prove unequivocally.

**Table 2.** Susceptibility loci for T1D, chromosome location as well as candidate genes or microsatellite markers (23)

| LOCUS | CHROMOSOME | CANDIDATE GENES OR MICROSATELLITES |
|---|---|---|
| IDDM1 | 6p21 | HLA-DR/ DQ |
| IDDM2 | 11p15.5 | INS-VNTR |
| IDDM3 | 15q26 | D15S107 |
| IDDM4 | 11q13 | MDU1, ZFM1, RT6, FADD, LRP5 |
| IDDM5 | 6q25 | ESR, MnSOD |
| IDDM6 | 18q12-q21 | D18S487, D18S64, JK (Kidd locus) |
| IDDM7 | 2q31 | D2S152, IL-1, NEUROD, GALNT3, HOXD8 |
| IDDM8 | 6q25-27 | D6S264, D6S446, D6S281 |
| IDDM9 | 3q21-25 | D3S1303, D3S1589, D3S3606 |
| IDDM10 | 10p11-q11 | D10S193, D10S208, D10S588, D10S1426 |
| IDDM11 | 14q24.3-q31 | D14S67 |
| IDDM12 | 2q33 | CTLA-4, CD28 |
| IDDM13 | 2q34 | D2S137, D2S164, IGFBP2, IGFBP5 |
| IDDM14 | 2q34-q35 | NCBI # 3413 |
| IDDM15 | 6q21 | D6S283, D6S434, D6S1580 |
| IDDM16 | 14q32 | IGH |
| IDDM17 | 10q25 | D10S1750-D10S1773 |
| IDDM18 | 5q31.1-33.1 | IL12B |

### 2.2.2 Race and ethnicity

One of the most striking characteristics of T1D is the marked geographic and ethnic variability in incidence (34-36). T1D predominantly affects europid populations in Europe and other continents but is less frequent in African, Asian, and Native North American populations. Notable differences, however, also exist within ethnic groups in neighboring countries like the Nordic countries and Estonia. Considerable differences exist in the annual T1D incidence in children, ranging from 0.1 per 100,000 in China and Venezuela to over 50 per 100,000 in Finland in children ≤14 years of age (36). This represents hundreds-fold variation between different populations. Clear geographical patterns such as the polar-equatorial gradient, cannot be seen in the incidence rates (36). The distribution of diabetes-associated HLA-genotypes explains a part of the variation (37), but environmental and life-style associated factors must contribute to the remarkable variation in incidence.

Some examples exist of a large difference in incidence between populations with relatively similar ethnic and genetic constitution. The Scandinavian countries are characterized by a high disease incidence. In contrast, the neighboring Russian Karelia and the Baltic countries display a low incidence rate (38; 39). A six-fold gradient has been observed in the incidence of T1D between Finland and Russian Karelia, where one can also find one of the sharpest welfare gradients in the world (40). The population in Russian Karelia lives under entirely different socioeconomic circumstances than the Finnish population, although they partly share the same ancestry. This suggests that environmental triggers that vary among these countries contribute to the steep differences in the incidence rate.

Noticeable within-country variations have also been observed. More than a six-fold variation in risk exists in Italy between the mainland and the island of Sardinia (41). Also in China a nearly 50-fold within-country variation has been observed (36).

### 2.2.3 Gender and age

A sex bias is a characteristic of autoimmune diseases (42). In contrast to most autoimmune diseases, male predominance occurs in T1D. The overall sex ratio is roughly equal in T1D with only a minor male excess observed in children diagnosed under the age of 15. After puberty males seems to be more commonly affected than females (43-45). Several mechanisms for these differences have been explored, such

as the effects of sex hormones on the immune system, but the reasons are not yet known (42). The earlier observed association between a male excess and a high incidence of T1D, in contrast to a female excess in the low incidence countries was not confirmed in a relatively recent studies (36; 46).

T1D like many other multifactorial diseases shows a variable age of onset. T1D can develop at any age, but predominantly occurs in children and young adults. The disease status of unaffected family members cannot be determined with certainty without a long follow-up time. The peak of T1D expression is seen between ages 10-14, but several countries have reported that the age at onset has showed a trend towards an earlier onset (47-51). T1D may represent a heterogeneous disorder with a different pathogenesis in patients with earlier onset compared to later onset of the disease. A very young age at onset of T1D may be under a different genetic control than a later onset of T1D (52-54). A significantly higher percentage of non-DR3/non-DR4 genotypes and a lower percentage of DR3/4 genotypes has been found in patients with later onset (55). Clinical subtypes of T1D can be divided into acute onset that mainly occurs in young patients or a slowly progressive form of β-cell destruction (56). A greater relative increase in incidence in individuals under five supports the importance of exposures operating early in life.

### 2.2.4 Environmental risk factors

During the recent years, the role of several environmental factors in the etiology of T1D has been extensively researched. Despite this no environmental agent responsible for triggering T1D has been conclusively identified. Table 3 summarizes the environmental factors that have been suggested to associate with T1D, either causative or protective. Seasonal variation in the onset of T1D has been observed worldwide with the largest proportion of cases diagnosed during fall and winter and the lowest during the summer, which has been interpreted as coincidence with the time of enterovirus infections (57-60). Many reports of epidemiological association between enterovirus infections and T1D have been brought out (61; 62). A new observation in the Finnish DIPP Project is a similar seasonality in the appearance of autoantibodies in non-diabetic, genetically susceptible children parallel the seasonal pattern of enterovirus infections (63). One hypothesis is that the increase in the incidence of T1D in Finland could be due to a parallel change in the epidemiology of enterovirus infections (64). The DIPP study further showed that the frequency of

enterovirus infections has rapidly decreased in the Finnish population as a consequence of modern life-style and increased hygiene.

A number of other viruses have also been associated with T1D. These include rubella, mumps, cytomegalovirus, Epstein-Barr virus, retrovirus, and rotavirus (65-67). Viruses are capable of causing a direct cytolytic effect or of triggering an autoimmune process that leads to the destruction of β-cells (68). The mumps virus was the first to be implicated in the development of human T1D. Several cases were reported in whic the mumps infection preceded the onset of T1D (67).

Like the virus infections, vaccinations also affect the immune system early in life. A link between childhood vaccinations and the development of T1D has been investigated, but most studies have not been able to find an increased risk of T1D associated with vaccination (69-71). Vaccines may have a biological effect by themselves or an impact on the incidence of T1D due to the elimination of certain viral infections. A follow-up study in Finnish children did not find an increased risk of T1D associated with Haemophilus influenzae type b vaccination or with the number of vaccinations received (72). Neither did a large Danish study (70).

A number of studies have investigated the relationship between dietary factors and the risk of T1D. Such factors include early introduction of cow's milk supplement, duration of breast-feeding, vitamin D, vitamin E, zinc, nitrate, and nitrite compounds (73-75). The possible protective role of breast-feeding and harmful effect of early introduction of cow's milk in the development of T1D have been proposed, but the results have remained controversial (75-78). Vitamin D supplement in the first year of life and already during prenatal life is associated with a decreased risk of T1D (79-82).

Toxic substances in the environment, other than infectious agents or exposures that stimulate an immune response, are associated with the occurrence of T1D (83). Among toxins, the nitrates, nitrites and N-nitroso compounds are the main candidates playing an environmental role in the etiology of T1D (74; 84; 85). Nitrate can convert to nitrite and further into nitrosamines, which are known to be toxic substances capable of damaging the pancreatic β-cells (85). Nitrates are found in vegetables, and nitrate and nitrite are used in meat products as food additives. In addition to food, nitrates are also found in drinking water due to natural occurrence and agricultural sources. Associations between regional water nitrate levels and the incidence of T1D have been reported in some ecologic studies (84; 86; 87). Finnish study, however, did not find such an association between the variation of T1D and the concentration of nitrates in the drinking water (85).

Novel potential environmental factors that may be associated with T1D are a bacterial toxin, the macrolide bafilomycin A1 produced by Streptomyces spp and antimicrobials (88; 89). An Australian study reported that the antibiotic bafilomycin A1 may induce changes in the pancreatic β-cells and a dysregulation of the insulin secretion in mice (88). Streptomyces species are found in the soil worldwide and they can produce many toxic compounds that infect vegetables and beets. Thereby humans could be exposed to high concentrations of bafilomycin A1. A recent Finnish study investigated the relationship between the use of antimicrobials before or during pregnancy or during childhood and the subsequent risk of T1D (89). Although no relationship between the use of antimicrobials in general and the risk of T1D was found, the maternal use of some specific antimicrobials, like the macrolides, before pregnancy was related to risk of T1D in the child.

The relatively young age at onset and the long preclinical phase of T1D suggest that environmental risk factors may play a role in the fetal or perinatal period of life, when the immune system is maturing. The most commonly studied perinatal factors include birth weight, parental age at birth, birth order, pre-eclampsia, Caesarean section, and fetal-maternal blood group incompatibility (90-95). The most consistent finding is an association between an increased maternal age and an increased risk of T1D (90; 91; 94-97). A positive association between birth weight and T1D has also been found (92; 94; 98; 99). A study from Norway found an almost linear correlation between incidence rate and birth weight. The risk of T1D was more than two-fold higher in children with birth weights > 4500 g in comparison to newborns with the lowest birth weight (< 2000 g) (99). It is difficult to envisage whether the perinatal factors have a direct effect on the variation in risk of T1D or are they only potential markers of other unmeasured variables.

**Table 3.** Environmental factors proposed to be involved in the pathogenesis of T1D

| **VIRAL INFECTIONS** (62; 67; 100) | **CHILDHOOD VACCINATIONS** (69-72) | **DIETARY FACTORS** (75) | **TOXINS** (83; 85; 88; 89) | **PERINATAL RISK FACTORS** (90; 94; 95; 99) |
|---|---|---|---|---|
| Mumps | MMR | Early introduction of cow's milk | N-nitroso compounds | Maternal and paternal age at onset |
| Enteroviruses | BCG | Breast feeding | Bacterial toxin, bafilomycin A1 | Maternal weight gain during pregnancy |
| Rubella | Haemophilus influenzae type b | Nitrate, nitrite, N-nitroso compounds | Antimicrobials | Intrauterine infections |
| Rotaviruses | Polio | Fats | Polychlorinated biphenyls (PCBs) | Birth weight |
| Retroviruses | Tetanus | Gluten | | Birth order |
| Cytomegaloviruses | Diphtheria | Vitamin D | | pre- eclampsia |
| Epstein-Barr viruses | Pertussis | Vitamin E | | Caesarean section |
| | | Zinc | | Feto- maternal blood group incompatibility |

### 2.2.5 Type 1 diabetes in Finland

Finland has the highest incidence of T1D in the world and the incidence rate has increased linearly since the 1950s (101), predominantly increasing in the younger age-groups (47). The incidence of T1D in children under 15 was predicted to reach 50/100,000 cases per year around 2010, if the calendar-period effect would have been linear from the 1950s to 2010 (101). The increase in the incidence, however, has in fact shown nonlinearity and already exceeded the milestone in the beginning of the millennium. The incidence increased linearly from 1965 to the beginning of the 1990s approximately by 2.4% per year, but thereafter the increase has turned out to be even faster (Figure 3).

A within-country variation has been observed in the risk of T1D in Finnish children. Notably, the incidence is higher in the rural heartland areas than in the urban areas suggesting that some geochemical, socio-economic, or behavioural factors may explain the geographic variation (102). Substantial regional variations in the HLA genotypes and allele frequencies among different geographical regions of Finland also exist despite the highly homogenous population (103; 104).

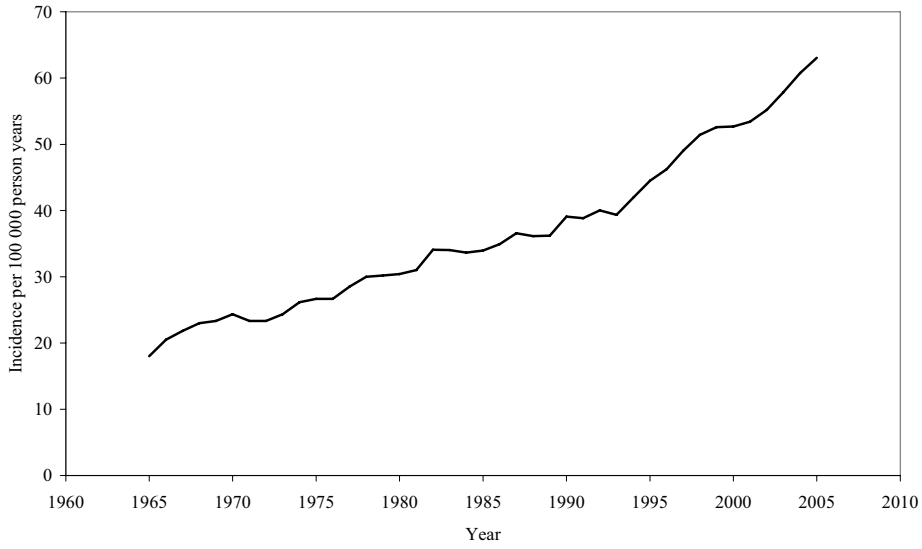


**Figure 3.** Incidence of type 1 diabetes in Finland during 1965-2005, 3-years moving averages, based on data from the Central Drug Register (CDR)

## 2.3 Risk of T1D in the first-degree relatives

### 2.3.1 Historical review of family studies on diabetes

More than 80% of T1D cases are sporadic and occur in an individual without a family history of diabetes at the time of diagnosis, but the remaining 20% clusters in families. As early as 1696, Richard Morton described a family of seven children, four of whom were diabetic (105).

Repeated attempts, to characterize the occurrence risk of first-degree relatives of individuals with T1D have continued since the 1930s, have tried to determine the mode of inheritance from family studies. Pincus and White from the Joslin Diabetes Unit, first claimed in 1933 that diabetes mellitus is an inherited disease and showed that the disease was considerably more frequent among siblings and parents of a series of diabetic patients than among those of a series of non-diabetic subjects (106; 107).

The very wide range of variation both in respect to severity and age at onset made the genetics of diabetes particularly difficult to elucidate. Different hypotheses about the transmission of diabetes have been suggested, of which the first and most popular suggested that the disease is due to a single recessive gene (106-109). It was suggested that late-onset mild cases could be regarded as heterozygous for a gene which, in its homozygous form, gives rise to the early-onset severe type of diabetes (105). Smith et al. concluded that early-onset and late-onset diabetes represent different levels of liability to the same disease rather than being distinct diseases (110). Expected Mendelian ratios were, however, not found in family studies and a multifactorial inheritance of diabetes was finally concluded (111). One of the strongest arguments for the multifactorial hypothesis was the observation that when both parents were affected, the incidence of the disease among the siblings of the proband was greater than when one parent was affected (112).

In the early family studies diabetic patients were not differentiated into type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes. Harris (1950) was the first to show that early and late onset forms of diabetes do not have the same genetic background (105). In the 1960s several authors proposed that the genetic basis might be different for the early-onset type and the late-onset type of diabetes (109; 111; 113). The classification of the two common types of diabetes, as well as some additional forms of diabetes, was made at the end of the 1970s by two expert committees (114; 115).

In the first reported family studies, early-onset and late-onset diabetes were kept together (108; 112), although in some studies the age at onset of diabetes in the probands was restricted to concern early-onset diabetes with subsequent insulin treatment. These criteria were, however, not required for the relatives. The original family studies were hospital-based and the family history material, of diabetes occurrence in relatives, was collected by questioning the patients treated at the diabetes clinics. Importantly, these investigations included only living probands and were thus prone to biases since deceased diabetics from the same cohort as the living ones could represent more serious cases of diabetes. Some studies combined all categories of first-degree relatives into one group with no possibility of differentiation between morbidity risk between parents, siblings, and children (116).

### 2.3.2 Type 1 diabetes in twins

The main interest of the first studies on diabetes in twins was focused on identical twins (117-121). Results that surprised the research community revealed that the early-onset diabetes (< 40 years) might not be entirely genetic in origin. Tattersall and Pyke suggested that the concordance for juvenile onset diabetes in MZ twins may never reach 100% (120). In addition, they concluded that the risk of the unaffected twin becoming diabetic declines with increasing duration of discordance (120).

Many studies have attempted to determine the concordance rate for T1D in MZ twins. The main weakness of these studies has been that most of them had ascertained diabetic twin pairs by advertising or sampling from diabetes clinics. This kind of ascertainment can easily lead to an excess of concordant pairs and thus biased estimates of the concordance rates. In addition, any comparison between MZ and DZ twins has not been possible by studying only MZ twins. Few of the studies were longitudinal, relying instead on cross-sectional analysis. The concordance rate in MZ twins in these studies (117; 118; 120; 122; 123) was estimated to be 25-50%. Later investigations on the topic used population based twin registries from Finland and Denmark, independently of the zygosity and diabetes status in the co-twin. In these studies the concordance rate was estimated to be between 13 and 38% (124; 125).
Johnston et al. (126) provided the first demonstration of a genetic heterogeneity between concordant and discordant MZ twins. They showed that more of the concordant rather than the discordant MZ twin pairs were heterozygous for HLA-DR3 and HLA-DR4. The increase in concordance was first seen in the younger (< 20 years) compared with the older twin pairs (> 20). Such an increased risk in co-twins of index twins diagnosed early in life was later confirmed (127-129). In the recently published study, Metcalfe et al. (130) found the disease associated high-risk INS genotype to increase the likelihood of MZ twins being concordant for T1D. For individual HLA-DQ antigens statistical differences also occurred between concordant and discordant twin pairs. An increased frequency of no high-risk HLA-DQB1 alleles in discordant compared with concordant twins was seen. They therefore concluded that the load of both major histocompatibility complex (MHC) and non-MHC susceptibility genes have an impact on the disease penetrance. Importantly, besides these two studies (126; 130) very little information is available on differences in concordance to T1D caused by genetic heterogeneity.

To date, all studies have found that the concordance rate of MZ twins with T1D is much higher than that in DZ twins, indicating that genetic factors contribute to the disease. The vast majority of MZ twin pairs are, however, discordant for T1D providing the evidence that non-genetically determined factors also influence on

susceptibility to the disease. The investigation of the Finnish Old Twin Cohort showed the probandwise concordance to be 23% and the pairwise concordance to be 13% in MZ twins and 4.8% and 2.5% in DZ twins. The heritability was estimated to be 74% in the Finnish Old Twin Cohort (124).

The concordance rate for T1D in MZ twins may change over time. Finland is one of the few countries with a population-based twin registry that consists of two temporally consecutive cohorts of twins, the Old Twin Cohort (born 1940-1957) and the Young Twin Cohort (born 1958-1986). This offers a rare opportunity to provide unbiased data on T1D among twins as well as to compare the concordance rates for T1D in two successive cohorts of twins throughout the recent decades when the incidence of T1D has changed substantially. If the increase of T1D that has been observed in Finland during the past decades is due to increasing exposure to the environmental risk factors, the concordance rate for T1D among MZ twins must have become higher over time.

The risk seems to be the highest in the two first years after a diagnosis of diabetes in the index twin, but declines sharply at least in young onset twins. The median discordance time among MZ twins is about four years. If a MZ twin pair remains discordant more than six years it is unlikely that the unaffected twin will ever develop diabetes (127), although a significant late progression to diabetes after six years of discordance has been detected. It is noteworthy that a discordance periods of even 36 and 39 years before the outbreak of diabetes in the co-twin have been reported (129; 131).

### 2.3.3 Sibling recurrence risk

Table 4 shows some of the central studies reporting sibling recurrence risk of T1D in different populations. These investigations are not entirely comparable due to methodological differences. The risk of T1D for siblings, however, does not vary to a great extent from study to study. The risk of T1D for siblings is found to be between 4% and 6.5% with a recurrence risk ratio of 10-26 (45; 53; 132-142).

Degnbol et al. concluded that the risk in siblings of probands with diabetes diagnosed at 20 or under was about ten times that in the general population, whereas the risk of the sibling of probands developing diabetes later in life does not differ from the risk in the general population (132). Based on this, they concluded that the early-onset and late-onset diabetes cannot share the same genetic background. Chern

et al. divided siblings according to the age at onset of diabetes in the probands into two groups under and over ten years in order to test the degree of heterogeneity in each group and found that the different risk of diabetes in these two groups is a function of the age at diagnosis of the probands (136). Later in the 1990s, Allen et al. confirmed this connection between progressively increasing risk and the decreased age at onset of diabetes in the proband (140). The coexistence of a diabetic child and at least one diabetic parent in the family was found to increase the risk in the siblings up to 10.5% (137). Further evidence was provided by a German study in which the risk for siblings of the T1D proband was four times higher if a parent also had T1D (138).

Familial aggregation of T1D has been studied using a cross-sectional study design at the time of diagnosis of the proband or if the follow-up time has been limited. A long follow-up time is needed to elicit the phenotype of unaffected family members, because a large part of individuals with T1D are affected by the disease after 15. Longitudinal follow-up studies on this topic are rare.

**Table 4.** Studies investigating the sibling recurrence risk for T1D

| STUDY | COUNTRY | PROBANDS (n) | SIBLINGS AT RISK (n) | T1D RISK IN SIBLINGS |
|---|---|---|---|---|
| Degnbol et al., 1978 (132) | Denmark | 185 | 391 | 6.2% |
| West et al., 1979 (133) | Canada | 518 | 1,080 | 4.1%* |
| Gottlieb et al., 1980 (134) | Boston, US | 259 | 614 | 4.5%* |
| Gamble et al., 1980 (135) | UK | 4,868 | - | 5.6% |
| Chern et al., 1982 (136) | US | 493 | 1,433 | 5.5% |
| Wagener et al., 1982 (137) | Pittsburg, US | 1,128 | 2,578 | 4.4% |
| Tillil et al., 1987 (138) | Germany | 554 | 982 | 6.6% |
| Dahlquist et al., 1989 (139) | Sweden | 3,503 | - | 4.3%* |
| Allen et al., 1991 (140) | Wisconsin, US | 194 | 300 | 12% |
| Gavard et al., 1992 (45) | Pittsburg, US | 1,774 | 3,966 | 6.3% |
| Pociot et al., 1993 (141) | Denmark | 1,419 | - | 5.0%* |
| Lorenzen et al., 1994 (142) | Denmark | 291 | 533 | 6.4% |
| Gillespie et al., 2002 (53) | UK | 1,299 | 1,430 | 4.3% |
| Steck et al., 2005 (143) | Colorado, US | 1,586 | 2,081 | 4.4% |

* at the time of diagnosis of patients with newly diagnosed T1D, T1D=type 1 diabetes

### 2.3.4 Offspring recurrence risk

It became possible to study the frequency of T1D in offspring after insulin therapy became available and enabled survival and reproduction in patients with diabetes. Several family studies concerning sibling risk and offspring risk have overlapped. Table 5 summarizes the studies from the literature during the last three decades where offspring risk of T1D parents has been evaluated. The risk ranges from 3-6% depending on the study design, follow-up time, and the population where the study has been conducted.

Gender-related effects seem to be present in the transmission of T1D from one generation to the next. The first support of such a preferential transmission of T1D was provided by epidemiological studies that demonstrated that fathers of T1D children were more likely to be affected than mothers (132; 137; 141; 144-147). Subsequent studies on recurrence risk in offspring of parents with T1D also revealed preferential sex-specific transmission of T1D. The offspring of fathers with T1D have a 2-3 times increased risk of T1D compared to the offspring of affected mothers. Warram et al. first described the differences in the risk of offspring according to the sex of the affected parent. By 20, 6.1% of the offspring of diabetic men, while only 1.3% of the offspring of diabetic women, were affected (148). The tendency that children of male probands have a higher risk than children of female probands was also found in studies published later (138; 149; 150). A large Finnish population-based study, as well as study from Denmark, confirmed the rather large difference in the risk transmitted from the diabetic mother and father (151; 152). It has further been observed that T1D fathers are more likely to transmit diabetes to their daughters and mothers to their sons, a phenomenon called preferential cross-sex transmission (151; 153). Conflicting results are, however, also reported concerning the preferential cross-sex transmission (148; 152).

The nature of the factors responsible for the preferential transmission is unclear. Several theories to explain the observed preferential transmission have been proposed, but so far none of them are unequivocally proven. These include selective loss of fetuses that carry T1D susceptible genes in women with diabetes, leading to a lower prevalence of T1D in the offspring of affected women than men (148). The rate of miscarriage among diabetic women is higher than in the general population, reported to be 15-30% (154-157). The main reason for spontaneous abortions in patients with diabetes is considered to be hyperglycemia at conception and early pregnancy, but selective loss of fetuses bearing diabetogenic genes can not be

excluded (158; 159). Fetuses exposed to maternal diabetes could also be protected from being affected with T1D in some way (148). Fetal exposure to islet autoantibodies in children born to mothers with T1D may be protective against future islet autoimmunity and diabetes. The large BABYDIAB studying the offspring of T1D mothers found that the offspring who were GAD or IA-2 autoantibody positive at birth had a significantly lower T1D risk than offspring who were autoantibody negative at birth (160).

Another explanation suggests that fetal β-cell development is accelerated in the hyperglycemic environment of a diabetic pregnancy. This earlier maturation of beta cells during the diabetic pregnancy might protect against diabetes in later life (161). Support for this hypothesis comes from the observations that children born to mothers who develop T1D after pregnancy have a higher risk of T1D than those born to mothers who already have diabetes (152). The risk of T1D in offspring could, however, also be a consequence of heterogeneity of the genetic background in the early-onset and late-onset T1D.

Transmission ratio distortion, mainly of HLA-DR4-linked diabetes susceptibility, has also been suggested (162). It has been claimed that the transmission of HLA-encoded susceptibility is influenced by parental sex. Fathers are reported to transmit HLA-DR4-positive haplotypes more frequently to their diabetic offspring than mothers. Contradictory evidence regarding a possible transmission rate distortion has been described for HLA genes in Finnish T1D families (163). Finally, it has been postulated that phenomenon of genomic imprinting, i.e. differential behaviour of genes depending on their parental origin, might be responsible for the preferential transmission of T1D, but this has not yet been convincingly demonstrated in complex diseases (164).

It is thus not fully clear, if the observed preferential transmission really exists or if it is only a consequence of faulty study design and ascertainment biases. Many bias-causing factors have been described that can lead to an apparent preferential transmission, even though there is no preferential transmission at all (165). When the study population is ascertained through the affected offspring and then the affection status of their parent examined, the higher prevalence of T1D in males in the background population can lead to the observation of more affected fathers than mothers. When the study population is ascertained through the affected parents, there may be a misclassification of mothers with gestational diabetes as T1D, fewer offspring in T1D mothers than in their male counterparts and a possible birth order effect (165).

Confirmation or refutation of the preferential transmission of T1D would have important and practical implications for the understanding of the etiology of T1D and for the design of genetic studies. It is important to eliminate bias-causing factors in order to resolve the existence of preferential transmission of T1D. Population-based studies with optimal study design, where the ascertainment of T1D in the offspring is complete and through the diabetic parents hardly exist.

**Table 5.** Studies investigating the offspring recurrence risk of T1D

| STUDY | COUNTRY | FAMILIES (n) | OFFSPRING AT RISK (n) | RISK IN OFFSPRING OF T1D MOTHER | RISK IN OFFSPRING OF T1D FATHER | RISK IN OFFSPRING OF T1D PARENT |
|---|---|---|---|---|---|---|
| Degnbol et al., 1978 (132) | Denmark | 187 | 178 | - | - | 5.4% |
| Gottlieb et al., 1980 (134) | Boston, US | 259 | 420 | - | - | 3.1% |
| Köbberling et al.,1980 (166) | Germany | 311 M | 464 | 3.4% by 25 years | - | - |
| Warram et al., 1984 (148) | Boston, US | 88 F / 99 M | 244 / 175 | 1.3% by 20 years | 6.1% by 20 years | 3.1% |
| Tillil et al., 1987 (138) | Germany | 278 F / 276 M | 104 / 545 | 4.0% | 7.4% | 4.9% |
| Bleich et al., 1993 (149) | Boston, US | | | 3.4% | 8.9% | 4.8% |
| Lorenzen et al., 1994 (142) | Denmark | 290 | 359 | - | - | 6.3% |
| El-Hashimy et al., 1995 (150) | Boston, US | 453 F / 696 M | 1,084 / 1,391 | 2.1% by 20 years | 5.4% by 20 years | - |
| Tuomilehto et al., 1995 (151) | Finland | 2,295 F / 2,960 M | 4,202 / 5,251 | 3.5% by 20 years | 7.6% by 20 years | 5.3% |
| Lorenzen et al., 1998 (152) | Denmark | 1,422 F / 1,304 M | 1,541 / 1,285 | 2.3% by 30 years | 5.7% by 30 years | 4.7% |

F=father, M=mother

### 2.3.5 Family studies and age-dependent variation in the risk of T1D

Genetic, immunological, and clinical studies have attempted to investigate, whether heterogeneity between familial and non-familial forms of T1D exist. It has been suggested that familial cases are more genetically susceptible than non-familial. One of the most important risk factors in siblings is the sharing of both HLA-haplotypes with the proband. The risk of T1D in HLA-identical sibling is 15-20%, when it is 2-9% for HLA haploidentical and 0-2% for HLA nonidentical sibling (26; 167; 168).

Age-related genetic factors seem to strongly influence the risk of T1D in first-degree relatives (169). Table 6 shows different kinds of family studies that report the effect of age at onset of diabetes in the proband on the risk of T1D in the first-degree relatives. The cut-off point of age at onset of T1D in the proband differs in these studies, but generally, the earlier the onset of diabetes the greater the risk in the first-degree relatives. This may indicate varying liability to diabetes that corresponds to the varying age at onset and may be due to more penetrant alleles or more severe forms of T1D at an earlier age. Age dependent HLA heterogeneity has been observed in Caucasian T1D patients. T1D patients diagnosed in early childhood are more likely to have HLA genes associated with high disease susceptibility. It seems that the both the rate of T1D and age at onset are directly related to the HLA-DR,DQ genotypes (53; 55; 170-174). In a large family study, Gillespie et al. detected that half of the children who were diagnosed before five were heterozygous for HLA DRB1*03-DQA1*0501-DQB1*02/DRB1*04-DQA1*0301-DQB1*0302, and the frequency of the genotype decreased with an increase in the age at onset (53). Similarly in the recent US study, nearly half of patients who had an age at onset before five possessed the high-risk genotype DR3/DR4,DQ*0302 (173).

The effect of parental age at onset of T1D on the recurrence risk of T1D in their offspring has been reported (139; 150; 152), but only few studies have distinguished between maternal and paternal differences. Some studies have suggested that the maternal age at onset has only a minor impact compared with the paternal age (150; 152).

There is an increasing interest to find out whether age-at-onset itself also exhibits familial clustering. Tattersall and Pyke already concluded in their early study that the genetic factors determine both the presence of diabetes and the time of its appearance based on the results of their identical twin study (120). Identical twins

tend to develop T1D at a similar age, age at onset seems to be highly correlated (r=0.95) (175). Heritability for age at diagnosis of T1D has been evaluated to be 74% (175). In addition, some studies have reported significant correlation between the ages at onset in the first-degree relatives with diabetes (141; 175; 176), but some studies have detected only moderate or no correlation (135; 147; 172). Too short follow-up times, however, may cause an artificial correlation between the ages at onset of T1D. Fava et al. suggested that variation in the incubated period of T1D is strongly genetically influenced (175). A strong association in the ages at onset between affected sibling pairs was observed, if the sibling pair shared both DR-DQ haplotypes (172).

**Table 6.** Studies investigating the recurrence risk for T1D in the first-degree relatives according to age at onset of diabetes in the proband

| STUDY | COUNTRY | TYPE OF RELATIVE | AGE AT ONSET OF T1D IN PROBAND | RECURRENCE RISK OF T1D IN RELATIVE |
|---|---|---|---|---|
| Wagener et al., 1982 (137) | Pittsburg, US | Sibling | <5 vs. 5-17 | 3.0% vs. 3.2% |
| Chern et al., 1982 (136) | US | Sibling | <10 vs. ≥10 | 8.5% vs. 4.6% |
| Allen et al., 1991 (140) | Wisconsin, US | Sibling | 0-9 | 18.5% |
| | | | 10-14 | 10.1% |
| | | | 14-29 | 7% |
| Gillespie et al., 2002 (53) | UK | Sibling | 0-4 | 11.7% |
| | | | 5-9 | 3.6% |
| | | | 10-14 | 2.3% |
| Steck et al., 2005 (143) | US | Sibling | 0-6 vs. 7-16 | 6.9% vs. 2.9% |
| Olmos et al., 1988 (127) | UK | MZ twin | <15 vs. ≥15 | 47.6% vs. 18% |
| Kumar et al., 1993 (128) | US and Canada | MZ twin | 0-4 | 65% |
| | | | 5-9 | 44% |
| | | | 10-14 | 31% |
| | | | 15-29 | 13% |
| Redondo et al., 2001 (129) | | MZ twin | <25 vs. ≥25 | 38% vs. 6% |
| Bleich et al., 1993 (149) | Boston, US | Offspring of T1D mother | <8 vs. ≥8 | 13.9% vs. 2.4% |
| el-Hashimy et al., 1995 (150) | Boston, US | Offspring of T1D mother | <11 vs. ≥11 | 11.9% vs. 4.9% |
| | | Offspring of T1D father | <11 vs. ≥11 | 2.7% vs. 1.8% |
| Lorenzen et al., 1998 (152) | Denmark | Offspring of T1D mother | <17 vs. ≥17 | 8.5% vs. 3.6% |
| | | Offspring of T1D father | <15 vs. ≥15 | 2.0% vs. 2.5% |

MZ=monozygotic, T1D=type 1 diabetes



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## 2.4 Diabetic nephropathy

### 2.4.1 Natural history of DN

Diabetic nephropathy (DN) with a multifactorial pathogenesis is the major life-threatening late microvascular complication of T1D and it is a leading cause of end stage renal disease (ESRD) in the Western world (177). The progression of DN includes three stages. The earliest clinical evidence is the appearance of an increased amount of albumin in the urine (albumin excretion rate [AER] ≥30 mg/day or 20 μg/min), referred to as microalbuminuria. The second stage is overt nephropathy, also called macroalbuminuria or proteinuria (AER ≥300 mg/day or 200 μg/min), culminating in renal failure with uremia (177). DN increases the risk of cardiovascular diseases such as coronary heart disease, acute myocardial infarction, and stroke (178; 179). The relative mortality is extremely high in individuals with T1D and DN compared to individuals with T1D and normoalbuminuria (180). Only a minority, approximately one third, of patients with T1D are expected to develop DN (181; 182). The incidence of DN first increases linearly with the duration of diabetes, but 20-25 years post diagnosis of diabetes it starts to decline (181; 182). This indicates that a subset of individuals with T1D is at an especially high risk to develop DN. The reasons why only some patients develop DN, even if they have a long duration of diabetes, have remained unclear. The observed incidence pattern suggests that genetic factors predisposing to DN may play a role in regulating the processes that lead to DN.

### 2.4.2 Risk factors related with DN

The risk of microvascular complications is known to increase with the duration of diabetes (181; 182). Hyperglycaemia is the major risk factor for DN (183; 184). The Diabetes Control and Complications Trial (DCCT) has shown that an intensive therapy for diabetes can reduce the risk of DN (185). Glycemia cannot, however, explain all of the variability in the risk of DN (181; 182). Some patients with a poor long term glycemic control do not develop DN if they have not done so by 30 years after the onset of diabetes, and vice versa many patients with a relatively tight glycemic control develop DN within a relatively short time after diagnosis of diabetes

(186). Hypertension is also closely associated with nephropathy (181; 187; 188), but whether it is primary or secondary to DN is not clear. Lipid abnormalities play a remarkable role in the rapid deterioration of kidney function (189; 190). Parental history of hypertension, type 2 diabetes (T2D), and cardiovascular diseases appear to be the risk factors for DN (191-193). Men with DN outnumber women (181).

The effect of age at onset of diabetes on the risk of DN is not studied much. Puberty has been used as a boundary, dividing the onset of diabetes before, during, or after puberty. It has been suggested that pubertal and postpubertal duration of diabetes contribute more to the risk of diabetic complications than do the years before puberty (194-197). Conflicting results have been reported. Some studies have argued that it is solely the total duration of diabetes that affects the risk of DN (196; 198). A possible reason for the inconsistent results might be that all the years before puberty have been supposed to have an equal impact on the development of complications. It has been noticed, in later studies, that subjects diagnosed before five have a delayed onset of early retinopathy and microalbuminuria compared to those diagnosed after five (195; 197). Two very recent studies reported a reduced risk of ESRD in the patients whose diagnosis occurred at four or under (199; 200).

Since the incidence of T1D is increasing the proportion of patients with severe complications like DN is also likely to increase. Because the age at onset of diabetes has become younger than before, the patients might also develop complications at a younger age than before. The pattern of progression to DN according to different ages at onset of diabetes is very little studied.

### 2.4.3 Familial clustering of diabetic nephropathy

Familial clustering of DN has been observed earlier in some studies (201-204). Data from the DCCT have also confirmed a familial aggregation of DN (205). However, most studies have been cross-sectional and/or clinic- rather than population-based, and the sample sizes have been relatively small. Seaquist et al. (201) showed a strong concordance of DN among sibling pairs with T1D in their pioneer study. This study was, however, based on only 37 families and the 26 probands with DN were drawn from the kidney transplant clinic. Although they showed for the first time the presence of familial aggregation of DN, the estimate of its magnitude might be severely biased. A Danish study estimated a nearly five-fold increased risk of DN in diabetic siblings of patients with DN compared to diabetic siblings of

normoalbuminuric patients (203). The third study on this topic from the Joslin Diabetes Clinic confirmed that the risk in siblings varied according to the renal status of the probands. This study found an incidence rate ratio of 2.5 for the diabetic siblings of probands with and without DN. Truly population-based sibling DN recurrence risk studies do not exist and the risk differences according to the severity of DN in the probands has not been studied.

Familial aggregation of DN and its co-aggregation with other pathogenetically important factors, particularly hypertension and cardiovascular diseases, make a genetic component likely. Hypertension, T2D, and cardiovascular diseases have been observed to cluster in families (192). Despite the lack of concordance for glycemia, strong concordance in severity and patterns of glomerular lesions was seen in sibling pairs with T1D (204). Snieder et al. (206) showed that levels of $HbA_{1c}$, a measure of blood glucose regulation, are largely genetically determined. Although these observations highlight the important role of genetic factors in the development of DN, whether factors contributing to the familial clustering are due to the genetic effects, similarity of shared environment among the siblings, or both, have remained unclear.

### 2.4.4 Candidate genes on diabetic nephropathy

Several candidate genes have been proposed in relation to the development of DN. The two major approaches in the search for susceptibility genes have been linkage analyses and association studies. Genome-wide scan is also conducted (207). The results have, however, been incompatible and the association has been found to be weak. Most of the genes considered as candidate genes for DN are the same that have been studied in hypertension, T2D, cardiovascular diseases, glucose metabolism, and dyslipidaemia (208). The genes coding for components of the renin-angiotensin system have received special attention, due to the central role that this system plays in the regulation of blood pressure, sodium metabolism, and renal haemodynamics. The ACE insertion/deletion polymorphism is the most studied candidate gene (209; 210). Despite the intensive research, the role of most of these candidate genes has remained controversial and thus far not a single gene has been unequivocally shown to be causally related to DN.

### 2.4.5 Changing incidence of DN

Diabetes management has improved greatly in the recent decades. Self-monitoring of the blood glucose, measurement of $HbA_{1c}$, development of diabetes care equipments, and the frequent use of antihypertensive medications have emerged. These developments are presumed to manifest in improved prognosis of diabetic patients, reduced incidence of diabetic complications, and lowered mortality. Randomised clinical trials demonstrate that the risk of developing microvascular complications has fallen in recent years (183; 185). Bojestig et al. (211) were the first to report a dramatic decline in the incidence of DN in Sweden as a consequence of improved glycemic control. A remarkable decrease was actually observed between the cohort with diabetes onset from 1961 to 1965 and the subsequent cohorts. The following two groups, patients with onset of diabetes from 1966 to 1970 and from 1971 to 1975, seemed to have an equal risk. Little can be said about the last diagnosis group, onset of diabetes between 1976 and 1980, because of insufficient follow-up time. The authors noticed that in their follow-up study of the same cohorts a significant decrease in the incidence of DN from that in the oldest cohort to that in all the other cohorts, but no significant difference appeared between the latter cohorts (212).

Many other studies have also noticed a decrease in the incidence of DN (213; 214), but after the first observations many have also reported contradictory results. A nation-wide study from Iceland did not show any decline in the cumulative incidence of DN over time, when patients were compared according to the year of onset of T1D (215). A Danish study also did not reveal any decline with an increasing calendar year of onset of diabetes (216). A Finnish study did not either show any changes in the hospitalization rate due to DN (217).

Temporal trends of ESRD have also been investigated recently (199; 214). A significant decline was observed in the 20-year cumulative incidence rates of ESRD for patients diagnosed from 1965 to 1969, 1970 to 1974, and 1975 to 1979 in the Pittsburgh cohort of patients with diabetes (214). A large Finnish population-based study reported a decreased incidence of ESRD (199). Only a sufficient follow-up time regarding the latest diagnosis group will reveal, if the decline in the incidence rate is a reality or if the onset of ESRD just shows a small time-shift.

# 3 AIMS OF THE STUDY

The main aims were:

1. To determine the concordance rates of T1D in MZ and DZ twins and estimate heritability for T1D in the Finnish Young Twin Cohort and evaluate the possible changes in the concordance for T1D in the MZ twins. The objective was also to study the length of discordance time of twin pairs and how does it depend on the zygosity and the age at onset of diabetes in the index twin.

2. To obtain long-term empirical estimates of the risk of T1D among the siblings of T1D patients and to find out the factors influencing the risk including birth cohort effect, the age at onset of diabetes in the proband, sex, parental history of young-onset diabetes, and maternal and paternal age at delivery.

3. To establish if DN is aggregated in the Finnish cohort of families with multiple cases of T1D and assess the magnitude of aggregation first time in the population-based cohort. This study was also going to define whether the risk of DN in the T1D siblings is varying according to the severity of DN in the proband and according to the age at onset of diabetes. The aim was also to study the sex differences and effects of calender year at onset of diabetes on the risk of DN in the T1D siblings. Also the effect of parental history of T1D, T2D, and hypertension were studied as risk factors for DN.

4. To assess the cumulative incidence of T1D in the offspring of T1D parent and find out the extent to which the sex of the T1D parent influence on the risk of T1D in the offspring and to identify the possible modifying factors, especially the effect of parental age at diagnosis of diabetes. The aim was also to find out whether the parent-offspring transmission of T1D has changed over time and compare the incidence of T1D in the offspring to that of T1D incidence in the background population.

# 4 MATERIALS AND METHODS

## 4.1 Study Population

### 4.1.1 Finnish Young Twin Cohort

The Finnish Twin Cohort was established in 1974, which in its first phase comprised of over 17,000 like-sex twin pairs born before 1958, with both members alive in 1967 (*The Old Twin Cohort*) (218). In 1987, the twin study was expanded to all like-sex and opposite-sex twin births from 1958 to 1986 comprising 22,650 twin pairs (*The Young Twin Cohort*) (219). The cohorts were compiled from the Central Population Registry (CPR) of all Finnish residents. The Young Twin Cohort formed the study population in the study I (Table 7).

**Table 7.** Finnish Young Twin Cohort: estimated zygosity distribution

| TYPE OF TWIN PAIRS | NUMBER OF TWIN PAIRS |
|---|---|
| OPPOSITE-SEX PAIRS | 8,133 |
| LIKE-SEX PAIRS | 14,517 |
| LIKE-SEX DIZYGOTIC PAIRS | 8,133 |
| MONOZYGOTIC PAIRS | 6,384 |
| ALL PAIRS | 22,650 |

### 4.1.2 Sibling and offspring recurrence risk of T1D and familial DN

The studies II, III, and IV included family data from the nationwide Finnish population-based cohort of patients that had been diagnosed with T1D before 18 between 1965 and 1979 (n=5,144 T1D cases, designated original DERI probands). This cohort was initially employed in the Diabetes Epidemiology Research

International (DERI) Mortality Study (220; 221). The case ascertainment in this cohort was virtually complete (222; 223).

Figure 4 illustrates a three-generation pedigree of a DERI family. In studies II and III the proband (index case) within the sibship was defined as the sibling that was diagnosed first with T1D chronologically. Thus, if there were two original DERI cases (with onset of T1D during 1965-1979) in the family the first affected was the proband and the other was considered as the affected sibling. Descriptive statistics of the siblings in the DERI families are described in Table 8. All siblings (n=10,168) of DERI families were born in 1990 or before.

The study population in study III was composed of the families with at least two children with T1D (multiplex families) that were identified in study II.

The study population in study IV consisted of the offspring of the original DERI probands. By the end of 2001 there were 5,291 offspring. Of these 2,297 (43.4%) were born to female probands and 2,981 (56.4%) to male probands. Offspring were mainly born between 1970 and 2001. Descriptive statistics of the offspring are described in Table 9.

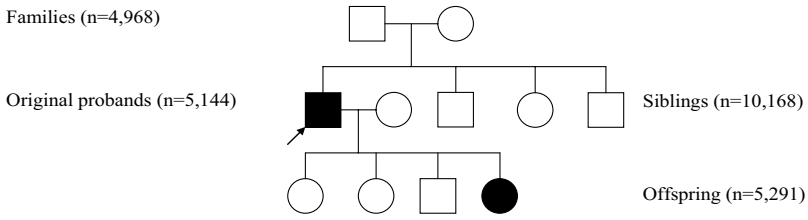


**Figure 4**. Pedigree of a DERI family with three generations

**Table 8.** Subject characteristics of study population (II, III)

| CHARACTERISTICS | | NUMBER OF SIBLINGS |
|---|---|---|
| Sex of the sibling | | |
| Male | | 5,202 |
| Female | | 4,966 |
| Family size | Families (n) | |
| 1 child | 682 | - |
| 2 children | 1,606 | 1,606 |
| 3 children | 1,230 | 2,460 |
| 4 children | 678 | 2,034 |
| ≥5 children | 772 | 4,068 |
| Total | 4,968 | 10,168 |
| Birth year of the sibling | | |
| <1960 | | 5,151 |
| 1960-69 | | 3,838 |
| 1970-79 | | 971 |
| ≥1980 | | 208 |
| Age at onset of T1D in the proband | | |
| 0-4 years | | 1,306 |
| 5-9 years | | 2,811 |
| 10-14 years | | 4,156 |
| ≥15 years | | 1,895 |

**Table 9.** Descriptive data of the study population (IV)

| CHARACTERISTICS | FEMALE PROBAND | MALE PROBAND | ALL |
|---|---|---|---|
| Number of probands | 2,313 | 2,831 | 5,144 |
| Number of probands with offspring | 1,335 (57.7%) | 1,440 (50.9%) | 2,775 (53.9%) |
| Number of offspring | 2,310 | 2,994 | 5,291 (13)* |
| Girls | 1,157 | 1,444 | 2,595 (6)* |
| Boys | 1,153 | 1,550 | 2,696 (7)* |
| Year of birth of offspring | | | |
| <1970 | 3 | 1 | 4 |
| 1970-79 | 253 | 269 | 522 |
| 1980-89 | 906 | 1,124 | 2,028(2)* |
| 1990-99 | 1,032 | 1,416 | 2,438(10)* |
| 2000-01 | 116 | 184 | 299(1)* |

* Both parents with T1D

## 4.2 Identification of family members and diabetes status -record linkages

Family members were identified from the Central Population Registry (CPR), in siblings and parents of the probands (studies II and III) and offspring and the other parent of the offspring (study IV). In the cases in Study II that one or both parents could not be found from the CPR, the family trees were traced from the church parishes by genealogical study. The vital status of the probands and their first-degree relatives were obtained by linkage of the records with the National Death Registry.

The diabetes status of the twins, the first-degree relatives of the DERI-cases, and the other parent of the offspring of the DERI-cases were ascertained through several sources. The data were linked to the National Hospital Discharge Register (HDR), the Social Insurance Institution Central Drug Register (CDR), and the nationwide Finnish Diabetes Register for Children and Young Adults. Since 1970 (nationwide since 1972) the HDR lists discharges of all hospital patients and includes each patient's ID code, dates of hospitalization admission and discharges, and up to four International Classification of Diseases (ICD) codes. Medication for diabetes is free of charge for diabetic patients in Finland. All patients receiving free medication for chronic diseases including diabetes are entered into the CDR.

It is not always possible to unambiguously distinguish between the different types of diabetes based on the CDR or HDR. In addition, the date of diagnosis is not always reliable based on these registers. Therefore, the primary use of the HDR and CDR in Studies I-III was to trace the cases with diabetes as well as the places of their health care. Copies of the original medical records for all diabetic twins, DERI probands and their siblings with diabetes, and for the other diabetic parent of the offspring were obtained and reviewed in order to verify the type of diabetes and to define the date of manifestation of diabetes. Nonetheless, in study IV, where the first case of T1D occurred in 1983 and over 90% of cases with diabetes were diagnosed at 14 or before the data from the CDR and HDR were considered reliable. Practically all children with newly-diagnosed diabetes are hospitalized in Finland (146) and the date of onset of diabetes could be defined in Study IV as the date of the first hospital admission because of diabetes or the approval date for the free-of-charge medication for diabetes.

In order to find diabetic parents in DERI families, the data on the parents were also linked to the HDR and CDR. The onset of diabetes in parents was defined as the

date of the first hospital admission due to diabetes or the approval date for free-of-charge medication for diabetes, whichever was earlier. Parents with early-onset diabetes were defined as those whose attained age at diagnosis of diabetes was 40 years or under.

## 4.3 Zygosity determination

Questionnaires were mailed to all twins with diabetes and their co-twins with questions on zygosity, diabetes type, date of onset, start of insulin therapy, and history of diabetes among family members and they were also invited to give blood samples for confirmation of zygosity and ongoing genetic studies. Twin zygosity was determined by examining the responses of the twins to questions on the similarity of appearance and confusion between twins at school by using a set of decision rules (224). The same questions were earlier used in the Finnish Twin Cohort Study and this method has been validated in a subsample by the use of 11 genetic markers (224). In addition, zygosity was confirmed by DNA analysis of 10 highly informative polymorphic microsatellite loci (D3S1358, vWA, FGA, AMEL, THO1, TPOX, CSF1PO, D5S818, D13S317, D7S820) in 14 of 15 same-sex concordant pairs for T1D and when there was any doubt about zygosity as used routinely in paternity testing procedures at the National Public Health Institute. Only one diabetic pair with both members having moved abroad and both parents being deceased remained unclassified.

## 4.4 Classification of diabetic nephropathy

A potential diagnosis of DN was followed among sibling pairs with T1D until the end of 2001. In order to identify patients with DN, copies of original medical records, death certificates, and autopsy data for the probands and their T1D siblings were systematically reviewed. Overt nephropathy was defined as a urinary albumin excretion rate (AER) repeatedly exceeding 200 μg/min or 300 mg/24 h, or as a 24-hour urinary protein excretion rate >0.5 g, or as a positive urinanalysis for protein using a reagent strip. Microalbuminuria was defined as an AER of 20-200 μg/min or 30-300 mg/24 h. Normal AER was defined as an AER <20 μg/min or <30 mg/24 h. If AER was elevated due to pregnancy, urinary tract infections or other renal diseases the result was not considered diagnostic for DN. If the patient used any

antihypertensive medication, the classification of DN was based on the findings prior to the initiation of drug treatment. End-stage renal disease (ESRD) was present if the patient was undergoing dialysis or had received a kidney transplant.

## 4.5 Ethical aspects of the study

This study was approved by The National Advisory Board on Health Care Ethics**.** All subjects in the twin study who were asked to give blood samples received written information about the study prior to their participation and gave their verbal consents. Medical records were reviewed with the permission of the Ministry of Social Affairs and Health.

## 4.6 Statistical methods

### 4.6.1 Study I

Concordance was estimated using both crude pairwise and probandwise concordance rates, each calculated separately for MZ and DZ twin pairs. (10). Because probandwise concordance can be interpreted as the recurrence risk in a co-twin of an affected twin and thus comparable with the cumulative incidence of T1D in the co-twins Kaplan-Meier survival analysis was also used to estimate the probandwise concordance. Furthermore, in the survival analyses the co-twins were divided into two groups: those in which the index twin was diagnosed at 10 or younger and those in which the index twin was older than 10. The log-rank test served to test risk difference between groups. Intra-class correlations (tetrachoric) were calculated to estimate the degree of similarity within a twin pair.

A polygenic, multifactorial liability model was fitted to the twin data to estimate the contribution of environmental and genetic factors to the susceptibility to T1D. It assumes that there is a normally distributed liability to the disease (12). When a certain level of threshold of liability is reached, the disease manifests. Analysis based on structural equation models permit formulation of models with components of variance; additive genetic variance (A), genetic variance due to dominance (D), environmental effects common to the twins (C) or unique environmental (E) sources of variation in the underlying liability to disease. Different combinations of models,

i.e. AE, ACE, ADE, and CE were fitted to the 2x2 contingency tables (disease present/absent in twin 1 versus disease present /absent in twin 2).

The superiority of alternative hierarchically nested models was assessed by using the –2 log-likelihood statistic: the likelihood-ratio of alternative hierarchically nested models was calculated by the difference in their $\chi^2$-values which itself is $\chi^2$-distributed with degrees of freedom equal to the of models compared (13). In addition, parsimony (as few parameters as possible) of the models was examined using AIC-value (Akaike's information criteria). The model with the lowest AIC-value indicates the best goodness-of-fit and the parsimony. Analyses were carried out with the Mx-software package (225), which has been developed especially for twin analyses.

### 4.6.2 Study II

Kaplan-Meier analyses were used to provide the long-term cumulative risk for the development of T1D in siblings. Person-years were calculated from birth to the date of diagnosis of T1D, until death, or until the end of follow-up at the end of 2001. Analyses were also performed stratifying by sex, age of diabetes onset in the proband, year of birth (cohort effect), and parental age at delivery. The effect of several independent risk factors on the risk of T1D for the siblings was evaluated by a Cox regression analysis using a forward selection procedure. The predictors studied were sex of the sibling, sex of the proband, diagnosis age in the proband (0-4, 5-9, 10-14, ≥15), parental history of young-onset diabetes (≤40 years), birth year (≤1970, 1970-1979, ≥1980), number of offspring in the family as continuous variable, and parental age at delivery (≤24, 25-29, 30-34, 35-39, ≥40). Model selection was based on likelihood ratio tests. Relative risks were calculated with a univariate analysis and with the best fitting multivariate model.

### 4.6.3 Study III

For each sibling, the person-years at risk was calculated from the onset of diabetes to the date of DN diagnosis, death, or until the last urine screening test found in the medical records before the end of the year 2001. The cumulative incidence of DN, according to the duration of diabetes, was estimated by using the Kaplan-Meier survival analysis method. This was also conducted according to the presence and severity of DN in the proband and according to age at onset of diabetes. To identify

prognostic factors for the development of DN in diabetic siblings, the Cox's proportional hazards model was fitted to the data. Sex, the age at the onset of diabetes (0-4, 5-9, 10-14, ≥15), the calendar year at the onset of diabetes (≤1970, 1971-79, ≥1980), the DN status of the proband, the parental history of T2D and hypertension, and maternal age at delivery were entered into the model. Age-specific incidence rates of DN were calculated per 1,000 person years for the diabetic siblings of the probands with and without DN separately and according to sex. Incidence rates were also calculated by duration of diabetes, separately for men and women.

### 4.6.4 Study IV

Follow-up started at birth and ended at the diagnosis of T1D, death, or the end of the year 2003. The data were grouped by age and calendar time (period) in 1-year classes. Poisson regression analysis was used to evaluate temporal trends in incidence. Standardized Incidence Ratios (SIRs) of T1D were calculated to determine the increase in the risk of T1D in the offspring of the parents with T1D compared with that in the background population for the period 1985-2003 for each of 5-year periods. The expected numbers of cases were derived by applying the age-specific incidence rates of T1D observed at the same time in the background population, i.e. in Finland nationwide. The data on the newly diagnosed T1D cases nationwide were derived from the CDR.

Kaplan-Meier analysis was used to estimate the cumulative incidence of T1D. The analyses were also carried out stratifying the data by the age at diagnosis of T1D in the parent and the birth year of the offspring, separately in the offspring of fathers with T1D and mothers with T1D. To assess the effect of several independent risk factors on the risk of T1D among the offspring, univariate and multivariate regression analyses of the data were performed using the Cox proportional hazards modelling. In addition to the sex of the T1D parent, the predictors studied were sex of the offspring, year of birth of the offspring (< 1985, 1985-89, 1990-1994, ≥1995), age at diagnosis of the parent (0-4, 5-9,10-14,15-17), the parental age at delivery and the birth order effect. Interaction between variables was also tested.

# 5 RESULTS

## 5.1 Genetic liability of T1D (Study I)

The record-linkages yielded a total of 303 cases with any type of diabetes by the end of the follow-up in 1998. A total of 247 twins (in 228 twin pairs) with T1D, 28 with T2D, 15 with secondary diabetes, and 13 with gestational diabetes were identified. In the last group three patients with gestational diabetes were co-twins of T1D twins and were classified as discordant for T1D. Table 10 shows the number of T1D twin pairs and the concordance rates by sex and zygosity. The concordance rate was notably higher among MZ twins than DZ twins. The crude pairwise concordance rate was 27.3% (95% CI 22.8-31.8%) in MZ and 3.8% (95% CI 2.7-4.9%) in DZ twins.

For the concordant pairs the twins in four MZ pairs were diagnosed within half a year and one pair was diagnosed on the same day. The longest discordance time was 6.9 years. The median discordance time was 2.0 years for MZ twins and 6.0 years for DZ twins. A higher risk of progression to T1D was observed for the MZ co-twin in the survival analysis if the index twin had been diagnosed with diabetes at the age of 10 years under than if the index twin had been diagnosed later ($p=0.06$, log-rank test). Half of the co-twins in whom the MZ index twin had been diagnosed with T1D at the age of 10 years or under were predicted to develop diabetes within 7 years compared to 23% of those with the index twin diagnosed >10 years.

Table 11 summarizes the results of the model fitting procedures. A model with only environmental effects (CE) fitted the data poorly. A model including additive genetic effects and non-shared environmental effects (AE) fitted best with a heritability estimate of 88%. A model that also included shared environmental effects (ACE) fitted second best (AIC=-3.23, $p=0.43$), with the same estimates as in the AE- model.

The striking correlation with age at onset among the concordant MZ twin pairs was evident throughout the age range, $r=0.95$ ($p<0.001$), but it was much lower among the concordant DZ pairs, $r=0.38$ ($p=0.41$).

**Table 10.** Number of concordant and discordant twin pairs and crude concordance rates for T1D in Finnish Young Twin Cohort

| Gender | Zygosity | Concordant pairs (n) | Discordant pairs (n) | CONCORDANCE RATE % Paiwise | Probandwise |
|---|---|---|---|---|---|
| Male | MZ | 7 | 19 | 27.0 | 42.4 |
| | DZ | 2 | 47 | 4.1 | 7.8 |
| Female | MZ | 5 | 13 | 27.8 | 43.5 |
| | DZ | 1 | 43 | 2.3 | 4.4 |
| Opp. sex | DZ | 4 | 86 | 4.4 | 8.5 |
| Total* | MZ | 12 | 32 | 27.3 (22.9-31.8) | 42.9 (26.7-59.2) |
| | DZ | 7 | 176 | 3.8 (2.7-4.9) | 7.4 (2.2-12.6) |

*Plus one discordant pair with unknown zygosity

**Table 11.** Results from fitting threshold models to T1D data in the Finnish Young Cohort Twins

| MODEL | COMPONENTS OF VARIANCE | | | | GOODNESS OF FIT | | | |
|---|---|---|---|---|---|---|---|---|
| | A | C | D | E | $\chi^2$ | df | p-value | AIC |
| ACE | 0.88 | 0.00 | - | 0.12 | 2.77 | 3 | 0.43 | -3.23 |
| AE | 0.88 | - | - | 0.12 | 2.77 | 4 | 0.60 | -5.23 |
| ADE | 0.79 | - | 0.09 | 0.12 | 2.88 | 3 | 0.40 | -3.20 |
| CE | - | 0.60 | - | 0.40 | 28.8 | 4 | 0.00 | 20.8 |

**A**, additive genetic effects; **C**, shared (family) environmental effects; **D**, due to dominance; **E**, unshared environmental effects. The best fitting model is generally one with the lowest AIC-value. AIC=Akaike's information criteria

## 5.2 Sibling recurrence risk of T1D and related factors (Study II)

The data linkage yielded a total of 715 cases with some type of diabetes among the siblings at risk. A total of 647 (6.4%) of the 10,168 siblings were diagnosed with T1D by 2001. Fifty siblings had T2D, six had gestational diabetes, and 12 had secondary diabetes such as steroid-induced diabetes or diabetes associated with Down's syndrome. The median age at diagnosis of T1D among the siblings was 15.1 years (range 0.8-56 years). Of non-diabetic living siblings, 99% were ≥20 at the end of the follow-up in 2001, and all had reached ≥11 years of age.

In the siblings, the overall cumulative risk of T1D up to ages 10, 20, 30, 40, and 50 years was 1.5 (95% CI 1.3-1.8), 4.1 (3.7-4.4), 5.5 (5.1-5.9), 6.4 (5.9-6.8), and 6.9% (6.4-7.4), respectively. There was a male predominance, 57% of the siblings with T1D were males. Males had a greater secondary attack rate of T1D at older ages than females. Males and females had an equal risk through the first 14 years, after which the risk for males began to slightly increase compared to females. The overall risk ratio was 1.3 (95% CI 1.1-1.5). Risk was not affected by adjustment for other risk factors (Table 12).

The risk was significantly ($P<0.0001$) higher in the siblings of those probands that had been diagnosed with T1D at four years or younger compared to the siblings in the families where the probands had been diagnosed at the age of 15 or more. Figure 5A shows the Kaplan-Meier curves for T1D in the siblings stratified by age at onset of diabetes in the proband. The cumulative risk of T1D by age 40 for a sibling whose proband was diagnosed at the ages of 0-4, 5-9, 10-14, and 15 or more was 13.2 (95% CI 11.4-14.8), 7.8 (6.9-8.8), 4.7 (4.1-5.3), and 3.4% (2.6-4.2). The T1D risk for the sibling was 4.1 (95% CI 3.1-5.4) times higher if the proband was diagnosed at four years or younger than if the proband was diagnosed at 15 years or more. The relative risk was even higher 4.6 after adjustment for other variables (Table 12).

Figure 5B shows the cumulative risk curve stratified by the year of birth of the sibling. Of siblings born in 1980 or after, 11.4% (95% CI 7.4-15.2) had developed T1D by the age of 15, while only 5.5% (95% CI 4.1-6.8) of those who were born between 1970-79 and 2.7% (95% CI 2.4-3.0) of those who were born before 1970 ($p<0.0001$, log-rank test) had developed T1D. SIRs were 11.0, 12.6, and 12.7 for the years 1965-74, 1975-84, and 1985-94, respectively.

A significant increase in the risk of T1D with increasing maternal and paternal age at delivery exist ($p < 0.0001$, log-rank test). The RR of T1D was 1.85 (95% CI 1.35-2.55) when maternal age at delivery over 35 years was compared with those below 25 years. The corresponding RR comparing paternal age at delivery was 1.5 (95% CI 1.07-2.11). The results were not considerably affected by adjustment for the other risk factors, but both maternal and paternal age at delivery remained significant predictors.

**Table 12.** Estimated risk ratios (95% confidence intervals) for T1D in siblings of childhood-onset T1D patients fitting explanatory variables

| VARIABLE | UNIVARIATE ANALYSIS | | | ADJUSTED FOR OTHER VARIABLES | | |
|---|---|---|---|---|---|---|
| | RR | 95% CI | p-value | RR | 95% CI | p-value |
| **SEX** | | | | | | |
| Female | 1.00 | | | 1.00 | | |
| Male | 1.26 | 1.08-1.47 | 0.004 | 1.29 | 1.10-1.50 | 0.002 |
| **AGE AT DIAGNOSIS IN THE PROBAND** | | | | | | |
| ≥15 | 1.00 | | | 1.00 | | |
| 10-14 | 1.35 | 1.03-1.77 | 0.03 | 1.43 | 1.09-1.88 | 0.009 |
| 5-9 | 2.27 | 1.73-2.96 | <0.0001 | 2.50 | 1.90-3.27 | <0.0001 |
| 0-4 | 4.05 | 3.06-5.36 | <0.0001 | 4.60 | 3.46-6.10 | <0.0001 |
| Test for trend | | | <0.0001 | | | <0.0001 |
| **YOUNG-ONSET DIABETES IN FATHERS** | | | | | | |
| Yes | 1.00 | | | 1.00 | | |
| No | 2.11 | 1.33-2.32 | 0.001 | 1.95 | 1.23-3.10 | 0.004 |
| **MATERNAL AGE AT DELIVERY** | | | | | | |
| <25 | 1.00 | | | 1.00 | | |
| 25-29 | 1.40 | 1.13-1.72 | 0.002 | 1.31 | 1.03-1.66 | 0.03 |
| 30-34 | 1.58 | 1.27-1.98 | <0.0001 | 1.33 | 1.00-1.76 | 0.05 |
| ≥35 | 2.23 | 1.77-2.80 | <0.0001 | 1.85 | 1.35-2.55 | 0.0002 |
| Test for trend | | | <0.0001 | | | 0.02 |
| **PATERNAL AGE AT DELIVERY** | | | | | | |
| <25 | 1.00 | | | 1.00 | | |
| 25-29 | 1.00 | 0.77-1.31 | 0.98 | 0.96 | 0.72-1.27 | 0.78 |
| 30-34 | 1.50 | 1.16-1.94 | 0.002 | 1.38 | 1.02-1.87 | 0.04 |
| ≥35 | 1.81 | 1.41-2.33 | <0.0001 | 1.50 | 1.07-2.11 | 0.02 |
| Test for trend | | | <0.0001 | | | 0.002 |

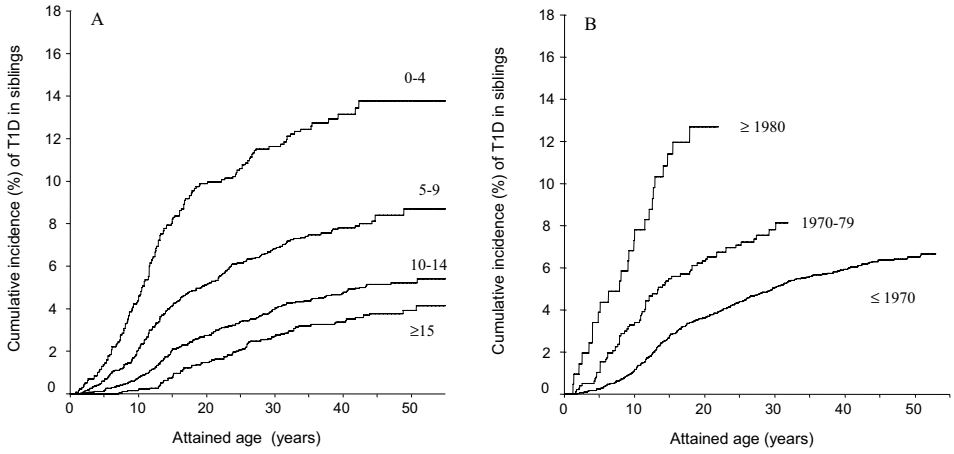


**Figure 5.** Cumulative incidence (%) of T1D in siblings of childhood-onset T1D patients according to age at onset of diabetes in the index case (A) and birth cohort (B)

## 5.3 Familial aggregation of DN (Study III)

The probands had a mean duration of diabetes of 31.4±6.5 years, whereas diabetic siblings had a mean disease duration of 21.6±9.0 years. The vast majority, 97% of the probands had had diabetes for over 20 years and none of those alive at the end of the follow-up had a duration of diabetes of less than 22 years. Duration of diabetes among 60% of siblings was at least 20 years and among 87% of siblings it was 10 years or more.

By the end of 2001, during 10,913 person-years of follow-up, 323 cases of DN were identified, 176 among the probands (33%) and 147 among the diabetic siblings (24%), with a total of 274 sibling pairs affected by DN. Significant differences in the presence of DN were found between the groups of diabetic siblings according to the presence of DN in the proband. DN was present in 77 (38%) of 204 siblings of the probands with DN, while 70 (17%) of 409 siblings of the probands without DN had

also DN. ESRD was present in 46 (8.6%) probands and in 36 (5.9%) diabetic siblings. Ten concordant pairs for ESRD (13.9% of all sib-pairs with ESRD) existed.

The peak incidence of DN occurred at ages of 25-29 years. After reaching the peak, the incidence decreased in the siblings of the probands without DN, whereas new DN cases continued to appear beyond these ages in the siblings of the probands with DN. The overall 25-year cumulative risk of DN in the siblings was 34.5% (95% CI 31.1-37.3%). Figure 6A indicates that not only the DN status of the proband, but also the severity of DN in the proband significantly affected the risk of DN in the siblings ($P < 0.0001$, log-rank test). The risk of progression to DN after a 25 years duration of diabetes in the siblings of probands with DN, but not ESRD, was 43.2% (95% CI 36.9-48.8), while the risk was 24.8% (95% CI 20.9-30.0) for siblings of probands without DN. If the proband suffered from ESRD the corresponding risk of DN in the siblings was even higher, 58.0% (95% CI 51.4-63.7). The risk ratio for DN in the siblings of probands with DN was 2.0 (95% CI 1.3-2.9) and in the siblings of probands with ESRD 2.9 (95% CI 1.8-4.5) compared with the siblings of normo- or microalbuminuric probands. The risk ratio was 2.3 (95% CI 1.4-2.7) when probands with DN and ESRD were combined.

Women tended to develop DN earlier after the onset of diabetes compared to men, the peak in incidence rate in women occurred five years earlier. Figure 6B shows the development of DN in siblings stratified by the age at the onset of diabetes. Siblings diagnosed with diabetes before age 5 years or at the age of 15 years or later had a smaller risk of developing DN than patients diagnosed at the age groups 5-9 and 10-14 years ($P = 0.001$, log-rank test). The 20-year progression rate of DN for the siblings diagnosed with diabetes at the ages of ≤4, 5-9, 10-14, and ≥15 were 18.7 (95% CI 6.8-29.0), 28.2 (21.3-34.4), 30.7 (25.3-35.7), and 15.9 (10.8-20.6), respectively.

Multivariate analysis by Cox regression analysis revealed that the independent prognostic factors predicting DN in the siblings were DN status of the proband, sex, the age at onset of diabetes in the siblings and parental T2D.

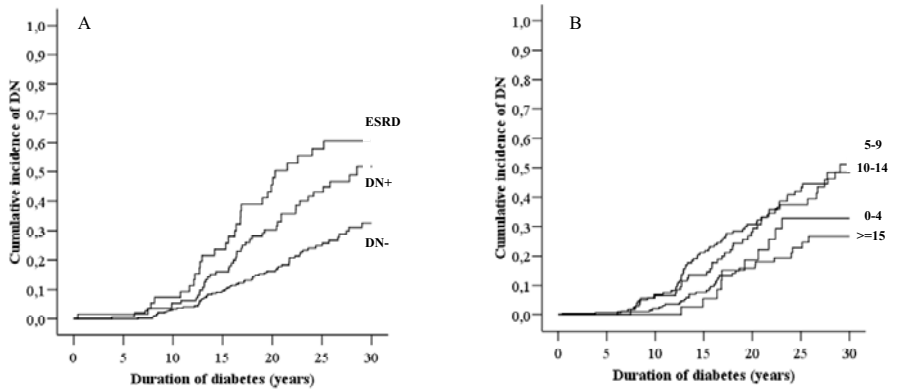


**Figure 6.** (A) Cumulative incidence of DN in diabetic siblings of T1D probands according to the DN status of probands. DN=diabetic nephropathy, ESRD=end-stage renal disease, DN-= DN absent; DN+=DN present, excludes ESRD. (B) Cumulative incidence of DN in diabetic siblings of T1D probands according to age at onset of diabetes in siblings

## 5.4 Offspring recurrence risk of T1D (Study IV)

A total of 259 offspring, 121 girls and 138 boys were affected with T1D by the end of the year 2003, of which 236 were diagnosed at the age of 14 or under. Thirteen (22.4%) of the 58 offspring with two T1D parents were affected. In all offspring with one diabetic parent, the overall cumulative incidence to develop T1D by the age of 15 years was 5.6% (95% CI 4.9-6.4) and by the age of 20 6.7% (95% CI 5.9-7.5). The cumulative incidence was similar in boys and girls up to 14 years but started to diverge thereafter. However, the difference did not reach statistical significance until 20 years.

Fathers with T1D transmitted diabetes to their offspring 1.7 times more frequently than mothers with T1D. This sex difference in the transmission rate manifested itself through the higher risk for fathers who were young when diagnosed with T1D. The risk of T1D in the offspring was especially high, when the father had been diagnosed with T1D at the age of four or under: 11.0% (95% CI 6.6-15.3) of the

offspring became affected during their first 10 years of life compared with only 2.4% (95% CI 0.2-4.4) in the offspring of the T1D mothers whose age at onset of T1D was four years or under (Figure 7A). A young age at onset of T1D in diabetic mothers did not increase the risk of T1D in the offspring (Figure 7B, Table 13).

During 1980 to 2003 the overall incidence of T1D in the offspring showed an average increase of 5.3% ($p<0.001$) per year. In the offspring of 0-4 years at diagnosis the annual increase was 7.3% ($p<0.001$), while in the offspring aged 5-9 and 10-14 years it was 4.7% ($p=0.04$) and 1.7% ($p=0.5$), respectively. Of the offspring of T1D fathers born in 1995 or after, 4.2% (95% CI 2.8-5.6) had developed diabetes by five years of age, whereas only 1.5% (95% CI 0.5-2.5) of those of T1D mothers had developed diabetes. It took on average 17.5, 10.1, 9.1, and 6.8 years for the offspring born in 1985 or before, 1985-1989, 1990-1994, and 1995 or later, respectively, to reach a 4% cumulative incidence of T1D.

The offspring of patients with T1D had approximately a 10-fold excess risk compared with the T1D incidence in the general population of Finland. SIRs in a five-year time periods were 9.7 (95% CI 8.5-11.0) during 1985 to 2003. In the first period, during 1985 to 1989, it was 7.6 (95% CI 4.9-11.3) and remained fairly stable during the subsequent years.

In the multivariate analysis the sex of the diabetic parent, the age at onset of diabetes in the T1D parent, interaction between them, and the birth year of the offspring were statistically significant predictors of T1D in the offspring.

**Table 13.** Multivariate Cox regression analysis for the factors influencing the risk of T1D in offspring of parents with T1D, final model

| **OFFSPRING OF MALE PROBAND** | | | | **OFFSPRING OF FEMALE PROBAND** | | |
|---|---|---|---|---|---|---|
| Variable | RR | 95% CI | p-value | RR | 95% CI | p-value |
| **Year of birth** | | | | | | |
| <1980 | 1.00 | - | | 1.00 | - | |
| 1980-89 | 1.47 | 0.94-2.28 | | 1.87 | 1.01-3.49 | |
| 1990-94 | 1.48 | 0.91-2.41 | | 2.97 | 1.49-5.91 | |
| ≥1995 | 2.94 | 1.71-5.03 | 0.001 | 2.21 | 0.86-5.69 | 0.02 |
| **Age at onset of T1D** | | | | | | |
| 0-4 | 2.66 | 1.48-4.79 | | 0.76 | 0.28-2.01 | |
| 5-9 | 1.45 | 0.90-2.33 | | 0.96 | 0.48-1.92 | |
| 10-14 | 1.17 | 0.77-1.79 | | 0.94 | 0.51-1.72 | |
| 15-17 | 1.00 | - | 0.007 | 1.00 | - | 0.95 |

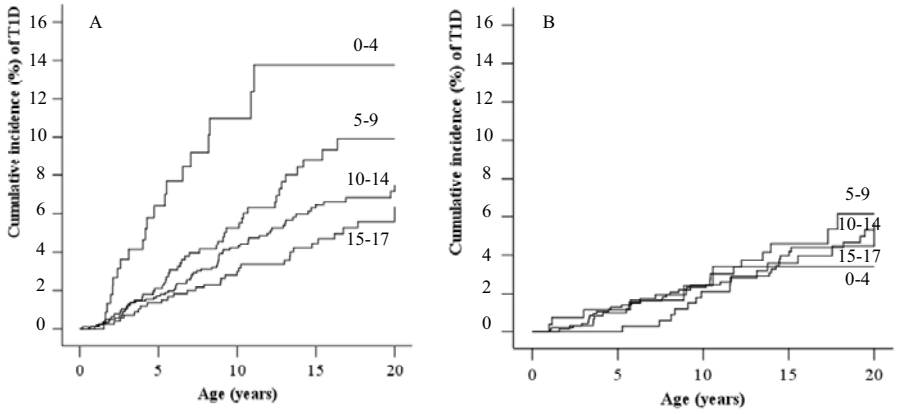


**Figure 7.** Cumulative incidence of T1D in the offspring of childhood-onset T1D patients according to the age at onset of diabetes in T1D fathers (A) and the age at onset of diabetes in T1D mothers (B)

# 6 DISCUSSION

## 6.1 Study population and methods

The aims of the present study were to determine concordance rates of T1D in twins, heritability of T1D, recurrence risk of T1D in siblings and offspring of patients with young onset T1D, temporal changes in the risk, and the factors related to the risk. Furthermore, the objective was also to assess the presence of familial aggregation of DN and its magnitude. The indisputable strengths of this study were that the study used uniquely large, population-based cohorts of twins, patients with young onset T1D, and their first-degree relatives and therefore represents unbiased data on families with multiple cases of patients with T1D. Opportunities to study the etiology of T1D are ideal in Finland because the population is genetically homogenous, health care for patients with diabetes is well organized, and nationwide registries exist. Therefore virtually all the cases with T1D can be ascertained. The magnitude of familial aggregation of DN has thus far not been specified in a population-based cohort.

At the time of compilation of the Young Twin Cohort the completeness of the ascertainment of twin pairs was very high. Especially, the younger portion of the cohort, twins born from the early 1970s onwards, can be regarded as virtually complete (218). The DERI cohort of patients with T1D diagnosed at the age of 17 or under is a part of the Finnish T1D register, which has been shown to have a degree of ascertainment of nearly 100% (222; 223). The case ascertainment of T1D subjects in all the studies was extremely high because of several nationwide data sources that were available: the CDR, the HDR and the Diabetes Register of National Public Health Institute. The validity of type of diabetes and the age at onset was ensured from the medical records.

One minor limitation of this study was that any subjects with diabetes that had been diagnosed at a very young age and deceased before 1964, when the CPR was founded, could not be detected. Such cases must however have been very few. Another limitation concerns the grading of the type of diabetes. Besides secondary and gestational diabetes, diabetes in siblings was classified into two types, T1D and T2D, and recorded in the medical records by the attending physician. Some of the siblings had a slowly progressive form of insulin-dependence and they were initially treated without insulin and thus might have been considered to have latent

autoimmune diabetes in adults (LADA) or type 1.5 diabetes (226; 227). All cases with autoimmunity, however, were considered as T1D irrespective of the length of the pre-diabetic period, if they finally showed insulin deficiency. No complete agreement on whether LADA and adult onset T1D are different diseases or not exists yet (226; 227). The vast majority of cases in this study, were however acute onset and the patients required insulin immediately at diagnosis. There were 50 cases with T2D, of which 32 had onset of diabetes at the age of 35 or under. Some of these cases could have been misclassified although they might later prove to be T1D cases. Several studies suggest the presence of a slowly progressive β-cell destruction in a part of patients, who were classified as T2D at diagnosis (52; 56).

Some limitations in the classification of DN can be brought up. Cases with DN and the date of the diagnosis were documented from the medical records, laboratory sheets, death certificates, and autopsy reports as precisely as it was possible. The data were, however, not always very solid and thus the grading of DN could not always be performed adequately. One confounding factor in the classification of patients with DN was the use of antihypertensive medication. ACE-inhibitors became widely used for the treatment of microalbuminuria in the 1990s. Most of the DN cases were, however, diagnosed before that. In addition, ACE-inhibitors were often prescribed after the diagnosis of DN and not in the microalbuminuric state, and there was also some variety depending on the residence of T1D patients.

Changes in medical care practices between the earliest and the more recent cohorts of T1D patients also caused some non-uniformity. Overt nephropathy was detected by dipsticks in the oldest cohorts, whereas more recently microalbuminuria testing was available and the screening was more regular. Some of the patients with DN could only be classified based on the death certificates or autopsy reports but in these cases the age at onset of DN could not be defined accurately. On the other hand, those cases were for the most part the probands in which DN was specified only as present or absent, whereas the age at onset of DN was a necessary tool only in the siblings. The data that encompass such a long time period can naturally not be coherent. The vast majority of DN grading, however, can be considered accurate.

A great effort was made to determine the zygosity of all twin pairs with T1D. Several sources were used and only one twin pair remained unclassified by zygosity. The alidity of the method used to determine zygosity has been ascertained earlier (224).

## 6.2 Heritability and concordance rate of T1D

The estimate of heritability for T1D in this study was 88% whereas it was 74% in the Finnish Old Twin Cohort. The concordance rates, pairwise and probandwise, in the MZ twins were substantially higher in the present study than those in the Finnish Old Twin Cohort, 27% vs. 13% and 43% vs. 23%, respectively (124). One could find the observed increase in heritability of T1D and the environmental factors influencing the increase in incidence paradoxical. Any estimate of heritability is valid only in a particular population, in a particular environment, and at a particular time. If either the genetic or the environmental component of the variance changes, then the heritability changes. Greater diversity of environmental factors present in a population will lower an estimate of heritability, while population with a more homogenous environment will increase an estimate of heritability, even though the biological mechanism underlying the trait is identical (228).

It is important to emphasize that heritability estimates are based on a calculation of the genetic variation relative to the total phenotypic variation. The calculation does not take into account a potential gene-environment interaction that means that different genotypes respond differently to the same environment. Some genotypes are more sensitive to changes in the environment than others. Gene-environment interaction, however, seems to be important in T1D. Increase in the concordance of T1D in the MZ twins, in the incidence of T1D in the population, and the recurrence risk of T1D in later cohorts of siblings and offspring all indicate the importance of the effects of environmental changes in genetically prone individuals and thus gene-environmental interaction.

If environmental conditions become equalized, the remaining variance must then become increasingly genetic. The development of Finland from a poor agricultural society into a modern welfare state has had an influence everyday life of all citizens. During the past decades, substantial changes have occurred everyday life towards an affluent Western style, as a consequence of the breakthrough of the consumer society, changes in nutrition, physical activity, vaccinations, personal hygiene, etc. Despite these changes, they have occurred coherently in the whole of the country and have possibly homogenized the living conditions. As a consequence environmental variation might be diminished.

The increased heritability might also be interpreted as a changed penetrance of the diabetes susceptibility genes. Some of the earlier low risk genes may have become more penetrant under the changing environmental conditions. It is possible that the

environment that has become more conducive to the diabetes development is reflected in the distribution of HLA genotypes among patients diagnosed during different time periods. The topic on temporal changes in HLA genotype frequencies in patients with T1D has not been studied much. The proportion of patients carrying high-risk HLA-genotypes has been reported to decrease while the frequency of low or moderate risk HLA-genotypes has increased over the last 50 years in Finland (229; 230). Similar findings have been reported in a recent study from the UK (231). Since T1D was, however, associated with high mortality in the earlier decades, the distribution of HLA haplotypes, that represent those who have survived, may differ from those of deceased patients. Thus those kinds of studies are subject to bias and interpretation should be made with caution.

## 6.3 Dynamic temporal changes in the recurrence risk of T1D in the first-degree relatives

When a child becomes affected with T1D, a common concern for the parents is, what is the risk in their other children to get the same disease, or if the parent has T1D him/herself what is the risk to the offspring. One can wonder if a customary answer of a 5-6% risk is a proper answer anymore. This study clearly showed that the lifetime risk in the first-degree relatives is dynamic and the cumulative risk of T1D is considerable higher in the more recent birth cohort than it was in those who were born some decades ago.

The incidence of T1D has increased during the last decades quickly and the increase seems to be accelerated in Finland. The increase has been about 2.4% per year, a turning point to an accelerated increase appears to have taken place in the beginning of the 1990s. Such a rapid increase highlights the importance of interaction of environmental factors with genetic factors, obviously affecting the penetrance of susceptibility genes. This study showed that the recurrence risk for T1D in siblings and offspring is dynamic and changes over time as does the incidence in the background population. Instead, the recurrence risk ratio seems to remain constant. The SIRs for siblings were about 12 and for offspring it was 10 and varied only slightly during the observation period. The cumulative incidence, however, was higher in the more recent birth cohorts of siblings and offspring. Siblings born during 1980 or later had a strikingly higher cumulative risk compared with the earlier birth cohorts. A cumulative risk of 4% in the offspring born in the year 1995

or later was reached already in 7 years, whereas such a high cumulative risk was reached in 17.5 years in the oldest birth cohort, offspring born in 1985 or earlier.

Very little information is available on birth cohort effects on the recurrence risk of T1D in first-degree relatives in different populations. Thus it is not possible to compare the results between studies. The previous Finnish study on recurrence risk in the offspring also reported a significant birth cohort effect. Offspring born in the year 1976 or after had a higher cumulative risk than those born before the year 1976 (151). If the SIR between the first-degree relatives and the background population is constant, irrespective of the population, and the incidence is varying then differences in the lifetime risk among the first-degree relatives should be seen across populations. Positive association between the prevalence of familial T1D and the population incidence of T1D was observed in the European ACE study that examined the hypothesis that the risk of T1D among the first-degree relatives varies across populations mirroring the pattern of disease incidence (232). The present study agrees with this pattern and showed that within one population that the recurrence risks in the siblings and offspring of patients with T1D changes in a similar manner as the changes in the incidence of the background population.

Regardless of high recurrence risk in the first-degree relatives, the vast majority of them do not develop diabetes during their lifetime. A much lower concordance rate in MZ twins than 100% proves that individuals with an apparent genetic susceptibility for T1D rarely develop the clinical disease. Only 10% of individuals bearing the highest-risk MHC genotype, the heterozygous DQB1*0302-DRB1*0401/ DQB1*0201-DRB1*03 genotype, develop T1D (233). Despite harmful effects of T1D-associated alleles they are common in many populations. For example, high-risk HLA-DR4 allele is quite abundant in Finnish population (234). Penetrance of the T1D susceptibility genes is low, typical for most diseases with polygenic inheritance.

Contrary to the environmental factors as a trigger of T1D, it is also possible that the exposure to some environmental factors prevents an individual from developing T1D. In such cases T1D may develop in a person who lacks the necessary protective exposure that non-affected individuals experience (235; 236). This pattern, known as the hygiene hypothesis, proposes that the incidence of T1D has risen due to a reduced stimulation of the immune system by early infections (237). It is assumed that something protective has been lost from the childhood environment over recent decades. People that are living in a cleaner environment have decreasing

chances of natural infections and their developing immune systems are not exposed to the necessary stimulation.

The association of enteroviruses with T1D has received much attention (61; 64; 238). Their role in the risk of T1D has been found to be paradoxical. A recent study suggested that the frequency of enterovirus infections has decreased during the past 30 years in the Finnish population in parallel with the doubled incidence of T1D (64). Notably, a substantial decrease occurred in the 1980s and the 1990s at the time when the incidence of T1D in Finland increased most rapidly. An interesting analogy has been found between the epidemiology of poliomyelitis and T1D, named as the polio hypothesis (64). The epidemiological pattern where the risk of poliovirus induced motor-neuron damage increased when the frequency of polio infections started to decrease rapidly in the population could be applicable to the observed pattern of decrease of enterovirus infections and the increase in the incidence of T1D in the Finnish population. The explanation for this phenomenon could be that the child's first infections are delayed and occur later in childhood when the protective maternal antibodies have disappeared and thus the exposure to enteroviruses has severe consequences, similar to poliomyelitis. The role of infectious diseases in general, however, in the etiology of T1D is far from clear.

## 6.4 Effect of age at onset of diabetes in the proband

The twin study, as well as the studies on recurrence risk of T1D in the sibling and the offspring, all confirmed a remarkable impact of the young age at onset of diabetes in the probands on the increased risk of T1D in their first-degree relatives. The only exception was the absence of this pattern in the offspring of mothers with T1D.

A correlation between the ages at onset of diabetes among affected siblings was lower (r=0.30) than had been suggested earlier (141; 175; 232). Among the concordant DZ twins it was of the same magnitude as in the siblings (r=0.38). The lower correlation coefficient could be a subsequent consequence of the much longer follow-up time in the current study than in the other studies. A sufficiently long follow-up time enables a larger disease-free interval among the siblings and makes it possible to get a more precise estimate of the correlation between the ages at onset between the siblings. Contrary to DZ twins and sibling pairs, MZ twins tended to develop diabetes at a similar age in this study (r=0.95). This result is in full agreement with the observation by Fava et al. (r=0.96) (175).

Based on this and other studies it seems that if a MZ twin pair does not become concordant within a relatively short time after the diagnosis of the first affected twin then they do not become at all (127-129). In this study, all concordant MZ pairs were diagnosed within 7 years and the longest discordance time for the MZ twin pairs was 30 years without diabetes in the co-twin. Some observations exist, however, that MZ twin can still be affected after a long discordance period, even after 36 years (131).

The low correlation between the onset ages in the affected siblings has been interpreted to indicate that non-genetic factors play an important role in determining the age at diagnosis of T1D. Fava et al. concluded, however, that the age at onset of diabetes is highly genetically determined (175). A greater than a two-fold difference in the correlation between DZ and MZ twins in the current study supports the importance of genetic effects and even suggests a possible non-additive genetic effect in determining the age at onset of T1D.

## 6.5 Sex differences

A sex bias in the autoimmune diseases is well-known (239). Unlike in the other autoimmune diseases there seems to be a male predominance in T1D (42; 46). The incidence of T1D shows a minor sex bias up to the puberty, but more males are diagnosed after that (43; 45). The reason for this is unknown. This study showed a striking divergence in the sex ratio in adulthood in concordance with earlier observations. Male excess was present in the siblings with T1D after the age of 14. In the offspring of patients with T1D the risk ratio was equal until the age of 14, but the cumulative risk of males starts to diverge from that of females after that. In both the siblings and the offspring the cut-off point seems to be 14 years, after which the sex difference becomes apparent. The reasons for the male sex bias from the puberty is unclear, but it has been suggested that hormonal changes during puberty might have an influence the immune system and thus also an effect on the risk of developing T1D (42).

## 6.6 Differential transmission of T1D from fathers vs. mothers

This study confirmed the sex difference in the parental-offspring transmission rate of T1D. It was also the first to confirm this sex difference while avoiding the sources

of biases. The most important strength of the study was the optimal design: ascertainment was done through the parents with T1D and the case ascertainment was virtually complete. In the past women with T1D were strongly recommended not to have children. Improvements in diabetes care, however, that have occurred in the recent decades have encouraged women with T1D to plan their pregnancies. In this cohort of patients with T1D, in fact more female than male probands had children. The average number of the offspring in the female probands with progeny was, however, slightly smaller than that of the male probands. The reduction of the fecundity in the females with T1D was only 5.6% compared with males. Taking into account this minor difference in the fecundity the expected ratio of the incidence of T1D in the offspring of the fathers and the mothers with T1D was minimal whereas the observed ratio was considerable higher. This study clearly showed that the observed sex-related preferential transmission of T1D is a reality and there must be other reasons than biases behind it. The excess risk in the offspring of male fathers with T1D manifested itself through a higher the risk the younger the father was when diagnosed with T1D. The risk of T1D in the offspring of the fathers with T1D followed the pattern of progressively increased risk the younger the proband was when diagnosed with T1D. Unexpectedly, this pattern was totally absent in the offspring of the mothers with T1D. Offspring of the mothers with T1D who were diagnosed at the age of four or under had in fact the lowest risk, although no statistically significant differences appeared in the risk according the age at onset of diabetes in the mothers.

The effect of the parental age at onset of T1D on the recurrence risk of T1D in offspring has been reported, but only few studies have analysed the data stratifying the offspring data by the sex of the T1D parents. Besides, none of them have used a cut-off point for the parental age at onset that is comparable with the current study. Two earlier studies provided the first observations that young maternal age at onset of diabetes might have a minor impact on the recurrence risk of T1D in their offspring (150; 152). The study from the Joslin Diabetes Clinic reported the highest risk of T1D in the offspring of both fathers and mothers who developed diabetes before age 11. The risk difference between before and after the age of 11 was, however, lower in the offspring of mothers with T1D than that of fathers with T1D (150). Another study, however, also conducted in the Joslin Diabetes Clinic reported contrary results: the mothers who developed diabetes before age 8 years transmitted diabetes at the same rate as fathers with T1D (149). The most consistent with the results of the current study were provided by a Danish study. Lorenzen et al. reported an over two-fold increased risk of T1D in the offspring of fathers who were

diagnosed before the age of 17 years compared with that of older ages, but no such relation was found in the maternal offspring (152).

The observed sex difference in the T1D transmission can be partly explained by a decreased transmission rate in mothers who have been diagnosed with diabetes at a very young age, but the mechanism that might be responsible for this remains unclear. Findings of this study suggest that genetic susceptibility to T1D might be modified by certain aspects of diabetic pregnancies. Fetal exposure to islet autoantibodies in children born to the mothers with T1D has been suggested to have a protective effect against future islet autoimmunity and diabetes (148; 160). Warram et al. suggested that if the mechanism requires exposure to a diabetic pregnancy, the difference in the transmission of T1D would not be seen in the offspring that were born before the onset of diabetes in the mother (148). A Danish study observed a decreased risk of T1D in the offspring of female probands compared to the risk in the offspring of male probands, but only if the parents had been diagnosed with T1D before the birth of their offspring (152). The effect might be genetic, however, a consequence of heterogeneity of the genetic background in the early-onset and late-onset T1D

It has been shown that perinatal mortality is not responsible for the decreased risk in the offspring of T1D mothers (148). Meanwhile, the outcome of diabetic pregnancies in their very early stage could be a matter of speculation. Given the fact, that the rate of miscarriages among women with diabetes is much higher than that among the general population, the possibility of selective loss of fetuses that bear a high-risk HLA haplotypes associated with young onset diabetes cannot be excluded.

Other explanations that have been put forward are a possible distortion of transmission from the expected segregation pattern of the diabetic haplotypes and genomic imprinting. Fathers have been described to transmit a certain diabetic HLA alleles or haplotypes more often to their offspring than mothers (162; 240). Some evidence of an increased transmission of a Finnish high-risk haplotype [A2, Cw1, B56, DR4, DQ8] was found (241), but has not been confirmed (242). The evidence concerning the existence of non-Mendelian inheritance of HLA alleles is conflicting (163; 243).

## 6.7 Familial aggregation of diabetic nephropathy

The strength of this study on familial clustering of DN was that this was population-based, free of family ascertainment bias and it was longitudinal. The study population included all the multiplex T1D families during the defined time period without prior knowledge of the DN status of the patients with T1D. Most of the attempts to determine the sibling recurrence risk of DN have been based on small, clinical-based series. This study confirmed the earlier suggestion of the existence of strong familial aggregation of DN. The first observations on familial clustering of DN was made by Seaquist et al. (201). The magnitude of familial clustering was, however, presumably overestimated due to the small sample size, selection bias toward sibling pairs that were concordant for DN and due to restriction on the severest form of DN. The magnitude of the risk ratio for DN in the siblings of patients with and without DN in this study, 2.3, was closer to the estimates of Borch-Johnsen et al. (203) and Quinn et al. (202) that were 2.5 and 4.9, respectively.

Familial aggregation has been shown in almost all human diseases. The familial aggregation is generally taken as evidence for the existence of a genetic background for a disease. It is also possible that clustering of strong environmental risk factors for a disease among family members can lead to the excess risk of a disease. Khoury et al., however, argued that without any genetic susceptibility, familial clustering of high environmental risk factors is unlikely to fully account for aggregation of a disease among the siblings and that genetic factors may indeed play a major role in causing familial clustering (244). This issue was later reviewed by Guo (245). He agrees with the main conclusion that remarkable familiality is basically genetic, but pointed out the importance to avoid ascertainment bias. Thus it seems more plausible that the greater environmental similarity in the siblings enhances the familial aggregation, but that it is not the main source.

No data on environmental risk factors, such as smoking or dietary habits, shared by siblings were collected in this study. Neither were the data on glycemic control available. It is also sometimes difficult to say if environmental factors are in real terms environmental. Such a factor may be glycemic control that plays an essential role in the development of microvascular complications. A twin study by Snieder et al. revealed that there is a remarkable genetic component in the variation of HbA1c levels (206). There might be a potential genetic link between glycemic control and DN, and furthermore between DN and other features that are closely related in DN such as hypertension, T2D, cardiovascular diseases, and insulin resistance.

This study showed for the first time that the risk in the sibling was dependent on the severity of DN in the proband suggesting a probable genetic heterogeneity in the DN predisposition. The risk of DN was two times higher in the siblings of the probands with DN (ESRD excluded) and three times higher in the siblings of the probands with ESRD compared with the siblings of normo- or microalbuminuric probands.

One of the aims of this study was to define if the pattern of progression to DN is different in those who were diagnosed with diabetes very young or if the pattern is similar in all cases and rather being related to the duration of diabetes. It has been speculated that the pubertal years with diabetes may have a greater impact on the risk of microvascular complications and premature death than do the years before puberty (194; 246). Few studies have distinguished the early childhood years from the years during the transition from prepuberty to puberty or they have focused on early signs of microvascular complications.

The risk of DN was the lowest in the patients whose diabetes was diagnosed at the age of four or under and in those with diabetes onset after puberty whereas the risk for siblings whose age of onset of diabetes was between 5 and 9 years was similar to the peripubertal risk. This observation is in accordance with two recently published population-based studies, one from Finland and another from Sweden. Both countries reported a reduced risk of ESRD in those who had an onset of diabetes at four years or under (199; 200). In addition, there are some observations that an onset of diabetes in the youngest age group, i.e. diagnosis of diabetes before the age of 5 years, significantly prolonged the time to early retinopathy and microalbuminuria and those patients had also less hospitalizations due to DN compared with the age groups 5–14 (197; 217; 247; 248).

The mechanism behind this effect of age at onset is unclear. It has been speculated that rapid growth and hormonal changes, and worsening glycemic control in the puberty may have an impact on the development of complications (249; 250). Children who were younger than 5 years at the onset of diabetes, however, experience the same hormonal changes during puberty as those diagnosed with diabetes later in childhood. One explanation might be that children at an early age have got used to better self-management than those diagnosed later. There is, however, no evidence that children with an early-onset diabetes can maintain better glycemic control during the pubertal years than those diagnosed during or shortly before puberty (248; 251). Hormonal changes start their influence years before visible pubertal changes (252; 253). Those changes that occur simultaneously with the onset of diabetes might have an impact on the risk of DN later in life.

# 7 SUMMARY AND CONCLUSIONS

The present study has provided new knowledge on the T1D recurrence risk in the first-degree relatives and the risk factors modifying the risk.

Study I showed that the vast majority of affected MZ twin pairs remained discordant. The model with additive genetic and individual environmental effects was the best-fitting liability model, with 88% of phenotypic variance due to additive genetic factors. These nationwide twin data demonstrated high genetic liability for T1D. The concordance rates of MZ twins were substantially higher in the Finnish Young Twin Cohort (probandwise 43%, pairwise 27%) than in the Old Twin Cohort (probandwise 23%, pairwise 13%) and actually doubled in the successive birth cohorts. Increased heritability over time reveals the possibility of increased penetrance of T1D genes. This study demonstrated that the penetrance of the susceptibility genes for T1D may be low, although strongly influenced by environmental factors. Correlation with age at onset among the concordant MZ pairs was very high, r=0.95, denoting short discordance time between concordant MZ twin pairs.

In Study II, the cumulative incidence of T1D in the siblings of T1D patients by ages 20, 30, 40, and 50 years was 4.1, 5.6, 6.5, and 6.9%, respectively. The age at diagnosis in the proband considerably influenced the risk of T1D in the siblings; if the proband was diagnosed at the age of 0-4, 5-9, 10-14, and 15 or more, the cumulative incidence of T1D in the siblings by the age of 40 was 13.2%, 7.8%, 4.7%, and 3.4%, respectively. The brothers had a progressively higher risk of T1D after the age of 14 compared to the sisters. Both increasing maternal and paternal age at delivery were significant risk factors for T1D in the siblings. Cumulative incidence of T1D in the siblings increased with increasing birth year mirroring the incidence of T1D in the background population. Siblings born in the year 1980 or after had 15-year cumulative risk of 11.4%, whereas siblings born 1970-79 and 1970 or before had 5.5% and 2.7%, respectively. SIRs among siblings aged 14 years or under, however, was approximately 12 throughout the follow-up.

Study III revealed that although the majority of sibling pairs with T1D were discordant for DN, its presence in one sibling doubles and presence of ESRD triples the risk of DN in the siblings with T1D. Truly population-based studies on the sibling DN recurrence risk did not exist before the current study. The familial aggregation of DN emphasizes the involvement of a genetic component in the

development of DN, but shared environmental risk factors cannot be completely excluded in the familial clustering of DN.

Since the incidence of T1D is increasing a greater number of patients is supposed to suffer from diabetic complications in the future. Because the age at onset of diabetes has decreased, the mean duration of diabetes will increase alike. An encouraging observation is that children diagnosed at the age of 4 or earlier seem to have a decreased risk of DN or they have at least a delayed onset of DN and may develop DN rather at the same age than after the same duration of diabetes than those with diabetes diagnosed at a later stage.

Study IV provided epidemiological evidence of sex-related preferential transmission of T1D. Offspring of fathers with T1D had 1.7 times higher risk to be affected than offspring of mothers with T1D. It was mediated by the different effect of the age at onset of diabetes in the fathers and mothers. A young age at onset of diabetes in the fathers with T1D increased the risk of T1D in the offspring, but such a pattern was not seen in the offspring of T1D mothers. This result reinforces the possibility that genetic susceptibility to T1D might be modified somehow in diabetic pregnancies. This study also indicated that the incidence in the offspring of T1D patients was 10 times higher than that in the general population.

This study revealed dynamic temporal changes in the recurrence risk of T1D in the first-degree relatives. The increase of the incidence in the first-degree relatives seemed to follow that of the background population: SIRs between the siblings of patients with T1D and the background population was fairly constant throughout the follow-up as well as between the offspring and the background population.

This study provides new knowledge on etiology of T1D and DN. The final goal in the research objectives is to achieve the prevention or cure T1D and its complications and it is possible only with good understanding of the etiology as well as the natural progression of the diseases.

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# 9 REFERENCES


1. Khoury M, Beaty T, Cohen B: *Fundamentals of genetic epidemilogy*. New York, Oxford University Press, 1993

2. Burton PR, Tobin MD: *Epidemiology and Genetic Epidemiology*, John Wiley & Sons Ltd., 2003

3. Zhao LP, Hsu L, Davidov O, Potter J, Elston RC, Prentice RL: Population-based family study designs: an interdisciplinary research framework for genetic epidemiology. *Genet Epidemiol* 14:365-388, 1997

4. Risch N: Linkage strategies for genetically complex traits. I. Multilocus models. *Am J Hum Genet* 46:222-228, 1990

5. Hartl DL, Clark AG: *Principles of Population Genetics*, Pennsylvania State University, 1997

6. Weiss K: *Genetic variation and human diseases: principles and evolutionary approaches*. Cambridge, The Press Syndicate of the University of Cambridge, 1993

7. Galton F: The history of twins, as a criterion of the relative powers of nature and nurture. *J Anthropol Inst.* 12:566-576, 1875

8. Boomsma D, Busjahn A, Peltonen L: Classical twin studies and beyond. *Nat Rev Genet* 3:872-882, 2002

9. Guo SW: Does higher concordance in monozygotic twins than in dizygotic twins suggest a genetic component? *Hum Hered* 51:121-132, 2001

10. Smith C: Concordance in twins: methods and interpretation. *Am J Hum Genet* 26:454-466, 1974

11. Witte JS, Carlin JB, Hopper JL: Likelihood-based approach to estimating twin concordance for dichotomous traits. *Genet Epidemiol* 16:290-304, 1999

12. Falconer D, MacKay T: *Introduction to quantitative genetics*. New York, Addison-Wesley, 1995

13. Neale M, Cardon L: *Methodology for Genetic Studies of Twins and Families*. Dordrecht, Kluwer Academic, 1992

14. Rewers M, LaPorte RE, King H, Tuomilehto J: Trends in the prevalence and incidence of diabetes: insulin-dependent diabetes mellitus in childhood. *World Health Stat Q* 41:179-189, 1988

15. Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 15:539-553, 1998

16. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183-1197, 1997

17. Lipton R, LaPorte RE: Epidemiology of islet cell antibodies. *Epidemiol Rev* 11:182-203, 1989

18. Singal DP, Blajchman MA: Histocompatibility (HL-A) antigens, lymphocytotoxic antibodies and tissue antibodies in patients with diabetes mellitus. *Diabetes* 22:429-432, 1973

19. Nerup J, Platz P, Andersen OO, Christy M, Lyngsoe J, Poulsen JE, Ryder LP, Nielsen LS, Thomsen M, Svejgaard A: HL-A antigens and diabetes mellitus. *Lancet* 2:864-866, 1974

20. Cudworth AG, Woodrow JC: Evidence for HL-A-linked genes in "juvenile" diabetes mellitus. *Br Med J* 3:133-135, 1975

21. Klein J, Sato A: The HLA system. Second of two parts. *N Engl J Med* 343:782-786, 2000

22. Klein J, Sato A: The HLA system. First of two parts. *N Engl J Med* 343:702-709, 2000

23. Pugliese A, Eisenbarth GS: Type I Diabetes Mellitus of Man: Genetic Susceptibility and Resistance. In *Type 1 Diabetes: Molecular, Cellular, and Clinical Immunology*, Kluwer Academic/Plenum Publishers, 2006

24. Pociot F, McDermott MF: Genetics of type 1 diabetes mellitus. *Genes Immun* 3:235-249, 2002

25. Cavender D, Orchard TJ, Wagener D, LaPorte RE, Rabin B, Eberhardt M: The development of type 1 diabetes in HLA identical siblings of type 1 diabetic patients: associations with specific HLA antigens]. *Diabetologia* 23:379, 1982

26. Tarn AC, Thomas JM, Dean BM, Ingram D, Schwarz G, Bottazzo GF, Gale EA: Predicting insulin-dependent diabetes. *Lancet* 1:845-850, 1988

27. Concannon P, Gogolin-Ewens KJ, Hinds DA, Wapelhorst B, Morrison VA, Stirling B, Mitra M, Farmer J, Williams SR, Cox NJ, Bell GI, Risch N, Spielman RS: A second-generation screen of the human genome for susceptibility to insulin-dependent diabetes mellitus. *Nat Genet* 19:292-296, 1998

28. Mein CA, Esposito L, Dunn MG, Johnson GC, Timms AE, Goy JV, Smith AN, Sebag-Montefiore L, Merriman ME, Wilson AJ, Pritchard LE, Cucca F, Barnett AH, Bain SC, Todd JA: A search for type 1 diabetes susceptibility genes in families from the United Kingdom. *Nat Genet* 19:297-300, 1998

29. Nerup J, Pociot F: A genomewide scan for type 1-diabetes susceptibility in Scandinavian families: identification of new loci with evidence of interactions. *Am J Hum Genet* 69:1301-1313, 2001

30. Cox NJ, Wapelhorst B, Morrison VA, Johnson L, Pinchuk L, Spielman RS, Todd JA, Concannon P: Seven regions of the genome show evidence of linkage to type 1 diabetes in a consensus analysis of 767 multiplex families. *Am J Hum Genet* 69:820-830, 2001

31. Concannon P, Erlich HA, Julier C, Morahan G, Nerup J, Pociot F, Todd JA, Rich SS: Type 1 diabetes: evidence for susceptibility loci from four genome-wide linkage scans in 1,435 multiplex families. *Diabetes* 54:2995-3001, 2005

32. Redondo MJ, Fain PR, Eisenbarth GS: Genetics of type 1A diabetes. *Recent Prog Horm Res* 56:69-89, 2001

33. Hirschhorn JN: Genetic epidemiology of type 1 diabetes. *Pediatr Diabetes* 4:87-100, 2003

34. Diabetes Epidemiology Research International Group: Geographic patterns of childhood insulin-dependent diabetes mellitus. *Diabetes* 37:1113-1119, 1988

35. Karvonen M, Tuomilehto J, Libman I, LaPorte R: A review of the recent epidemiological data on the worldwide incidence of type 1 (insulin-dependent) diabetes mellitus. World Health Organization DIAMOND Project Group. *Diabetologia* 36:883-892, 1993

36. Karvonen M, Viik-Kajander M, Moltchanova E, Libman I, LaPorte R, Tuomilehto J: Incidence of childhood type 1 diabetes worldwide. Diabetes Mondiale (DiaMond) Project Group. *Diabetes Care* 23:1516-1526, 2000

37. Ronningen KS, Keiding N, Green A: Correlations between the incidence of childhood-onset type I diabetes in Europe and HLA genotypes. *Diabetologia* 44 Suppl 3:B51-59, 2001

38. Tuomilehto J, Podar T, Brigis G, Urbonaite B, Rewers M, Adojaan B, Cepaitis Z, Kalits I, King H, LaPorte R, et al.: Comparison of the incidence of insulin-dependent diabetes mellitus in childhood among five Baltic populations during 1983-1988. *Int J Epidemiol* 21:518-527, 1992

39. Podar T, Solntsev A, Karvonen M, Padaiga Z, Brigis G, Urbonaite B, Viik-Kajander M, Reunanen A, Tuomilehto J: Increasing incidence of childhood-onset type I diabetes in 3 Baltic countries and Finland 1983-1998. *Diabetologia* 44 Suppl 3:B17-20, 2001

40. Kondrashova A, Reunanen A, Romanov A, Karvonen A, Viskari H, Vesikari T, Ilonen J, Knip M, Hyöty H: A six-fold gradient in the incidence of type 1 diabetes at the eastern border of Finland. *Ann Med* 37:67-72, 2005

41. Carle F, Gesuita R, Bruno G, Coppa GV, Falorni A, Lorini R, Martinucci ME, Pozzilli P, Prisco F, Songini M, Tenconi MT, Cherubini V: Diabetes incidence in 0- to 14-year age-group in Italy: a 10-year prospective study. *Diabetes Care* 27:2790-2796, 2004

42. Gale EA, Gillespie KM: Diabetes and gender. *Diabetologia* 44:3-15, 2001

43. Nyström L, Dahlquist G, Ostman J, Wall S, Arnqvist H, Blohme G, Lithner F, Littorin B, Schersten B, Wibell L: Risk of developing insulin-dependent diabetes mellitus (IDDM) before 35 years of age: indications of climatological determinants for age at onset. *Int J Epidemiol* 21:352-358, 1992

44. Vandewalle CL, Coeckelberghs MI, De Leeuw IH, Du Caju MV, Schuit FC, Pipeleers DG, Gorus FK: Epidemiology, clinical aspects, and biology of IDDM patients under age 40 years. Comparison of data from Antwerp with complete ascertainment with data from Belgium with 40% ascertainment. The Belgian Diabetes Registry. *Diabetes Care* 20:1556-1561, 1997

45. Gavard JA, Dorman JS, LaPorte RE, Orchard TJ, Drash AL, Trucco MM, Kelsey SF, Kostraba JN, Becker DJ: Sex differences in secondary attack rate of IDDM to siblings of probands through older ages. Pittsburgh Etiology of IDDM Study. *Diabetes Care* 15:559-561, 1992

46. Karvonen M, Pitkäniemi M, Pitkäniemi J, Kohtamäki K, Tajima N, Tuomilehto J: Sex difference in the incidence of insulin-dependent diabetes mellitus: an analysis of the recent epidemiological data. World Health Organization DIAMOND Project Group. *Diabetes Metab Rev* 13:275-291, 1997

47. Karvonen M, Pitkäniemi J, Tuomilehto J: The onset age of type 1 diabetes in Finnish children has become younger. The Finnish Childhood Diabetes Registry Group. *Diabetes Care* 22:1066-1070, 1999

48. Gardner SG, Bingley PJ, Sawtell PA, Weeks S, Gale EA: Rising incidence of insulin dependent diabetes in children aged under 5 years in the Oxford region: time trend analysis. The Bart's-Oxford Study Group. *Bmj* 315:713-717, 1997

49. Green A, Patterson CC: Trends in the incidence of childhood-onset diabetes in Europe 1989-1998. *Diabetologia* 44 Suppl 3:B3-8, 2001

50. Feltbower RG, McKinney PA, Parslow RC, Stephenson CR, Bodansky HJ: Type 1 diabetes in Yorkshire, UK: time trends in 0-14 and 15-29-year-olds, age at onset and age-period-cohort modelling. *Diabet Med* 20:437-441, 2003

51. Charkaluk ML, Czernichow P, Levy-Marchal C: Incidence data of childhood-onset type I diabetes in France during 1988-1997: the case for a shift toward younger age at onset. *Pediatr Res* 52:859-862, 2002

52. Horton V, Stratton I, Bottazzo GF, Shattock M, Mackay I, Zimmet P, Manley S, Holman R, Turner R: Genetic heterogeneity of autoimmune diabetes: age of presentation in adults is influenced by HLA DRB1 and DQB1 genotypes (UKPDS 43). UK Prospective Diabetes Study (UKPDS) Group. *Diabetologia* 42:608-616, 1999

53. Gillespie KM, Gale EA, Bingley PJ: High familial risk and genetic susceptibility in early onset childhood diabetes. *Diabetes* 51:210-214, 2002

54. Tuomilehto-Wolf E, Tuomilehto J: Is the high incidence of diabetes in young children diagnosed under the age of 4 years determined by genetic factors in Finland? The DIME Study Group. *Diabete Metab* 19:167-172, 1993

55. Caillat-Zucman S, Garchon HJ, Timsit J, Assan R, Boitard C, Djilali-Saiah I, Bougneres P, Bach JF: Age-dependent HLA genetic heterogeneity of type 1 insulin-dependent diabetes mellitus. *J Clin Invest* 90:2242-2250, 1992

56. Seissler J, de Sonnaville JJ, Morgenthaler NG, Steinbrenner H, Glawe D, Khoo-Morgenthaler UY, Lan MS, Notkins AL, Heine RJ, Scherbaum WA: Immunological heterogeneity in type I diabetes: presence of distinct autoantibody patterns in patients with acute onset and slowly progressive disease. *Diabetologia* 41:891-897, 1998

57. Karvonen M, Jäntti V, Muntoni S, Stabilini M, Stabilini L, Tuomilehto J: Comparison of the seasonal pattern in the clinical onset of IDDM in Finland and Sardinia. *Diabetes Care* 21:1101-1109, 1998

58. Padaiga Z, Tuomilehto J, Karvonen M, Dahlquist G, Podar T, Adojaan B, Urbonaite B, Zalinkevicius R, Brigis G, Virtala E, Kohtamäki K, Cepaitis Z, Tuomilehto-Wolf E: Seasonal variation in the incidence of Type 1 diabetes mellitus during 1983 to 1992 in the countries around the Baltic Sea. *Diabet Med* 16:736-743, 1999

59. Laron Z: Incidence and seasonality of type 1 diabetes mellitus--what now? *J Pediatr Endocrinol Metab* 15:573-575, 2002

60. Durruty P, Ruiz F, Garcia de los Rios M: Age at diagnosis and seasonal variation in the onset of insulin-dependent diabetes in Chile (Southern hemisphere). *Diabetologia* 17:357-360, 1979

61. Hyöty H: Enterovirus infections and type 1 diabetes. *Ann Med* 34:138-147, 2002

62. Haverkos HW, Battula N, Drotman DP, Rennert OM: Enteroviruses and type 1 diabetes mellitus. *Biomed Pharmacother* 57:379-385, 2003

63. Kimpimäki T, Kupila A, Hämäläinen AM, Kukko M, Kulmala P, Savola K, Simell T, Keskinen P, Ilonen J, Simell O, Knip M: The first signs of beta-cell autoimmunity appear in infancy in genetically susceptible children from the general population: the Finnish Type 1 Diabetes Prediction and Prevention Study. *J Clin Endocrinol Metab* 86:4782-4788, 2001

64. Viskari HR, Koskela P, Lönnrot M, Luonuansuu S, Reunanen A, Baer M, Hyöty H: Can enterovirus infections explain the increasing incidence of type 1 diabetes? *Diabetes Care* 23:414-416, 2000

65. Jun HS, Yoon JW: A new look at viruses in type 1 diabetes. *Diabetes Metab Res Rev* 19:8-31, 2003

66. Hyöty H, Taylor KW: The role of viruses in human diabetes. *Diabetologia* 45:1353-1361, 2002

67. Gamble DR: Relation of antecedent illness to development of diabetes in children. *Br Med J* 281:99-101, 1980

68. Knip M, Veijola R, Virtanen SM, Hyöty H, Vaarala O, Åkerblom HK: Environmental triggers and determinants of type 1 diabetes. *Diabetes* 54 Suppl 2:S125-136, 2005

69. Schattner A: Consequence or coincidence? The occurrence, pathogenesis and significance of autoimmune manifestations after viral vaccines. *Vaccine* 23:3876-3886, 2005

70. Hviid A, Stellfeld M, Wohlfahrt J, Melbye M: Childhood vaccination and type 1 diabetes. *N Engl J Med* 350:1398-1404, 2004

71. Classen JB, Classen DC: Clustering of cases of type 1 diabetes mellitus occurring 2-4 years after vaccination is consistent with clustering after infections and progression to type 1 diabetes mellitus in autoantibody positive individuals. *J Pediatr Endocrinol Metab* 16:495-508, 2003

72. Karvonen M, Cepaitis Z, Tuomilehto J: Association between type 1 diabetes and Haemophilus influenzae type b vaccination: birth cohort study. *Bmj* 318:1169-1172, 1999

73. Haglund B, Ryckenberg K, Selinus O, Dahlquist G: Evidence of a relationship between childhood-onset type I diabetes and low groundwater concentration of zinc. *Diabetes Care* 19:873-875, 1996

74. Virtanen SM, Jaakkola L, Räsänen L, Ylönen K, Aro A, Lounamaa R, Åkerblom HK, Tuomilehto J: Nitrate and nitrite intake and the risk for type 1 diabetes in Finnish children. Childhood Diabetes in Finland Study Group. *Diabet Med* 11:656-662, 1994

75. Virtanen SM, Knip M: Nutritional risk predictors of beta cell autoimmunity and type 1 diabetes at a young age. *Am J Clin Nutr* 78:1053-1067, 2003

76. Borch-Johnsen K, Joner G, Mandrup-Poulsen T, Christy M, Zachau-Christiansen B, Kastrup K, Nerup J: Relation between breast-feeding and incidence rates of insulin-dependent diabetes mellitus. A hypothesis. *Lancet* 2:1083-1086, 1984

77. Atkinson MA, Ellis TM: Infants diets and insulin-dependent diabetes: evaluating the "cows' milk hypothesis" and a role for anti-bovine serum albumin immunity. *J Am Coll Nutr* 16:334-340, 1997

78. Couper JJ, Steele C, Beresford S, Powell T, McCaul K, Pollard A, Gellert S, Tait B, Harrison LC, Colman PG: Lack of association between duration of breast-feeding or introduction of cow's milk and development of islet autoimmunity. *Diabetes* 48:2145-2149, 1999

79. The EURODIAB Substudy 2 Study Group: Vitamin D supplement in early childhood and risk for Type I (insulin-dependent) diabetes mellitus. *Diabetologia* 42:51-54, 1999

80. Hyppönen E, Läära E, Reunanen A, Järvelin MR, Virtanen SM: Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet* 358:1500-1503, 2001

81. Stene LC, Ulriksen J, Magnus P, Joner G: Use of cod liver oil during pregnancy associated with lower risk of Type I diabetes in the offspring. *Diabetologia* 43:1093-1098, 2000

82. Stene LC, Joner G: Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset type 1 diabetes: a large, population-based, case-control study. *Am J Clin Nutr* 78:1128-1134, 2003

83. Longnecker MP, Daniels JL: Environmental contaminants as etiologic factors for diabetes. *Environ Health Perspect* 109 Suppl 6:871-876, 2001

84. Kostraba JN, Gay EC, Rewers M, Hamman RF: Nitrate levels in community drinking waters and risk of IDDM. An ecological analysis. *Diabetes Care* 15:1505-1508, 1992

85. Moltchanova E, Rytkönen M, Kousa A, Taskinen O, Tuomilehto J, Karvonen M: Zinc and nitrate in the ground water and the incidence of Type 1 diabetes in Finland. *Diabet Med* 21:256-261, 2004

86. Parslow RC, McKinney PA, Law GR, Staines A, Williams R, Bodansky HJ: Incidence of childhood diabetes mellitus in Yorkshire, northern England, is associated with nitrate in drinking water: an ecological analysis. *Diabetologia* 40:550-556, 1997

87. Van Maanen JM, Albering HJ, Van Breda SG, Curfs DM, Ambergen AW, Wolffenbuttel BH, Kleinjans JC, Reeser HM: Nitrate in drinking water and risk of childhood diabetes in The Netherlands. *Diabetes Care* 22:1750, 1999

88. Myers MA, Hettiarachchi KD, Ludeman JP, Wilson AJ, Wilson CR, Zimmet PZ: Dietary microbial toxins and type 1 diabetes. *Ann N Y Acad Sci* 1005:418-422, 2003

89. Kilkkinen A, Virtanen SM, Klaukka T, Kenward MG, Salkinoja-Salonen M, Gissler M, Kaila M, Reunanen A: Use of antimicrobials and risk of type 1 diabetes in a population-based mother-child cohort. *Diabetologia* 49:66-70, 2006

90. Bingley PJ, Douek IF, Rogers CA, Gale EA: Influence of maternal age at delivery and birth order on risk of type 1 diabetes in childhood: prospective population based family study. Bart's-Oxford Family Study Group. *Bmj* 321:420-424, 2000

91. Stene LC, Magnus P, Lie RT, Sovik O, Joner G: Maternal and paternal age at delivery, birth order, and risk of childhood onset type 1 diabetes: population based cohort study. *Bmj* 323:369, 2001

92. Dahlquist G, Bennich SS, Kallen B: Intrauterine growth pattern and risk of childhood onset insulin dependent (type I) diabetes: population based case-control study. *Bmj* 313:1174-1177, 1996

93. Larsson K, Elding-Larsson H, Cederwall E, Kockum K, Neiderud J, Sjöblad S, Lindberg B, Lernmark B, Cilio C, Ivarsson SA, Lernmark A: Genetic and perinatal factors as risk for childhood type 1 diabetes. *Diabetes Metab Res Rev* 20:429-437, 2004

94. Dahlquist GG, Patterson C, Soltesz G: Perinatal risk factors for childhood type 1 diabetes in Europe. The EURODIAB Substudy 2 Study Group. *Diabetes Care* 22:1698-1702, 1999

95. Cardwell CR, Carson DJ, Patterson CC: Parental age at delivery, birth order, birth weight and gestational age are associated with the risk of childhood Type 1 diabetes: a UK regional retrospective cohort study. *Diabet Med* 22:200-206, 2005

96. Flood TM, Brink SJ, Gleason RE: Increased incidence of type I diabetes in children of older mothers. *Diabetes Care* 5:571-573, 1982

97. Wagener DK, LaPorte RE, Orchard TJ, Cavender D, Kuller LH, Drash AL: The Pittsburgh diabetes mellitus study. 3: An increased prevalence with older maternal age. *Diabetologia* 25:82-85, 1983

98. Podar T, Onkamo P, Forsén T, Karvonen M, Tuomilehto-Wolf E, Tuomilehto J: Neonatal anthropometric measurements and risk of childhood-onset type 1 diabetes. DiMe Study Group. *Diabetes Care* 22:2092-2094, 1999

99. Stene LC, Magnus P, Lie RT, Sovik O, Joner G: Birth weight and childhood onset type 1 diabetes: population based cohort study. *Bmj* 322:889-892, 2001

100. Honeyman MC, Coulson BS, Stone NL, Gellert SA, Goldwater PN, Steele CE, Couper JJ, Tait BD, Colman PG, Harrison LC: Association between rotavirus infection and pancreatic islet autoimmunity in children at risk of developing type 1 diabetes. *Diabetes* 49:1319-1324, 2000

101. Tuomilehto J, Karvonen M, Pitkäniemi J, Virtala E, Kohtamäki K, Toivanen L, Tuomilehto-Wolf E: Record-high incidence of Type I (insulin-dependent) diabetes mellitus in Finnish children. The Finnish Childhood Type I Diabetes Registry Group. *Diabetologia* 42:655-660, 1999

102. Rytkönen M, Moltchanova E, Ranta J, Taskinen O, Tuomilehto J, Karvonen M: The incidence of type 1 diabetes among children in Finland--rural-urban difference. *Health Place* 9:315-325, 2003

103. Siren MK, Sareneva H, Lokki ML, Koskimies S: Unique HLA antigen frequencies in the Finnish population. *Tissue Antigens* 48:703-707, 1996

104. Kukko M, Virtanen SM, Toivonen A, Simell S, Korhonen S, Ilonen J, Simel O, Knip M: Geographical variation in risk HLA-DQB1 genotypes for type 1 diabetes and signs of beta-cell autoimmunity in a high-incidence country. *Diabetes Care* 27:676-681, 2004

105. Harris H: The familial distribution of diabetes mellitus: a study of the relatives of 1241 diabetic propositi. *Annals of Eugenics* 15:95-119, 1950

106. Pincus G, White P: The inheritance of diabetes mellitus. II. Further analysis of family histories. *Am J Med Sci* 188:159-168, 1934

107. Pincus G, White P: On the heritance of diabetes mellitus. I. An analysis of 675 family histories. *Am J Med Sci* 186:1-14, 1933

108. Steinberg AG, Wilder RM: A study of the genetics of diabetes mellitus. *Am J Hum Genet* 4:113-135, 1952

109. Barrai I, Cann HM: Segregation Analysis of Juvenile Diabetes Mellitus. *J Med Genet* 42:8-11, 1965

110. Smith C, Falconer DS, Duncan LJ: A statistical and genetical study of diabetes. II. Heritability of liability. *Ann Hum Genet* 35:281-299, 1972

111. Falconer DS: The inheritance of liability to diseases with variable age of onset, with particular reference to diabetes mellitus. *Ann Hum Genet* 31:1-20, 1967

112. Simpson NE: Diabetes in the families of diabetics. *Can Med Assoc J* 98:427-432, 1968

113. Thompson GS: Genetic factors in diabetes mellitus studied by the oral glucose tolerance test. *J Med Genet* 2:221-226, 1965

114. WHO Expert Committee: WHO Expert Committee on Diabetes Mellitus: second report. *World Health Organ Tech Rep Ser* 646:1-80, 1980

115. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 28:1039-1057, 1979

116. Darlow JM, Smith C, Duncan LJ: A statistical and genetical study of diabetes. 3. Empiric risks to relatives. *Ann Hum Genet* 37:157-174, 1973

117. Harvald B, Hauge M: Selection in diabetes in modern society. *Acta Med Scand* 173:459-465, 1963

118. Gottlieb MS, Root HF: Diabetes mellitus in twins. *Diabetes* 17:693-704, 1968

119. Pollin W, Allen MG, Hoffer A, Stabenau JR, Hrubec Z: Psychopathology in 15,909 pairs of veteran twins: evidence for a genetic factor in the pathogenesis of schizophrenia and its relative absence in psychoneurosis. *Am J Psychiatry* 126:597-610, 1969

120. Tattersall RB, Pyke DA: Diabetes in identical twins. *Lancet* 2:1120-1125, 1972

121. Kumar D, Gemayel NS, Gill SK, Bray GA, Roy-Burman P, Deapen D, Mack TM: Type-specific concordance in young diabetic monozygotic twins. *Adv Exp Med Biol* 246:259-267, 1988

122. Barnett AH, Eff C, Leslie RD, Pyke DA: Diabetes in identical twins. A study of 200 pairs. *Diabetologia* 20:87-93, 1981

123. Committee on Diabetic Twins JDS: Diabetes mellitus in twins: a cooperative study in Japan. *Diabetes Res Clin Pract* 5:271-280, 1988

124. Kaprio J, Tuomilehto J, Koskenvuo M, Romanov K, Reunanen A, Eriksson J, Stengård J, Kesäniemi YA: Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. *Diabetologia* 35:1060-1067, 1992

125. Kyvik KO, Green A, Beck-Nielsen H: Concordance rates of insulin dependent diabetes mellitus: a population based study of young Danish twins. *Bmj* 311:913-917, 1995

126. Johnston C, Pyke DA, Cudworth AG, Wolf E: HLA-DR typing in identical twins with insulin-dependent diabetes: difference between concordant and discordant pairs. *Br Med J (Clin Res Ed)* 286:253-255, 1983

127. Olmos P, A'Hern R, Heaton DA, Millward BA, Risley D, Pyke DA, Leslie RD: The significance of the concordance rate for type 1 (insulin-dependent) diabetes in identical twins. *Diabetologia* 31:747-750, 1988

128. Kumar D, Gemayel NS, Deapen D, Kapadia D, Yamashita PH, Lee M, Dwyer JH, Roy-Burman P, Bray GA, Mack TM: North-American twins with IDDM. Genetic, etiological, and clinical significance of disease concordance according to age, zygosity, and the interval after diagnosis in first twin. *Diabetes* 42:1351-1363, 1993

129. Redondo MJ, Yu L, Hawa M, Mackenzie T, Pyke DA, Eisenbarth GS, Leslie RD: Heterogeneity of type I diabetes: analysis of monozygotic twins in Great Britain and the United States. *Diabetologia* 44:354-362, 2001

130. Metcalfe KA, Hitman GA, Rowe RE, Hawa M, Huang X, Stewart T, Leslie RD: Concordance for type 1 diabetes in identical twins is affected by insulin genotype. *Diabetes Care* 24:838-842, 2001

131. Verge CF, Gianani R, Yu L, Pietropaolo M, Smith T, Jackson RA, Soeldner JS, Eisenbarth GS: Late progression to diabetes and evidence for chronic beta-cell autoimmunity in identical twins of patients with type I diabetes. *Diabetes* 44:1176-1179, 1995

132. Degnbol B, Green A: Diabetes mellitus among first- and second-degree relatives of early onset diabetics. *Ann Hum Genet* 42:25-47, 1978

133. West R, Belmonte MM, Colle E, Crepeau MP, Wilkins J, Poirier R: Epidemiologic survey of juvenile-onset diabetes in Montreal. *Diabetes* 28:690-693, 1979

134. Gottlieb MS: Diabetes in offspring and siblings of juvenile- and maturity-onset-type diabetics. *J Chronic Dis* 33:331-339, 1980

135. Gamble DR: An epidemiological study of childhood diabetes affecting two or more siblings. *Diabetologia* 19:341-344, 1980

136. Chern MM, Anderson VE, Barbosa J: Empirical risk for insulin-dependent diabetes (IDD) in sibs. Further definition of genetic heterogeneity. *Diabetes* 31:1115-1118, 1982

137. Wagener DK, Sacks JM, LaPorte RE, Macgregor JM: The Pittsburgh study of insulin-dependent diabetes mellitus. Risk for diabetes among relatives of IDDM. *Diabetes* 31:136-144, 1982

138. Tillil H, Kobberling J: Age-corrected empirical genetic risk estimates for first-degree relatives of IDDM patients. *Diabetes* 36:93-99, 1987

139. Dahlquist G, Blom L, Tuvemo T, Nyström L, Sandström A, Wall S: The Swedish childhood diabetes study--results from a nine year case register and a one year case-referent study indicating that type 1 (insulin-dependent) diabetes mellitus is associated with both type 2 (non-insulin-dependent) diabetes mellitus and autoimmune disorders. *Diabetologia* 32:2-6, 1989

140. Allen C, Palta M, D'Alessio DJ: Risk of diabetes in siblings and other relatives of IDDM subjects. *Diabetes* 40:831-836, 1991

141. Pociot F, Norgaard K, Hobolth N, Andersen O, Nerup J: A nationwide population-based study of the familial aggregation of type 1 (insulin-dependent) diabetes mellitus in Denmark. Danish Study Group of Diabetes in Childhood. *Diabetologia* 36:870-875, 1993

142. Lorenzen T, Pociot F, Hougaard P, Nerup J: Long-term risk of IDDM in first-degree relatives of patients with IDDM. *Diabetologia* 37:321-327, 1994

143. Steck AK, Barriga KJ, Emery LM, Fiallo-Scharer RV, Gottlieb PA, Rewers MJ: Secondary attack rate of type 1 diabetes in Colorado families. *Diabetes Care* 28:296-300, 2005

144. Dahlquist G, Gustavsson KH, Holmgren G, Hagglof B, Larsson Y, Nilsson KO, Samuelsson G, Sterky G, Thalme B, Wall S: The incidence of diabetes mellitus in Swedish children 0-14 years of age. A prospective study 1977-1980. *Acta Paediatr Scand* 71:7-14, 1982

145. O'Leary LA, Dorman JS, LaPorte RE, Orchard TJ, Becker DJ, Kuller LH, Eberhardt MS, Cavender DE, Rabin BS, Drash AL: Familial and sporadic insulin-dependent diabetes: evidence for heterogeneous etiologies? *Diabetes Res Clin Pract* 14:183-190, 1991

146. Tuomilehto J, Lounamaa R, Tuomilehto-Wolf E, Reunanen A, Virtala E, Kaprio EA, Åkerblom HK: Epidemiology of childhood diabetes mellitus in Finland--background of a nationwide study of type 1 (insulin-dependent) diabetes mellitus. The Childhood Diabetes in Finland (DiMe) Study Group. *Diabetologia* 35:70-76, 1992

147. Metcalfe MA, Baum JD: Family characteristics and insulin dependent diabetes. *Arch Dis Child* 67:731-736, 1992

148. Warram JH, Krolewski AS, Gottlieb MS, Kahn CR: Differences in risk of insulin-dependent diabetes in offspring of diabetic mothers and diabetic fathers. *N Engl J Med* 311:149-152, 1984

149. Bleich D, Polak M, Eisenbarth GS, Jackson RA: Decreased risk of type I diabetes in offspring of mothers who acquire diabetes during adrenarchy. *Diabetes* 42:1433-1439, 1993

150. el-Hashimy M, Angelico MC, Martin BC, Krolewski AS, Warram JH: Factors modifying the risk of IDDM in offspring of an IDDM parent. *Diabetes* 44:295-299, 1995

151. Tuomilehto J, Podar T, Tuomilehto-Wolf E, Virtala E: Evidence for importance of gender and birth cohort for risk of IDDM in offspring of IDDM parents. *Diabetologia* 38:975-982, 1995

152. Lorenzen T, Pociot F, Stilgren L, Kristiansen OP, Johannesen J, Olsen PB, Walmar A, Larsen A, Albrechtsen NC, Eskildsen PC, Andersen OO, Nerup J: Predictors of IDDM recurrence risk in offspring of Danish IDDM patients. Danish IDDM Epidemiology and Genetics Group. *Diabetologia* 41:666-673, 1998

153. EURODIAB ACE Study Group: Familial risk of type I diabetes in European children. *Diabetologia* 41:1151-1156, 1998

154. Miodovnik M, Lavin JP, Knowles HC, Holroyde J, Stys SJ: Spontaneous abortion among insulin-dependent diabetic women. *Am J Obstet Gynecol* 150:372-376, 1984

155. Dorman JS, Burke JP, McCarthy BJ, Norris JM, Steenkiste AR, Aarons JH, Schmeltz R, Cruickshanks KJ: Temporal trends in spontaneous abortion associated with Type 1 diabetes. *Diabetes Res Clin Pract* 43:41-47, 1999

156. Lorenzen T, Pociot F, Johannesen J, Kristiansen OP, Nerup J: A population-based survey of frequencies of self-reported spontaneous and induced abortions in Danish women with Type 1 diabetes mellitus. Danish IDDM Epidemiology and Genetics Group. *Diabet Med* 16:472-476, 1999

157. Penney GC, Mair G, Pearson DW: Outcomes of pregnancies in women with type 1 diabetes in Scotland: a national population-based study. *Bjog* 110:315-318, 2003

158. Rosenn B, Miodovnik M, Combs CA, Khoury J, Siddiqi TA: Glycemic thresholds for spontaneous abortion and congenital malformations in insulin-dependent diabetes mellitus. *Obstet Gynecol* 84:515-520, 1994

159. Greene MF, Hare JW, Cloherty JP, Benacerraf BR, Soeldner JS: First-trimester hemoglobin A1 and risk for major malformation and spontaneous abortion in diabetic pregnancy. *Teratology* 39:225-231, 1989

160. Koczwara K, Bonifacio E, Ziegler AG: Transmission of maternal islet antibodies and risk of autoimmune diabetes in offspring of mothers with type 1 diabetes. *Diabetes* 53:1-4, 2004

161. Buschard K, Jorgensen M, Aaen K, Bock T, Josefsen K: Prevention of diabetes mellitus in BB rats by neonatal stimulation of beta cells. *Lancet* 335:134-135, 1990

162. Vadheim CM, Rotter JI, Maclaren NK, Riley WJ, Anderson CE: Preferential transmission of diabetic alleles within the HLA gene complex. *N Engl J Med* 315:1314-1318, 1986

163. Pitkäniemi J, Onkamo P, Arjas E, Tuomilehto-Wolf E, Tuomilehto J: Estimation of transmission probabilities in families ascertained through a proband with variable age-at-onset disease: application to the HLA A, B and DR loci in Finnish families with type 1 diabetes. The DiMe Study Group. *Hum Hered* 50:308-317, 2000

164. Hall JG, Solehdin F: Genomic imprinting. *Zhonghua Min Guo Xiao Er Ke Yi Xue Hui Za Zhi* 37:401-404, 1996

165. Guo SW, Tuomilehto J: Preferential transmission of type 1 diabetes from parents to offspring: fact or artifact? *Genet Epidemiol* 23:323-334, 2002

166. Köbberling J, Bruggeboes B: Prevalence of diabetes among children of insulin-dependent diabetic mothers. *Diabetologia* 18:459-462, 1980

167. Cudworth AG: Type I diabetes mellitus. *Diabetologia* 14:281-291, 1978

168. Contu L, Deschamps I, Lestradet H, Hors J, Schmid M, Busson M, Benajam A, Marcelli-Barge A, Dausset J: HLA haplotype study of 53 juvenile insulin-dependent diabetic (I.D.D.) families. *Tissue Antigens* 20:123-140, 1982

169. Leslie RD, Delli Castelli M: Age-dependent influences on the origins of autoimmune diabetes: evidence and implications. *Diabetes* 53:3033-3040, 2004

170. Shield JP, Wadsworth EJ, Baum JD: The genetic contribution to disease pathogenesis in childhood diabetes is greatest in the very young. *Diabet Med* 12:377-379, 1995

171. Komulainen J, Kulmala P, Savola K, Lounamaa R, Ilonen J, Reijonen H, Knip M, Åkerblom HK: Clinical, autoimmune, and genetic characteristics of very young children with type 1 diabetes. Childhood Diabetes in Finland (DiMe) Study Group. *Diabetes Care* 22:1950-1955, 1999

172. Valdes AM, Thomson G, Erlich HA, Noble JA: Association between type 1 diabetes age of onset and HLA among sibling pairs. *Diabetes* 48:1658-1661, 1999

173. Emery LM, Babu S, Bugawan TL, Norris JM, Erlich HA, Eisenbarth GS, Rewers M: Newborn HLA-DR,DQ genotype screening: age- and ethnicity-specific type 1 diabetes risk estimates. *Pediatr Diabetes* 6:136-144, 2005

174. Hathout EH, Hartwick N, Fagoaga OR, Colacino AR, Sharkey J, Racine M, Nelsen-Cannarella S, Mace JW: Clinical, autoimmune, and HLA characteristics of children diagnosed with type 1 diabetes before 5 years of age. *Pediatrics* 111:860-863, 2003

175. Fava D, Gardner S, Pyke D, Leslie RD: Evidence that the age at diagnosis of IDDM is genetically determined. *Diabetes Care* 21:925-929, 1998

176. Dahlquist GG, Mustonen LR: Clinical onset characteristics of familial versus nonfamilial cases in a large population-based cohort of childhood-onset diabetes patients. *Diabetes Care* 18:852-854, 1995

177. American Diabetes Association: Diabetic Nephropathy. *Diabetes Care* 26:S94-S98, 2003

178. Borch-Johnsen K, Kreiner S: Proteinuria: value as predictor of cardiovascular mortality in insulin dependent diabetes mellitus. *Br Med J (Clin Res Ed)* 294:1651-1654, 1987

179. Tuomilehto J, Borch-Johnsen K, Molarius A, Forsén T, Rastenyte D, Sarti C, Reunanen A: Incidence of cardiovascular disease in Type 1 (insulin-dependent) diabetic subjects with and without diabetic nephropathy in Finland. *Diabetologia* 41:784-790, 1998

180. Borch-Johnsen K, Andersen PK, Deckert T: The effect of proteinuria on relative mortality in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 28:590-596, 1985

181. Andersen AR, Christiansen JS, Andersen JK, Kreiner S, Deckert T: Diabetic nephropathy in Type 1 (insulin-dependent) diabetes: an epidemiological study. *Diabetologia* 25:496-501, 1983

182. Krolewski AS, Warram JH, Christlieb AR, Busick EJ, Kahn CR: The changing natural history of nephropathy in type I diabetes. *Am J Med* 78:785-794, 1985

183. The Diabetes Control and Complications Trial Research Group (DCCT): The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977-986, 1993

184. Orchard TJ, Forrest KY, Ellis D, Becker DJ: Cumulative glycemic exposure and microvascular complications in insulin-dependent diabetes mellitus. The glycemic threshold revisited. *Arch Intern Med* 157:1851-1856, 1997

185. The Diabetes Control and Complications (DCCT) Research Group: Effect of intensive therapy on the development and progression of diabetic nephropathy in the Diabetes Control and Complications Trial. *Kidney Int* 47:1703-1720, 1995

186. Anderson CE, Hodge SE, Rubin R, Rotter JI, Terasaki PI, Irvine WJ, Rimoin DL: A search for heterogeneity in insulin dependent diabetes mellitus (IDDM): HLA and autoimmune studies in simplex, multiplex and multigenerational families. *Metabolism* 32:471-477, 1983

187. Feldt-Rasmussen B, Borch-Johnsen K, Mathiesen ER: Hypertension in diabetes as related to nephropathy. Early blood pressure changes. *Hypertension* 7:II18-20, 1985

188. Parving HH, Hommel E: High blood pressure is a major factor in progression of diabetic nephropathy. *J Diabet Complications* 2:92-95, 1988

189. Jenkins AJ, Steele JS, Janus ED, Best JD: Increased plasma apolipoprotein(a) levels in IDDM patients with microalbuminuria. *Diabetes* 40:787-790, 1991

190. Groop PH, Elliott T, Ekstrand A, Franssila-Kallunki A, Friedman R, Viberti GC, Taskinen MR: Multiple lipoprotein abnormalities in type I diabetic patients with renal disease. *Diabetes* 45:974-979, 1996

191. 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

192. Earle K, Walker J, Hill C, Viberti G: Familial clustering of cardiovascular disease in patients with insulin-dependent diabetes and nephropathy. *N Engl J Med* 326:673-677, 1992

193. 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

194. Kostraba JN, Dorman JS, Orchard TJ, Becker DJ, Ohki Y, Ellis D, Doft BH, Lobes LA, LaPorte RE, Drash AL: Contribution of diabetes duration before puberty to development of microvascular complications in IDDM subjects. *Diabetes Care* 12:686-693, 1989

195. Schultz CJ, Konopelska-Bahu T, Dalton RN, Carroll TA, Stratton I, Gale EA, Neil A, Dunger DB: Microalbuminuria prevalence varies with age, sex, and puberty in children with type 1 diabetes followed from diagnosis in a longitudinal study. Oxford Regional Prospective Study Group. *Diabetes Care* 22:495-502, 1999

196. McNally PG, Raymond NT, Swift PG, Hearnshaw JR, Burden AC: Does the prepubertal duration of diabetes influence the onset of microvascular complications? *Diabet Med* 10:906-908, 1993

197. Donaghue KC, Fairchild JM, Craig ME, Chan AK, Hing S, Cutler LR, Howard NJ, Silink M: Do all prepubertal years of diabetes duration contribute equally to diabetes complications? *Diabetes Care* 26:1224-1229, 2003

198. Donaghue KC, Fung AT, Hing S, Fairchild J, King J, Chan A, Howard NJ, Silink M: The effect of prepubertal diabetes duration on diabetes. Microvascular complications in early and late adolescence. *Diabetes Care* 20:77-80, 1997

199. Finne P, Reunanen A, Stenman S, Groop PH, Grönhagen-Riska C: Incidence of end-stage renal disease in patients with type 1 diabetes. *Jama* 294:1782-1787, 2005

200. Svensson M, Nyström L, Schon S, Dahlquist G: Age at onset of childhood-onset type 1 diabetes and the development of end-stage renal disease: a nationwide population-based study. *Diabetes Care* 29:538-542, 2006

201. Seaquist ER, Goetz FC, Rich S, Barbosa J: Familial clustering of diabetic kidney disease. Evidence for genetic susceptibility to diabetic nephropathy. *N Engl J Med* 320:1161-1165, 1989

202. Quinn M, Angelico MC, Warram JH, Krolewski AS: Familial factors determine the development of diabetic nephropathy in patients with IDDM. *Diabetologia* 39:940-945, 1996

203. Borch-Johnsen K, Norgaard K, Hommel E, Mathiesen ER, Jensen JS, Deckert T, Parving HH: Is diabetic nephropathy an inherited complication? *Kidney Int* 41:719-722, 1992

204. Fioretto P, Steffes MW, Barbosa J, Rich SS, Miller ME, Mauer M: Is diabetic nephropathy inherited? Studies of glomerular structure in type 1 diabetic sibling pairs. *Diabetes* 48:865-869, 1999

205. The Diabetes Control and Complications Trial Research Group (DCCT): Clustering of long-term complications in families with diabetes in the diabetes control and complications trial. *Diabetes* 46:1829-1839, 1997

206. Snieder H, Sawtell PA, Ross L, Walker J, Spector TD, Leslie RD: HbA(1c) levels are genetically determined even in type 1 diabetes: evidence from healthy and diabetic twins. *Diabetes* 50:2858-2863, 2001

207. Österholm AM, He B, Pitkäniemi J, Albinsson L, Berg T, Sarti C, Tuomilehto J, Tryggvason K: Genome-wide scan for type 1 diabetic nephropathy in the Finnish population reveals suggestive linkage to a single locus on chromosome 3q. *Kidney Int*, 2006

208. Dogra G, Watts GF: Genes and diabetic nephropathy: what have we learnt so far? *Practical Diabetes International: the international journal for diabetes care teams worldwide* 17:84-90, 2000

209. Andersen S, Jacobsen P, Parving HH: ACE insertion/deletion genotypes and angiotensin II receptor blockade in diabetic nephropathy: is there a light at the end of the tunnel? *Diabetes Care* 26:2962-2963, 2003

210. Merta M, Reiterova J, Rysava R, Kmentova D, Tesar V: Genetics of diabetic nephropathy. *Nephrol Dial Transplant* 18 Suppl 5:v24-25, 2003

211. Bojestig M, Arnqvist HJ, Hermansson G, Karlberg BE, Ludvigsson J: Declining incidence of nephropathy in insulin-dependent diabetes mellitus. *N Engl J Med* 330:15-18, 1994

212. Nordwall M, Bojestig M, Arnqvist HJ, Ludvigsson J: Declining incidence of severe retinopathy and persisting decrease of nephropathy in an unselected population of Type 1 diabetes-the Linkoping Diabetes Complications Study. *Diabetologia* 47:1266-1272, 2004

213. Hovind P, Tarnow L, Rossing K, Rossing P, Eising S, Larsen N, Binder C, Parving HH: Decreasing incidence of severe diabetic microangiopathy in type 1 diabetes. *Diabetes Care* 26:1258-1264, 2003

214. Nishimura R, Dorman JS, Bosnyak Z, Tajima N, Becker DJ, Orchard TJ: Incidence of ESRD and survival after renal replacement therapy in patients with type 1 diabetes: a report from the Allegheny County Registry. *Am J Kidney Dis* 42:117-124, 2003

215. Tryggvason G, Indridason OS, Thorsson AV, Hreidarsson AB, Palsson R: Unchanged incidence of diabetic nephropathy in Type 1 diabetes: a nation-wide study in Iceland. *Diabet Med* 22:182-187, 2005

216. Rossing P, Rossing K, Jacobsen P, Parving HH: Unchanged incidence of diabetic nephropathy in IDDM patients. *Diabetes* 44:739-743, 1995

217. Tuomilehto J, Borch-Johnsen K, Molarius A, Jormanainen V, Lounamaa R, Grönhagen-Riska C, Reunanen A, Sarti C: The unchanging incidence of hospitalization for diabetic nephropathy in a population-based cohort of IDDM patients in Finland. *Diabetes Care* 20:1081-1086, 1997

218. Kaprio J, Sarna S, Koskenvuo M, Rantasalo I: The Finnish Twin Registry: formation and compilation, questionnaire study, zygosity determination procedures, and research program. *Prog Clin Biol Res* 24 Pt B:179-184, 1978

219. Kaprio J, Koskenvuo M, Rose RJ: Population-based twin registries: illustrative applications in genetic epidemiology and behavioral genetics from the Finnish Twin Cohort Study. *Acta Genet Med Gemellol (Roma)* 39:427-439, 1990

220. Diabetes Epidemiology Research International Mortality Study Group: International evaluation of cause-specific mortality and IDDM. *Diabetes Care* 14:55-60, 1991

221. Diabetes Epidemiology Research International Mortality Study Group: Major cross-country differences in risk of dying for people with IDDM. *Diabetes Care* 14:49-54, 1991

222. Lounamaa R: Mortality in Finnish Patients With Insulin-Dependent Diabetes Mellitus: A Follow-Up Study of Patients Diagnosed When Under Twenty Years of Age. Helsinki, Finland, Publication of the Social Incurance Institution, 1993

223. Tuomilehto J, Rewers M, Reunanen A, Lounamaa P, Lounamaa R, Tuomilehto-Wolf E, Åkerblom HK: Increasing trend in type 1 (insulin-dependent) diabetes mellitus in childhood in Finland. Analysis of age, calendar time and birth cohort effects during 1965 to 1984. *Diabetologia* 34:282-287, 1991

224. Sarna S, Kaprio J, Sistonen P, Koskenvuo M: Diagnosis of twin zygosity by mailed questionnaire. *Hum Hered* 28:241-254, 1978

225. Neale M: Mx: Statistical Modeling. Richmond, 1991

226. Fourlanos S, Dotta F, Greenbaum CJ, Palmer JP, Rolandsson O, Colman PG, Harrison LC: Latent autoimmune diabetes in adults (LADA) should be less latent. *Diabetologia* 48:2206-2212, 2005

227. Palmer JP, Hampe CS, Chiu H, Goel A, Brooks-Worrell BM: Is latent autoimmune diabetes in adults distinct from type 1 diabetes or just type 1 diabetes at an older age? *Diabetes* 54 Suppl 2:S62-67, 2005

228. JKhoury MB, TH. Cohen BH.: *Fundamentals of genetic epidemiology*, 1993

229. Hermann R, Knip M, Veijola R, Simell O, Laine AP, Åkerblom HK, Groop PH, Forsblom C, Pettersson-Fernholm K, Ilonen J: Temporal changes in the frequencies of HLA genotypes in patients with Type 1 diabetes--indication of an increased environmental pressure? *Diabetologia* 46:420-425, 2003

230. Kontiainen S, Scheinin T, Schlenzka A, Mäenpää J, Groop L, Koskimies S: Differences in HLA types in children with insulin-dependent diabetes diagnosed in 1960s, 1970s, and 1980s. *Lancet* 2:219, 1988

231. Gillespie KM, Bain SC, Barnett AH, Bingley PJ, Christie MR, Gill GV, Gale EA: The rising incidence of childhood type 1 diabetes and reduced contribution of high-risk HLA haplotypes. *Lancet* 364:1699-1700, 2004

232. The Eurodiab Ace Study Group and The Eurodiab Ace Substudy 2 Study Group: Familial risk of type I diabetes in European children. *Diabetologia* 41:1151-1156, 1998

233. Todd JA: From genome to aetiology in a multifactorial disease, type 1 diabetes. *Bioessays* 21:164-174, 1999

234. Ikäheimo I, Silvennoinen-Kassinen S, Tiilikainen A: HLA five-locus haplotypes in Finns. *Eur J Immunogenet* 23:321-328, 1996

235. Palmer JP: Prediction, Prevention and Genetic Counseling in IDDM. Chichester, John Wiley & Sons Ltd, 1996

236. Todd JA: A protective role of the environment in the development of type 1 diabetes? *Diabet Med* 8:906-910, 1991

237. Kolb H, Elliott RB: Increasing incidence of IDDM a consequence of improved hygiene? *Diabetologia* 37:729, 1994

238. Viskari H, Ludvigsson J, Uibo R, Salur L, Marciulionyte D, Hermann R, Soltesz G, Fuchtenbusch M, Ziegler AG, Kondrashova A, Romanov A, Knip M, Hyoty H: Relationship between the incidence of type 1 diabetes and enterovirus infections in different European populations: results from the EPIVIR project. *J Med Virol* 72:610-617, 2004

239. Whitacre CC, Reingold SC, O'Looney PA: A gender gap in autoimmunity. *Science* 283:1277-1278, 1999

240. Field LL: Genes predisposing to IDDM in multiplex families. *Genet Epidemiol* 6:101-106, 1989

241. Tuomilehto-Wolf E, Tuomilehto J, Cepaitis Z, Lounamaa R: New susceptibility haplotype for type 1 diabetes. DIME Study Group. *Lancet* 2:299-302, 1989

242. Pitkäniemi J, Onkamo P, Arjas E, Tuomilehto-Wolf E, Tuomilehto J: Estimation of transmission probabilities in families ascertained through a proband with variable age-at-onset disease: application to the HLA A, B and DR loci in Finnish families with type 1 diabetes. The DiMe Study Group. *Hum Hered* 50:308-317, 2000

243. Martin-Villa JM, Vicario JL, Martinez-Laso J, Serrano-Rios M, Lledo G, Damiano A, Hawkins F, Regueiro JR, Arnaiz-Villena A: Lack of preferential transmission of diabetic HLA alleles by healthy parents to offspring in Spanish diabetic families. *J Clin Endocrinol Metab* 70:346-348, 1990

244. Khoury MJ, Beaty TH, Liang KY: Can familial aggregation of disease be explained by familial aggregation of environmental risk factors? *Am J Epidemiol* 127:674-683, 1988

245. Guo SW: Familial aggregation of environmental risk factors and familial aggregation of disease. *Am J Epidemiol* 151:1121-1131, 2000

246. Nishimura R, Tajima N, Matsushima M, LaPorte RE: Puberty, IDDM, and death in Japan. Diabetes Epidemiology Research International Study Group. *Diabetes Care* 21:1674-1679, 1998

247. Kullberg CE, Abrahamsson M, Arnqvist HJ, Finnström K, Ludvigsson J: Prevalence of retinopathy differs with age at onset of diabetes in a population of patients with Type 1 diabetes. *Diabet Med* 19:924-931, 2002

248. Svensson M, Eriksson JW, Dahlquist G: Early glycemic control, age at onset, and development of microvascular complications in childhood-onset type 1 diabetes: a population-based study in northern Sweden. *Diabetes Care* 27:955-962, 2004

249. Hamilton J, Daneman D: Deteriorating diabetes control during adolescence: physiological or psychosocial? *J Pediatr Endocrinol Metab* 15:115-126, 2002

250. Lane PH: Diabetic kidney disease: impact of puberty. *Am J Physiol Renal Physiol* 283:F589-600, 2002

251. Kordonouri O, Danne T, Enders I, Weber B: Does the long-term clinical course of type I diabetes mellitus differ in patients with prepubertal and pubertal onset? Results of the Berlin Retinopathy Study. *Eur J Pediatr* 157:202-207, 1998

252. Korth-Schutz S, Levine LS, New MI: Serum androgens in normal prepubertal and pubertal children and in children with precocious adrenarche. *J Clin Endocrinol Metab* 42:117-124, 1976

253. Albertsson-Wikland K, Rosberg S, Karlberg J, Groth T: Analysis of 24-hour growth hormone profiles in healthy boys and girls of normal stature: relation to puberty. *J Clin Endocrinol Metab* 78:1195-1201, 1994