GENETIC ANALYSIS OF TYPE 1 DIABETES

Karin Åkesson

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ABSTRACT

Type 1 diabetes is one of the most common chronic diseases among children and young adults in Sweden today. Type 1 diabetes is classified as an autoimmune disease caused by a destruction of the beta-cells in the endocrine pancreas. The disease is thought to be caused by interaction between genetic and environmental factors. The HLA region on chromosome 6 accounts for about 40-50% of the genetic risk to develop type 1 diabetes but other genes also contribute to the risk.

In paper I I have, through a questionnaire sent to almost 4000 individuals been able to show that women developing type 1 diabetes between 15-34 years of age have more first degree relatives with the disease than men developing type 1 diabetes in the same age group. This suggests that more genetic risk factors are needed when a woman, compared to when a man develops type 1 diabetes. This finding is consistent with the fact that in this age group 60% of the persons developing type 1 diabetes are men and only 40% are women.

In paper II we performed a genome-wide linkage analysis of families with two or more siblings with type 1 diabetes from Scandinavia, France and USA. We scanned for regions where risk genes for type 1 diabetes are located. Apart from verifying the HLA and Insulin regions, we could confirm a locus on chromosome 6q21. Suggestive linkage was also seen on chromosome 2p, 5q, and 16p.

There is an increased risk for a child to develop type 1 diabetes if the father rather than the mother has the disease. We have tried to find explanations for this.

In paper III we examined whether the HLA type that the child does not inherit from the mother affects the risk for the child to develop type 1 diabetes. In children that have inherited one high risk HLA type and one neutral HLA type, it seems to be protective if the HLA haplotype not inherited from the mother is a high risk haplotype. The mechanism for this could be the shaping of the immune repertoire during fetal life and we speculate that the child might be partly protected against the disease later in life when he/she is predisposed to the mother’s high risk HLA haplotype intrauterine.

In paper IV, the hypothesis that the difference in risk to develop type 1 diabetes between children to mothers and fathers with type 1 diabetes is due to imprinting mechanisms is tested. The hypothesis is that the genes are expressed in a parent of origin specific manner and that there is an increased risk to develop disease if the risk gene is inherited from one of the parents. We show in this study that the risk to develop type 1 diabetes seems to be influenced by imprinting mechanisms in two regions. One is located on chromosome 6q (IDDM15) and here we see an increased risk for the child to develop type 1 diabetes if the risk allele is inherited from the father. This is consistent with the finding that there is a greater risk for children to develop type 1 diabetes if the father rather than the mother has the disease. The other region is on chromosome 13q12 and this region shows a maternal effect. The maternal effect might also be explained by the fact that a woman need more risk genes to develop type 1 diabetes. This increases the risk for the child to inherit a risk gene from the mother than from the father.

In summary, the overall aim of this thesis was to identify genetic factors that influence the risk to develop type 1 diabetes. By identifying genetic risk factors these studies hopefully will contribute to the understanding of the genesis of type 1 diabetes.
LIST OF PUBLICATIONS


IV. K. Åkesson, P. Nikamo, J. Ludvigsson, H. Luthman, I. Kockum for the European Consortium for IDDM Genome Studies. Analysis of parent of origin effects in a genome wide linkage analysis for type 1 diabetes using both non-parametric and parametric MOD score analysis. (Manuscript)

“There is only one thing about which I am certain, and that is that there is very little about which one can be certain.”

W. Somerset Maugham
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<tbody>
<tr>
<td>bp</td>
<td>Base pair</td>
</tr>
<tr>
<td>cM</td>
<td>Centi Morgan</td>
</tr>
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<td>CTLA4</td>
<td>Cytotoxic T lymphocyte associated-4</td>
</tr>
<tr>
<td>DISS</td>
<td>Diabetes Incidence Study in Sweden</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>HLA</td>
<td>Human Leukocyte Antigen</td>
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<td>GAD</td>
<td>Glutamic Acid Decarboxylase</td>
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<td>GWS</td>
<td>Genome-Wide association Study</td>
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<tr>
<td>IAA</td>
<td>Insulin Autoantibodies</td>
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<tr>
<td>IA-2</td>
<td>Insulin associated Antigen-2</td>
</tr>
<tr>
<td>IBD</td>
<td>Identical by Descent</td>
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<tr>
<td>ICA</td>
<td>Islet Cell Antibodies</td>
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<tr>
<td>ITPR3</td>
<td>Inositol 1, 4, 5-triphosphate receptor 3</td>
</tr>
<tr>
<td>LOD</td>
<td>Logarithm of Odds</td>
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<td>LYP</td>
<td>Lymphoid protein tyrosine Phosphatase</td>
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<tr>
<td>MHC</td>
<td>Major Histocompatibility Complex</td>
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<tr>
<td>MLS</td>
<td>Maximum Likelihood Score</td>
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<tr>
<td>Mmc</td>
<td>Maternal microchimerism</td>
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<tr>
<td>MODY</td>
<td>Maturity-onset Diabetes of the Young</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>PTPN22</td>
<td>Protein tyrosine phosphatase N22</td>
</tr>
<tr>
<td>RIA</td>
<td>Radio Immuno Assay</td>
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<tr>
<td>RFLP</td>
<td>Restriction Fragment Length Polymorphism</td>
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<tr>
<td>SNP</td>
<td>Single-nucleotide Polymorphism</td>
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<tr>
<td>SSO</td>
<td>Sequence Specific Oligo</td>
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<tr>
<td>TDT</td>
<td>Transmission Disequilibrium Test</td>
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<tr>
<td>TREGS</td>
<td>Regulatory T-cells</td>
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</table>
In remembrance of my father, Lars-Gösta Elfvin
1. BACKGROUND

INTRODUCTION

Type 1 diabetes is a chronic disease where the insulin producing beta-cells in the endocrine pancreas are destroyed. This causes an insulin deficiency, which results in high blood sugar and a need of daily insulin injections. In the studies underlying this thesis I have tested different hypothesis trying to find explanations for the difference in risk between persons to develop type 1 diabetes. I have analyzed genetic markers all over the genome in order to find new genetic susceptibility regions. I have also been looking at differences in gender of the patients with type 1 diabetes as well as differences in risk for children, to mothers and fathers with type 1 diabetes, to develop the disease. My wish is that the findings in some way may contribute to solve the riddle of type 1 diabetes.

HISTORY

Already more than 3500 years ago type 1 diabetes was described on papyrus from Egypt. The patients were in these years diagnosed by the sweet taste of the urine and in India doctors described “honesweet urine” (mellitus=honey sweet) 600 B.C. About 2000 years ago an ancient Greek doctor Aretaios of Cappadocia first named the disease diabetes=passing through, as he described the body of a diabetes patient as a water-pipe – “the liquids do not stay in the body but just pass through”. He described the symptoms of diabetes as large urine amounts and excessive thirst. From the 17th century and until modern time there were attempts to treat diabetes mellitus with starving cures, excessive fatty diets, vomiting cures, hot baths och opium cures. But despite these treatments the patients never survived for a long time. In 1869 Paul Langerhans described islets in the pancreas that were involved in the metabolism and in experiments where the pancreas had undergone an operation in dogs, the dogs developed diabetes. It was not until 1922 that Banting and Best managed to extract a substance from the pancreas – insulin – that had a blood sugar lowering effect. This made it possible to start to treat type 1 diabetes patients with insulin (1, 2). A 14-year old boy was the first person that was treated. The patient could survive!

Since then thousands of studies have been performed and still no one has managed to fully understand: Why do some people develop type 1 diabetes? Many researchers have speculated and we need to generate hypotheses that we can test in order to solve the mystery of type 1 diabetes. A common view of how type 1 diabetes develops is that genetically predisposed individuals might get an infection during foetal life or later in life and this starts an autoimmune process which sometimes stops but in some cases the process continues. If more risk factors such as infections, poisons and stress are added, further steps towards the disease are taken and finally the disease is clinically manifested (Figure 1).
**Figure 1.** β-cell destruction. The way to the type I diabetes diagnosis. Type 1 diabetes is a multifactorial disease and combination of different factors lead to the disease. It is also suggested that there is a possibility for the beta-cells to regenerate if for instance a genetic risk factor is combined with a protective factor in the environment. Modified from J Ludvigsson 1981.

**SYMPTOMS**

It is thought that symptoms are seen when roughly 80% of the beta cells are destroyed. The blood sugar is raised, the kidneys can no longer absorb the sugar so the patients get sugar in the urine. This raises the liquid elimination through the kidneys out of the body. Increased urine amounts, increased thirst, loss of weight and tiredness are symptoms at the disease onset. Tiredness and loss of body weight are also effects of serious disturbances in the metabolism caused by the lack of insulin.

**CLASSIFICATION**

In the ancient years the diabetes diagnosis was based on the finding of sweet taste of the urine and it was first in the 20th century that it was understood that diabetes is a group of diseases. The classification of diabetes is today based on a WHO-report with principles of definition and classification of diabetes (1998) (3)

Clinical manifest diabetes mellitus is defined by a fasting blood glucose (fB-glucose) 6.1 mmol/l or more measured at two separate occasions or one non-fasting blood glucose >11 mmol/l (WHO 1998).
Disturbances in the glucose metabolism are divided into:

A. Type 1-diabetes, beta-cell destruction usually leading to a total insulin deficiency divided into:
   a) immunemediated (autoimmune)
   b) idiopathic

B. Type 2-diabetes, range from predominantly insulin resistance with relative insulin deficiency to predominantly secretory defect with insulin resistance

C. Other specific types of diabetes.
   a) Genetic defects of beta-cell function (formerly MODY-maturity onset diabetes in the young)
   b) Endocrinopathies
   c) Genetic defects in insulin action
   d) Drug- or chemical-induced insulin deficiency

D. Gestational diabetes mellitus (GDM) - onset or recognition of glucose intolerance in pregnancy.

C-peptide, analyzed with Radio Immuno Assay (RIA) -methods, can be used to easier classify the type of diabetes, especially in the middle age, to quantify the endogenous insulin production and to estimate the need of insulin.

INCIDENCE/ PREVALENCE

The incidence is the number of new cases of a disease in a population during a given time interval, usually one year. It can be expressed as a proportion or as a rate. The prevalence of a disease in a population is defined as the total number of cases of the disease in the population at a given time, or the total number of cases, divided by the number of individuals in the population.

The risk to develop diabetes during life is 15% in Sweden and the disease is most common among elderly people. About 20% of the Swedish population above 80 years has diabetes (www.socialstyrelsen.se). Type 1 is more common among individuals who develop diabetes early in life and the older a person is the more likely it is that his or her diabetes is not type 1 diabetes. Type 1 diabetes is one of the most common chronic diseases among children and youngsters in Sweden. The prevalence of type 1 diabetes is about 0.5% representing about 15% of all diabetes regardless of age (www.socialstyrelsen.se). The last years the incidence of type 1 diabetes has been almost 700 children between 0-14 years (the Swedish Childhood Diabetes Register, 2006). In the agegroup 15 to 34 years the number has, during the last 25 years, been varying between 350 in 1984 and 250 in 1995 (20).
During the last two decades there has been a 50% increase in incidence of type 1 diabetes among children and young people (figure 2A and 2B) (4, 5). The increase was until year 2000 most pronounced among children in the 5-9 year age group but was seen in all age groups (6). The last years the increase is mainly seen in age group 10-14 years (figure 2B)

![Figure 2](image)

**Figure 2.** Increase of incidence of type 1 diabetes among 0 to 14 year olds during the past 25 years. A Subgroups divided due to gender. B Subgroups divided due to age at diagnosis (the Swedish Childhood Diabetes Register, 2006, coordinator G. Dahlqvist).

In 1978 21.1/100000 Swedish children (0-14 years) were diagnosed per year and in 1997 it had increased to 31.9/100000. (7). In 2006 44/100000 children were diagnosed (Swedish Childhood Diabetes Register, figure 2). There is a large geographical variation in the incidence of type 1 diabetes (figure 3). The overall age-adjusted incidence of type 1 diabetes varied from 0.1/100,000 per year in China and Venezuela to 36.8/100,000 per year in Sardinia and 36.5/100,000 per year in Finland. This represents a >350-fold variation in the incidence among 100 populations worldwide (6, 8). There is a connection between the incidence and the distance to the equator with a higher incidence the further from the equator you come. One exception to this correlation is the high incidence of type 1 diabetes in Sardinia and this has been a subject of extensive investigations (9).

10
In many parts of the world the incidence of type 1 diabetes among children and teenagers is increasing with about 2-5% per year. The increasing incidence is seen in both high and low incidence countries, but a bit more pronounced in the low incidence countries (12). It has been suggested that the major increase of type 1 diabetes among children is due to a shift to a younger age group developing the disease (13) It has even been suggested that the incidence of type 1 diabetes in the age group 15-34 years in Sweden is decreasing which is compatible with the finding that there are more small children developing type 1 diabetes (14).

Before puberty the incidence among girls and boys is almost the same but among the young adults about 60% are men and 40% women (15).

Until recently type 2 diabetes mellitus has been rare among children and young adults. During the last decade there has been an increased incidence of type 2 diabetes even in this age group (16). The proportion of obese children and teenagers has increased and overweight is an important risk factor for type 2 diabetes and perhaps even for type 1 diabetes in genetically predisposed individuals (17-19). The development of type 2 diabetes in young people often leads to complications with an increased morbidity and mortality.

Figure 3. The latest published incidence numbers where the incidence is compared between countries is from the EURODIAB study in 2000 (8-11.) Since 1999 the numbers are continuing to increase and there is today unpublished data from Finland exceeding 60/100000 cases per year. The SWEDIABKIDS is reporting 44 new cases /10000/year in Sweden (figure 2Aand 2B).
COMPLICATIONS

The goal with the diabetes treatment is to prevent acute and late complications with a maintained high quality of life for the patients.

Although the insulin treatment is getting better and better the treatment of type 1 diabetes is not good enough to avoid complications due to impaired blood glucose levels.

The most prominent acute complications are hyperglycemia, ketoacidosis, hyperosmolar conditions, and hypoglycemia. To be free from symptoms of diabetes such as large urine amounts, thirst, tiredness and visual disturbances as well as having a normal growth and development are also primary goals. The long term complications are caused by microangiopathy and macroangiopathy.

Despite the improved diabetic control and the better long time prognosis of late complications there is still a considerable risk of development of retinopathy, nephropathy and neuropathy. There is a clear connection between the blood glucose level, duration and the progression of these complications. Almost all patients with type 1 diabetes, unless they have a very good metabolic control, get some type of retinopathy, while about 30-40% suffer from diabetic kidney disease (21-24). The difference is thought to be an effect of different susceptibility to the damaging effect of hyperglycaemia (25). It has also been shown that even in a population with intensive insulin treatment from the beginning and fairly good metabolic control, the prevalence of neuropathy is high. The prevalence of subclinical neuropathy is almost 60% (25-26)

A faster development of atherosclerosis (macroangiopathy) is also seen and the risk for coronary disease is about 2-3 times the risk for non diabetic persons. There is even an increased risk of peripheral vessel disease with claudicatio intermittens as a symptom. The life expectancy among people that develop type 1 diabetes during childhood has been shown to be shortened with 10-20 years (27). The treatment of diabetes and complications due to the disease uses about 8% of the direct Swedish health care costs (www.socialstyrelsen.se).
GENETICS

We inherit genes from our parents and these genes play a major role (together with the environment) in the phenotype that each individual will develop. The human genome consists of 23 pairs of chromosomes. 22 of these are autosomes and one pair is the sex chromosomes. The inheritance of half of the genes from our mother and half from our father make a unique new mix in every newborn individual (except for monozygotic twins who inherit identical copies of all genes). It is random which of the two chromosomes in a pair that is transmitted to each offspring in the next generation. This is called Mendel’s first law because it was in the 1860s described by the Austrian monk Gregor Mendel. He was observing the frequency of different phenotypes in garden peas and postulated the existence of genes. The relevance of his work for human traits was not understood until 40 years later (28, classical article 1902).

However, in the meiosis, the cell division where the gametes are formed, the two chromosomes in a pair can exchange genetic material through a process called crossing-over. The crossover events can indirectly be measured by determining the parental origin of alleles of polymorphic markers. Several crossover events can occur per chromosome in each meiosis although on average there is one crossover event per chromosome arm per generation. A recombination is taking place when an odd number of crossover events have occurred between two genes. Then a new combination of alleles will be transmitted together on the haplotype.

Patterns of inheritance

Inherited diseases are usually divided into two groups based on their etiology: monogenic and multifactorial diseases. The two classical Mendelian inheritance patterns of monogenic diseases are dominant and recessive. For recessive diseases only individuals being homozygous (carrying two identical copies) for the disease allele, are at risk of developing the disease. Thus, in this case both parents must be carriers of the disease allele. An example of a recessive monogenic disease is cystic fibrosis (CF) where an alteration of a single gene, such as a mutation or a deletion is enough for disease development (figure 4).
Figure 4. This pedigree shows a Mendelian inheritance pattern in a family with healthy parents and four daughters. The parents give one of their alleles to each of their children and there are four possible combinations in the children. Hence, on average, 25% of the children will share both alleles, 50% will share one allele, and 25% will not share any of the alleles. In this example, you can see a recessive trait model. Both parents are carriers of a disease-causing allele, and the child needs two alleles to get the disease, hence the disease is recessive.

In diseases with a dominant inheritance, there are typically affected individuals in every generation, as inheriting one disease allele is sufficient for disease development. Examples of dominant diseases are Huntington’s disease and myotonic dystrophy. Today, approximately 6000 genetic diseases with Mendelian inheritance have been reported (29). In about 1500 monogenic diseases, the mutated gene has been identified (30). The majority of these monogenic diseases are very rare. However, in the majority of the diseases, no single gene is sufficient for disease development. The disease is presumably the result of a complicated interaction of several factors. No clear inheritance pattern can be identified but these types of diseases usually cluster in families, which indicate genetic components. These diseases are defined as complex or multifactorial and they are often common in the human population as the disease risk alleles are thought to be common variants (31, 32). Examples of multifactorial diseases are cardiovascular diseases, cancer, and type 1 diabetes. Although a person inherits a risk allele, the phenotype is not always expressed, this tells us that the penetrance of each allele is reduced. Many of these diseases are described as multifactorial because commonly even environmental factors are necessary in combination with genes to produce the phenotype.

Genetic maps

The genome maps can be separated into two types; physical maps - which gives the distance between two loci in base pairs (bp) and the genetic maps - which gives the distance in centi-Morgan (cM). One cM is a 1% probability that a meiotic crossover (recombination) has occurred between two loci on the same haplotype from one generation to the next. An estimation of the relation between genetic and physical distance can be approximated to 1 cM = 1 Mb but this relationship varies across the
Genetic analysis of type 1 diabetes

Genetic markers

In family studies we try to find co-inheritance between disease phenotypes and genetic markers. There are different types of genetic markers; 1) Mini- and Micro-satellite markers consist of tandem repeats and differ depending on the number of these repeats. The repeats are flanked by unique basepair-sequences. The fragments are amplified and size-fractioned and different alleles are represented by different fragment lengths. 2) Single nucleotide polymorphisms (SNPs) is another type of marker to detect the difference in sequence by analyzing the polymorphism on a single basepair. More SNPs need to be used to get the same information as with the micro satellite markers, on the other hand the SNPs are more common and it is easier to automate their genotyping and finally they are more likely to represent the true disease-associated polymorphism. The SNPs can be analyzed using the RFLP method (restriction fragment length polymorphism); variations in the sequence make it possible for a restriction enzyme to cleave or not cleave a DNA fragment. The resulting fragments can then be separated on an agarose gel depending on the fragment length. This makes it possible to detect polymorphism (33). Nowadays more automated methods are used.

Linkage analysis

When the phenotype is based upon which genes that are inherited at a specific locus, there is a genetic linkage between the phenotype and the locus. This correlation can be seen when persons with a certain phenotype more often than expected share a certain allele. Linkage is the tendency for genes and other genetic markers to be inherited together because of their location near one another on the same chromosome. During meiosis the two homologous chromosomes exchange genetic material, a process known as recombination. The closer two loci are placed the less often are they separated by recombination. The recombination fraction is a measure of a genetic distance between loci. Recombination is necessary for the individual to develop and to be able to meet a changing environment. To measure the recombination fraction at a specific gene in correlation to a close gene one can tell whether these two are linked and not inherited independently of one another. In humans we have variability in the family structure, a limit in family size and a difficulty in defining the correct inheritance of an allele when parents are uninformative. The probability of a recombination to have occured is called the recombination fraction (θ). Recombination enables the construction of genetic maps with the use of genetic markers. Defined genetic markers are used to find the genes involved in a disease. A genetic marker close to the disease locus will be inherited together with the disease more often than expected by random segregation. When trying to map a susceptibility gene an inter-marker distance of about 10 cM is needed, otherwise we risk having a too large distance between the marker and the unknown disease causing gene and we might not detect linkage to any marker despite a disease locus being present.
When making linkage analysis we try to localize positions on the genome that are linked to type 1 diabetes. To make this type of analysis we have examined families where there are affected sibpairs.

Linkage analysis can be performed in a single-point or a multipoint fashion. In single-point analysis, linkage analyses are performed for each marker in relation to the disease locus, independent of the surrounding markers. Multipoint analysis refers to the simultaneous analysis of several markers with known location on a genetic map, which increases information in the region.

**Parametric linkage analysis**

In a parametric linkage analysis a genetic model is assumed detailing the disease gene allele frequency and penetrance. Evidence of linkage is then estimated for several different values of recombination fractions. This is a useful analysis when the genetic model is known and when the alleles of the parents and even grandparents are known (figure 5). The probability of linkage is given in the form of a logarithm of odds (LOD) score (to the base 10), this is the odds ratio between the alternate hypothesis (linkage exists, recombination fraction \( \theta < 0.5 \)) and the null hypothesis (no linkage exists, recombination fraction \( \theta = 0.5 \)). The null hypothesis (\( H_0 \)) is the prediction that an observed difference is due to chance alone and not due to a systematic cause. This is the case if the marker and the disease gene segregate independently of each other in the family, because there is no linkage between them. The frequency of recombination is then 50%, which is the definition of unlinked loci (34). The alternative hypothesis (\( H_1 \)) is that linkage exists between the marker and the gene. These two alternatives are compared in a likelihood ratio. The LOD score is the statistical estimate of whether two loci (the marker and the disease gene) are likely to lie near each other on a chromosome and are therefore likely to be inherited together as a package. A LOD score of three or more is generally taken to indicate that two gene loci are close to each other on the chromosome. A LOD score of three means that the odds are a thousand to one in favour of genetic linkage in the studied dataset and given that the genetic model used is correct. This corresponds to a significance level of 5% (35).

The linkage analysis results in a number of LOD scores at various values of \( \theta \) (between 0 and 0.5). The more positive the LOD is the stronger evidence for linkage while the more negative LOD score the more unlikely it is that linkage exists at that specific locus. In a set of families, the LOD scores can be added across families as the overall probability is the product of probabilities obtained in each existing family. To perform a parametric linkage analysis the genetic model of the disease needs to be known as it needs to be specified in order to estimate the number of recombinations between the marker and the disease locus. If the disease model is not known several different models need to be tested and there is an increased risk for false linkage or exclusion of linkage.
Figure 5. Parametric linkage analysis

The parametric linkage analysis. Loci are inherited independently of each other if they are located far apart. They are then not linked (θ=0.5). Loci located close to each other are not inherited independently of each other, they are linked. The number of recombinant (R) and non-recombinant (NR) offspring can be used for the calculation of linkage. In the example in this figure we have a dominant disease with 100% penetrance and known linkage phase. The LOD-score for this data at θ=0.2 is as follows:

\[
\text{LOD-score}(\theta) = \log_{10}\left(\frac{\theta R (1-\theta) NR}{0.5 R 0.5 NR}\right)
\]

If θ=0.2; LOD-score(0.2) = \log_{10}\left(\frac{(0.2) R (0.8) NR}{(0.5) R 0.5 NR}\right) = 0.42

The LOD-score for these data is 0.42 at θ=0.2. The LOD-score can be calculated for different values of the recombination fraction, (θ), and the disease locus is located at the distance corresponding to the maximum LOD-score. Thus θ is the hypothetical distance between the marker and the disease locus and the more recombinations the larger is θ at the maximum LOD-score.

R = recombinant, NR = non-recombinant, D = disease causing allele, d = normal allele

In many cases the true disease model parameters are unknown, especially in the case of genetically complex traits. The power of the linkage test is sensitive to the degree of dominance, the frequency of the disease allele and slightly to the penetrance, but not to the gene frequency (36). The estimation of the recombination fraction may be strongly affected by an error on any genetic parameter.

Therefore, a procedure called "MOD-score" analysis has been developed (37, 38). Hence, not only information about evidence for linkage and the disease-locus position can be calculated, but also a valid, ascertainment-assumption–free method is provided to estimate the disease model parameters. The linkage is estimated on every single locus on the chromosome by varying the model of inheritance as we for most multifactorial diseases do not know the correct genetic model.
Non-parametric linkage analysis

In the non-parametric linkage analysis the allele sharing between relatives, for instance sibpairs, with a certain phenotype is compared. This method does not demand a complete specification of a genetic model for the trait under investigation. Non-parametric linkage analysis is used when the mode of inheritance is unknown (figure 6). This is often a useful model for multifactorial diseases. The disadvantage with non-parametric linkage compared to parametric linkage is that the power to detect linkage is reduced assuming that a correct disease model was used in the parametric linkage analysis.

Affected sib-pairs are often used when making a non-parametric linkage analysis. Alleles can be inherited identical by state, which means that the affected sibs have inherited the same allele, but it does not say that the allele is inherited from the same parent. If the sharing of alleles is identical by descent (IBD) the ancestral origin has been determined. The sibpair analysis determines whether each sibpair shares 0, 1 or 2 alleles IBD. This is not always possible to determine due to uninformative relatives for instance because of homozygosity. Probability methods can then be used to maximize the probability of the unknown polymorphic genotype data with consideration to sharing 0, 1 or 2 alleles IBD. This method is referred to as maximum lod score (MLS) in a Mendelian model 25% of sibpairs share 0, 50% share 1 and 25% share 2 alleles IBD. The aim is to find loci that are inherited together more often than the expected 50% among the affected pairs. An increase in allele sharing between siblings will be identified as an increased MLS.

Figure 6. Non-parametric linkage analysis. Here we assume that all four parental alleles can be distinguished. The possible IBD sharing for a sibpair at a marker are 0, 1 or 2 alleles, with a probability of 25%, 50% and 25% respectively. The \( H_0 \) hypothesis expects that this will be true. The alternative hypothesis \( H_1 \) is excess allele-sharing and this is what we study in sibpair analysis among affected sibpairs. The MLS score is calculated as \( \log_{10}(L(H_1)/L(H_0)) \), where \( L \) stands for likelihood. For example in a population of 100 affected sibpairs; 20 pairs share 0 alleles IBD (20/100), 50 pairs share 1 allele IBD (50/100) and 30 pairs share 2 alleles IBD (30/100). Giving: \( L(H_0)=0.2^{20} \times 0.5^{50} \times 0.3^{30} \) and \( L(H_1)=0.25^{20} \times 0.5^{50} \times 0.25^{30} \). Thus \( \log_{10}(L(H_1)/L(H_0)) = 0.44 \) (Revised from Nyholt 2000 (39)).
Significance levels

It is important to define the significance levels for linkage well in these types of studies. A LOD-score of 3 tells us that the probability that the tested locus is linked is 1000 times greater than that it is not. This mostly corresponds to the level of significance used in conventional statistical analysis. P<0.05 is the most commonly used limit for positive results and it tells us that if we repeat the same study a hundred times we will obtain an outcome which was at least as significant as the observed one, five times due to chance.

When making genome wide linkage scans it is important to distinguish between the point-wise (nominal) significance level and the genome wide significance level. The point-wise significance level is corresponding to the observed value at a specific locus and is not taking into consideration that many analysis are being performed. The genome wide significance level is showing the probability that the observed value, by chance, will be exceeded anywhere on the genome. (40). The point-wise significance level must be set to a much more stringent value to reach a genome-wide significance of p<0.05. A genome-wide significance threshold of p=0.05 is achieved at a pointwise p value of p = 2.2 x 10^{-5}, which is equivalent to a LOD score of approximately 3.6. LOD score 2.19 (equivalent to pointwise P-value threshold of p = 7.4 x 10^{-4} is evidence for suggestive linkage this is expected to occur one time at random in a genome scan (34, 40-42).

<table>
<thead>
<tr>
<th>Significance Thresholds</th>
<th>LOD/MLS</th>
<th>MLS*</th>
<th>X-MLS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>p&lt;0.05</td>
<td>0.59</td>
<td>0.74</td>
<td>1.18</td>
</tr>
<tr>
<td>p&lt;0.01</td>
<td>1.18</td>
<td>1.38</td>
<td>1.90</td>
</tr>
<tr>
<td>p&lt;0.005</td>
<td>1.44</td>
<td>1.66</td>
<td>2.21</td>
</tr>
<tr>
<td>p&lt;0.001</td>
<td>2.07</td>
<td>2.32</td>
<td>2.93</td>
</tr>
<tr>
<td>p&lt;7.4x10^{-4}</td>
<td>2.19</td>
<td>2.45</td>
<td>3.06</td>
</tr>
<tr>
<td>p&lt;2.2x10^{-5}</td>
<td>3.63</td>
<td>3.93</td>
<td>4.62</td>
</tr>
<tr>
<td>p&lt;3x10^{-7}</td>
<td>5.3</td>
<td>5.76</td>
<td>6.52</td>
</tr>
</tbody>
</table>

*Programs based on methods restricted to biological possible allele-sharing (39, 43).
GENETIC PREDISPOSITION

That genetic factors play an important role in the risk to develop to type 1 diabetes is quite clear, but the mode of inheritance is complicated and still unclear. Almost nine of ten of persons developing type 1 diabetes do not have any first degree relative with the disease (44, 45). In the higher incidence countries the risk for an identical or monozygotic twin of a child with type 1 diabetes is between 30%-50% while it is about 4.8%-27% among dizygotic twins, and it is about 4.4%-12.5% risk for a sibling. (46-54). This is also supported by familial clustering, as illustrated by an estimated recurrence risk in siblings ($\lambda_s$) of 15 (55). $\lambda_s$ is the risk for a sibling to develop disease compared to the risk for a child in the general population.

The relatively low concordance between monozygotic and dizygotic twins tells us that environmental factors also play an important role in the development of the disease. A child of a parent with type 1 diabetes has less than 5% risk to get the same disease and if both parents have type 1 diabetes the risk is increased to between 10-20%.

HLA association

Autoimmune disease is the result when the immune system launch an attack against the body's tissues. The risk of developing autoimmune disease is often related to the alleles of HLA genes. Type 1 diabetes is quite unique among these diseases in that HLA alleles may increase the risk of diabetes, have no effect, or even be protective. Recently this phenomenon has also been shown also in multiple sclerosis (56). HLA (Human Leukocyte Antigen)-typing is made to determine which antigen within the HLA-system that is present in an individual. The HLA genes are located on chromosome 6. There are at least six loci that code for class 1 antigens and they are named HLA-A, HLA-B, HLA-C, HLA-E, HLA-F and HLA-G and there are allelic genes for each locus (figure 8). The genes encoding class II MHC proteins are most strongly associated with type 1 diabetes, and these genes are called HLA-DR, HLA-DQ, and HLA-DP and they also show polymorphism in the population (table 2). The polymorphism in these genes affects the ability to present antigens in the immunesystem.
The inheritance of certain alleles in the IDDM1 region can account for almost half of the genetic risk of developing type 1 diabetes (57). Associations between HLA alleles and diabetes began to be documented in the 1970s when serological markers were used (58-60). This association was later confirmed with DNA-based genotyping (61-65) and in genome-wide linkage and association scans (66-69). In the general population, only half of the people inherit either the DRB1*03 or the DRB1*04 alleles, and less than 3% of the people have both these alleles. However, in type 1 diabetes at least one of the DRB1*03 or the DRB1*04 allele is found in 90% of Caucasians. Individuals with both DRB1*03 and DRB1*04 are particularly susceptible to type 1 diabetes (70). The presence of both these alleles is found among 36.5% of type 1 diabetes patients in the Swedish population (table 3) (71, 72).

Conversely, the DRB1*15 allele is protective (73). Similar to the DR gene, certain alleles of the DQB1 gene are risk factors for developing the disease, whereas other alleles of DQB1 are protective. There is a strong linkage disequilibrium (LD) in the MHC region on chromosome 6 thus there is also a tendency for people who inherit DRB1*03 or DRB1*04 to inherit certain DQ alleles namely DQA1*0201, DQB1*0501, DQA1*0301, DQB1*0302 respectively. This adds to their genetic risk of developing diabetes. On the other hand, the protective alleles of DR and DQ also tend to be inherited together, so that DRB1*15 often is inherited together with DQA1*0102, DQB1*0602 (74, 75). These tendencies have complicated the study of the effects of individual HLA-DR or HLA-DQ genes. The HLA region (IDDM1 locus) contains several diabetes susceptibility genes, and it is difficult to identify the specific risk alleles because of this linkage disequilibrium; certain alleles tend to be co-inherited with other alleles, making it difficult to distinguish between the effects.

*Figure 8. Simplified map over the MHC genetic region on chromosome 6.*
Genetic analysis of type 1 diabetes

of either on diabetes susceptibility. Fine mapping of these regions suggests that the two genes DRB1 and DQB1 are the most important (76). One of the protective HLA haplotypes is, as mentioned above, DRB1*15-DQA1*0102, DQB1*0602. Approximately 20% of Americans and Europeans have this haplotype, whereas less than 1% of children with type 1 diabetes do (77). In Sweden, there are no DR15 positive children that have developed type 1 diabetes under the age of ten (75, 78, 79).

Type 1 diabetes, although called the juvenile-onset type of diabetes, has its onset after the age of 20 years in 50% of the cases (80). Type 1 diabetes with an adult onset shows quite different HLA class II gene profile, with a significantly higher percentage of non- DRB1*03/non- DRB1*04 in older patients compared to paediatric patients. Although non- DRB1*03/non- DRB1*04 patients are presented clinically as type 1 diabetes, they show a lower frequency of islet cell antibodies (ICA) at diagnosis and a significantly milder insulin deficiency. The Diabetes Prevention Trial-type I has identified 100 islet cell antibody (ICA)-positive relatives with the protective, DRB1*15-DQA1*0102-DQB1*0602 haplotype, far exceeding the number of such subjects reported in other studies worldwide (81). These results suggest that the immune response to different beta-cell auto antigens may be mediated via HLA class II molecules from different loci (82). In Norway, a study using the transmission disequilibrium test to analyze haplotypes for association and linkage to diabetes within families showed that protection was associated with the DQ alleles rather than DRB1*15 in linkage disequilibrium with DQA1*0102-DQB1*0602. DR15-DQA1*0102-DQB1*0602 was transmitted to 2 of 313 (0.6% vs. the expected 50% transmission) of affected offspring (P <0.0001) (77).

<table>
<thead>
<tr>
<th>Allele or haplotype</th>
<th>Patients %</th>
<th>Controls %</th>
<th>OR (95% CI)</th>
<th>p</th>
<th>AR</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1<em>04-DQA1</em>0301-DQB1*0302</td>
<td>74</td>
<td>28</td>
<td>14.0</td>
<td>0.0001</td>
<td>1/222</td>
</tr>
<tr>
<td>DRB1<em>03-DQA1</em>0501-DQB1*02</td>
<td>52</td>
<td>22</td>
<td>11.8</td>
<td>0.0001</td>
<td>1/255</td>
</tr>
<tr>
<td>DRB1<em>03-DQA1</em>0501-DQB1<em>02/DRB1</em>04-DQA1<em>0301-DQB1</em>0302</td>
<td>36.5</td>
<td>3.0</td>
<td>8.9</td>
<td>0.0001</td>
<td>1/48</td>
</tr>
</tbody>
</table>

Table 3. Odds ratio (OR) and absolute risk (AR) of DR and DQ phenotypes for type 1 diabetes in Swedish 0- to 15-year-olds (71, 72).
Genetic analysis of type 1 diabetes

Table 2. Frequency of common HLA DRB-DQA1-DQB1 haplotypes in the general Swedish population, Kockum et al 1999.

<table>
<thead>
<tr>
<th>DRB1*</th>
<th>DQA1*0</th>
<th>DQB1*0</th>
<th>Haplotype frequency %</th>
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</thead>
<tbody>
<tr>
<td>15</td>
<td>102</td>
<td>602</td>
<td>14.0</td>
</tr>
<tr>
<td>03</td>
<td>501</td>
<td>201</td>
<td>11.8</td>
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<td>01</td>
<td>101</td>
<td>501</td>
<td>8.9</td>
</tr>
<tr>
<td>401</td>
<td>301</td>
<td>302</td>
<td>8.3</td>
</tr>
<tr>
<td>13</td>
<td>103</td>
<td>603</td>
<td>7.7</td>
</tr>
<tr>
<td>11</td>
<td>501</td>
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<td>5.9</td>
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</tr>
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<td>08</td>
<td>401</td>
<td>402</td>
<td>3.8</td>
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<tr>
<td>07</td>
<td>201</td>
<td>303</td>
<td>3.0</td>
</tr>
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<td>09</td>
<td>301</td>
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<td>0.2</td>
</tr>
<tr>
<td>15</td>
<td>103</td>
<td>601</td>
<td>0.2</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td>5.5</td>
</tr>
</tbody>
</table>

A study from Norway clearly demonstrates that HLA associations in type 1 diabetes extend far beyond the well-known associations with the DR3 DQA1*0201, DQB1*0501 and DR4 DQA1*0301, DQB1*0302 haplotypes. The study suggest that there is a hierarchy of HLA class II haplotypes conferring risk to develop type 1 diabetes. Case-control analysis including the relative predispositional effect test, and transmission disequilibrium test (TDT) analysis in Norwegian type 1 diabetes families revealed that the DQA1*03-DQB1*0301, DQA1*0401-DQB1*0402, DQA1*0101-DQB1*0501, DQA1*03-DQB1*0303 and DQA1*0102-DQB1*0604 haplotypes may also confer risk (83).

The highest risk to get type 1 diabetes is if you are heterozygote for DR3 and DR4 (DRB1*03-DQA1*0501-DQB1*02/ DRB1*04-DQA1*0301-DQB1*0302).

The impact of HLA on the development of type 1 diabetes is also shown by studying the frequency of type 1 diabetes among siblings to type 1 diabetes patients. As mentioned above the risk for a sibling is about 4.4%-12.5% while for siblings carrying a high risk HLA haplotype, the risk is increased to between 12 and 18% (84, 85).
Non-HLA susceptibility genes

Other genes outside of the MHC have shown to be associated with type 1 diabetes and other autoimmune diseases. In addition other genetic regions have been reported to be linked to type 1 diabetes when analyzing sibpairs with the disease.

Association of the insulin gene

The contribution of the insulin gene region to type 1 diabetes susceptibility has been widely studied and the effect of the polymorphisms upon disease risk within the region is well established, although their exact function in the disease etiology is still to be unraveled. The IDDM2 allele that is associated with type 1 diabetes is common in the population and this has made it more difficult to show linkage to type 1 diabetes hence the relative risk then is low. IDDM2 maps to the insulin gene (INS) VNTR (variable number of tandem repeats) minisatellite on chromosome 11p15, located 596 bp upstream of the insulin gene (INS) translation starting codon (86-91). The Insulin VNTR consists of repeat units of 14–15 base pairs (92). Class I VNTR alleles contain 28 to 44 repeats and class III alleles 138 to 159 repeats. Intermediate class II alleles are rare in non-African populations (86, 93).

Several studies have shown that type 1 diabetes risk is associated with homozygosity for the class I VNTR haplotypes within the insulin gene region. The -23HphI and +1140A/C SNPs are effectively in complete linkage disequilibrium (LD) with the VNTR (93), which allows them to be used as surrogate markers for the VNTR. SNPs adjacent to VNTR were excluded as aetiological polymorphisms (87), but a recent association study of 177 polymorphisms in and around the IDDM2 region reinstated two SNPs, -23HphI and +1140A/C, as potential candidates for causal variant (94). For both SNPs, (-23HphI and +1140A/C) the VNTR class III homozygote (the protective alleles) showed a stronger protective effect than the heterozygote. Heterozygotes carrying a class III haplotypes together with a class I haplotype had a significantly lower risk of developing type 1 diabetes than the class I homozygote in the Finnish and Swedish populations (95). It has also been found that HLA-DR4-positive diabetes patients showed an increased risk associated with common variants at polymorphic sites in a 19-kb segment spanned by the 5-prime INS VNTR and the third intron of the gene for insulin-like growth factor II. In multiplex families the type 1 diabetes-associated alleles for polymorphisms in this region were transmitted preferentially to HLA-DR4-positive diabetic offspring from heterozygous parents. The effect was strongest in paternal meioses, suggesting a possible role for maternal imprinting (88). The results strongly support the existence of a gene or genes affecting HLA-DR4 type 1 diabetes susceptibility in a 19-kb region of INS-IGF2.

The polymorphism of the insulin gene seems to play a critical role in the pathogenesis of type-1 diabetes. The expression of insulin in human fetal thymus, a critical site for tolerance induction to self proteins, has been analysed (96, 97). Insulin was detected in all thymus tissues examined and class III VNTR alleles were associated with 2- to 3-fold higher INS mRNA levels than the class I VNTR. It is therefore proposed that the mechanism for the dominant protective effect of class III alleles is higher levels of thymic INS expression, facilitating immune tolerance induction. This suggests that diabetes susceptibility and resistance associated with IDDM2 may derive from the VNTR influence on INS transcription in the thymus. On the other hand a low expression of insulin in thymus allow autoreactive T-cells
to be released and to be expressed in the pancreas which leads to destruction of the B-cells and the developing of type 1 diabetes (96, 97).

**Association of the CTLA4 gene**

The CTLA4 (Cytotoxic T Lymphocyte antigen 4)/CD28 gene region on chromosome 2q33 (IDDM12) demonstrates association and linkage to the type 1 diabetes. The A/G SNP in exon 1 (49 A/G) of the CTLA4 gene encoding a regulatory molecule in the immune system associate with type 1 diabetes and autoimmune thyroid diseases. An alanine at codon 17 of CTLA4 is associated with genetic susceptibility to these diseases (98-101). The 3' untranslated region of exon 4 on this gene is another polymorphic site of the CTLA4. This site determines the level of soluble CTLA-4 (102). A third polymorphism is a C to T transition at position -318 of the promoter sequence (103). CTLA4 polymorphism has also been associated with celiac disease (104) Grave's disease, rheumatoid arthritis and multiple sclerosis and they all demonstrate associations to the A/G single nucleotide polymorphism (SNP) in exon 1, position +49 of the CTLA4 gene (105-108).

**Association of the PTPN22 gene**

In 2004 it was discovered that a single-nucleotide polymorphism (1858C/T) in the protein lymphoid tyrosine phosphatase (LYP), encoded by the PTPN22 gene on chromosome 1p13, is associated to type 1 diabetes (109). One function of Lyp is down regulation of T-cell signalling through its interaction with the negative regulatory kinase C-terminal Src tyrosine kinase (Csk). Individuals lacking the C allele of PTPN22 may have reduced capacity to down regulate T-cell responses and may therefore be more susceptible to autoimmunity (110). Several studies have confirmed that PTPN22 is associated with type 1 diabetes (111-116). The T allele of the 1858(C/T) polymorphism is the major risk variant for type 1 diabetes in the PTPN22 locus, but it has been suggested that additional infrequent coding variants at PTPN22 may also contribute to type 1 diabetes risk (117). Disease association has been reported also in studies of other autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, Graves' disease and generalized vitiligo (118-120).

**Association of the IL2RA gene.**

The candidate gene IL2RA, the CD25 region of chromosome 10p15, encodes a subunit (IL-2R alpha) of the high-affinity interleukin-2 (IL-2) receptor complex and has, been suggested to be associated to type 1 diabetes. (121, 122). The two SNPs (rs706778 and rs3118470) that have shown association with type 1 diabetes are located in the 5' end of the long intron 1 within 3 kb of each other and are in high linkage disequilibrium. The A-C haplotype is indicating association with increased risk of developing type 1 diabetes (123).
Genetic analysis of type 1 diabetes

Association of the IFIH1 gene.

There is also support for a type 1 diabetes locus in the innate immunity viral RNA receptor gene region IFIH1 (mda-5 or Helicard) on chromosome 2q24. The association is found in an interim analysis of a genome-wide nonsynonymous SNP (nsSNP) scan, and validated in a case-control collection and replicated it in an independent family collection. The risk ratio for the minor allele of the nsSNP rs1990760 A --> G (A946T) was 0.86 (95% confidence interval = 0.82-0.90) at \( P = 1.42 \times 10^{-10} \) (124).

Association of the ITPR3 gene

In the region centromeric to the MHC, a peak of association within the inositol 1, 4, 5-triphosphate receptor 3 gene (ITPR3; formerly IP3R3) has been identified. The most significant single SNP in this region was at the center of the ITPR3 gene. Two-locus regression analysis supports an influence of ITPR3 variation on type 1 diabetes that is independent of from that of any MHC class II gene (125).

Association of the FOXP3 gene.

Associations between common polymorphisms in FOXP3 and type 1 diabetes have resulted in conflicting results. A Japanese study found a disease association with the \((GT)_{35}\) allele at the microsatellite located in intron zero of the FOXP3 gene (126). Type 1 diabetes families from Sardinia have been genotyped for twelve polymorphisms in the FOXP3 gene including the two microsatellites from the study of Bassuny et al, without finding any evidence of association with type 1 diabetes (127). One study has studied if the previously shown evidence of association between the FOXP3 polymorphisms and type 1 diabetes was seen in the Caucasian population and their results suggest that the tested FOXP3 markers do not have any major impact on susceptibility for this disease (128, 129).

The involvement of the FOXP3 gene in the development of regulatory T-cells is obvious, but whether it has an impact in the risk of developing type 1 diabetes remains to be shown (130).

Other type 1 diabetes susceptibility loci

In 2007 a large genome wide association study was presented (68, 69). SNPs were placed all over the genome. The study confirmed the previously shown association with type 1 diabetes and MHC, INS, CTLA4, PTPN22, IL2RA and IFIH1. These loci can only explain some of the familial associated clustering and more genes are assumed to be involved in the pathogenesis of type 1 diabetes. Four chromosome regions not previously associated with type 1 diabetes were showing strong association in the genome scan and was replicated in independent populations. These regions were 12q13, 12q24, 16p13 and 18p11. The 12q13 and the 12q24 regions map to more than ten genes that are suggested to have a role in insulin and immune signaling. One of these genes is the PTPN11, a regulatory phosphatase gene that is suggested to play a major role in the immune signaling. The 16p13 region contains two genes of which one is the KIAA03050. The 18p11 region maps to the PTPN2 gene, which is involved in immune regulation. The scan also found association with the chromosome 10p15 region. This region contains CD25 which encodes the IL-2
receptor. The CD25 region is shown to be associated also with Graves disease (131). Evidence of association is also seen on chromosome 4q27, a region containing genes encoding IL-2. In independent populations this region showed weak association with type 1 diabetes (69). Neither the chromosome 12p13 (CLEC2D gene) nor the 5q14 association found in the genome wide association study were able to replicate in the independent populations (69).

Figure 9 and table 4 show the genetic regions that have been linked and/or associated to type 1 diabetes. HLA (IDDM1) is the region that in all studies has shown the strongest linkage and association. In the table is listed each regions contribution to the familial aggregation ($\lambda_s$ or Odds Ratio). The linkage analysis was made with sib pair analysis in informative families, a “maximum likelihood test” was used in families that were not totally informative (55). The association studies were made in patient families and patients and controls.

Figure 9. The type 1 diabetes risk gene loci placed over the genome.
Stars are the loci that have shown evidence of association to type 1 diabetes (69).
Dots are the loci identified in linkage studies.
Table 4. Chromosome regions that show evidence for linkage and/or association to Type 1 diabetes mellitus.

The markers are the ones that show evidence for linkage and/or association in the analysis.

† Locus specific ($\lambda_s$ is a measure of how big part of the familial aggregation that is caused by a specific gene)

* $\lambda_s$ value taken from Todd et al (69).
ENVIRONMENTAL FACTORS

Together with genetic factors, environmental factors are involved in the pathogenesis of type 1 diabetes. The rapid increased incidence suggests that environmental factors play an important role. It has also been shown that there is a broadening of HLA genotypes among diabetes patients and this also speaks for the importance of environmental factors (152, 153).

Dietary factors

Dietary factors have been suggested as possible risk factors for type 1 diabetes. There have been studies showing a relation between a short period of breast feeding and a high risk of type 1 diabetes (154-157), but other studies have not been able to confirm this (158). An early introduction of cow’s milk has been discussed as a risk factor (159, 160). A high gain in weight early in life has also been shown to be associated with type 1 diabetes (161).

In a study of 7208 2 ½ year old children from south-east of Sweden it has been shown that short duration of breast-feeding, an early introduction of cows milk formula and a late introduction of gluten, as well as a high consumption of milk at the age of 1 year, were dietary risk factors for the induction of beta-cell autoantibodies in the 2 ½ year old children (162).

Many other dietary factors have also been suggested as risk factors; meat, protein, coffee, cereals, sugar (163-165), whereas vitamin D has been shown to have a protective effect (156).

Viruses

Viral infections with enteroviruses (166-169) and even for instance cytomegal-(170.171), rubella-(172-174), rota-(175) and ljunganviruses (176-178) during fetal life or later have been suggested to be risk factors of type 1 diabetes. It is not known whether the infection causes a direct damage in the beta-cells or if it triggers an autoimmune reaction that will injure the beta-cells. One theory is that these factors stress the beta-cells to produce more insulin and that this stress leads to inflammation and death of the beta-cells.

It has been shown that more persons are diagnosed with type 1 diabetes during the winter (9, 179-181). One theory is that this is due to an increased number of viral infections during the cold winter months. The infections cause stress which leads to in increased blood sugar, causing an extra burden on the B-cells and if many of the cells are already damaged the rest can not make a sufficient amount of insulin to keep the blood sugar at a satisfying level and the diabetes symptoms will appear.

Living conditions and hygiene

It has also been proposed that improved living conditions, including a better hygiene, causes a lower frequency of infections in the background population which leads to a lower level of protective antibodies against viruses in the mothers and children. This can cause more severe infection and an increased risk for type 1 diabetes. There is a large variation in incidence between different countries with a higher incidence in the countries with the best living conditions (182).
Psychological stress

Psychological stress, measured as psychosocial strain in the family, seems to be involved in the induction or progression, of diabetes-related autoimmunity in the child during the 1st year of life (183).

To better understand which combinations of genes and different environmental factors that will lead to a high risk to develop type 1 diabetes is one of the major targets in the type 1 diabetes research.
**PATHOGENESIS:**

That type 1 diabetes is an autoimmune disease seems quite clear (82, 184-189). Antibodies and auto reactive T-cells cause lymphocyte infiltration of the islet cells in pancreas and this inflammation is associated with a loss of the beta-cells in the pancreas. Insulin deficiency is a result of the autoimmune destruction of the insulin producing pancreatic beta cells.

Almost every cell in the body is technically an antigen presenting cell, since it can present antigen to the T-cells via MHC molecules. But there are cells, for instance macrophages and phagocytes, specialized for this purpose. They can activate and stimulate the T-cells to become helper (CD4+) cells as well as cytotoxic (CD8+) T-cells.

If T cells bind to the antigen presented on an MHC molecule, the T-cell is powerfully attacking the antigen presenting cell. Ideally, the body only contains T-cells that bind to antigens from infectious organisms (viruses, bacteria, etc.) and tumour cells. The alternative is found in autoimmune diseases such as type 1 diabetes: T-cells bind to antigens from the body's healthy cells and starts reacting against these healthy cells. This is the result when autoreactive T-cells have passed through the selection in thymus causing the individuals to carry T-cells able to react against the body's own tissues.

If a T-helper cell meets an antigen on a MHC-class two molecule the cell will be activated which causes a large amount of T-memory cells to be made. These will meet the combination antigen and MHC wherever in the body they are and they will start a reaction similar to delayed sensitivity, which means that the T-helper cells will be activated, start proliferation and make cytokines. The cytokines will recruit and activate other immune system cells that will produce aggressive substances that damage the tissue (figure 10).

Insulin deficiency in type 1 diabetes is usually the result of a destruction of the B-cells in the islet of Langerhans in pancreas. When a patient is getting symptoms of type 1 diabetes with hyperglycemia there is an infiltration of B- and T-lymphocytes and macrophages which bring reactivity to islet cell autoantigens.
Figure 10. The antigen presenting cell has different HLA alleles presented on the surface and depending on which allele that is presented; there is different affinity of peptide binding of the insulin molecule. If Lyp and CTLA do not down regulate the T-cell activity then it will more easily be activated by the antigen presenting cell.

It is thought that not only autoreactive T-cells but also autoreactive B-cells are important in the development of type 1 diabetes.

Antibodies, also called immunoglobulin, are proteins, normally produced in response to a foreign substance - the antigen-, for instance an infectious organism within the body. Normally, the immune system is able to ignore the body's own cells and to not overreact to non-threatening substances in the environment. Sometimes, however, the immune system does not manage to recognize one or more of the body's normal constituents, and this leads to production of autoantibodies. These autoantibodies attack the body's own cells, causing inflammation and damage.

At time of diagnosis of type 1 diabetes about 60% of new onset children show signs of insulinitis (190) and islet autoantibodies may be present in more than 90% of the patients. There are four main autoantibodies associated with type 1 diabetes (191) IAA (Insulin Autoantibodies) (192-194), GAD (Glutamic Acid Decarboxylase) (195, 196), ICA (Islet Cell Autoantibodies) (197, 198) and IA2 (protein tyrosine phosphatase) antibodies (199). To have one or more antibodies is one of the criteria to get the diagnosis autoimmune type 1 diabetes. The autoantibodies will during the first years of disease disappear. This is due to the fact that all autoreactive B-cells have died because there are no antigens left to stimulate them.

We do not know exactly today what triggers the inflammatory process, but most authors agree that it is a combination of genes and environmental factors that are involved.

When there have been attempts to transplant beta cells to a patient with type 1 diabetes the beta cells are being destroyed in a cell mediated reaction without any
newly produced autoantibodies (200-202) and the body also tries to reject the transplanted beta cells despite the patient taking immunosuppressive drugs. Therefore this is still not a useful way to cure type 1 diabetes.

There is a prolonged prediabetic stage, in which the autoantibodies are present and measurable (figure 1). This gives the opportunity to predict and prevent the clinical onset of disease. While the presence of a single autoantibody has been shown to be a weak predictive marker for the eventual onset of type 1 diabetes, the presence of additional antibodies suggests a greater risk (203,204).
DIFFERENT NUMBER OF CHILDREN WITH TYPE 1 DIABETES TO MOTHERS AND FATHERS WITH TYPE 1 DIABETES.

There are many questions left to be answered and many problems yet to be solved before we can understand the development of type 1 diabetes.

When looking into the genetics one can see many interesting parts to deal with and I started to wonder about the difference in risk for children born to men and women with type 1 diabetes. There are several studies reporting that it is 2-4 times more common that fathers compared to mothers of children with type 1 diabetes are diagnosed with type 1 diabetes. The risk for child with a father with type 1 diabetes to develop the disease is estimated to be 7%, while the risk for a child with a mother with type 1 diabetes is estimated to be 2% (52, 205-209). What can be the reason for this phenomenon?

The Eurodiab Ace Study Group and the Eurodiab Ace Substudy Group have studied families in which more than one affected first-degree relative was diagnosed before the age of 15 years and found a positive association between the population incidence rate of type 1 diabetes and the prevalence of type 1 diabetes in fathers of affected children. Pooling results from all centres showed that a greater proportion of fathers (3.4%) of affected children had type 1 diabetes than mothers (1.8%) giving a risk ratio of 1.8. Affected girls were more likely to have a father with type 1 diabetes than affected boys, and there was no evidence of a similar finding for mothers or siblings (210).

Several hypotheses have been tested in order explain the differences in frequency of type 1 diabetes in fathers and mothers with type 1 diabetes.

Do women with type 1 diabetes choose to have fewer children than men with type 1 diabetes? This could, if it is the case, be due to the difficulties in keeping a normal blood sugar during pregnancy and the increased risk of lesion of the fetus (211, 212). Could there be an increased risk of spontaneous abortions for the fetus carrying the type 1 diabetes risk genes if the risk genes are inherited from the mother? This would result in fewer children with type 1 diabetes to type 1 diabetes mothers compared to fathers.

As there are more men than women that develop type 1 diabetes this would lead to more fathers than mothers with type 1 diabetes. Harjutsalo et al ascertained the families through the diabetic parents and not through the affected offspring which avoid the bias due to type 1 diabetes being more common among men than women (60% vs. 40% for adult onset type 1 diabetes). They also took into account other factors that may lead to an apparent preferential transmission, for example misclassification of gestational diabetes as type 1 diabetes, coupled with fecundity difference between the two sexes. They showed that the risk of type 1 diabetes in the offspring of the diabetic fathers was 1.7 times higher than that in the offspring of the type 1 diabetes mothers even after correcting for these factors (206).

Studies have also been performed to test the hypothesis that increased transmission by the father of genes predisposed to diabetes may explain this phenomenon (213). The parent-to-offspring transmission of HLA haplotypes and DR alleles in 107 nuclear families in which a child had type 1 diabetes was examined. They found that fathers with a DR4 allele were significantly more likely to transmit this allele to their diabetic or nondiabetic children than were mothers with a DR4 allele (214). But this finding has not been replicated (75).
There might also be a protective environment for the fetus having a diabetic mother. Bleched et al show decreased risk of type 1 diabetes in offspring of mothers who acquire diabetes during adrenarchy, but they can not determine that presence of diabetes in mothers before pregnancy has an effect on the risk of diabetes in their offspring (215). One can speculate that if the mother still has autoantibodies towards insulin producing cells it could lead to the development of tolerance towards type 1 diabetes later in life. The fact that the child is exposed to the mother’s high risk HLA haplotype might also have this effect. This would be consistent with the hypothesis that an immunological process is starting during fetal life and that this would affect us many years after birth.

Other possible explanations for the difference in risk for offspring to men and women with type 1 diabetes include an effect of non-inherited maternal HLA-haplotype and imprinting of type 1 susceptibility genes. I have studied these phenomenons and they are therefore discussed in more detail below.

**NIMA**

Many studies are suggesting that NIMA (Non Inherited Maternal Haplotype/Antigen) but not NIPA (Non Inherited Paternal Haplotype/Antigen) influences the immunological repertoire and that this is affecting the response when a person is challenged with an antigen. A few studies have been made on type 1 diabetes and the influence of NIMA.

A study concerning the impact of feto-maternal tolerance in haematopoietic stem cell transplantation shows that the NIMA-specific tolerance is maintained by an immunological mechanism (216). It has been shown that female cells were detected in multiple tissues when studying four male infants who never received a blood transfusion and died during the first week of life. They conclude that maternal cells enter fetal circulation and that this is of importance with regard to postnatal development of autoimmune disease (217). It has also been shown that kidney transplants from a sibling donor who is mismatched with the recipient for one HLA haplotype, have a higher graft survival if the donor is matched for NIMA compared to when it is matched for NIPA (218). Interestingly even breast feeding was in an early study shown to be associated with a more favourable renal post transplant course (219). It has also been demonstrated that oral and intravenous exposure to NIMA in the fetus and newborn is much higher than previously thought. In a review article it was discussed that there is evidence that three HLA haplotypes, those inherited from the parents plus NIMA, shape the immune response. It is suggested that NIMA has a life-long effect on allograft recognition that could influence tolerance of organ grafts and that NIMA also appear to influence disease susceptibility (220). It was also indicated that HLA-disparate maternal cells can persist in immunocompetent offspring well into adult life (221). The conclusion was that there is biological significance of maternal microchimerism and whether it might contribute to autoimmune disease requires further investigation.

A study has also been performed where NIMA among type 1 diabetes patients and healthy controls was compared. This study showed increased high type 1 diabetes risk NIMA haplotypes among healthy individuals compared to type 1 diabetes patients (222). On the other hand one study shows significantly increased positively associated NIMA compared to positively associated NIPA among type 1 diabetes patients (223) but two other studies have not confirmed any of these findings (224,
In these studies NIMA in healthy individuals was not compared to type 1 diabetes patients’ NIMA.

Imprinting

Another possible contributing explanation for the risk in the offspring to male and female type 1 diabetes patients, is genomic imprinting which leads to an unequal expression of the maternal and paternal alleles for a diploid locus. In our genome we have two copies of every somatic cell. The autosomal genes are therefore represented by two copies, or alleles, with one copy inherited from each parent at fertilization. For the vast majority of autosomal genes, expression may occur from either allele. However, in a small proportion of genes (<1%) the expression occurs from only one allele. The expressed allele is dependent upon its parental origin. These genes are imprinted.

For example, the gene encoding insulin-like growth factor II (IGF2/Igf2) is only expressed from the allele inherited from the father (226). It is now known that there are approximately 80 imprinted genes in humans and mice (29 in both human and mice), many of which are involved in embryonic and placental growth and development (227-230). The maternal (inherited from the mother) allele is expressed exclusively because the paternal (inherited from the father) allele is imprinted or vice-versa. Imprinting is a dynamic process. Imprinted regions are observed to be more methylated and less transcriptionally active (286). There are two major mechanisms that are involved in establishing the imprint; these are DNA methylation and histone modifications. Methylation in vertebrates typically occurs at cytosine-phosphate-guanine sites; that is, where a cytosine is directly followed by a guanine in the DNA sequence. The methylation state of these CpG sites can have a major impact on gene expression. Methylation of promoters seems to prevent binding of transcription factors to the promoter thus shutting down expression of the gene. In biology, histones are the chief protein components of chromatin. They act as spools around which DNA winds and they play a role in gene regulation.

Examples of genetic disease due to imprinting are Prader-Willi and Angelman syndromes. They map to 15q11 in humans. This region is differently imprinted in maternal and paternal chromosomes, and both imprints are needed for normal development. If neither copy of 15q11 has paternal imprinting, the result is Prader-Willi syndrome (characterized by hypotonia, obesity, and hypogonadism). If neither copy has maternal imprinting, the result is Angelman syndrome (characterized by epilepsy, tremors, and a perpetually smiling facial expression). Studies have been performed concerning imprinting mechanisms in type 1 diabetes. It has been shown that in families from the USA, the class I allele of the insulin gene (the high risk allele in type 1 diabetes) is transmitted preferentially from fathers. However, in families from the UK, Denmark and Sardinia, the maternal transmission is shown to be stronger (231). At IDDM10, evidence for excess sharing of the maternal allele has also been detected, while IDDM8 and a locus on chromosome 16q have been showing a paternal origin effect (232).
1. AIMS AND HYPOTHESIS

Type 1 diabetes is an autoimmune disease resulting from the destruction of beta cells in the endocrine pancreas. The process probably starts early in life or during fetal life, but there are no clinical signs of the disease until about 80% of the beta cells have been destroyed. Why do some people develop type 1 diabetes? What make the disease process start? What different factors, genetic and environmental, need to be combined before a patient develops symptoms? Why is there a difference in prevalence between gender and between the incidence in children to mothers and fathers with type 1 diabetes?

AIMS OF THE STUDY

I To estimate the frequency of first degree relatives with diabetes among patients diagnosed with type 1 diabetes between 15 and 34 years of age in Sweden. To test the hypothesis that there is a difference in the number of relatives with diabetes between women and men with type 1 diabetes.

II To identify non-HLA type 1 diabetes risk loci by performing a genome-wide linkage analysis.

III To test the hypothesis that the HLA-haplotype that is not inherited from the mother affects the risk of developing type 1 diabetes.

IV To test the hypothesis that there are imprinted type 1 diabetes susceptibility genes.
MATERIALS

I  Since January 1983, all newly diagnosed cases of diabetes mellitus in Sweden in the age group 15–34 years have been prospectively reported to the DISS (the Diabetes Incidence Study in Sweden) registry (5). During the 11-year period 1983–1993, 4466 patients were reported. At the time of follow-up (15 April 1994) 63 had died, 30 had emigrated and 72 did not have a known address (Fig. 1, paper I). A questionnaire was sent to the remaining 4301 patients in December 1994. The questionnaire asked the index case to give the date of birth of their first-degree relatives and if they were diagnosed with diabetes we asked for the date of diagnosis and their current treatment (insulin, oral or diet treatment). The patients were also requested to indicate their current treatment and date of diagnosis. The response rate was 78.7% and we received 3343 correctly filled in questionnaires.

II and IV DNA from families with at least two affected individuals other than parent-child pairs was collected in Sweden, Norway, and Denmark (table 5). Individuals were considered affected (1) if they had been diagnosed with diabetes before the age 15 years, required insulin treatment at the time of onset, and had remained on insulin treatment subsequently or (2) if diabetes with either ketoacidosis or requirement of insulin therapy from time of onset had been diagnosed after the age of 15 years. The Swedish families were identified through the two nationwide incidence registries: the Swedish Childhood Registry (4, 233, 234) and the Diabetes Incidence Study in Sweden (DISS) (5, 15). The childhood diabetes registry has since 1977 included about 99% of all children below the age of 16 diagnosed with type 1 diabetes in Sweden. Three hundred of these had in 1994 at least one sibling with type 1 diabetes at the time when they were diagnosed. We have blood samples from 184 families with at least two siblings with type 1 diabetes.

The Norwegian families were recruited during 1993–95, through advertisements, in the journal of the Norwegian Diabetes Association. The Danish families were identified through the Danish Study Group of Diabetes in Childhood (235), and the Danish IDDM Epidemiology and Genetics Group (236). In all, 408 multiplex families from Scandinavia, comprising 464 affected full-sibpairs, were studied.

126 families with 158 affected sibpairs were available from France, and 255 families with 310 affected sibpairs were available from the United States (141). The French and American families were only included in paper II in the analysis of IDDM15. To get a larger material the populations were combined in this analysis.
III  Four hundred and seventy nine Swedish type 1 diabetes families were included in this investigation. Among the families, 222 were multiplex families with at least two affected individuals from all over Sweden. We identified these multiplex families through the childhood diabetes registries in Sweden (4, 233, 234) and the Diabetes Incidence Study in Sweden (DISS) (5, 15). Most of these families were also included in paper II and IV. There were 237 simplex families also identified through DISS as well as from the pediatrics clinics in Lund and Jönköping. A total of 55 control families were studied. The control families were from two Swedish clinics (Jönköping and Linköping) located in the type 1 diabetes high risk part of the country. The Linköping children were schoolmates to a child with type 1 diabetes and the Jönköping controls were healthy 12 year-old children. The control group included both healthy siblings within the families where one or more children were affected with type 1 diabetes and the children in the healthy control families. These groups were also analyzed both separately and together. The result for each group was similar.

IV  The same Scandinavian subjects that were used in the genomescan analysis were investigated (paper II).

METHODS
I  A questionnaire was sent to patients in the Diabetes Incidence Study in Sweden registry diagnosed between 1983 and 1993 to determine the presence of first-degree relatives with diabetes. Comparisons of frequencies between groups were made using
\( \chi^2 \) tests. Analysis of survival times was carried out using the LIFETEST procedure in the SAS (SAS Institute, Cary, NC, USA) computer program package. The product-limit or Kaplan–Meier method was used as recommended (287). The log rank test was used to test whether the observed difference between the survival curves was due to random variation or not. Confidence intervals (CIs) were calculated as 95% CIs.

II DNA was extracted by standard procedures, by either phenol/chloroform extraction or a salting-out method (237).

We made the genome-wide linkage study using the Medical Research Council (MRC) microsatellite panel (144). These microsatellite markers were characterized by PCR amplification with fluorescence-labeled primers followed by size separation on polyacrylamide gels in ABI373 or ABI 377 sequencing machines where the alleles were separated depending on the amplified fragments size followed by analysis using GENESCAN. The allele assignments were made by GENOTYPER. In all, 324 markers were genotyped and included in the scan before quality control was performed. Family structures were corrected on the basis of the genome-wide-scan data, by the SIBERROR program (238). Half-sibs and MZ (monozygotic) twins were identified, and one member in each MZ pair was excluded from further analysis. The quality-control tests were performed for each marker using the PEDCHECK program (239). The observed number of homozygotes was compared with the number expected on the basis of the observed allele frequencies, by a \( \chi^2 \) test. A cut-off of \( P = 0.001 \) was used. These tests led to the exclusion of six microsatellite markers and we could use a total of 318 microsatellite markers for analysis.

The genetic map was assessed by both the CRIMAP program and the ASPEX program (240, 241). The average spacing between the 318 markers was 11.3 cM.

The HLA-DRB1 (MIM 142857) genotype was determined by allele-specific PCR for the DR3 and DR4 alleles (242, 243) and the INS polymorphism INS-23/HphI was genotyped by PCR amplifying followed by cleavage using a restriction enzyme and separated due to size on an agarose gel to determine the susceptibility status at the INS (IDDM2) locus (89). Linkage was assessed by nonparametric linkage methods. First, the data were scanned by programs including only nuclear families. We used the ANALYZE package and the SIBIBD program from ASPEX (244). The DOWNFREQ program was used to estimate allele frequencies in all family members. For chromosome X, MAPMAKER/SIBS (245) was also used for linkage calculations, as suggested (39). We further investigated loci reaching a LOD score >2.0 in ALLEGRO (246), which allowed the inclusion of all affected family members (246). Locus-specific \( \lambda S \) was estimated as described by Risch (55).

TDT analysis was performed by the ANALYZE program, with all affected siblings in each family (247). We stratified based on the high risk HLA haplotypes. For IDDM15 on chromosome 6q, analysis was performed to correct for linkage to the HLA region (141). Marker D6S283 was chosen as the marker locus for the IDDM15 locus (141).

III Experimental analysis The HLA DR typing is made with either RFLP or sequence specific amplification and genomic typing by PCR (Polymerase Chain Reaction) (75, 79, 248, 249). The HLA DQA1 and DQB1 genotyping was performed
either with Synthetic sequence-specific oligonucleotide (SSO) or Perkin Elmer Hybridization Assay DELFIA®. In the SSO typing the PCR products were dot-blotted onto nylon membranes. SSO probes were 3’ end-labelled with $^{33}P$-dCTP and used for hybridization. This was followed by stringency washes as described (250). The DQA1 and DQB1 genotyping of approximately 160 of the families was made with the Perkin Elmer Hybridization Assay DELFIA® (251). The basic probeset allowing for detection of DQB1*02, 0301, 0302, 0304, 0602, 0603 and 0604 was used. We also analyzed individuals carrying DQB1*02 DQA1*03, DQA1*0201 and DQA1*0501 in order to distinguish between the DQB1*02 –DQA1*0501 and DQB1*02-DQA1*0201 haplotypes.

**Statistical analysis** The differences of distribution of haplotypes between groups were tested by chi square analysis with Yates correction in 2x2 contingency tables. A p-value <0.05 was considered significant. The 95% confidence intervals of frequency estimations were calculated using the Bernoulli Model for categorical populations with two categories as described (252). The analysis was stratified based on the haplotypes present among the children. A meta-analysis of the current and previously published studies was performed using Woolf’s method for testing for heterogeneity (253).

**IV** We have used 318 microsatellite markers placed with an average distance of 11.3 cM. For details concerning genotyping methods see paper II. In the current investigation we estimated male, female and sex average genetic maps in the studied families using CRIMAP v2.4 (240). As expected the female genetic map was longer (on average 1.80 times longer). Two separate linkage analyses were performed; a non-parametric (model-free) allele sharing analysis using ASPEX v2.3 (254) and a parametric linkage analysis using GENEHUNTER MODSCORE v2.0 where a maximization of linkage is performed by varying the genetic model (255, 256). Sibpairs formed with the first affected sibling in the family were included in the analysis and the IBD sharing among affected sibpairs was limited to 50% or more. Estimated sex specific maps were used and we performed linkage analysis both for paternal and maternal meioses separately and for all meioses combined. Allele frequencies were estimated in all families included in the linkage analysis. The GENEHUNTER MODSCORE v2.0 program can maximize the LOD score with respect to the disease allele frequency and the penetrances. The program can perform a parametric multipoint linkage analysis and take imprinting into account. This is done by distinguishing individuals who are heterozygous at the disease locus with respect to which parent who transmitted the disease allele. A separate maximization of the disease model was performed for each position along the chromosome using the modcalc single command. First we performed a parametric (LOD-score) analysis of traits caused by imprinted genes, i.e. showing a parent-of-origin effect. By specification of two heterozygote penetrance parameters, paternal and maternal origin of the mutation can be treated differently in terms of probability of expression of the trait. Therefore, a single-disease-locus-imprinting model includes four penetrances (homozygotes for wildtype allele, homozygotes for disease allele and heterozygotes with the disease allele inherited from mother and father respectively) instead of only three (37). I gives a measure of the level of imprinting. Values of the index I close to 1 indicate maternal imprinting whereas values of the index I close to -1 indicate paternal imprinting (37).
RESULTS AND DISCUSSION

Many studies have been performed in order to establish the risk for type 1 diabetes among first degree relatives of children with type 1 diabetes. The risk is estimated to be approximately 10% (4, 233, 234). Among young adults, type 1 diabetes is also a common disease with about 300 individuals (15-34 years of age) developing the disease every year in Sweden (14, 15). For comparison more than 600 children between 0-15 years of age develop type 1 diabetes in Sweden every year (SWEDIABKIDS, 2007).

We wanted to determine the risk to develop diabetes among first degree relatives to young adults with type 1 diabetes. We were able to get the address of 96.3% of the 4466 patients that had developed type 1 diabetes between in the age of 15-34 years from 1983 to 1993. (78.7%) persons returned their questionnaires and of these, 3087 were treated with insulin at time of the questionnaire. One of the major difficulties in the beginning of this study was to classify the patient correctly. We could not check medical records or visit each relative with diabetes. We were, however, able to use data concerning the distribution of type 1 and type 2 diabetes in 2084 insulin-treated patients by age at onset collected in Sweden in a separate study during the same decade as the current investigation. Insulin-treated patients were classified as type 1 (26.9%), type 2 (71.2%) or secondary/unclassified (1.8%) diabetes by the treating physician. The distribution of type 1 diabetes among insulin-treated patients varied between 100% (for patients with current age 0–10 years) to 0.7% (for patients with current age 71–80 years). We adjusted the number of insulin-treated cases among the relatives to expected counts of type 1 diabetes, using these data. Even after this adjustment there was a significantly higher risk for relatives of female compared with male patients ($P = 0.03$).

We analyzed the number of relatives with diabetes among the type 1 diabetes patients and we made a subgroup of the patients classified as having type 1 diabetes at the time of diagnosis that still were on insulin treatment at follow up (n=2537). At follow up a total of 3087 patients were treated with insulin, 24.7% of them reported that they had a first degree relative with diabetes. in this group there might be some patients with type 2 or other types of diabetes. Among patients classified with type 1 diabetes at diagnosis and on insulin treatment 16.5% reported a first degree relative with insulin treated diabetes. In the analysis we saw a significantly higher frequency of relatives to female compared to male type 1 diabetes patients. The main difference was seen in the parents of the patients; 11.5 % parents of female compared to 8.4 % ($p=0.018$) of parents to male insulin treated patients classified as type 1 diabetes patients at diagnosis. Also when performing life table analysis we saw this difference (Figure 2 and 3, paper1 ). The higher frequency of relatives with diabetes in this age group compared to the younger age group could of course be due to the relatives being older and therefore they have had a longer time to develop diabetes. It might also be affected by the fact that more genes need to be involved when you develop diabetes at a higher age which would be consistent with the fact that the age of onset is higher in multiple than single sibling case families (257, 258). In this age group more men than women develop type 1 diabetes but they have fewer affected relatives. Thus it seems that women need more genes to develop the disease and therefore transmit more predisposing genes to their children. This is called the Carter effect and has also been observed in atopic manifestations where atopic asthma is seen more often among children of women than among children of men affected with
atopic asthma. The male to female ratio in atopic asthma is about 2:1 (259) It has even been shown in MS, but in MS the sex effect is in the opposite direction (260) and it has not been replicated (261).

II We made a genome-wide linkage search for susceptibility genes for type 1 diabetes in 408 multiplex families (464 affected sibpairs). We used 318 microsatellite markers with an average success rate of 84.9% and the average heterozygosity of 0.79.
As well as in all previous and later performed genome-wide linkage analysis we confirmed IDDM1 (HLA) as the major risk locus for type 1 diabetes. The multi-locus LOD value in our study was 42.6 (p=7x10^{-45}) and the \( \lambda_s \) (the observed locus-specific sibling recurrence risk) was 3.6. All other type 1 diabetes susceptibility loci identified so far have much smaller \( \lambda_s \) values. When stratifying for the insulingene we observed heterogeneity in the linkage to HLA with an increased evidence for linkage to HLA in the subgroup that did not carry the type 1 diabetes high risk allele of the insulin gene (IDDM2). Fourteen regions, apart from HLA, were detected with a LOD score of 1.0 in multilocus analysis, 8 of these also showed significant levels in single-locus analyses. In the primary linkage analysis no linkage to the IDDM2 region was observed, but in the presence of association was confirmed by TDT, with an excess transmission of INS+ (the high risk allele) to diabetic children from heterozygous parents (p=10^{-6}). The INS+ allele is found in 71% of healthy individuals in Sweden (262) and this is complicating the detection of linkage and is probably why only association can be detected to the insulin gene.

Linkage was observed to the IDDM15 (markers D6S300-D6S283) region on chromosome 6q, with a LOD score of 4.8. This region is linked to HLA with a recombination rate of 0.32 in male and 0.47 in female meiosis between IDDM1 and IDDM15. Suggestive linkage was yielded in the Scandinavian families even with the contribution from HLA take into account. When combining the data with the French and US families the significance of linkage to IDDM15 reached p=7 10^{-7}.

On chromosome 2, (marker D2S113) near EIF2AK3 (involved in the risk of neonatal diabetes) showed suggestive linkage, with a LOD score of 2.1. If the families were stratified based on their HLA type, the evidence of linkage was increased. We selected extra markers to include the EIF2AK3 region in the analysis, but we could not find any association to this locus.
A LOD score of 2.2 (p=8 10^{-4}) was seen on chromosome 5 (5p11-q13, close to marker D5S407), in the Scandinavian families. This region has not previously been shown to be linked to type 1 diabetes. We typed markers (D5S2112, D5S412 and D5S1352) in the IDDM18 locus on chromosome 5q31.1-q33.1 (263) and we did not observe any evidence of linkage in this region. The same finding is shown in other investigations on IDDM18 locus (264-266).

On chromosome 16p, a LOD score of 2.8 was reached in a wide region from D16S407 to D16S287. The support for linkage increased when we stratified the families based on the genotypes at HLA, with LOD=3.6 in the HLA DR3/DR4 subgroup and to LOD=3.4 in the INS- subgroup. D16S3113 demonstrates significant evidence for heterogeneity when stratification based on INS genotypes was performed (p=0.0005). The region on chromosome 16p that is linked to type 1 diabetes is very wide and is split into two separate loci based on the stratification for HLA and INS families. The
location of linkage peaks in multifactorial diseases might occur some distance from the susceptibility gene (267), but it is likely that this region contains two risk loci for type 1 diabetes.

On chromosome 4, at marker D4S412 we saw linkage with a LOD score of 2.6 in the group without HLA DR3 and/or DR4. On chromosome 13 we found a LOD score of 3.2 in the DR4 subgroup. On chromosomes 4, 9, 10, 12, 13, 19, 21 and X LOD scores of 1.0 in single- or multilocus analyses were observed.

We could exclude 61% of the genome from containing genes with a locus specific λ<sub>s</sub> of 1.5. When using a locus specific λ<sub>s</sub> of 2.0 we could exclude 89% of the genome. However, most non-HLA susceptibility genes that have been identified have a λ<sub>s</sub> < 1.2 (69) and could therefore not be excluded in the current analysis due to lack of power.

**What could be indicating that these regions do have an impact of the developing of type 1 diabetes?**

The MHC class II molecules are presenting antigens on the cell surface, T-cell reactivity against beta-cells can be triggered and could influence the possibility to prevent the body from type 1 diabetes. When carrying the high risk HLA haplotypes, autoantigens have a higher affinity to the HLA molecule leading to an increased risk of autoimmunity. The T-helper cells will be activated, start proliferation and make cytokines. The cytokines will recruit and activate other immune system cells that will produce aggressive substances that damage the tissue. Insulin deficiency in type 1 diabetes is the result of a destruction of the B-cells in the islet of Langerhans in pancreas.

One way that the insulin gene could affect the risk to develop type 1 diabetes would be by affecting the tolerance to insulin and it has been shown that in persons having the risk allele of the insulin gene (class 1 VNTR) low levels of insulin are expressed in the thymus than in persons carrying the protective allele (class III) (96). As the T-cells learn between self and not-self in the thymus, this could make them react against the body’s own insulin. It might also be so that changes in the insulin expression could influence the development of the pancreas.

Two independent studies of Multiple Sclerosis (MS) (268-269), and a study of systemic lupus erythematosus (SLE) (270) have shown linkage to the region on chromosome 5 that we find linked to type 1 diabetes. This could reflect a common autoimmunity susceptibility gene on chromosome 5. The EIF2AK3 gene, on chromosome 2, has been implicated in Wolcott-Rallison syndrome, a rare form of early onset insulin dependent diabetes (271). We could not detect any association in this region and Wolcott-Rallison syndrome is not known to be an autoimmune disease. The MHC2TA gene which is the main regulator of expression of HLA molecules maps to the region on chromosome 16 which we found to be linked in Scandinavian families. This gene has been shown to be associated with MS, RA and cardiac infarction (272-273). We have also found that it is associated with type 1 diabetes in one case control study but this finding has not been replicated in other case controls studies of type 1 diabetes (274). The KIAA0305, that is shown to be associated both with type 1 diabetes and multiple sclerosis (69, 275) in genome wide association studies, is mapping next to MHC2TA. In a large genome wide analysis by Concannon et al in
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2005 they show, apart from the HLA region, nine regions with nominal evidence supporting linkage to type 1 diabetes (p<0.001). 2q31-q33 (IDDM7, IDDM12), 10p14-q11 (IDDM10) also showed genome wide significance and 16q22-q23 had a genome wide p<0.075. The HLA and the IDDM15 region are located close to each other but despite this Concannon et al also show results supporting a HLA independent effect in the IDDM15 region. The results from the genome-wide linkage analysis that we provided are included in this study. They can not confirm a locus on chromosome 1p13 although there are earlier studies showing both linkage and association to this locus (109, 114, 276). The absence of evidence of linkage in this study is explained to be mainly due to a $\lambda_s$ of 1.05 and to detect linkage at p<0.001 with 50% power, a sample of >8000 affected sibpair families is needed (using a fully informative genetic map). In the genome-wide linkage that we performed, we found evidence of linkage on chromosome 2p, this finding was not replicated in the genome-wide linkage analysis made by Concannon et al where the LOD score was less than one in this region (66). The linkage that we found on chromosome 5p11-q13 was not seen in the study by Concannon (66), but on the other hand the gene for the IL7R is mapping on 5p13. This region is shown association to in a newly performed genome-wide association study (69). It has also been identified a possible gene-gene interaction between 5q11-q13 and 7q32 and these loci together seem to constitute a significant susceptibility factor for type 1 diabetes(137).

III We wanted to examine whether the HLA haplotype that was not inherited from the mother could affect the risk for a child to develop type 1 diabetes. We compared the frequency of inherited and non-inherited positively (DR4-DQA1*0301-B1*0302 and DR3-DQA1*0501-B1*0201), negatively (DR15-DQA1*0102-B1*0602) or neutrally (all other) type 1 diabetes associated HLA haplotypes in 563 type 1 diabetes children and 286 non-diabetic control children from Sweden. We stratified the persons based on their inherited haplotype. In individuals who had inherited one neutral haplotype (DRX) and either a DR3 or DR4 haplotype the frequency of type 1 diabetes positively-associated NIMA was higher among healthy individuals than patients with type 1 diabetes (DR3/X p<0.009; DR4/X p<0.00003). In DR3 /X patients NIMA, type 1 diabetes negatively associated haplotypes, were increased compared with control NIMA (p<0.05). Neutral haplotypes were more frequent among patients than among controls in the DR4/X group (p<0.0005). There were no significant differences when comparing NIPA in these two groups.

We also compared NIMA and NIPA in the healthy children and observed significantly more (p= 0.02) type 1 diabetes positively-associated haplotypes among NIMA than NIPA in DR4/X- positive subjects. Among DR4/X positive persons the neutral NIMA were significantly lower than the neutral NIPA (p<0.02). In the type 1 diabetes patients, the frequencies of DR3 and DR4 haplotypes did not differ between NIMA and NIPA.

The patient families were initially divided in two groups; one with the simplex families in which only one child had type 1 diabetes and the other with the multiplex families where two or more of the siblings had type 1 diabetes. The result for each group was similar. The control group was divided into healthy children from healthy families (n
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for NIMA=80) and healthy siblings in type 1 diabetes families (n for NIMA=188). We
observed a significant difference between patient and control NIMA when the healthy
siblings to the type 1 diabetes patients were used in the control group and when all the
controls were used. When looking at the haplotypes that are not transmitted to type 1
diabetes children, one would expect these to have a lower frequency of type 1 diabtes
risk genes than the parental haplotypes as a whole, since the risk haplotypes are more
frequently transmitted to type 1 diabetes patients. To avoid this bias we stratified the
patients and the healthy children haplotype by haplotype. This was done in order to
avoid the bias introduced because the haplotype that is transmitted to patients will
affect the distribution of non-inherited high and low-risk haplotypes. A stratification
based on inherited haplotypes also avoids the bias that the healthy siblings more often
have a non inherited high risk haplotype, because they have inherited a low risk
haplotype from their parents.

Earlier studies have compared the risk for children to develop type 1 diabetes
depending on if the mother or the father has the disease (205-209, 236). These studies
all show an increased risk if the father compared to the mother has type 1 diabetes. One
contributing explanation to this phenomenon may be that the HLA haplotype that is
not transmitted from the mother influences the risk of developing type 1 diabetes. Thus
if the NIMA is positively associated, this seems to protect from type 1 diabetes
whereas the NIPA does not seem to influence the risk for type 1 diabetes.

One explanation is that the father transfer disease susceptibility genes in higher
frequency, but this remains to be proven and NIMA can be an important factor when
explaining the difference in risk to develop type 1 diabetes between children to
mothers and fathers with type 1 diabetes. The mechanism could be a protecting affect
of NIMA as we detect a highly significant increase in positively associated NIMA
among healthy persons compared to type 1 diabetes patients.

IV In this study we wanted to look upon imprinting mechanisms as a contributing
factor for type 1 diabetes.

This linkage analysis which was performed using the same data as in paper II is
divided into two parts. In the first part we compared the ability to detect linkage
between a non-parametric- (Aspex) and a parametric- (GENEHUNTER MODSCORE)
analysis in 408 multiplex families from Scandinavia. There was a good correlation
between the two methods. The MOD scores were higher than the MLOD in the Aspex
analysis for some markers. This was expected as the power to detect linkage in the
parametric analysis with a correctly specified model is higher than for a non-parametric
model free analysis. We observed linkage to IDDM1 on chromosome 6p21 and to
IDDM15 on chromosome 6q. We also observed evidence of linkage on chromosome
2p11, 4p15, 5q11-12, 16p12-13 and 21q22 as reported previously. With parametric
linkage analysis we detect linkage to 2q36 and 14q31 which has not been reported
previously. On chromosome 2; D2S159 and D2S206, chromosome 4; D4S403,
chromosome 5; D5S647, and chromosome 14; D14S51, D14S267 the MOD from the
GENEHUNTER was higher than the MLOD from Aspex. In linkage studies of
complex traits suggest that, chance variation in the location may lead to linkage peaks
some distance from the causative genetic effect and for chromosome 2 the linkage peak
at 2p11 appears at slightly different locations in the two analyses (267). Next we
performed the MOD score analysis allowing for different penetrance depending on whether an allele was inherited from the mother or the father. This allows for the detection of imprinted loci. We also compared the paternal and maternal IBD sharing, which would differ for imprinted loci. Markers mapping close to IDDM15 (D6S402-D6S283) all showed increased paternal sharing, indicated by higher MODimp than MOD values and values of the index I approaching 1. The difference between MODimp and MOD was ≥ 2 close to D6S402 and D6S286 at the IDDM15 locus.

There are no known loci for type 1 diabetes between IDDM and IDDM 15 but it is shown in Transient Neonatal Diabetes Mellitus (TND) that there is a locus mapping to marker D6S286 that shows a parental effect. The hypothesis is that overexpression of a putative gene located on chromosome 6 alters pancreatic maturation and insulin secretion when the maternal gene is imprinted. It has also been demonstrated that the microsatellite D6S310 (6q16.3-q27) showed linkage with TND in 1 multiple family (281), and a duplicated segment of paternal 6q22-q23 has been identified (282). Three additional cases with TND and parental effect of chromosome 6 have been reported (283-285). Our finding of maternal imprinting of the D6S286 marker in T1DM is consistent with these results and therefore there may be shared genetic effects between transient neonatal diabetes mellitus and T1DM.

A significant difference in IBD sharing between the maternal and paternal meioses was also seen for markers on chromosome 6 close to the IDDM1 (D6S470) and IDDM15 loci (D6S402, D6S286) in ASPEX single point analysis. This was not observed for markers mapping exactly at IDDM1 (e.g., D6S273 and TNFA). Two other markers showed a significant difference in maternal and paternal IBD sharing (D13S192 and D22S264) in the Aspex single-point analysis. Similar results were found in the ASPEX multipoint analysis, where the difference between MLOD in maternal and paternal meioses was ≥ 2 for markers close to IDDM1, IDDM15 and D13S192 but not for D22S264. The difference between MODimp and MOD was 1.52 close to the D13S192 marker. We did not see a parent of origin effect for marker D22S264 with the MOD SCORE analysis.

The female map was longer than the male map both on the centromeric and the telomeric side of TNFA on chromosome 6. The genetic distance between IDDM1 (TNFA) and IDDM15 (D6S283) was 88 cM in the female map and 35 cM in the male map (i.e. differs by a factor of 2.5.). However the female map was also longer than the male map on the telomeric side of IDDM1, (46 cM in the female map and 19 cM in the male map (i.e. differs by a factor of 2.4). This indicates that the effect seen on IDDM15 is not due to a stronger linkage between IDDM15 and HLA in the male compared to the female map.

We performed a separate analysis of MODscore among sibpairs sharing one or two alleles IBD at HLA in an attempt to correct for a possible tighter linkage to HLA in males. This analysis showed a difference between MODimp and MOD among those that shared HLA IBD and not among those that did not share alleles IBD at HLA. Finally, we performed an analysis of parental sharing of discordant or unaffected sibpairs for the loci found in the affected sibpairs and could not detect any significant linkage or similar differences in parental sharing among discordant or unaffected
sibpairs. We suggest that our findings are related to imprinting mechanisms as a contributing factor in developing type 1 diabetes and not due to a parental origin transmission ratio distortion. The effect of imprinting at IDDM15 locus seems to be dependent on HLA.

In many investigations the INS locus has been shown to be associated with type 1 diabetes (277) but we did not detect linkage to the insulin gene. The power to detect linkage as well as POO-effects in this region is low because the type 1 diabetes-associated allele in the INS loci is common in the population (71% in the Swedish population) (87, 232, 278, 262, 279).

A substantial proportion of genes that control fetal growth in placental mammals are imprinted. The IGF2 gene where maternal imprinting is shown (280) is located close to the insulin gene and there have been studies showing a parental effect on the insulin gene (231, 232).

On chromosome 13q14 close to the locus where we observe paternal imprinting the HTR2A gene coding for the serotonin receptor is showing paternal imprinting in patients with bipolar affective disorder (229). In type 1 diabetes 6p21, 6q25–q27, 10p11-q11 and 16p12 have been showing a parental effect (229). If a mother can have more risk genes than a father without developing type 1 diabetes, the genetic risk that a mother can pass to her child may be higher, thus simulating imprinting and the gender specific penetrances must be taken into consideration when trying to explain the maternal effect seen on chromosome 13. On the other hand in the IDDM15 region we observed a paternal effect and this can not be an effect of gender specific penetrances.
CONCLUSIONS

The principal conclusions of the individual studies underlying this thesis are as follows:

I In the age group 15-34 years there is a higher frequency of relatives with diabetes compared to the younger age group. This is at least in part an effect of the that fact that the relatives are older, but may also be due to that more genes need to be involved when you develop diabetes at a higher age. This would be consistent with the fact that the age of onset is higher in multiple than single sibling case families. We also see that among young adults more men than women develop type 1 diabetes and that there is a higher frequency of relatives with type 1 diabetes to the women than to the men. This may reflect that women need more genes to develop the disease as young adults.

II We confirmed in our genome-wide linkage analysis linkage to IDDM1, IDDM2 and IDDM15 and suggestive linkage on chromosome 2p, 5p11-q13 and 16p. On chromosome 2 and 16 the linkage was increased if we stratified based on HLA or INS. We conclude that apart from HLA other genetic regions are of importance in risk of developing type 1 diabetes and that there is a gene-gene interaction in the feature of genetic predisposition. To detect loci with small effects investigations in large materials are needed.

III The risk to develop type 1 diabetes in children to fathers with type 1 diabetes is higher than in children to mothers with the disease. This may in part be explained by the fact that the HLA haplotype that is not transmitted from the mother influences the risk of developing type 1 diabetes. Thus if the NIMA is positively associated, this seems to protect from type 1 diabetes in the children carrying one risk and one neutral HLA allele, whereas the NIPA does not seem to influence the risk for type 1 diabetes. The reason for this could be an effect of an immunological process starting during fetal life.

IV The risk for children to develop type 1 diabetes may also in part be an effect of imprinting mechanisms as we show that there is an increased risk for the child to develop the disease if IDDM15 is inherited from the father compared to if it is inherited from the mother. The effect of imprinting at IDDM15 loci seems due to an interaction with HLA as we observed imprinting at IDDM15 in the HLA linked families. We also show a maternal effect on chromosome 13 but it might be explained by gender specific penetrances, as it seems as if a mother can have more risk genes than a father without developing type 1 diabetes, thus simulating imprinting. We also conclude that parametric maximization methods might be effective to identify susceptibility loci in multifactorial diseases and that it may be of importance to maximize the models taking POO effects into account.
3. FUTURE PERSPECTIVES AND THE USE OF THESE STUDIES FOR THE TYPE 1 DIABETES PATIENTS

The studies performed in this thesis are all dealing with the genetics of type 1 diabetes. Many questions are left to be answered and many studies need yet to be performed. Concerning the genome-wide linkage analysis there has been finemapping performed on chromosome 5 and 16 to find candidate genes in these regions. Right now a large international cooperation, the Type 1 Diabetes Genetics consortium (www.T1DGC.org), is performing a genomescan in order to screen other regions that might include risk loci for type 1 diabetes. The larger the material is, the higher is the possible power of the investigation and by cooperation between countries it is possible to get results with increased power that are even more reliable. It is also interesting to study other autoimmune diseases such as multiple sclerosis and rheumatoid arthritis to search for genes that are risk genes in more than one of these autoimmune diseases.

I want to keep on trying to understand the difference in risk of type 1 diabetes between children to mothers and fathers with type 1 diabetes. Can the answerer be that women with type 1 diabetes choose to have fewer children than men with the disease do? There might also be an increased number of spontaneous abortions among women with type 1 diabetes compared to among healthy women. If we look at pregnant women who were pregnant a number of decades ago these might contribute to explain the difference, but today the treatment of diabetes during pregnancy is very carefully regulated and women with type 1 diabetes, deliver healthy children almost in the same proportion as healthy women. The environment for the fetus might still be one factor that can explain the difference. One can always speculate that the risk of spontaneous abortion could be increased if the fetus is carrying type 1 diabetes risk genes that the mother also has. If this would be the case, children that have inherited the risk genes from the father would have greater chance to survive and later in life develop type 1 diabetes. Another possible explanation could be that the presence of autoantibodies in the fetus environment would affect the risk and then it would matter when the woman is diagnosed with type 1 diabetes in relation to her pregnancy. If she still has autoantibodies this might protect the child from future type 1 diabetes.

We have permission from the ethics committee to perform a study where we ask type 1 diabetes women when they were diagnosed with type 1 diabetes, how many children they have, at what age they had there children and if they have gone through any miscarriage(s). We hope that this study will contribute to the knowledge of the difference in risk of type 1 diabetes between children to mothers and fathers with type 1 diabetes. In two of the studies presented in this thesis we are dealing with this question. I want to collect more controls to examine the difference in the non inherited maternal haplotype between healthy children and children with type 1 diabetes.
Concerning the study on imprinting mechanisms we want to perform a permutation analysis for each marker a pre-specified number of times. The times we make the analysis is depending on the threshold p-value. For instance if we want a p-value of 0.001 to be the level of significance, we have to make the analysis 1000 times. By using this analysis we also take into consideration the large number of analysis performed as we have had many markers (possible loci) all over the genome. This gives us genome-wide p-values instead of nominal p-values and makes the reliability of the significance thresholds higher. We randomly mix the genotypes in each analysis and measure what p-values we would get by chance. By comparing with these p-values we can determine the level of significance. We also want to perform this analysis to be able to better valuate the p-values of the MOD and MODimp analysis. We are also asking for permission to use the T1DGC material to have a larger number of persons when performing the study.

In my work as a pediatrician I meet children suffering from a chronic disease that interfere with their daily activities and my wish is to help these children to a high quality of life. The best would be to find ways to prevent and cure type 1 diabetes and I think that one way to come closer to this goal is to better learn the reasons why some people but not others develop the disease. We know that there is a mix of both genetic and environmental factors. By trying to understand the inheritance patterns within families, the difference in incidence between men and women and between relatives to type 1 diabetes patients we might get one step closer to understand how type 1 diabetes mellitus develops.
Typ 1 diabetes är den en av de vanligaste kroniska sjukdomarna hos barn och ungdomar i Sverige. Typ 1 diabetes klassificeras som en autoimmun sjukdom som orakas av att B-cellerna i bukspottkörteln förstörs. Sjukdomen är i viss mån genetiskt betingad och risken för en enäggsatt Trilogy att få sjukdomen om den andra tvillingen är sjuk är 21-38%, medan den är 7-15% för tvåäggsatt Trilogy och syskon. HLA regionen på kromosom 6 står för cirka 40-50% av den genetiska risken att insjukna i typ 1 diabetes men också andra gener påverkar risken. Omgivningstillförsel har också betydelse för risken att insjukna och genom en samverkan mellan flera faktorer utvecklas sjukdomen.

I min första studie har jag genom en enkät som skickades till nästan 4000 individer (som insjuknade i typ 1 diabetes mellan 15 och 34 års ålder) kunnat konstatera att kvinnor som insjuknar i typ 1 diabetes har signifikant fler första gradssläktingar med sjukdomen än vad män har. Detta talar för att det behövs fler genetiska riskfaktorer för att en kvinna ska insjukna. Detta syftar även att det mätt vara att 60% av dem som insjuknar i denna åldersgrupp är män och endast 40% är kvinnor.


Det finns en ökad risk för barn att insjukna i typ 1 diabetes om pappa, jämfört om mamma, har typ 1 diabetes. Vi har försökt hitta förklaringar till detta.

I den tredje studien i avhandlingen undersöker vi om det HLA anlag som barnet inte ärver från sina föräldrar påverkar riskan att insjukna i typ 1 diabetes. Hos barn som har ärvt ett HLA riskanlag och ett neutralt HLA anlag visar vår studie att det verkar skydda mot att insjukna om modern har ett riskanlag som barnet inte ärvt. Mekanismer för detta skulle kunna vara att den immunologiska processen startar redan i fosterlivet och om mamma har riskanlag som barnet inte har så kan barnet genom att utsättas för dessa intrauterint ha ett visst skydd mot att utveckla sjukdomen senare i livet.

Den fjärde studien i avhandlingen testar en annan tänkbar orsak till skillnaden i risk att insjukna i typ 1 diabetes hos barn till pappor och mammor med sjukdom. Hypotesen är att det föreligger imprinting, vilket innebär att sjukdom endast utvecklas om anlaget nedärvs från ena föräldern. Vi kan i denna studie visa att risken att insjukna i typ 1 diabetes verkar påverkas av imprinting på två regioner. En är lokalisert på kromosom 6q (IDDM15) och en region finns på kromosom 13q12. IDDM 15 visar på ökad risk för typ 1 diabetes om detta riskanlag nedärvs från pappan och det är överensstämmande med att risken för barn att insjukna i typ 1 diabetes är ökad om pappa har sjukdom. Området på kromosom 13 visar däremot att risken är ökad om anlaget kommer från mamman, vilket också skulle kunna förklaras av att det föreligger en könsspecifik penetrans. Om en kvinna behöver kan
ha fler riskgener än en man utan att utveckla sjukdom är det större risk att hon ger en genetisk riskfaktor till sitt barn och detta kan simulera imprinting.

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