Karolinska Institutet

Thesis for doctoral degree (Ph.D.) 2009

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Stockholm 2009

Published by Karolinska Institutet.

ISBN 978-91-7409-665-1

Printed by
REPROPRINT AB
Stockholm 2009
www.reproprint.se
Gårdsvägen 4, 169 70 Solna

To Therese, David, Amelie,
Simon, Malin, and Annie

“Problemet med goda idéer är
att de snabbt urartar till hårt arbete”

1 ABSTRACT

Diseases caused by disturbances in the function of the immune system are a heterogeneous group of disorders, including e g allergic and autoimmune diseases. Several of these diseases have become increasingly common during the last decades. The reasons for this increase in prevalence are mostly unknown. However, environmental factors must contribute, since no genetic changes can explain the rapid changes in occurrence. Many of the diseases are supposed to have a long latency period before manifest symptoms. Thus the perinatal period, during which the immune system undergoes a rapid development, may be an especially vulnerable period for the influence of environmental factors causing immunological disturbances.

The aim of this thesis was to study the associations between factors and events during the fetal and neonatal period, and the risk of diseases during childhood caused by inappropriate immune responses. We have studied autoimmune diabetes mellitus (T1DM), coeliac disease, inflammatory bowel disease (IBD), and bronchial asthma (asthma) by use of large, population based, Swedish health registers. These registers make it possible to identify prospectively collected risk determinants even for rare diseases.

We have found a significant positive trend in odds ratio for T1DM and birth weight, with intrauterine growth retardation being associated with a decreased risk. Low birth weight and being small for gestational age are instead associated with an increased risk of asthma and coeliac disease. Severe infections during the neonatal period are associated with an increased risk of coeliac disease, IBD, and asthma. Maternal smoking during early pregnancy is associated with a decreased risk of IBD, but an increased risk of asthma and coeliac disease. Low maternal age is linked to an increased risk of coeliac disease and hospitalization due to asthma. High maternal educational level is instead associated with a decreased risk of hospitalization for asthma. Being the first born child is linked to a decreased risk of coeliac disease, but an increased risk of asthma. Several complications during the fetal and neonatal period are associated with an increased risk of asthma, including pre-eclampsia, maternal diabetes during pregnancy, prematurity, caesarean section, instrumental vaginal delivery, low Apgar score, and respiratory problems. Phototherapy and/or neonatal icterus are risk determinants for asthma.

In conclusion, there are several possible fetal and neonatal risk factors for diseases caused by immunological disturbances. These associations warrant further research, which may provide help to prevent disease in childhood.

Key words: diabetes mellitus, coeliac disease, inflammatory bowel disease, asthma, fetal, neonatal, risk, intrauterine growth, infections, smoking, icterus, phototherapy.

2 LIST OF PUBLICATIONS

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

I. Dahlquist G, Sandberg Bennich S[1], Källén B. Intrauterine growth pattern and risk of childhood onset insulin dependent (type 1) diabetes: Population based case-control study. BMJ 1996: 313: 1174-7.

II. Sandberg Bennich S[1], Dahlquist G, Källén B. Coeliac disease is associated with intrauterine growth and neonatal infections. Acta Paediatr 2002: 91: 30-3.

III. Aspberg S, Dahlquist G, Kahan T, Källén B. Fetal and perinatal risk factors for inflammatory bowel disease. Acta Paediatr 2006: 95: 1001-4.

IV. Aspberg S, Dahlquist G, Kahan T, Källén B. Is neonatal phototherapy associated with an increased risk for hospitalized childhood bronchial asthma? Pediatr Allergy Immunol 2007: 18: 313-9.

V. Aspberg S, Dahlquist G, Kahan T, Källén B. Confirmed association between neonatal phototherapy or neonatal icterus and risk of childhood asthma. Submitted.

[1] former name, now Aspberg S.

Contents

3 LIST OF ABBREVIATIONS

CI	Confidence interval
GAD	Glutamic acid decarboxylase
OR	Odds ratio
P value	Probability value
PCR	Polymerase chain reaction
SEM	Standard error of the mean
SD	Standard deviation
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
UV light	Ultraviolet light

4 INTRODUCTION

4.1 BACKGROUND

Diseases caused by disturbances in the function of the immune system are characterized by inadequate immunological reactions against internal and external antigens. These reactions cause tissue and organ damage, resulting in various symptoms and sequele. Among diseases caused by such immunological disturbances are autoimmune and allergic diseases. The aetiology of these diseases is mostly unknown, but both genetic and environmental factors are supposed to contribute. The interactions between genes and environmental factors are complex, meaning that different combinations of genes under different unfavourable circumstances can cause the same syndrome or disease. Some kind of genetic predisposition is then supposed to be a necessary, but not sufficient, prerequisite for the development of the diseases.

Autoimmune and allergic diseases have become increasingly common during the last decades, especially among children, and most obvious in the Western world (*1-5*). Most of these diseases are chronic and some are nowadays very common, and are of importance not only for the child and family but also for the society. The causes of the increase in prevalence are to a major extent unknown despite much effort to find explanations. Most of these diseases are supposed to have a long latency period before manifest symptoms appear. Among children this latency period has directed the interest towards events early in life, especially during the fetal and perinatal period. During this time period the immune system undergoes a rapid development from a fetal phenotype characterized of tolerance to a childhood phenotype characterized of defence towards infections, a development that continues into adulthood. The T-lymphocyte activity, which dominates in the newborn child is mainly of Th-2 type, the same type of activity that is supposed to be of importance in allergic disease. In interaction with the environment, the older child develops an immune response mainly of Th-1 type (*6,7*). According to the so called hygiene hypothesis, proposed by Strachan in 1989, a decreased exposure for external antigens during the neonatal and infant period, e g by fewer infections, can result in a delay of this switch in immune activity. The delay could in turn increase the risk of allergic diseases (*8-10*). Events and factors with possible influence on the early immunological development may thus be of importance for the risk to develop diseases caused by disturbances in immunological function.

In Sweden nationwide health registers of comparatively high quality give good possibilities to study early risk determinants for different diseases, also for diseases that are rare, and risk determinants that only moderately influence the risk. In complex diseases the health registers make it possible to effectively study prospectively collected risk determinants. We have taken advantage of the health registers to study four diseases, or groups of diseases, caused by disturbances in the function of the immune system, namely bronchial asthma, autoimmune or type 1 diabetes mellitus, coeliac disease, and inflammatory bowel disease. The diseases are further described below.

Autoimmune or insulin dependent (type 1) diabetes mellitus (T1DM) is caused by an autoimmune destruction of insulin producing β-cells in the pancreas. This destruction leads to a more or less complete insulin deficiency and often to complications, both early and in the long term. The incidence has rapidly increased in children in many countries, and tends to shift to younger age at onset (*3*). T1DM is more common among children who have a first degree relative with the disease, but only around 10% of children with T1DM have a first degree relative with the disease (*11*). The concordance in monozygotic twins has been reported to be only 30 to 50%, clearly indicating a low gene expression and an important role for non-genetic factors (*12,13*). The gene specificities associated with T1DM were early found in the HLA system of chromosome 6 known to be involved in autoimmune regulation (*14*). Molecular genetic studies have later on identified several genes of possible importance for the development of T1DM. A recent large meta-analysis on genome wide scans identified more than 40 loci for significant associations, indicating the complex nature of the disease (*15*). Nearly 90% of all newly diagnosed cases in Sweden carry the HLA alleles DR4-DQ8 or DR3-DQ2. A single copy of the allele DQ6 instead offers protection against T1DM. On the other hand, the high risk alleles are common in Sweden, and far from everyone who carry them will develop the disease (*16*). Environmental factors thus contribute to a large extent to disease initiation and progression.

Since autoantibodies against islet cell antigens can be identified in children long before overt T1DM (*17,18*), events during early life have been of special interest. An early study from our group showed associations with several fetal and perinatal risk

determinants, the strongest association was with a child diagnosis of blood group incompatibility (*19*). The findings were in part confirmed in a smaller British case-control study and one large European multi centre study (*20,21*). It was unclear if phototherapy as a treatment, rather than blood group incompatibility or hyperbilirubinemia *per se* was responsible for the increase in risk, as suggested both from the first study (*19*) and a later study (*22*). Pre-eclampsia, caesarean section and complications during partus have also been linked to an increased risk (*21,23-25*). Some fetal and perinatal infections (e g rubella or enteroviruses, including Coxsackie B during the fetal period) are associated with an increased risk of T1DM (*26,27*). Older mothers seem to have an increased risk of having a child who develops T1DM in several studies (*28,29*). However, in one interesting study the risk of T1DM in the child depended more on paternal than maternal age. The risk was increased for older fathers, and when controlling for the age of the father the effect of maternal age disappeared. Controlling for maternal age, on the other hand, did not eliminate the increase in risk among older fathers (*30*). The first born child seems to have the highest risk of T1DM, while the risk decreases with the number of children born (*31*). Short duration of breastfeeding is a rather stable risk determinant for the disease (*32-34*).

In 1991 Barker et al (*35*) showed an association between reduced birth weight and non-insulin dependent diabetes mellitus (T2DM) and carbohydrate metabolism in 64-year-old men. This study led to the so called Barker hypothesis arguing that intrauterine starvation will affect future carbohydrate metabolism. Thus events during the fetal period can affect risk of disease years later in life. This is supported by several later studies, showing e g inverse associations between birth weight and blood pressure (*36-38*).

Coeliac disease is characterized by a lifelong intolerance to gluten (so called prolamines including gliadine) in cereals. These substances induce an immunologically mediated enteropathy in the small intestines. The disease has a wide variety of symptoms, from asymptomatic cases to severe intestinal inflammation and malnutrition. The diagnosis is based on analyses of antibodies directed towards gliadine and autoantigens in the intestinal mucosa, especially against transglutminase, and on intestinal biopsies. Coeliac disease is basically treated with withdrawal of gluten from the food, a measure which leads to a healing of the intestinal inflammation (*39,40*).

Genetic factors are important for the development of coeliac disease. Some HLA loci are of special importance. More than 90% of the patients with coeliac disease are DQ2 positive and most of the remainders carry the HLA allele DQ8. Other gene loci of importance for immunological disturbances have recently also been linked to coeliac disease (*41*). The HLA haplotypes that predispose for coeliac disease are to some extent associated with T1DM, and T1DM is consequently more common among patients with coeliac disease (*42,43*). There are also associations between coeliac disease and other autoimmune diseases, e g Addison's disease and autoimmune thyreoiditis. The risk of malignant lymphoma is increased (*44,45*). Coeliac disease also occurs in an increased frequency in children with Turner's syndrome and Down's syndrome (*39*).

Although genetic factors are a prerequisite for development of coeliac disease, no more than 60 to 70% of monozygotic twin pairs are concordant for the disease. HLA DQ2 is also widespread in the population, while coeliac disease has a prevalence of around 1% at least in the Western world (*39,46*). Environmental factors may thus contribute to the development of the disease. The most obvious environmental factor of importance is exposure to gluten. The significance of amount and time of introduction of gluten in the food has been of much controversy (*47-49*). Breast feeding is in this sense supposed to be protective (*50*). Other environmental factors that have been proposed as risk factors for coeliac disease are infections, e g hepatitis C, adenovirus, enterovirus and rotavirus (*51*). Associations with low socioeconomic class, maternal smoking, and older siblings have also been observed (*52*).

Inflammatory bowel disease constitutes a group of disorders characterized of relapsing, intestinal inflammation. The principal diseases are ulcerative colitis and Crohn's disease. These diseases resemble each other closely. Although they can sometimes not even be distinguished pathologically, they differ enough clinically to be regarded as independent entities. The prevalence of inflammatory bowel disease is higher in Northern than in Southern countries, and less marked in Western than in Eastern countries. Ulcerative colitis is slightly more common among men, and Crohn's disease among women (*1*). The incidence of inflammatory bowel disease has been rising during the last decades, although mainly the incidence of Crohn's disease, while the incidence of ulcerative colitis has been more stable (*1,53*). When inflammatory bowel disease develops during childhood the natural history seems to be more severe, with a more extensive and complicated disease (*54,55*).

Mutations in a gene on chromosome 16 coding for the protein CARD 15/NOD2 were described some years ago, and seem to be of importance for the development of inflammatory bowel disease. CARD 15/NOD2 is expressed in monocytes, and is required for bacterial lipopolysaccharide-dependent induction of nuclear factor κ B. This substance in turn participates in immunological reactions (*56*). Several other genetic risk markers have been identified after this discovery (*57*).

Possible environmental risk factors for inflammatory bowel disease have been studied with somewhat divergent results. Smoking in patients is associated with an increased risk of Crohn's disease, but a decreased risk of ulcerative colitis (*58-60*). The results concerning passive smoking have been more contradictory (*62-63*). Appendectomy, at least before the age of 20, seems to be protective for ulcerative colitis (*64-66*), but has been associated with an increased risk of Crohn's disease (*67*). Several microbial agents have been proposed to be associated with an increased risk of inflammatory bowel disease, mostly Crohn's disease. Among them are e g Mycobacteria, Yersinia, Campylobacter, Clostridium, Chlamydia, and herpes, rota and measles viruses (*1,68*). Helminths have instead been supposed to decrease the risk, and have even been tried as therapy for IBD (*69*). Non-intestinal, bacterial infections early in life have in some studies been linked to an increased risk of inflammatory bowel disease (*55,70*). Breastfeeding can be a protective factor (*71*). Delivery by caesarean section, urban location, and higher socioeconomic status have been associated with an increased risk of Crohn's disease (*72*).

Bronchial asthma (asthma) is a heterogeneous disease, maybe better described as a syndrome, with different causes and pathogenesis. The different types of asthma have in common inflammation and reversible obstruction of the airways. Among children asthma is often associated with atopy, and the airway obstruction can be induced by the release of allergen-specific IgE antibodies after exposure for the allergen. Asthma is in part a genetic disease, since the risk of developing childhood asthma is increased if one or both of the parents are affected by the disease. Some chromosome loci have been associated with the risk of asthma (*73,74*). The high prevalence of asthma in many countries, and the rapidly increasing prevalence in others (*5,75,76*) have resulted in numerous attempts to identify environmental factors contributing to the development of the disease. However, there are few environmental factors which have constantly been

associated with an increased risk of the disease (*77*). Among perinatal factors maternal age, parity, maternal educational level, low gestational age, low birth weight, complications during partus, caesarean section, and respiratory problems have all been associated with an increased risk of asthma in some studies (*78-81*), while others have failed to find some of these associations or have opposite results (*82,83*). Also studies of maternal smoking during pregnancy (84-87) and breastfeeding (*88-90*) have given divergent results concerning the risk of childhood asthma. Furthermore several infectious agents, e g respiratory syncytial-, influenza-, and rhinoviruses, have been linked to an increased risk of asthma (*91,92*).

4.2 ASSUMPTIONS

Our research is based on the hypothesis that perinatal events may be of importance for later development of immunological disturbances. In view of Barker's hypothesis (*35,93*) we decided to study intrauterine growth and risk of T1DM, an association that had not been studied at that time. Our group (*19*) had previously been able to identify blood group incompatibility, exchange transfusion, neonatal icterus, and phototherapy as perinatal risk markers for T1DM. We wanted to study also these associations further based on our hypothesis that neonatal phototherapy can influence the early development of the immune system.

Coeliac disease is more common among patients with T1DM and these two diseases share some genetic risk alleles. Thus, we decided to study possible associations between coeliac disease and the risk determinants we had identified for T1DM. Little was known about perinatal factors and risk of coeliac disease when we started this study. There was also limited knowledge about perinatal factors and other diseases caused by intestinal inflammation with supposed autoimmune origin, e g ulcerative colitis and Crohn's disease. In our third study we continued the research with focus on these diseases.

The importance of the perinatal period for the risk of childhood asthma had been shown in several studies when we started our fourth project, in which we planned to study fetal and neonatal events and the risk of asthma. We had the specific purpose to study neonatal icterus and phototherapy, and the risk of asthma. This aim was based on the observation of an increased risk of T1DM after neonatal icterus and/or phototherapy. We assumed that the possible effect of phototherapy could be more important for risk

of asthma than for T1DM due to the supposed importance of Th2 immune responses in atopic diseases. The hypothesis concerning phototherapy is further described below (Discussion).

5 AIMS

The overall aim of this thesis was to study possible fetal and neonatal risk factors for diseases caused by disturbances in the function of the immune system. The specific aims were;

1. to study the influence of intrauterine growth disturbances on the risk of childhood autoimmune diabetes mellitus (T1DM);
2. to study the influence of blood group incompatibility, exchange transfusion, neonatal icterus and phototherapy on the risk of T1DM during childhood;
3. to study the importance of intrauterine growth disturbances and other perinatal events for the risk of childhood coeliac disease;
4. to study possible fetal and neonatal risk factors for inflammatory bowel disease during childhood;
5. to study factors and events during the fetal and neonatal period and the risk of childhood bronchial asthma, with special focus on neonatal icterus and phototherapy.

6 MATERIALS AND METHODS

The registers, variables used, and the study designs are described below.

6.1 REGISTERS

The Swedish Medical Birth Register (*94,95*) started in 1973. It contains information on the pregnancy, delivery and neonatal period for nearly all children born in Sweden. Since 1982 the information is based on copies of the medical records from the antenatal care units, the delivery units, and the paediatric examinations of the newborns.

The Hospital Discharge Register (now part of the National Patient Register, which also includes outpatients) contains information on all hospitalizations in Sweden with discharge diagnoses and covers the whole country since 1987 (*96*).

The Swedish Childhood Diabetes Register (*97*) includes incident childhood cases of T1DM. In Sweden all children with newly diagnosed T1DM are referred to paediatric clinics. The clinics report the cases to the register since 1977. The level of ascertainment has been shown to be 96 to 99 % (*98,99*).

The Swedish Prescribed Drug Register contains information on all prescriptions that have been redeemed in Sweden since 1999. Drugs are coded by ATC (Anatomical Therapeutic Chemical) codes. Patient identifications are included in the register since July 1, 2005, thus giving possibilities for pharmacoepidemiological research (*100,101*). Using this register, it is possible also to identify children with asthma who have not been hospitalized.

Registers were linked with the use of the personal identification numbers, which everyone living in Sweden receives, and which are widely used in society and in all health care. Linkage may fail for various reasons: errors in such numbers or that the child was not born in Sweden and therefore was not registered in the Medical Birth Register.

6.2 VARIABLES

The variables reflect exposures during the pregnancy and the neonatal period. In some instances socio-economic factors of possible importance for the development of the

diseases have been included in the analyses. Small for gestational age was determined by use of parity and sex specific standard growth curves based on data from the Swedish Medical Birth Register (*102*). The variables are summarized in Table 1, papers are given in roman numbers. Broader classes are given when numbers are few. The prescribed classes of drugs used in paper V are given in Table 2. Variables of high validity are year of birth, maternal age, infant sex, and birth weight. Information on parity is obtained by linkage with the Birth Register of Statistics Sweden, and has a relatively high validity. Diagnoses and treatments must be expected to have a lower validity since clinical classification and registration may sometimes be inaccurate, or forgotten. This error will, however, occur both among the infants who will develop the diseases under study and the control infants. Maternal smoking refers to early pregnancy and may be underreported, since this is based on information given by the mother during the first visit to maternal care, often during weeks 10 to 12. It is, however, prospectively collected information, and can hardly be biased by late development of certain diseases.

Comparisons were made with children without the disease under study. Children who died were excluded as controls, but information on children who have emigrated are missing. Some children classified as controls may thus actually have the disease under study, which will bias risk estimates towards 1.

6.3 DESIGN

6.3.1 Paper I

This is a case-control study based on the Swedish Medical Birth Register and the Swedish Childhood Diabetes Register. From the latter register we identified 4 702 children born 1973 or later, who were recorded in the register from 1978 to 1992. Linkage between the two registers was possible for 4 584 children. Thus, linkage was successful for 97.5 % of the children. For each of the children with diabetes three controls were randomly selected among all children born the same year and at the same hospital as the proband child. The purpose of this matching was to eliminate the possible confounding effect of different hospitals and age groups. Only living children were accepted as controls. Data from the pregnancy and neonatal period were then compared between cases and controls.

6.3.2 Previously unpublished results

This is a case-control study based on medical records. By linkage of the Swedish Childhood Diabetes Register and the Swedish Medical Birth Register we could identify 93 children with T1DM who had been treated with phototherapy and/or exchange transfusion during the first weeks of life. For each of these children we selected two controls who had also been treated with phototherapy and/or exchange transfusion but had not developed T1DM. The cases and controls were matched according to maternal age and year of birth. The medical records were possible to find for 84 cases and 183 controls. Thus, 12 children were lost to follow up. Among the cases 80 had been treated with phototherapy and 14 with exchange transfusion, and among the controls 161 had been treated with phototherapy and 39 with exchange transfusion. The analysis was restricted to triplets with information on the case and at least one control. Data on the pregnancy and neonatal period were collected from medical records in delivery and paediatric care units from a total of 58 hospitals. All medical records were reviewed by the author of this thesis. Hospitals with fewer than five patients sent copies of the medical records for the review, the remaining hospitals were visited. Although most of the medical records could be identified, there was a considerable amount of incomplete records and missing data.

Frequencies of blood group incompatibility and blood transfusion were compared among all cases and all controls with data (after breaking the triplets). Measurement data on bilirubin/serum at start of treatment, maximum bilirubin/serum values, and duration of treatment in hours were analysed. Analyses were first made after breaking the triplets and comparing all cases with all controls, then within triplets as differences between case and control mean values.

6.3.3 Papers II - IV

These are all case-control studies based on the Hospital Discharge Register and the Swedish Medical Birth Register. The whole population in the Swedish Medical Birth Register during specified time intervals serves as control. Twins are excluded in papers II and III due to difficulties in interpretation of the results when children sharing both environmental exposures and genetic risk are mixed with singletons. In paper IV we included twins and triplets with the purpose to study asthma risk among multiple births.

In paper II we used the Hospital Discharge Register to identify children who had been hospitalized due to coeliac disease. Among all children born in 1987 to 1997 we found 3 482 children with the diagnosis coeliac disease in the register. After exclusion of multiple births, 3 392 children of the children remained. The Hospital Discharge Register was then linked to the Swedish Medical Birth Register. Fetal and neonatal data for children with coeliac disease were compared with data for all children born during these years.

In paper III we identified a total of 497 children born in 1987 to 2000 who had been hospitalized for inflammatory bowel disease and were recorded in the Hospital Discharge Register. Linkage with the Swedish Medical Birth Register was possible for 455 children, i e 92%. After exclusion of multiple births 452 of the children remained, among whom 307 were assigned the diagnosis ulcerative colitis, and 172 the diagnosis Crohn's disease. Both diagnoses were given to 27 children. These were excluded from the final analysis.

In paper IV we included children who had been hospitalized due to asthma up to and including 2001 and who were born between 1987 and 1999. We excluded children younger than two years of age due to uncertainty about the diagnosis of asthma among such young children. A total of 15 409 children fulfilled these criteria in the Hospital Discharge Register. Linkage with the Swedish Medical Birth Register was possible for 14 803 children, 96% of the total. Among these children, 14 364 were born as singletons, 427 as twins and 12 as triplets.

6.3.4 Paper V

Paper V is based on The Swedish Prescribed Drug Register and the Swedish Medical Birth Register. One important limitation in papers II, III and IV is the fact that only hospitalized cases were included. By use of the Swedish Prescribed Drug Register we could identify children for whom anti-asthmatic drugs had been redeemed, whether or not they had been hospitalized due to the disease. We identified all children in the register who were born between January 1, 1990 and June 30, 2003 and for whom drugs with ATC code R03 had been redeemed during the last six months of 2005, the first half year with patient identification in the register. We included 64 204 children. Linkage with the Swedish Medical Birth Register was possible for 61 988 children

(97%). Data were analysed in comparison with data for all children born in Sweden during the same time period.

6.4 STATISTICS

Odds ratios (OR) were calculated by using Mantel-Haenzel procedure (*103*) after adjustments for various variables. Miettinen's test based method (*104*) was used to estimate (95%) confidence intervals (CI). Some of the analyses (paper III) were based on few observations and then observed and expected values were compared based on exact Poisson distributions. In the previously unpublished results OR were estimated by Fisher's exact test and are presented as OR and p-values. Differences between case and control mean values were analysed by *t*-tests. In paper I tests for homogeneity between strata were made with the Breslow and Day test (*105*), and for trends in the frequency tables exact trend statistics were used (StatExact software).

Table 1 Variables

Maternal characteristics
Age[1] (I, II, III, IV, V) • *In five-year classes (II, IV)* • *<25, 25-34, ≥35 years (III)* • *<20, 20-39, ≥40 years (V)*
Parity[1] (I, II, III, IV, V) • *1, ≥2 (III, V)* • *1, 2, 3, ≥4 (IV)*
Diabetes of any kind during pregnancy[1] (I, IV, V)
Smoking during pregnancy[1] (I, II, III, IV, V) • *0, >10 cigarettes/day (II)* • *0, any amount of cigarettes (III, V)* • *0, <10, ≥10 cigarettes/day (IV)*
Pre-eclampsia[1] (II, III, IV, V)
Education[2] (III, IV) • *Comprehensive school, continuation school, university or equivalent (III)* • *<compulsory school, compulsory school, <3 years gymnasium, 3-4 years gymnasium, <3 years post gymnasium, ≥ 3 years post gymnasium, university or equivalent (IV)*
Unwanted childlessness[1] (IV, V) *Years* • *1, 2, 3, 4, 5, any (IV)* • *any (V)*
Caesarean section[1] (IV, V)
Instrumental vaginal delivery[1] (IV, V)

Infant characteristics
Year of birth[1] (IV, V)
Multiple births[1] (IV)
Sex[1] (II, III, IV)
Gestational age[1] (I, II, III, IV, V) *Weeks* • *< 37 (III)* • *<32, <37 (IV,V)*
Birth weight[1] (I, II, III, IV, V) *Gram* • *< 2500 (III)* • *<1500, <2500, >4500 (IV, V)*
Birth weight by gestational week[3] (I, II, III, IV, V) *Multiples of standard deviations* *Small for gestational age defined as a weight below minus 2 standard deviations from the expected weight at that week, parity, and infant sex*

- *<2, -2 to -1, -1 to 1, 1 to 2, >2 (I, II)*

Apgar score[1] (II, III, IV, V)

- *Low Apgar score defined as <7 at 5 minutes*

Respiratory problems[1] (IV, V)
Mechanical ventilation[1] (IV, V)
Neonatal infection[1] (II, III, IV, V),

- *Sepsis and/or pneumonia*

Neonatal icterus[1] (II, III, IV, V, unpublished results)
Blood group incompatibility[1] (II, III, IV, V, unpublished results)

- *AB0, Rh, others* (VI)

Blood transfusion[1] (II, unpublished results)

- *Exchange transfusion* (II)
- *Exchange transfusion or transfusion due to anaemia* (unpublished results)

Phototherapy[1] (II, III, IV, V, unpublished results)
Infant bilirubin level at the beginning of phototherapy[4] (unpublished results)
µmol/l
Maximum bilirubin level[4] (unpublished results)
µmol/l
Phototherapy at the neonatal care unit or at the delivery clinic[4] (unpublished results)
Time span of phototherapy[4] (unpublished results)

[1] Source: The Swedish Medical Birth Register. Gestational age is mostly based on ultrasound in second trimester
[2] Source: The Education Register of Statistics Sweden. Swedish gymnasium corresponds to upper secondary school in the UK and to senior high school in the USA. Data on education only available up to and including 1995
[3] Source: A birth weight standard for gestational age based on data in the Swedish Medical Birth Register (*102*)
[4] Source: Medical records

Table 2 Anti-asthmatic drug classes examined in paper V

Drug	Number of patients
Adrenergics, inhalation	51 026
Steroids, inhalation	29 923
Anticholinergics	269
Cromoglicic acid	148
Adrenergics, systemic	10 360
Xantines	17 382
Leukotrien receptor antagonists	4 883
Steroids, systemic	4 173

7 RESULTS AND COMMENTS

7.1 TYPE 1 DIABETES MELLITUS (PAPER I)

Contrary to the findings of a strong inverse association between T2DM and birth weight (*35,93*), our study showed for the first time a significant positive trend in OR for T1DM and birth weight. After stratification for known confounders, such as maternal age, parity, maternal diabetes and maternal smoking, p for trend was <0.001 (Figure 1). Analysing small for gestational age as a group after the same stratifications showed a decreased risk of T1DM, OR 0.81, 95% CI (0.65-0.99). Being large for gestational age (>2 SD) was instead associated with an increased risk, OR 1.20, 95% CI (1.02-1.42). Interestingly, a later study from our group confirming the result for young onset T1DM cases (<10 years) could not find intrauterine growth disturbances as a risk factor for T1DM in young adults (*106*). Several more recent reports, including a meta-analysis, have confirmed our findings (*107*).

We found no significant association between the risk of T1DM and birth weight or gestational length *per se*, although there was a trend for an increased risk with preterm birth (< 37 completed weeks), OR 1.25, 95% CI (0.99-1.33). High birth weight has been associated with an increased risk of T1DM in several studies (*21,108,109*), but others have failed to show this association (*25,110*). Maternal diabetes during pregnancy has a known association with childhood diabetes due to the genetic trait of T1DM in particular (*19,25*). This association was confirmed in paper I. Classification of diabetes during pregnancy can be unclear due to changes in the ICD classification system. ICD 8 used the same code for all types of diabetes during pregnancy, ICD 9 differentiated between pre-existing and gestational diabetes, and ICD 10 gives a detailed division. Thus, in paper I maternal diabetes during pregnancy might include T1DM as well as T2DM and gestational diabetes.

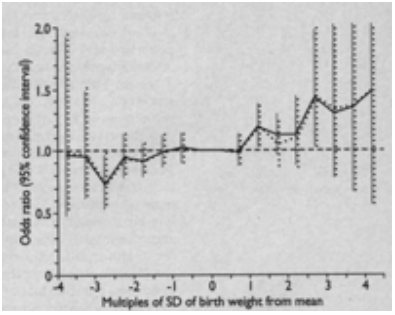
7.2 PREVIOUSLY UNPUBLISHED RESULTS

The analyses of medical records concerning T1DM revealed no increase in risk caused by blood group incompatibility. This diagnosis was registered in 8 among 52 cases and 15 among 77 controls, OR 0.75, 95 % CI (0.25-2.09), p=0.64. Neither did we find an association with blood transfusion, given to 7 (all exchange transfusions) among 52 cases, and 13 (10 of them exchange transfusions) among 77 controls, OR 0.77, 95 % CI (0.24-2.27), p=0.80. Only four controls and four cases had been treated at the delivery

care unit, making further comparison between treatment at the neonatal care unit and the delivery care unit inconclusive.

Quantitative analyses were made for serum bilirubin levels at the start of treatment, the maximum serum bilirubin level recorded, and the duration of treatment (including pauses between treatments). Data are presented after breaking the triplets (Table 3) and within the triplets (Table 4), which both gave similar results. Although data in many respects are incomplete there was an interesting increase in the risk of T1DM with increasing levels of bilirubin. No such increase in risk could be associated with duration and intensity of phototherapy. This suggests that icterus *per se* may cause an increase in risk of T1DM.

Figure 1 The risk of type 1 diabetes mellitus in relation to birth weight



Reference: −0.5 to +0.5 SD. Vertical lines give 95% CI. Dotted line represents OR after exclusion of infants whose mothers had diabetes.

From paper I, with permission

Table 3 Type 1 diabetes mellitus, bilirubin, phototherapy, breaking triplets

	Cases		Controls				
	n	Mean±SEM	n	Mean±SEM	Difference±SEM	t	p
Serum billirubin *µmol/l*							
At start	36	273.2±12.2	49	223.4±12.8	49.8±18.2	2.73	0.01
Maximum level	42	299.4±8.4	60	261.5±9.4	38.0±13.2	2.87	0.01
Treatment time *hours*	36	49.3±4.8	50	60.0±6.1	-10.6±8.2	1.29	0.17

Bilirubin levels and duration of phototherapy among cases and controls

Table 4 Type 1 diabetes mellitus, bilirubin and phototherapy, within triplets

	Mean difference±SEM	t	p
Bilirubin/s *µmol/l*			
At start	49.0±16.7	2.93	0.01
Maximum level	35.9±13.0	2.76	0.01
Treatment time *Hours*	-10.4±8.2	1.27	0.17

Bilirubin levels and duration of phototherapy among cases and controls

7.3 COELIAC DISEASE (PAPER II)

Low maternal age (adjusted for parity) was associated with an increased risk of having a child who develops coeliac disease. There was also a clear trend with decreasing risk for older mothers. The risk of coeliac disease was reduced for the first born child (adjusted for maternal age). Coeliac disease was less common among boys than girls, sex ratio 0.66, 95% CI (0.61-0.70). The disease was more common among children whose mothers had smoked during early pregnancy. Low birth weight was associated with an increased risk, OR 1.27, 95% CI (1.07-1.52). Being small for gestational age was associated with a more pronounced increase in risk, OR 1.45, 95% CI (1.20-1.75). Preterm birth (< 37 weeks), on the other hand, was weakly associated with a reduced risk. Severe neonatal infections, defined as sepsis and/or pneumonia, were linked to an increased risk of coeliac disease. We did not find any associations between coeliac

disease and neonatal phototherapy, blood group incompatibility, exchange transfusion, neonatal icterus, low Apgar score, or pre-eclampsia. For details concerning associations, see Table 5.

Table 5 Coeliac disease

	OR and 95% CI
Maternal age[1] years	
• *20-24*	1.30 (1.20-1.80)
• *25-29*	1.08 (1.01-1.16)
• *30-34*	0.79 (0.73-0.86)
• *35-39*	0.71 (0.62-0.81)
• *40-44*	0.73 (0.53-0.99)
Parity[2]	
• *First*	0.84 (0.78-0.90)
Smoking in early pregnancy[3]	
• *Any*	1.10 (1.01-1.19)
Pre-eclampsia[4]	1.12 (0.93-1.36)
Apgar score[4]	
• *<7 at 5 minutes*	1.21 (0.87-1.71)
Neonatal icterus[4]	0.82 (0.66-1.02)
Blood group incompatibility[4]	0.83 (0.49-1.40)
Neonatal phototherapy[4]	0.94 (0.73-1.20)
Severe neonatal infections[5]	
• *Sepsis and/or pneumonia*	1.52 (1.18-1.95)
Gestational length[4]	
• *<37 completed weeks*	0.82 (0.68-0.98)
Birth weight[4]	
• *<2500 g*	1.27 (1.07-1.52)
Small for gestational age[6]	1.45 (1.20-1.75)

The risk of coeliac disease. Odds ratios (OR) and 95% confidence interval (CI) are presented

[1] Adjusted for year of birth, parity, and maternal smoking
[2] Adjusted for year of birth, maternal age, and maternal smoking
[3] Adjusted for year of birth, maternal age, and parity
[4] Adjusted for infant sex, year of birth, maternal age, parity, and maternal smoking
[5] Adjusted for infant sex, year of birth, birth weight, maternal age, parity, and maternal smoking
[6] birth weight adjusted for gestational age, and neonatal infections

7.4 INFLAMMATORY BOWEL DISEASE (PAPER III)

The risk of inflammatory bowel disease was increased for boys compared to girls, sex ratio 1.47 95% CI (1.22-1.78). Maternal smoking during early pregnancy was associated with a decreased risk of inflammatory bowel disease, OR for Crohn's disease was 0.73, 95% CI (0.58-0.94), and for ulcerative colitis 0.70, 95% CI (0.56-0.86). Severe neonatal infections, defined as sepsis and/or pneumonia, were related to an increased risk of inflammatory bowel disease. The number of patients with severe infections and later development of inflammatory bowel disease were very few (n=3) and thus the results with a high formal relative risk 17.6, 95% CI (3.6-51.6), must be interpreted with caution.

The risk of inflammatory bowel disease was not affected by birth weight, preterm birth, pre-eclampsia, neonatal icterus, or parity. Neither did maternal age or educational level influence the risk, with the exception of a slightly decreased risk of ulcerative colitis among children to younger mothers. Associations are summarized in Table 6. Only 4 of the patients with inflammatory bowel disease had a low Apgar score which was close to the expected number 4.5, 2 had blood group incompatibility (expected number 2.5), and 9 had been given phototherapy (expected number 9.7).

Table 6 Inflammatory bowel disease

	Ulcerative colitis OR and 95% CI	**Crohn's disease** OR and 95% CI	**Inflammatory bowel disease** OR and 95% CI
Maternal age[1]			
<25 years	0.77 (0.63-0.94)	0.80 (0.63-1.01)	0.87 (0.69-1.10)
≥35 years	0.86 (0.65-1.13)	1.03 (0.77-1.39)	0.98 (0.72-1.32)
Parity[2]			
1	1.17 (0.99-1.39)	1.15 (0.95-1.40)	1.16 (0.96-1.41)
Smoking in early[3] pregnancy			
Any	0.70 (0.56-0.86)	0.73 (0.58-0.94)	0.71 (0.55-0.91)
Maternal education[4]			
Comprehensive school	0.97 (0.69-1.37)	1.09 (0.66-1.81)	0.92 (0.69-1.22)
University or equal	0.99 (0.74-1.34)	1.24 (0.84-1.84)	1.12 (0.89-1.40)
Preterm birth[6]			
<37 weeks	---	---	1.17 (0.80-1.72)
Birth weight[5]			
<2500 g	---	---	1.05 (0.65-1.72)
Small for gestational age[6]	---	---	0.74 (0.38-1.43)
Pre-eclampsia[6]	---	---	0.85 (0.42-1.72)
Neonatal icterus[6]	---	---	0.93 (0.53-1.66)

The risk of inflammatory bowel disease. Odds ratios (OR) and 95% confidence interval (CI) are presented

[1] 25-34 years serve as reference. Adjusted for parity, maternal smoking, birth weight, and maternal education
[2] ≥2 serve as reference. Adjusted for maternal age, maternal smoking, maternal education, and birth weight
[3] adjusted for maternal age, parity, maternal education, and birth weight
[4] up to an including 1995. Adjusted for maternal age, parity, maternal smoking, birth weight
[5] adjusted for maternal age, parity, maternal smoking, and maternal education
[6] adjusted for year of birth, maternal age, parity, and maternal smoking

7.5 ASTHMA (PAPERS IV AND V)

The results are given in Tables 7 and 8. The risk of childhood asthma increased with age of the mother (paper V). However, when only hospitalized children were included, teenage mothers seemed to have an increased risk of having a child with asthma (paper IV). It is possible that younger mothers tend to seek or are offered hospital care more often than older mothers. Mothers with higher educational level had children with fewer hospitalizations due to asthma. First born children had an increased risk of asthma, which can be in accordance with the hygiene hypothesis. However, this was obvious only in the study including outpatients (paper V) as no significant effect of parity was seen in hospitalized cases. Asthma was more common among boys than girls, the sex ratio was 1.83 95% CI (1.77-1.90) among hospitalized cases. Maternal smoking during early pregnancy was associated with an increased risk of childhood asthma, but only when smoking ≥10 cigarettes/day among hospitalized cases.

There was an association with involuntary childlessness prior to gestation and an increased risk of asthma, irrespective of whether a child had been hospitalized or been given outpatient care. Theoretically there can be an association between immunological disturbances causing unwanted childlessness and immunological disorders like asthma, both by inherited and fetal environmental factors. Maternal diabetes during pregnancy was also associated with an increased risk of childhood asthma. Pre-eclampsia, another disease characterized of immunological disturbances and with linkage to T1DM (*19*), also showed an association with an increased risk of asthma. Caesarean section was linked to an increased risk of asthma. Caesarean section has also been associated with an increased risk of T1DM (*24,111*). The prevailing theory has been that exposure to the normal intestinal bacterial flora during partus is important for the maturation of the immune system in the child. Instrumental vaginal delivery was also weakly associated with an increased risk of asthma.

Preterm birth was clearly associated with an increased risk of asthma. Low birth weight and being small for gestational age were also linked to an increased risk of asthma. Other perinatal complications including respiratory problems, mechanical ventilation, low Apgar score, and severe neonatal infections, defined as above, were also relatively strongly associated with an increased risk of asthma.

Asthma was more common among children who had been given neonatal phototherapy and the increase in risk remained after extensive adjustments and exclusions made to avoid that the effect was secondary to other possible risk factors, OR among hospitalized cases was 1.27, 95% CI (1.08-1.50) and when also including outpatients younger than 12 years, 1.30, 95% CI (1.16-1.47). There was obviously also an association between asthma and icterus, but no association between asthma and blood group incompatibility. In an effort to distinguish between the effect of icterus and of phototherapy, the OR for either exposure was determined. When a diagnosis of icterus was present but not phototherapy, the OR was 1.26, 95% CI (1.21-1.32). When both diagnoses were present the OR were higher 1.41, 95% CI (1.29-1.53). These two OR estimates differ significantly, $z=2.32$, $p=0.03$.

Table 7 Asthma, maternal characteristics

	Hospitalized astma OR and 95% CI	**Asthma including outpatients** OR and 95% CI
Maternal age, years[1]		
• *≤19*	1.28 (1.16-1.42)[7]	0.84 (0.79-0.90)
• *20-24*	1.11 (1.06-1.16)[7]	---
• *25-29*	1.00 (0.96-1.03)[7]	---
• *30-34*	0.91 (0.87-0.95)[7]	---
• *35-39*	0.94 (0.88-1.00)[7]	---
• *40-44*	1.04 (0.91-1.18)[7]	1.07 (1.01-1.13)[8]
• *≥45*	1.20 (0.64-2.24)[7]	---
Parity[2]		
• *1*	0.95 (0.90-1.00)[7]	1.14 (1.12-1.16)
• *2*	1.02 (0.98-1.06)[7]	---
• *3*	1.02 (0.97-1.07)[7]	---
• *≥4*	1.05 (0.98-1.13)[7]	---
Smoking in early pregnancy[3]		
• *<10*	1.02 (0.97-1.07)[7]	---
• *≥10*	1.29 (1.21-1.36)[7]	1.08 (1.05-1.10)[9]
Maternal education[4]		
• *<9 years compulsory school*	0.97 (0.84-1.12)[7]	---
• *compulsory school*	1.12 (1.06-1.18)[7]	---
• *<3 years gymnasium*	Reference	---
• *3-4 years gymnasium*	0.84 (0.79-0.90)[7]	---
• *<3 years postgymnasium*	0.91 (0.86-0.97)[7]	---
• *≥3 years postgymnasium*	0.76 (0.70-0.82)[7]	---
• *University or equal*	0.47 (0.27-0.83)[7]	---
Involuntary childlessness[5]		
• *Any (>1 year)*	1.10 (1.02-1.19)[7]	1.18 (1.14-1.22)
Pre-eclampsia[6]	1.18 (1.05-1.33)[7]	1.24 (1.19-1.29)
Diabetes during pregnancy		
• *(any type)*[6]	1.20 (1.02-1.42)[7]	1.19 (1.12-1.28)
Caesarean section[6]	1.44 (1.37-1.50)[7]	1.30 (1.27-1.33)
Instrumental vaginal delivery[6]	1.08 (1.01-1.17)[7]	1.09 (1.06-1.13)

The risk of asthma. Odds ratios (OR) and 95% confidence interval (CI) are presented. Data from Paper IV and V

[1] Adjusted for year of birth, parity, maternal smoking, and involuntary childlessness. Adjusted for education among children who had been hospitalized due to asthma
[2] Adjusted for year of birth, maternal age, maternal smoking, and involuntary childlessness. Adjusted for education among children who had been hospitalized due to asthma

[3] Adjusted for year of birth, maternal age, parity, and involuntary childlessness. Adjusted for education among children who had been hospitalized due to asthma
[4] Adjusted for year of birth, maternal age, parity, involuntary childlessness, and maternal smoking. Swedish gymnasium corresponds to upper secondary school in the UK and to senior high school in the USA. Data on education only available up to and including 1995
[5] Adjusted for year of birth, maternal age, parity, and maternal smoking.
[6] Adjusted for year of birth, maternal age, parity, maternal smoking, and involuntary childlessness. Adjusted for education among children who had been hospitalized due to asthma
[7] exclusion of children born preterm (<37 weeks), with low birth weight (<2500 g), or low Apgar score (<7 at 5 minutes)
[8] ≥40 years
[9] Any maternal smoking

Table 8 Asthma, infant characteristics

	Hospitalized asthma OR and 95% CI	**Asthma including outpatients** OR and 95% CI
Multiple births[1]	0.81 (0.13-5.00)	---
Gestational age[2]		
• *<32 weeks*	3.67 (3.28-4.11)	2.18 (2.02-2.35)
• *<37 weeks*	1.86 (1.62-2.15)	1.52 (1.47-1.57)
Birth weight[2]		
• *<1500 g*	3.79 (1.68-8.58)	2.23 (1.64-3.03)
• *<2500 g*	1.97 (1.63-2.37)	1.52 (1.42-1.63)
Small for gestational age[2]	1.43 (1.11-1.84)	1.22 (1.11-1.35)
Apgar		
• *<7 at 5 minutes*[2]	2.24 (2.00-2.50)	1.50 (1.40-1.60)
Severe neonatal infection[2]	1.74 (1.50-2.01)[3]	1.42 (1.33-1.52)
Respiratory problems[2]	1.58 (1.40-1.79)[3]	1.61 (1.54-1.68)
Mechanical ventilation[2]	2.95 (1.34-6.50)[3]	2.47 (2.09-2.92)
Blood group incompatibility[2]	1.09 (0.85-1.40)[3]	1.11 (0.99-1.24)
Icterus[2]	1.37 (1.22-1.54)[3]	1.45 (1.40-1.51)
Phototherapy[2]	1.34 (1.16-1.54)[3]	1.35 (1.28-1.42)

The risk of asthma. Odds ratios (OR) and 95% confidence interval (CI) are presented. Data from paper IV and V
[1] Exclusion of children born preterm (<37 weeks), with low birth weight (<2500 g), or low Apgar score (<7 at 5 minutes). Adjusted for maternal age, parity, maternal smoking, an involuntary childlessness
[2] Adjusted for year of birth, maternal age, parity, maternal smoking, and involuntary childlessness
[3] Exclusion of children born preterm (<37 weeks), with low birth weight (<2500 g), or low Apgar score (<7 at 5 minutes)

8 GENERAL DISCUSSION

8.1 METHODOLOGICAL ASPECTS

The results of this thesis are based on data from registers and medical records. There are some specific aspects to consider when using such information. The most important advantage of this methodological approach is the prospective collection of information. Data obtained long before the diagnosis of the disease can not be systematically biased by the outcome, e g by recall bias. Furthermore, a less well defined diagnosis will not lead to an overestimate of the risk, but to more conservative risk estimates, and will thus not give false-positive results. The only possible consequence is a slight underestimate of the risk. Another important advantage in our studies is the use of well validated, large, population based registers of relatively high quality including nearly all children in the country.

There are, however, potential disadvantages with register studies. Data may be incomplete. Diagnoses and information on exposures are not always well defined. When hospitalized cases are used, cases that have never been hospitalized will be wrongly classified as controls. This means both a reduction in the number of all cases available for study and a selection of potentially more severe cases, which may give a dilution of the risk estimates. The conclusions can furthermore only be made in context of the study base. The results may not without consideration be extrapolated to the whole population. The limited amount of information in the registers and medical records makes it possible to miss important confounders which then can not be controlled for. On the other hand, using variables in the registers as exposures without any prior hypothesis about the association with the outcome, a so called fishing expedition, leads to the risk to find false associations just by chance.

Another important remark is that the study design does not allow conclusions about causality, i e that the factor studied actually (in part) causes the disease. The associations found in register studies can be divided into risk markers, risk determinants, and risk factors, depending on the strength of the association. A risk marker is supposed to be an exposure associated with the risk of a disease, but without any causation. A risk determinant can be causal of the disease but the causality is not proven. A risk factor, finally, is probably causal of the disease and an intervention can modify the risk of developing the disease. Nevertheless, experimental or randomised

studies are necessary to prove causality. Register studies performed without a prior hypothesis can at best be used to create new hypotheses. However, they allow us to form hypotheses that would not be possible to do unless registers gave us the opportunity to describe and see the reality in a way that randomised studies can not do. The often large size of the register studies can make it possible to identify associations which would hardly be detectable in clinical studies of selected populations.

Taken these aspects in to consideration, we conclude that the methods are useful in research aimed for identification of possible risk factors for different diseases.

8.2 RISK DETERMINANTS

8.2.1 Intrauterine growth disturbances

One major result (paper I) is the trend for increased risk of T1DM with increasing birth weight, and a decreased risk of the disease among children born small for gestational age. In an attempt to explain the results we can assume that fetal adaptation to intrauterine starvation involves every organ system, including e g the brain regulation of appetite, lipid storage in the adipose tissue, and β-cell activity in the pancreas. There is no need for an excessive release of insulin and the β-cells will to be less active than would be the case if energy supply was sufficient. The expression of possible autoantigens, including glutamic acid decarboxylase (GAD) and other known β-cells antigens, will then be less pronounced. If the β-cell stays relatively quiet, the chance to escape the scanning of autoreactive T-lymphocytes will be enhanced. Thus, the risk of T1DM may decrease. It is well known that the incidence of childhood T1DM has its peak during times of excessive weight gain or rapid growth, e g during the rapid growth phase prior to puberty (*112,113*). This is in agreement with the result of an increased risk of T1DM for children born large for gestational age. The finding of a protective effect of intrauterine growth retardation is also supported by the observation of a low prevalence of T1DM among men born in Germany during the Second World War (*114*).

In contrast to the association between intrauterine growth disturbances and T1DM, being small for gestational age is associated with an increased risk of coeliac disease. Low birth weight is also associated with an increased risk. Maternal coeliac disease can be a confounder, since mothers with coeliac disease, perhaps undiagnosed, may have an increased risk of having a child with low birth weight. The direct effect of intrauterine

starvation on the developing intestinal mucosa and immune system in the fetus may also influence the risk of coeliac disease, by as yet unknown mechanisms. Furthermore, ulcerative colitis and Crohn's disease are also diseases characterized by intestinal inflammation, and are not at all associated with intrauterine growth disturbances in our results.

Asthma, finally, is also associated with low birth weight and being small for gestational age, conditions often associated with immature lung function and respiratory complications in the neonatal period. Very low birth weight (<1500 g) is strongly associated with an increased risk of asthma. Primary and secondary injuries to the airways have long term effects for lung function, and possibly also for the development of asthma.

In conclusion, intrauterine growth patterns influence the risk of childhood T1DM, coeliac disease, and asthma. Intrauterine growth retardation may decrease the activity, and thereby the antigen expression in the β-cells, which may decrease the risk of T1DM.

8.2.2 Infections

The hypothesis that infections cause autoimmune diseases has gained support for various reasons. It seems logical that a disease which causes injury to one or several tissues can reveal possible autoantigens, otherwise not detected by the immune system. Once seen the autoimmune destruction will start and self generate. Another possible mechanism can be autoantigenic mimicry between certain infectious microbes and self antigens. An immunological reaction towards the infection will then lead to a reaction towards the own body. It is also possible that e g viruses induce genetic changes that will direct the immune system to an autoimmune reaction. There are obvious examples of infection induced autoimmune reactions, e g intrauterine rubella infection, increasing the risk of childhood T1DM (*26*).

Our results of an increased risk of coeliac disease, inflammatory bowel disease and asthma after severe neonatal infections (papers II-IV) can be taken to support the hypothesis that infections cause autoimmunity or allergy. However, we may also observe two parallel phenomena, i e a dysregulated immune system that manifests itself both in an increased risk of infections and in an increased risk of autoimmune or

allergic reactions. There are some observations that illustrate the problem of causality. In 2008 Beasley et al published a study on the possible association between use of paracetamol during the first year of life and later risk of asthma, based on a large, worldwide cohort of children (ISAAC). The conclusion was that use of paracetamol for fever during the first year of life (and later during childhood with a dose-response relationship) was associated with an increased risk of allergic reactions of all kind. The authors suggested that exposure for paracetamol might be a risk factor for asthma (*115*). However, it is not possible in the study to differentiate the effect of paracetamol use from the effect of the infection itself. Furthermore, the risk of allergic reactions increased, whatever the cause of the fever, suggesting that no specific infection was causing the association. In one ambitious study (*116*) children at high genetic risk of developing asthma were prospectively followed with respect to infections. By the use of polymerase chain reaction (PCR), viral etiologies were identified in 90% of febrile diseases. The children who later developed asthma had on average more airway infections, "one every second week in infancy", and ordinary rhinoviruses were a common cause. Thus, the children seemed to have an unspecific sensitivity for infections. Even if the association between unspecific infections and asthma may be a risk marker for a defect immune system, rather than a risk factor for asthma, it does not exclude that some viruses can be especially harmful to the airways (e g respiratory syncytial, parainfluenza and influenza viruses) and that a pre-existing tendency of asthma might be worsened by airway inflammation forming a vicious circle.

The associations between severe neonatal infections and inflammatory bowel diseases or coeliac disease are also characterized of an absence of a specific infectious agent as underlying cause. The numerous microbes that have been associated with especially inflammatory bowel disease speak against a specific causative infection. The finding of a genetic marker encoding a protein involved in unspecific defence against bacteria (see Introduction) can support the idea of a defect immune system making the patient both prone to infections and at risk of inflammatory bowel disease.

In summary, these observations illustrate the problem with inference between association and causation. Neonatal infections may as well be risk markers as risk determinants for asthma, coeliac disease, and inflammatory bowel disease.

8.2.3 Maternal smoking

Smoking during early pregnancy is associated with a decreased risk of inflammatory bowel disease (paper III). It is not possible from our results to separate smoking during pregnancy from maternal (or parental) smoking post partum, due to the lack of information about postnatal smoke exposure in our data. Thus, the observed effect can be the result of antenatal or postnatal smoke exposure, or a combination of both.

Smoking has a well documented protective effect in ulcerative colitis in adults (*58*), whereas smoking seems to worsen Crohn's disease (*59*). We found that the effect of smoking during pregnancy was similarly protective for both diseases. These diagnoses are not always easy to differentiate, however, especially not during childhood. The exact mechanism by which smoking is protective for ulcerative colitis is not known. Anti-inflammatory mechanisms in the intestinal mucosa have been proposed, but in that case the anti-inflammatory effect in adults should be restricted to ulcerative colitis only. Any antenatal protective effect can furthermore not be caused by direct inhibition of inflammation, but must exert its influence by indirect mechanisms.

Maternal smoking during early pregnancy is associated with a slightly increased risk of coeliac disease (paper II) and an increased risk of asthma (papers IV and V). Smoking during pregnancy increases the risk of premature birth and low birth weight, and thus interacts with, or confounds, other factors of possible importance for childhood asthma or coeliac disease. During childhood the tobacco smoke directly irritates the airways and worsens an ongoing inflammation. The smoke also predisposes for airway infections. The effect of maternal smoking during pregnancy may also, as for inflammatory bowel disease, actually be an effect of postnatal smoke exposure, or a combined effect of both exposures. However, in one study based on questionnaires the risk of childhood wheezing and asthma was increased even if the mother had stopped smoking during pregnancy (*87*).

Finally, maternal smoking during pregnancy has been associated with a decreased risk of childhood T1DM (*19,117*), which, in part, may be explained by the decrease in risk of T1DM for children born small for gestational age.

There is no easy way to use the supposed protective effect of smoking for childhood inflammatory bowel disease and T1DM in clinical practice. The negative effects of

smoking are well known and overshadow any protective effect. However, if it would be possible to identify the compounds in tobacco smoke which inhibit the intestinal inflammation, the results can have clinical implications, though more for treatment than prevention.

8.2.4 Phototherapy and icterus

We find that neonatal icterus and/or phototherapy are associated with an increased risk of asthma. Phototherapy during the neonatal period is used to treat jaundice, a common condition among newborn children. The main reason for neonatal jaundice is the physiological break down of the increased amount of haemoglobin necessary for oxygen supply during fetal life. Several other conditions and complications during the perinatal period, including prematurity and infections, can worsen the icterus. Maternal fetal blood group incompatibility can result in very high bilirubin levels. High levels of bilirubin will result in deposition of the substance in different organs, including the brain, which can give irreversible injuries, so called kernicterus. Phototherapy has the capability to break down bilirubin in the skin and thus prevent further harmful effects. The therapy is thought to be safe and with few side-effects, except sometimes skin rashes and watery stools. In experimental studies, however, DNA strand breaks and mutations have been observed (*118*).

Phototherapy is not only used during infancy but also later in life with the purpose to treat skin diseases. The light has a known capability to suppress inflammation in the skin, in part by a direct effect on T-lymphocytes. The light used is usually in the ultra violet wave-length range, while the wave lengths used for neonatal phototherapy are in the visible range, although mainly in the blue area close to the UV-light range. However, it is possible to use also visible light to cure skin diseases (*119*). The light-induced immunosuppression is not restricted to the skin. There are also systemic effects if sufficient doses are used. Experimental asthma in mice has been successfully treated with phototherapy, and the treatment effect can furthermore be transferred to naïve mice by transplantation of UVB-light induced regulatory T-lymphocytes (*120*).

It seems reasonable to assume that phototherapy can influence T-lymphocytes also in the thin skin of the newborn child. A suppression of T-lymphocyte activity during the neonatal period can potentially slow the shift from Th2- to Th1-dominated immune responses. There can also be an impairment of the thymus dependent deletion of

autoreactive T lymphocytes. This deletion is based on the expression of surface antigens on the T lymphocytes. An inactivation of the T lymphocyte will result in less expression of surface markers and thus increase the possibility to escape deletion. These mechanisms can be of importance for the development of diseases based on immunological dysregulation.

One important confounder is the bilirubin level. The strong association between bilirubin and phototherapy, i e the disease and its treatment, makes it very difficult to separate the effect of the two exposures. A randomised study including children with icterus not treated with phototherapy is impossible to perform for ethical reasons. In paper V we found a significantly higher OR for childhood asthma if phototherapy had been given than if the child only had a diagnosis of icterus. This could suggest an effect of phototherapy itself, but most likely treated cases had higher bilirubin values than non-treated cases, and thus we cannot exclude a sole effect of icterus. A further complication is that in all large data bases, diagnoses or treatments can be unregistered, so the apparent effect of icterus in the absence of phototherapy could be due to an under-recording of the latter, if the true cause is phototherapy. T1DM has also been linked to neonatal phototherapy (*19,22*). Our study of medical records on children who had been treated with phototherapy was in part made in an attempt to differentiate between the influence of phototherapy and of icterus. This study revealed no dose-response effect of length or intensity of phototherapy for the risk of developing T1DM. If anything, there was a dose-response effect of bilirubin level on T1DM risk, suggesting that it actually is bilirubin or its degradation products that affects the immune system. However, the results are preliminary due to the small size of the study and incomplete data.

In summary, neonatal icterus and/or phototherapy are risk determinants for the development of childhood asthma. Clinical measures which decrease the risk of neonatal icterus may be of value, thus avoiding both phototherapy and high levels of bilirubin. However, further clinical implications must be based on research with the aim to study the influence of both exposures.

8.3 FUTURE PERSPECTIVES

Diseases caused by disturbances in the function of the immune system are obviously a very heterogeneous group of disorders. Altogether common causes are not to be

expected. Still, with growing knowledge about the immunological mechanisms (*121*) and the epidemiological patterns it is clear that a strict division, e g into allergic and autoimmune diseases, is no longer valid. The apparent importance of the perinatal period is another common feature, although with clear differences between diseases.

In this thesis we have been able to identify risk determinants and possible risk factors during the fetal and neonatal period for common diseases during childhood. Further research is warranted to clarify the mechanisms behind these associations. Is it e g possible to reduce the risk of childhood T1DM by prevention of maternal overweight leading to children being born large for gestational age? Can a restrictive use of caesarean section decrease the risk of asthma and T1DM? Which substances in tobacco smoke contribute to the prevention of inflammatory bowel disease and can these substances be used avoiding the otherwise harmful effects of smoking? Why are severe neonatal infections more common among children who later develop asthma and coeliac disease? Can we find a proper animal model for further studies of neonatal phototherapy? Can the use of phototherapy with stricter indications, and maybe by use of light with other wave-lengths, decrease the risk of asthma and T1DM or would the effect instead be an increase in risk due to higher levels of bilirubin?

These and other questions form the basis for further research. The answers will help us to better understand the pathogenesis of the diseases. Finally, and hopefully, the answers will give us the possibility to prevent new cases.

9 CONCLUSIONS

1. Intrauterine growth disturbances are associated with the risk of childhood T1DM. Being small for gestational age decreases, and being large for gestational age increases the risk of T1DM, with a clear trend in the increase of risk. Thus, intrauterine growth disturbances are considered as risk determinants for T1DM. It is still unclear whether neonatal icterus and/or phototherapy shall be considered risk determinants for T1DM.
2. Low birth weight, being small for gestational age, low maternal age, maternal smoking during early pregnancy, and severe neonatal infections are risk markers or possible risk determinants for coeliac disease during childhood.
3. Maternal smoking during early pregnancy is associated with a decreased risk of childhood inflammatory bowel disease and may be a protective factor. Severe neonatal infections are possible risk determinants for inflammatory bowel disease. Intrauterine growth disturbances are not associated with risk of inflammatory bowel disease.
4. Low Apgar score and instrumental vaginal delivery are risk markers for asthma during childhood. Maternal age, parity, involuntary childlessness, maternal smoking during early pregnancy, maternal diabetes during pregnancy, pre-eclampsia, prematurity, low birth weight, being small for gestational age, caesarean section, respiratory problems, mechanical ventilation, and severe infections during the neonatal period are possible risk determinants for asthma. Neonatal icterus and/or phototherapy are possible risk factors for asthma among children.
5. In conclusion, the fetal and neonatal period is of importance for diseases caused by disturbances in the function of the immune system.

10 SAMMANFATTNING PÅ SVENSKA

Sjukdomar orsakade av störningar i immunsystemets funktion karakteriseras av att immunsystemet reagerar felaktigt på inre och yttre antigen. Till denna heterogena sjukdomsgrupp hör allergiska och autoimmuna sjukdomar. Orsakerna till dessa sjukdomar är ofta okända. Gemensamt är dock en multifaktoriell genes där både ärftliga faktorer och faktorer i omgivningen fordras för sjukdomsutveckling. Ofta har sjukdomarna också en lång latensperiod. Flera av sjukdomarna har ökat i förekomst under de senaste decennierna. Orsakerna till denna ökning i prevalens är ofullständigt kända. Under fosterutvecklingen och nyföddhetsperioden genomgår immunsystemet en snabb utveckling. Omgivningsfaktorer som stör denna utveckling kan ha betydelse för risken att senare utveckla sjukdomar orsakade av immunologiska störningar.

Syftet med denna avhandling är att studera sambandet mellan faktorer och händelser under fetal- och neonatalperiod, och risken att under barndomen utveckla sjukdomar orsakade av störningar i immunsystemets funktion. Vi har studerat autoimmun diabetes mellitus (T1DM), glutenintolerans, inflammatorisk tarmsjukdom (IBD) och astma bronchiale (astma). Avhandlingen baseras på stora, svenska hälso- och sjukvårdsregister som ger goda möjligheter att identifiera prospektivt insamlade risk-determinanter för olika sjukdomar.

Vi har funnit en signifikant positiv trend för risk för T1DM och födelsevikt, innebärande att intrauterin tillväxthämning kan vara en skyddsfaktor för senare utveckling av T1DM. Intrauterin tillväxthämning och låg födelsevikt är istället associerade med en ökad risk för glutenintolerans och astma. Svåra infektioner under nyföddhetsperioden är kopplade till en ökad risk för glutenintolerans, IBD och astma. Rökning under tidig graviditet är associerad med minskad risk för IBD, men med ökad risk för glutenintolerans och astma. Att vara det förstfödda barnet är associerat med en minskad risk för glutenintolerans och en ökad risk för astma. Det finns en koppling med ökad risk för glutenintolerans för barn till yngre mödrar, med en trend för minskande risk med ökande mödraålder. Sjukhusvård för astma under barndomen är också associerat med låg mödraålder, medan högre utbildningsnivå hos mor är kopplat till en minskad risk. Ett flertal komplikationer under graviditet och nyföddhetsperiod är förenade med ökad risk för astma, såsom pre-eklampsi, diabetes hos mor, underburenhet, kejsarsnitt, instrumentell vaginal förlossning, lågt Apgar och andningsstörningar i nyföddhetsperioden. Nyföddhetsgulsot och/eller ljusbehandling är riskdeterminanter för astma.

Sammanfattningsvis finner vi flera möjliga riskfaktorer under graviditet och nyföddhetsperiod för sjukdomar orsakade av störningar i immunsystemets funktion. Dessa samband kan utgöra grunden för vidare forskning med syfte att förebygga nyinsjuknande.

11 ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to

Gisela Dahlquist, one of my two main supervisors. Thank you for sharing with me your deep knowledge in the fields of science, for all your encouragement, constructive criticism and ideas which form the basis for this thesis.

Bengt Källén, my other main supervisor and dear friend. The best part of this research has been the visits to Lund, working together with you at Tornblad Institutet. Thank you for the many hours we have spent on the project, for interesting discussions about analyses and results, and for your careful guidance. It has been a privilege to get your help and support. I also want to give my warmest thanks to you and your wife Ingegerd for your fantastic hospitality and kind welcome of me as a guest in your home.

Thomas Kahan. I have had the good fortune to have you as my co-supervisor. Besides your genuine interest in research and your great knowledge in science, you have a natural capability to support and encourage in a way that gives the feeling of having a supervisor always on your side. I very much look forward to our further research collaboration.

Karin Malmqvist, Head of the Department of Cardiology, Danderyd University Hospital, for the enthusiasm and energy by which you lead our clinical department to continuous improvements, for your interest in research and education, and for your kind attention to your staff.

Carl-Göran Ericsson, former Head of the Department of Medicine, Danderyd University Hospital, for providing good working and research conditions, and for giving the best possible support when I asked for your help.

Håkan Wallén and Peter Henriksson for showing interest in my research, for good advice, and a lot of practical help.

Nina Ringart, Birgitta Lindén, Beatrice Johansson, and Kathleen Norell for excellent help with all the practical details and administration during my time as a Ph D student. Special thanks to Kathleen Norell for skilful help with proof reading and editing the thesis.

Inge Björkander and Jonas Persson for your very kind efforts to help me during the last days before printing this thesis.

Mika Skeppholm and Kristina Hagvall, my room mates and friends, for patience with occupied computers and sheaves of papers, and for many interesting discussions.

To all other friends, colleagues and medical staff at the Departments of Medicine and Cardiology, Danderyd University Hospital for sharing sometimes hard clinical work and for contributing to the friendly atmosphere.

My dear parents Bengt and Birgitta Sandberg, and my sister Eva Sandberg with family for your support and all the memorable and happy events we have had together.

My children Therese, David, Amelie, Simon, Malin, and Annie for being my innermost happiness and joy, and for seeing to that I never, ever, lack things to do in my spare time.

Johannes, my beloved husband, for all your love and support, and for sharing our busy everyday life.

These studies were supported by K A Wallenbergs Foundation, the Swedish Research Council, the Swedish Diabetes Association, and Karolinska Institutet. We also thank the National Board of Health and Welfare for giving us access to the Swedish health registers.

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