KETOACIDOSIS AT DIAGNOSIS OF TYPE 1 DIABETES IN CHILDREN UNDER 15 YEARS OF AGE

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Hekkala, Anne, Ketoacidosis at diagnosis of type 1 diabetes in children under 15 years of age.
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Abstract
The aim of this work was to evaluate the frequency of diabetic ketoacidosis (DKA) in children <15 years of age at the time of diagnosis of type 1 diabetes (T1D) at Oulu University Hospital over a period of 33 years (1982−2014) and throughout Finland in 2002−2005. The aim was to assess the effect of certain host characteristics (age at diagnosis, family history of T1D, T1D-associated HLA risk genotypes and participation in T1D prediction and prevention trials) on the frequency on DKA. A further aim was to assess temporal changes in the frequency of DKA.

The overall frequency of diabetic ketoacidosis at the diagnosis of T1D in children <15 years was low both at Oulu University Hospital and over the whole country. A decrease in the frequency of DKA was observed at the university hospital during the years 1982−2001, but it then seemed to stabilize at just under 20.0%. The frequency in the whole of Finland during the period 2002−2005 was similar, i.e. 19.4%.

The frequency of DKA at diagnosis in very young children (<2 years of age) at Oulu University Hospital decreased markedly during the period in question, being 50.0% in 1982−1991, 39.1% in 1992−2001 and 17.1% in 2002−2014 (p=0.021), and a similar decrease was seen in children <5 years (32.1% in 1982−1991, 17.7% in 1992−2001 and 13.0% in 2002−2014, p=0.007). The children aged 10−14.9 years at diagnosis, however, had an increased risk of DKA over the whole period studied here, and more attention should definitely be paid to this group in the future to reduce its DKA frequency.

In the analysis of the data based on all children in Finland diagnosed with T1D in 2002−2005 the risk of DKA at diagnosis was lower in those with a first-degree relative affected by T1D. The children carrying a higher HLA-conferred risk of T1D had DKA less frequently at the manifestation of the disease.

Prospective studies based on screening for HLA-DQB1-associated genetic susceptibility to T1D from cord blood and subsequent regular clinical, immunological and metabolic follow-up have been going on in Oulu University Hospital since 1995, and the children taking part have been found to have a reduced frequency of DKA (5%) at diagnosis. Genetic screening without follow-up did not prevent DKA at disease presentation.

Keywords: age difference, childhood, diabetic ketoacidosis, diagnosis, frequency, temporal change, type 1 diabetes
Hekkala, Anne, Ketoasidoosin esiintyminen alle 15-vuotiailla lapsilla tyypin 1 diabeteksen diagnoosisivaiheessa.

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Tiivistelmä


Analysoitaessa kaikkia Suomessa 2002–2005 tyypin 1 diabetekseen sairastuneita lapsia, havaittiin lapsilla, joilla oli ensimmäinen asteen tyypin 1 diabetesta sairastava sukulainen (vanhemmat, sisarukset), ketoasidoosiriski matalammaksi. Lisäksi niillä lapsilla, joilla oli korkea sairastumisriski liittyvän HLA-genotyyppi, oli ketoasidoosin esiintyminen vähäisempää tyypin 1 diabetekseksi diagnoosihetkellä.


Asiasanat: ajallinen muutos, diagnoosi, esiintyminen, ikä, ketoasidoosi, lapsuus, tyypin 1 diabetes
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Abbreviations

ATG  antithymocyte globulin
BMT  bone marrow transplantation
CI   confidence interval
CMV  cytomegalovirus
CTLA4  the cytotoxic T-lymphocyte-associated protein 4
CVB  Coxsackie virus B
DAISY  The Diabetes Autoimmunity Study in the Young
DiMe  The Childhood Diabetes in Finland Study
DPT–1  The Diabetes Prevention Trial –1
DIPP  The Type 1 Diabetes Prediction and Prevention Study
DKA  diabetic ketoacidosis
ENDIT  The European Nicotinamide Diabetes Intervention Trial
ESPE  The European Society for Paediatric Endocrinology
FDR  first-degree relative
FFA  free fatty acid
FPIR  first-phase insulin response
GADA  autoantibodies against glutamic acid decarboxylase
GDP  gross domestic product
GWA  genome-wide association
HbA1c  haemoglobin A1c
HEV  human enterovirus
HLA  human leukocyte antigen
HR  hazard ratio
IAA  insulin autoantibodies
IA-2A  tyrosine phosphatase autoantibodies
ICA  islet cell antibodies
IDDM  insulin-dependent diabetes mellitus
IFG  impaired fasting glucose
IFIH1  the interferon induced with the helicase C domain 1 gene
ig  immunoglobulin
IGT  impaired glucose tolerance
IL2RA  the interleukin 2 receptor, subunit alpha gene
INS  the insulin gene
LWPES  The Lawson Wilkins Paediatric Endocrine Society
MODY  Maturity-Onset Diabetes of Young
$PTPN22$ the protein tyrosine phosphatase, non-receptor type 22 gene
RNA ribonucleic acid
SNPs single nucleotide polymorphisms
TEDDY The Environmental Determinants of Diabetes in the Young Study
T1D type 1 diabetes
T2D type 2 diabetes
VDR vitamin D receptor
List of original publications

This thesis is based on the following publications, which are referred to in the text by the respective Roman numerals.


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Introduction

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycaemia resulting from defects in insulin secretion, insulin action, or both and a global prevalence is estimated to be even 8.8%. Type 1 diabetes (T1D), previously defined as insulin-dependent or juvenile diabetes, is a common, multifactorial disease of autoimmune origin with a strong genetic component, affecting about 542,000 children in the world. In high income countries T1D is accounting for about 7-12% of all diabetes. However, the relative proportion of T1D has not been studied in great detail in low- and middle-income countries (Cavan D 2015). The incidence of T1D has been increasing both in industrialized and developing countries (Rewers et al. 2008). Very active research into the aetiology of T1D has taken place over the past 20 years, but the exact pathogenic process and contributing factors that are involved remain only partly understood.

The underlying metabolic abnormality in diabetic ketoacidosis (DKA) results from the combination of absolute or relative insulin deficiency with increased amounts of counterregulatory hormones. DKA is a common phenomenon in children with T1D, occurring in 12.8–80% of cases at presentation with the disease and correlating inversely with the regional incidence of T1D (Komulainen et al. 1996, Levy-Marchal et al. 2001, Rewers et al. 2008, Usher-Smith et al. 2012). The prevalence of DKA in children with established T1D varies between 1.4-15 episodes per 100 patient years, being the major cause of hospitalization in children and adolescents with T1D (Hanas et al. 2009, Levine et al. 2001). There are some indications that the frequency of DKA may be decreasing, however (Rewers et al. 2008, Samuelsson & Stenhammar 2005). DKA present at the diagnosis of T1D is more common in younger children, with children <5 years, and especially <2 years, singled out as having a high risk. Other high risk groups are children without any first-degree relative (mother, father or siblings) with T1D and children from families of lower socioeconomic status (Komulainen et al. 1999, Pinkney et al. 1994).

The purpose of the present work was to assess the frequency of DKA at the diagnosis of T1D among children <15 years of age in Finland, where the incidence of T1D is the highest in the world, and to find out whether the frequency of DKA is decreasing. In addition we wanted to evaluate the possible effect of certain host-related characteristics (age at diagnosis, family history of T1D, T1D-associated HLA risk genotypes and participation in prospective trials aimed at T1D prediction and prevention) on the frequency of DKA.
1 Review of the literature

1.1 Definition and symptoms of type 1 diabetes

According to the American Diabetes Association and the World Health Organization, the classification of T1D is primarily based on the pathogenesis of the disease. Most cases have been supposed to result from T-cell mediated destruction of the pancreatic insulin-producing beta cells. These are classified as type IA (autoimmune), while a minority of cases are of unknown aetiology and are classified as type 1B (idiopathic). These phenotypes are differentiated by the presence or absence of circulating diabetes-associated antibodies in affected patients (Craig et al. 2009). However, nowadays T1D is seen as a complex chronic disease involving several immune and non-immune elements, many of which remains obscure, resulting in progressive dysfunction of the pancreatic beta-cells and severe lack of insulin. Thus, the immune attack is not thought to orchestrated entirely by T-cells (Christoffersson et al. 2016).

In addition, the less common types of diabetes like monogenic diabetes are known and are important in the differencing diagnosis. Examples of monogenic diabetes include Maturity-Onset Diabetes of Young (MODY) or Neonatal Diabetes Mellitus, the latter occurring before the age of 6 months (Cavan D 2015). In these subtypes, the genetic defects due to mutations in genes important for beta-cell biology result in impaired insulin synthesis or secretion or reduced beta-cell mass. Monogenic diabetes can also cause by mutations in the insulin receptor and thus affecting insulin action. The symptoms of monogenic diabetes may vary from modest hyperglycaemia to severe diabetes (Tuomi et al. 2000). An accurate diagnosis remains important, because it might lead to change in the treatment of affected subjects and influence long-term complications (Schwitzgebel 2014).

The symptoms of T1D at diagnosis of the disease on account of hyperglycaemia include polydipsia, polyuria, weight loss, blurred vision and fatigue. Hyperglycaemia with ketoacidosis represents an acute, life-threatening consequence of uncontrolled diabetes, the symptoms of which including nausea, vomiting and abdominal pain mimicking an acute abdomen, rapid, deep sighing (Kussmaul respiration) and a progressive decline in consciousness (Wolfsdorf et al. 2009).
T1D is a considerable health care problem globally, with consequences for the patients, their families and society because of long-term complications including retinopathy with potential loss of vision, nephropathy leading to renal failure, peripheral neuropathy with a risk of foot ulcers, amputations and Charcot joints, and autonomic neuropathy causing gastrointestinal, genitourinary and cardiovascular symptoms and sexual dysfunction. Furthermore, patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease. Hypertension and abnormalities of the lipoprotein metabolism are also often seen in people with diabetes (Craig et al. 2009).

1.2 Epidemiology of type 1 diabetes

Considerable geographical and ethnic variability has been established in the incidence of T1D among children of age 0−14 years (Green et al. 1992, Karvonen et al. 1993, Rewers et al. 1988), with its incidence among different populations showing more than 350-fold variations worldwide. A very high incidence (≥ 20/100000 per year) has been observed in Finland, Sardinia, Sweden, Norway, Portugal, the U.K., Canada and New Zealand, while the lowest incidence rates (< 1/100000 per year) have been found in certain parts of China and South America. In most populations the incidence in childhood has been found to increase with age, being highest among children aged 10−14 years (Karvonen et al. 2000). Also, general increases in the incidence rates of T1D have been reported in many countries (Bendas et al. 2015, Jarosz-Chobot et al. 2011, Podar et al. 2001, Schober et al. 2009). In a study of 16 362 cases gathered from 44 centres representing most European countries during the period 1989−1994 the average annual increase in incidence was observed to be 3.4% (95% CI 2.5−44%), the most rapid increase, 6.3% (4.1−8.5%), being seen in children under 5 years and the lowest increase, 2.4% (1.0−3.8%), in children aged 10−14 years (EURODIAB ACE Study Group 2000). A male preponderance has been observed in countries with high T1D rates, while a slight female preponderance has been reported in low-incidence countries (Rewers et al. 1988). Consistent with this, Harjutsalo et al (2013) found that the incidence rate of T1D was 68.4/100 000 in boys and 55.4/100 000 in girls in 2006−2011 (Harjutsalo et al. 2013).

The incidence of T1D in Finland is the highest in the world (Karvonen et al. 2000). It has also increased during recent decades, being 31.3 per 100,000 per year in 1980 and increasing to 64.9 per 100,000 per year in 2006 (Harjutsalo et al. 2013).
2008, Harjutsalo et al. 2013). The average annual increase in incidence was 3.0%, being 1.6% from 1980 to 1991 and 4.1% from 1992 to 2005. The most rapid increase, 4.7% per year, has been seen in children aged 0–4 years (Harjutsalo et al. 2008). It has been suggested earlier that the number of genetically susceptible individuals may be increasing, thus explaining the increase in the incidence of T1D, but Pitkäniemi et al. (2004) concluded that non-Mendelian transmission of diabetic haplotypes, if present, could only partially account for this phenomenon, indicating that other, probably environmental factors are involved in modifying the incidence of T1D (Pitkäniemi et al. 2004). This theory is supported by the observation that the frequencies of HLA risk haplotypes and genotypes have been found to be higher and the frequencies of protective haplotypes lower among Finnish patients diagnosed in childhood before 1965 than among those diagnosed after 1990 (Hermann et al. 2003a). Fortunately, the incidence of T1D has actually levelled off since the peak year of 2006 (Harjutsalo et al. 2013).

A clear geographical variation in the incidence among children younger than 15 years of age was nevertheless observed during a 10–year period (1987–96), the high-risk areas being found in a wide belt crossing the central part of the country (Rytkönen et al. 2001).

1.3 Pathogenesis of type 1 diabetes

The clinical presentation of T1D is preceded by an asymptomatic period of variable duration. It is characterized by selective loss of insulin-producing beta-cells in the pancreatic islets by the autoimmune mediated process (Christoffersson et al. 2016). Aggressive beta-cell destruction may lead to clinical disease within only a few months in infants and younger children, while in some older individuals the process can proceed for more than 20 years before presentation with an overt disease (Knip & Siljander 2008). Recent studies have shown great variability in beta-cell mass decline in patients on their first year after diagnosis of T1D, arguing for the existence of an unknown amount of remaining beta-cells in the pancreas (Christoffersson et al. 2016).

Although the initiation of the process does not necessarily lead to progression to clinical disease, recent analyses with long-term follow-up of children positive for multiple islet autoantibodies indicate that the risk of developing clinical diabetes is more than 80% after a follow-up of 15 years (Ziegler et al. 2013). The risk determinants of T1D, the initiators of the autoimmune response, the mechanisms regulating progress towards beta-cell failure and the factors
determining the time of presentation with clinical diabetes, are poorly understood. According to current perceptions, both genetic risk factors and external determinants have an effect on the development of T1D. The genetic component cannot be assigned to any classical model of inheritance, but rather interactions between genes together with environmental factors contribute to T1D (Knip 2002b).

![Diagram of T1D progression](image)

**Fig. 1. Progression to type 1 diabetes (T1D) in individuals with genetic diabetes susceptibility.** The vertical axis represents insulin secretory capacity and the horizontal axis time. The disease process may be triggered and modified by a series of environmental factors. Symptoms of T1D occur when around 80-90% of the beta-cells have been destroyed. The figure is modified from Eisenbarth et al. (1985) and Knip et al. (2005).

The appearance of one or more islet autoantibodies is the first detectable sign of beta-cell autoimmunity. There are five autoantibodies that have been shown to predict T1D, including islet cell antibodies (ICA), insulin autoantibodies (IAAs), tyrosine phosphatase autoantibodies (IA-2A) and autoantibodies against glutamic acid decarboxylase (GADA) and zinc transporter 8 (ZnT8A) (Knip 2002a). The number of various types of detectable autoantibodies has been found in both family studies and surveys based on general population cohorts to be unequivocally related to the risk of progression to overt T1D. Family studies have
shown that positivity for three to four autoantibodies is associated with a 60–100% risk of developing clinical T1D over the next 5–10 years (Bingley et al. 1994), while general population studies indicate that the predictive value of multiple autoantibody positivity is comparable to that observed among first-degree relatives (Siljander et al. 2007).

The process of beta-cell autoimmunity may be induced early in infancy and the first autoantibodies may have already appeared before the age of 3 months (Kimpimäki et al. 2001b). The development of multiple autoantibodies (≥2) occurs in most cases during the subsequent 6-12 months after the appearance of the first autoantibody (Kupila et al. 2002, Parikka et al. 2012). On the other hand, if this spreading does not happen within one year, it will seldom occur later, implying that in most cases positivity for a single autoantibody represents harmless non-progressive beta-cell autoimmunity (Knip 2002b). The majority of children who present with overt T1D before the age of 10 years seroconvert to autoantibody positivity by the age of 2 years (Knip et al. 2010). It has been shown by observing subjects with increased HLA-conferred susceptibility to T1D from birth at intervals of 3-12 months that there is an unequivocal temporal variation in the appearance of the first autoantibodies, reflecting the initiation of the disease process. Most initial autoantibodies appear during the cold period in the autumn and winter but rarely in the spring or summer. The exact timing and rate of new seroconversions would nevertheless seem to vary from one year to another (Kimpimäki et al. 2001b).

The appearance of autoantibodies reflecting the autoimmune process has been described in a few studies. In the DIPP prospective birth cohort study (Ilonen et al. 2013), comprising children with HLA-conferred susceptibility to type 1 diabetes, the pattern of autoantibody appearance was analysed in 520 cases with advanced beta-cell autoimmunity associated with a high disease risk. In the 315 cases where a single biochemical autoantibody could be identified in the first positive sample this was IAA in 180 instances, GADA in 107 and IA-2A in 28. Also, the age of the children at seroconversion differed significantly between the three groups, IAA generally appearing as the first autoantibody during the second year of life, whereas GADA as the first autoantibody peaked later, between 3 and 5 years of age. The primary autoantigen in the development of beta-cell autoimmunity and T1D correlated closely with age and genetic factors, indicating heterogeneity in the initiation of the disease process. In the TEDDY study (Krischer et al. 2015), autoantibodies at 3 and 6 months were rare. Out of 549 children, 43.7% had only IAA, 37.7% had only GADA, 13.8% had both IAA and
GADA and 1.6% had IA2A as the first autoantibodies to appear. The incidence of IAA peaked within the first year of life and declined over the following 5 years, while GADA only increased until the second year and subsequently remained relatively constant.

1.4 Familial aggregation of type 1 diabetes

Although familial aggregation of T1D is a well-established phenomenon, as demonstrated in many epidemiological studies suggesting that genetic factors play an important role in disease susceptibility, more than 85% of patients lack any positive family history of the disease. In the Finnish DiMe study (Tuomilehto et al. 1992), 11.2% of the children with newly diagnosed T1D had at least one FDR (mother, father or a biological sibling) with T1D, and in 8.3% this was a parent, the prevalence being significantly higher in fathers (5.7%) than in mothers (2.6%). In a another study of 18 European centres, including one region from Finland (The EURODIAB ACE Study Group and The EURODIAB ACE Substudy 2 Study Group 1998), a positive association between the population incidence rate of T1D and the prevalence of T1D in fathers of affected children ($p<0.001$). A similar association was observed with the prevalence in sibling ($p<0.001$), but the association with prevalence in mothers of affected children was weaker and not significant.

The long-term risk of T1D in the FDRs of probands with T1D is considerably higher than in the background population. The cumulative incidence of T1D in 10 168 siblings of young-onset type 1 diabetic patients has been observed to be 6.4% in Finland during a follow-up lasting 30 years (Harjutsalo et al. 2005), and the concordance rates for T1D among monozygous twins with a long-term follow-up was higher than 50%, as compared with 6-10% among dizygous twins, a figure which is identical to that observed among siblings in general (Redondo et al. 2008). The cumulative incidence of T1D in the offspring of parents with adult-onset T1D was 4.0% (95% CI 3.1-4.8) by 20 years of age, the risk being similar for both parents (Harjutsalo et al. 2010). In a Danish population-based study of 2726 T1D probands whose 2826 offspring were followed up to age 30 years (Lorenzen et al. 1998), 69 offspring were affected by T1D (2.4%) and the cumulative risk was observed to be significantly lower in the maternal than in the paternal ones (2.3% vs. 5.7%, respectively). In another study (Lorenzen et al. 1994), in which the T1D probands (n=310) were diagnosed before the age of 20 years and were observed up to the age of 50 years or older, 25.1% of them had at
least one FDR with T1D by the end of the observation period, while the proportion at T1D diagnosis had been 10.4%. Seventeen percent of the probands had at least one affected sibling. The cumulative risks among siblings from the time of birth of the proband were estimated to be 6.4% up to age 30 years and 9.6% up to 60 years, while the risk among offspring was 6.3% up to the age of 34 years.

1.5 Genetics of type 1 diabetes

The basis of the genetic investigations of type 1 diabetes was the observations of the familial aggregation of the disease. Totally, about 10% of newly diagnosed patients have at least one family member affected by T1D (Knip et al. 2005). However, the concordance rate between monozygotic twins is significantly less than 100% (for T1D 13-65%), thus it is thought that nongenetic (environmental) factors contribute to the disease pathogenesis (Kaprio et al. 1992, Redondo et al. 2008).

The HLA gene region on the short arm of chromosome 6 (6p21) is the most important of the multiple gene loci affecting susceptibility to T1D and explains approximately one-half of the genetic susceptibility for the disease. The region contains genes encoding class I (HLA-A, B, C), class II (HLA-DR, DQ, DP) and as well as class III region which includes several other genes encoding molecules that are crucial to the immune system (MIC-A, TNFA, TNFB, Hsp70) (Bouqbis et al. 2003, Klein & Sato 2000a, Klein & Sato 2000b, Kumar et al. 2012, Mokhtari et al. 2009). Class II alleles define the main HLA effect on T1D, but there is an independent effect of certain class I alleles on disease susceptibility. Thus it has been proposed that class II genes determine the initiation of autoimmunity, whereas class I genes are involved in the progression of beta-cell damage (Lipponen et al. 2010, Tait et al. 2003). The highest risk DR/DQ haplotypes for T1D are DR3-DQA1*0501-DQB1*0201 (DR3) and DR4-DQA1*0301-DQB1*0302 (DR4), accounting for up to 30-50% of the genetic T1D risk (Noble et al. 1996). Although the heterozygous genotype including both of these risk haplotypes confers a high risk of T1D, there is a spectrum of risk associated with the HLA DR/DQ genotypes – from increased to neutral to protective (Hermann et al. 2003b, Pugliese et al. 1995). These risk markers are nevertheless to some extent dependent on the ethnic group studied. Some common alleles appear in nearly every population studied, for example DRB1*03:01, however others are population specific. For instance, the allele DRB1*04:03 unlike most DRB1*04
alleles, is protective for T1D, and is seen at far higher frequency in individuals of Asian descent than for European descent. Similarly, a designation for haplotypes including the allele DRB1*07:01 is commonly seen as DRB1*07:01-DQA1*02:01-DQB1*02:02 in Europeans and is known to be protective for T1D. However, a closely related version of DRB1*07:01-DQA1*0301-DQB1*02:02, found in African populations, is predisposing for T1D (Noble 2015).

In an analysis of 622 Finnish children <15 years of age diagnosed with T1D the major susceptibility haplotype was DRB1*0401-DQB1*0302, present in 52.5% of the patients, followed by (DR3)-DQA1*05-DQB1*02 (41.7%) and DRB1*0404-DQB1*0302 (16.9%). The haplotypes associated with significant protection from the disease in the Finnish population were (DR7)-DQA1*0201-DQB1*0303, (DR14)-DQB1*0503, (DR15)-DQB1*0602, DRB1*0403-DQB1*0302, (DR12)-DQB1*0603, (DR11/12/13)-DQA1*05-DQB1*0301 and (DR1)-DQB1*0501 (Hermann et al. 2003b). However, another study of Finnish patients diagnosed in the periods 1939–1965 and 1990–2001 pointed to a considerable temporal change in the distribution of T1D-associated HLA genotypes over 50 years, implying increased environmental pressure that had resulted in higher disease penetrance, especially among individuals with protective HLA genotypes (Hermann et al. 2003a).

Although HLA accounts for approximately one-half of T1D risk (Lambert et al. 2004, Noble et al. 1996), there remains substantial residual genetic risk, likely attributed to single nucleotide polymorphisms in genes outside the HLA region (non-HLA). Non-HLA T1D loci associated with T1D include the insulin gene (INS) on chromosome 11p15 (Bell et al. 1984), the polymorphic, cytotoxic T-lymphocyte-associated protein 4 (CTLA4) gene on chromosome 2q33 (Nistico et al. 1996), the protein tyrosine phosphatase, non-receptor type 22 (lymphoid) (PTPN22) gene on chromosome 1P13 (Bottini et al. 2004), the interleukin 2 receptor, alpha (IL2RA) and interferon induced with helicase C domain 1 (IFIH1) genes, and other loci discovered quite recently in genome-wide association (GWA) studies (Todd et al. 2007). In the Finnish population, insulin gene polymorphism appeared to contribute to the pathogenesis of T1D by initiating/modifying insulin-specific autoimmunity, whereas none of the autoantibodies showed any association with the CTLA4 gene (Hermann et al. 2005). In another study, however, a possible linkage of the CTLA4 region with T1D was observed in a Finnish population (Turpeinen et al. 2003).

It has been shown, that highest risk heterozygous genotype HLA-D3/D4 is present in 50% of cases of very early-onset diabetes (Gillespie et al. 2014).
Similarly, it has been reported that T1D which begins in adulthood is characterized by a longer asymptomatic period before diagnosis, better preservation of residual beta-cell function, and lower HLA-D3/D4 heterozygosity than T1D that begins in childhood or adolescence (Karjalainen et al. 1989). In the Finnish DiMe Study, the children who had T1D sibling were younger at diagnosis of T1D and had more often high risk HLA genotype than children with a parent or non FDR with T1D (Veijola et al. 1996).

1.6 Environmental risk factors for type 1 diabetes

Since it has been proposed that about a half of the risk of T1D is due to genetic factors (Noble et al. 1996), environmental factors must obviously also play an important role in the pathogenesis of the disease. This view is supported by observations that are not explainable by genetic factors, such as the fact that less than 10% of people with HLA-conferred susceptibility progress to overt T1D, the more than 15-fold difference in T1D incidence even among Caucasians, a several-fold increase in the incidence of T1D over the last 50 years in many developed countries and a simultaneous decrease in the mean age at diagnosis (Knip et al. 2005, Maclaren & Atkinson 1992). Furthermore, it has been observed in migrant studies that the incidence of T1D has increased in population groups who have moved from a low-incidence region to a high-incidence one, emphasizing the influence of environmental factors (Åkerblom & Knip 1998).

Environmental factors can either trigger the process of beta-cell autoimmunity or accelerate and promote an ongoing process. The progression to clinical T1D can result from several assaults on the beta cells by different environmental factors, each leading to gradual destruction and a decrease in insulin secretory capacity (Åkerblom et al. 2002, Knip et al. 2005). A clear feature in pathogenesis of T1D is beta-cell autoimmunity which most often occurs early in life, a peak at 1–3 years of age (Parikka et al. 2012). Thus, the trigger leading to autoimmunity may occur early in life. The prenatal and neonatal periods are probably very important to study considering the remarkable development of the immune system and gut microbiome. No individual contributory exogenous factor has yet been definitely identifier, however.
1.6.1 Microbial factors

Viral infections are regarded as potential major actors in the development of T1D. Direct destruction of beta cells by viruses, immune-mediated destruction by cross-reactive T cells or non-specific enhancement of antigen presentation have been implicated as underlying pathogenic mechanisms (Jun & Yoon 2003).

The incidence of T1D has increased in parallel with the reduced incidence of many infections due to improved standards of hygiene and medical care (use of antibiotics, vaccination campaigns and better socio-economic conditions). The working hypothesis proposing a causal link between these two factors is called the “hygiene hypothesis”. This predicts that increased hygienic living conditions, the use of antibiotics and sterile food preparation will result in the continuous segregation of the immune system from positive microbial pressure, thus favouring increased susceptibility to immune-mediated disorders (Chatenoud et al. 2010). In addition, there have also been several reports of an inverse relationship between the incidence of T1D and population density, population mixing and the proportion of children in the population (Parslow et al. 2001, Schober et al. 2003, Waldhor et al. 2000). In Finland, a strong inverse correlation has been reported between population density and the incidence of T1D (Karvonen et al. 1997, Rytkönen et al. 2003), and many studies have similarly shown the common childhood infections occurring during the first 6–12 months of life to reduce the risk of T1D (Blom et al. 1991, Gibbon et al. 1997, Pundziute-Lycka et al. 2000). Some authors have suggested that day-care protects children against T1D (Kaila & Taback 2001), while others have reported that the risk of T1D was inversely associated with having older siblings (OR 0.56; 95%CI 0.45-0.75, for 3 or more siblings) and with lower economic status (D’Angeli et al. 2010). These epidemiological findings support a possible protective role for environments in which infections are readily transmissible. However, one Finnish investigation into the influence of 9 forms of exposure (indoor and outdoor dogs and cats, farm animals, farming, visits to stables, day care and exposure to antibiotics during the first week of life), showed that only indoor exposure to a dog during the first year of life was inversely associated with the development of preclinical type 1 diabetes (Virtanen et al. 2014).

Enterovirus infections are one of the major candidates for the role of an environmental trigger of T1D, but there are still contradictory observations in this area. Since the discovery in 1969 of a significant correlation between the seasonal incidence of clinical T1D and the prevalence of coxsackie B4 virus infection

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(Gamble & Taylor 1969), there has been a constant search for evidence linking enterovirus infections to the aetiopathogenesis of T1D. Earlier studies suggested that enterovirus epidemics may be inversely associated with the incidence of T1D (Wagenknecht et al. 1991), as enterovirus antibodies have been found to be less frequent in countries with a high T1D rate (Finland, Sweden) than in countries with a low rate (Estonia, Germany, Hungary, Lithuania, Russia). This may indicate that a low frequency of enterovirus infections in the environment will increase the susceptibility of young children to the diabetogenic effect of enteroviruses due to impaired maternal protection (Viskari et al. 2004). This is supported by the finding that the frequency of maternal enterovirus antibodies was higher in countries with a low or intermediate incidence of T1D than in high-incidence countries (Viskari et al. 2005).

In the DIPP study 38 children who developed T1D and 140 control subjects were followed up and serum samples were screened for enterovirus RNA at 3 to 12-month intervals. Enterovirus RNA-positive samples were more frequent among the T1D subjects (5.1%) than among the control children (1.9%) before diagnosis of T1D. The strongest risk for T1D was related to enterovirus RNA positivity during the 6-month period preceding the first autoantibody-positive sample [OR 7.7 (95% CI 1.9-31.5)] (Oikarinen et al. 2011). In the DAISY-study, Stene et al. (Stene et al. 2010) found that progression from islet autoimmunity to clinical T1D may increase after an enterovirus infection characterized by the presence of viral RNA in the peripheral circulation. On the contrary, it was found in Norway that the frequency of human enterovirus RNA in stool samples did not differ between case and control subjects before seroconversion (Tapia et al. 2011b), nor was any correlation observed in the Babydiet study between the presence of human enteroviruses in the first year of life and the development of islet cell antibodies, or between human enterovirus infections and dietary intervention, maternal diabetes or clinical symptoms (Simonen-Tikka et al. 2011).

In the Finnish DIPP study, however, the effect of enterovirus infections on the appearance of T1D-associated autoimmunity was seen to be modified by exposure to cow’s milk proteins in early infancy, suggesting an interaction between these two factors. This observation may provide an explanation for the controversial findings obtained when analysing the effect of any single factor on the appearance of T1D-associated autoimmunity (Lempäinen et al. 2012). A new Finnish report has been published recently (Laitinen et al. 2014) in which the objective was to identify enterovirus serotypes that might be involved in the initiation of the disease process by screening neutralizing antibodies against 41
enterovirus types in children participating in the prospective DIPP study and testing those who were persistently positive for at least two autoantibodies that are predictive of diabetes. The participants comprised 183 cases and 366 matched autoantibody-negative control children. Coxsackie virus B1 was associated with an increased risk of beta-cell autoimmunity, the risk being strongest when the infection occurred a few months before the autoantibodies appeared. Conversely, Coxsackie viruses B3 and B6 were associated with a reduced risk. The analysis of potential interactions of CVB1 and other CVB serotypes indicated clear risk effect when the child had experienced CVB1 alone without protective serotypes (OR 2.5 [95%CI 1.4-4.7]; p=0.003), whereas children infected both CVB1 and one or more of the protective serotype were not at risk. These results were interpreted as a possible sign of immunological cross-protection against Coxsackie virus B1.

Little research has been done into the histopathology of the pancreas at the time of diagnosing T1D. Yoon et al. (1979) isolated the Coxsackie B4 virus from the pancreas of a 10-year-old boy who died of severe ketoacidosis at diagnosis. This virus also led to insulitis and beta-cell death in mice inoculated with it (Yoon et al. 1979). Willcox et al. (Willcox et al. 2010), who described seven patients with recent-onset T1D and insulitis and investigated the presence of the enteroviral capsid protein VP1 by immunohistochemistry, reported that this marker is preferentially present in the islets which contain beta-cells and show signs of enhanced replicative activity.

The association of cytomegalovirus (CMV) infections with T1D is contradictory. A close correlation has been reported between the CMV genome detectable in lymphocytes and ICA in patients with T1D (Pak et al. 1988), and a significant association has similarly been observed between high titres of anti-CMV IgG antibodies and ICA (Nicoletti et al. 1990). More recently, however, correlations have failed to emerge between the presence of CMV IgG antibodies and the appearance of ICA or other initial autoantibody specificities in children who later developed T1D (Aarnisalo et al. 2008, Hiltunen et al. 1995).

Other studies have focused on the possible relationship between T1D and virus infections other than the enteroviruses, such as picornaviruses (human parechovirus and Ljungan viruses), but no significant associations have been observed (Tapia et al. 2010, Tapia et al. 2011a, Tauriainen et al. 2007). There are fewer studies about the role of measles and mumps in the development of T1D. In an Italian study, a significant association was observed between T1D incidence
and mumps ($P = 0.034$) and rubella ($P = 0.014$) infections (Ramondetti et al. 2012).

The association of childhood vaccination and development of T1D have been studied particularly. The rationale may be related to the hypothesis that limited exposure to infections and more extensive use of vaccinations early in life can lead to an increased risk of T1D (Kaila & Taback 2001). However, in a meta-analysis of 23 observational studies investigating 16 forms of childhood vaccination was found no evidence of an association between routine vaccination and childhood T1D (Morgan et al. 2015).

1.6.2 Nutritional factors

Since beta-cell autoimmunity may appear during the first year of life, infant dietary characteristics, including breastfeeding and age at the first exposure to an infant formula and other supplementary foods, are likely candidate exposures. It has been hypothesized that T1D results from complex interactions between the gut microbiome, i.e. gut permeability and mucosal immunity (Vaaarala et al. 2008), and dietary exposures, particularly during infancy (Hansen et al. 2006, Penders et al. 2006). These studies have focused on the role of cow’s milk, wheat/cereals/gluten, omega-3 fatty acids, vitamin D and the maternal diet during pregnancy as well as on the overall role of the gut immune system. So far, no specific dietary factor has been shown to be an unequivocal risk factor for beta-cell autoimmunity or T1D, but there have also been a number of contradictory observations regarding the impact of various nutritional factors.

Numerous studies have been conducted to examine the association between age at the introduction of cow’s milk and T1D or beta-cell autoimmunity, but the results have been inconsistent. Some have suggested that young age at introduction of cow’s milk is associated with an increased risk (Gerstein 1994, Kimpimäki et al. 2001a), while others have found no association (Norris et al. 2003a, Savilahti & Saarinen 2009, Virtanen et al. 2006, Ziegler et al. 2003). In one Finnish study an enhanced humoral immune response to various cow’s milk proteins in infancy was found in children who later progressed to T1D (Luopajärvi et al. 2008). Breastfeeding has been reported to be protective, to have no effect or to predispose the infant to the development of T1D (Virtanen & Knip 2003), and one meta-analysis of retrospective case-control studies found that short-term breastfeeding (<3 months) was associated with an increased risk of T1D with OR 1.43 (Gerstein 1994). The results of the MIDIA study suggested
that breast-feeding for 12 months or longer predicted a lower risk of progression from islet autoimmunity to T1D among genetically predisposed children (Lund-Blix et al. 2015). Some prospective studies have reported no association between breastfeeding and beta-cell autoimmunity (Norris et al. 2003b, Virtanen et al. 2006), while others have indicated that short-term breastfeeding is a risk factor for the development of autoimmunity (Holmberg et al. 2007, Kimpimäki et al. 2001a). The TRIGR intervention study, assessing whether weaning to either an extensively hydrolysed formula or a conventional cow’s milk-based formula before the age of 8 months has any effect on the development of T1D in children carrying HLA-conferred disease susceptibility and a positive history of T1D in a FDR, showed in its Finnish pilot study that the cumulative incidence of autoantibodies was almost 50% lower in the experimental group, thus supporting the possibility for manipulating spontaneous beta-cell autoimmunity by dietary intervention in infancy (Åkerblom et al. 2005). Nevertheless, the first analysis of the 2159 participants at the 78 centres in 15 countries taking part in the full-scale TRIGR study showed the absolute risk of positivity for two or more islet autoantibodies by the age of 6 years to be 13.4% among those randomized to the casein hydrolysate formula vs 11.4% among those randomized to the conventional formula, suggesting no significant difference between the groups (Knip et al. 2014). The final outcome of the TRIGR study, i.e. figures for the progression to clinical T1D by the age of 10 years, will become available in 2017.

Some authors have suggested that a lack of vitamin D supplementation in infancy may increase the subsequent risk of T1D. In a Finnish birth cohort study regular high-dose supplementation with vitamin D (2000 IU/day) was associated with a decrease in the risk of T1D [OR 0.12 (95% CI 0.03-0.51)], but even irregular supplementation was linked to a reduced risk compared with cases receiving no supplementation [OR 0.16 (95% CI 0.04-0.74)] (Hyppönen et al. 2001). In a retrospective European case-control study supplementation with vitamin D was observed to reduce the risk of T1D [ OR 0.67 (95% CI 0.53-0.86)] (The EURODIAB Substudy 2 Study Group 1999), and a meta-analysis of retrospective studies indicated that vitamin D supplementation in infancy provided protection from T1D [OR 0.71 (95% CI 0.60-0.84)] (Zipitis & Akobeng 2008). Low levels of vitamin D have sometimes been found in children at the diagnosis of T1D, however (Franchi et al. 2014), and in one study the odds on T1D were more than twice as high for the offspring of women with low levels of 25(OH)D than for the offspring of those with levels in the upper quartile (Sorensen et al. 2011). It was evident in the population of the DAISY study
(Simpson et al. 2011), however, that neither vitamin D intake nor 25(OH)D levels throughout childhood showed any association with the risk of IAA development or progression to T1D. Recent data from the DIPP study show no differences in the circulating concentrations of 25(OH) D during a prospective follow-up between autoantibody-positive children who progressed to overt T1D and matched autoantibody-negative control children (Mäkinen et al. 2015). Furthermore, maternal intake of vitamin D either from food or supplements during pregnancy was not associated with beta-cell autoimmunity/T1D in Finnish offspring carrying increased genetic susceptibility to T1D (Marjamäki et al. 2010). Similarly, maternal use of multivitamin supplements containing vitamin D during pregnancy was not related to the risk of children in Sweden developing T1D before 14–16 years of age (Granfors et al. 2015). The preliminary results of another Finnish study suggest that the maternal genotypes of single nucleotide polymorphisms (SNPs) in the vitamin D receptor (VDR) may influence the in utero environment and thus contribute to the early programming of the foetal immune system to favour the development of T1D, and it is possible that such effects are operative only in the presence of vitamin D deficiency (Miettinen et al. 2015).

It has been hypothesized that probiotics may affect immunological responses to environmental exposures by supporting healthy gut microbiota and could thus be used to prevent the development of T1D-associated islet autoimmunity. In the TEDDY study early probiotic supplementation (at the age of 0–27 days) was found to be associated with a decreased risk of islet autoimmunity as compared with probiotic supplementation after 27 days or no probiotic supplementation (Uusitalo et al. 2015).

In a longitudinal observational study observing otherwise healthy children carrying an increased genetic risk of T1D the investigators reported that a higher omega-3 fatty acid intake and higher erythrocyte membrane omega-3 fatty acid levels were associated with a reduced risk of beta-cell autoimmunity (HR 0.45 and HR 0.63, respectively) (Norris et al. 2007). Similarly, in a case-control study from Norway (Stene & Joner 2003), the children with T1D were less likely to have been given cod liver oil, which contains vitamin D, omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA), during infancy than were the control children. It was also reported, however, that omega-3 or omega-6 fatty acid intake and omega-3 or omega-6 levels in the erythrocyte membranes were not associated with progression to T1D among the autoantibody-positive children (Miller et al. 2011).
The available data suggest that there are specific times in infancy when gluten or cereal exposure is associated with an increased risk of beta-cell autoimmunity. In two prospective studies of genetically susceptible children (Norris et al. 2003b, Ziegler et al. 2003), an increased risk of beta-cell autoimmunity was observed in those who were first introduced to cereals containing gluten before the age of 3 months relative to those with their first introduction between the ages of 3 and 6 months. Furthermore, interventions in the case of islet autoantibody-positive subjects have indicated that beta-cell function may be improved by a gluten-free diet for 6 months, although the intervention did not influence autoantibody titres (Pastore et al. 2003). On the other hand, a dietary intervention study among children with HLA-conferred susceptibility to T1D and having a FDR with T1D found no significant differences between the children exposed to gluten for the first time at the age of 6 months (control group) and those exposed to it at the age of 12 months (late-exposure group) (Hummel et al. 2011). Likewise, the results of the TEDDY study indicate that the dietary intake of soluble fibre is not associated with islet autoimmunity or T1D in early life (Beyerlein et al. 2015).

1.6.3 Maternal risk factors and infant growth

The relationship between maternal and neonatal factors and the subsequent risk of T1D has been studied extensively. There are several reports of a significant increased risk of T1D with increasing maternal age (Cardwell et al. 2010a, Haynes et al. 2007, Stene et al. 2001), while others have reported no association (Ievins et al. 2007). Maternal weight status and weight gain during the pregnancy were not associated with the development of advanced beta-cell autoimmunity in the offspring (Arkkola et al. 2011, Robertson & Harrild 2010). A reduced risk of T1D development was observed in children whose mothers smoked (Robertson & Harrild 2010, Svensson et al. 2005), whereas children born to mothers diagnosed with pre-eclampsia during pregnancy had a slightly increased risk of T1D (Henry et al. 2011). Caesarean section was observed to increase the T1D risk by around 20%, which have mostly thought to result from differences in exposure to bacteria in early life (Cardwell et al. 2008).

A high birth weight seems to increase the risk of T1D (Algert et al. 2009, Cardwell et al. 2010b), and there is even an “accelerator hypothesis” that proposes that weight gain and the associated insulin resistance accelerate the loss of beta cells in both T1D and T2D in such a way that the two disease entities are
distinguished only by their rate of progression (Wilkin 2001). Harder et al. found a 7% increase in the risk for each 1000 g in birth weight (Harder et al. 2009). In a prospective birth cohort study in which the growth of infants with an affected FDR was observed up to the age of 6 years, their weight gain in early life was observed to predict the risk of islet autoimmunity (Couper et al. 2009). Similarly, excess body weight was observed to be a risk factor for islet autoimmunity in Finnish children (Hyppönen et al. 2000). In the large prospective population-based study including data on 99 832 children in Norway and Denmark, their weight increase during the first year of life was positively associated with type 1 diabetes (Magnus et al. 2015). Contrary results have been reported in some studies, however (Cambuli et al. 2010, Vehik et al. 2009, Winkler et al. 2009), and increased linear height growth during childhood has also been shown to increase the risk of T1D (Lamb et al. 2009, Vehik et al. 2009).

1.6.4 Psychosocial factors

Serious life events have been studied retrospectively in children with newly diagnosed with T1D, and the data suggest that such events might be a risk factor for T1D. Negative life events such as parental divorce, serious illness within the family, or loss of a first-degree relative during the first 2 years of life increased the risk of T1D (Hagglof et al. 1991, Thernlund et al. 1995), while elsewhere psychosocial stress was found to be associated with the induction or progression of islet autoimmunity in infancy in the general population, leading to the “beta-cell stress hypothesis” (Sepa et al. 2005). Furthermore, maternal experiences of serious life events such as divorce or violence were observed to affect the induction or progression of islet autoimmunity in children by the age of 2.5 years (Sepa et al. 2005).

1.7 Prediction and prevention of type 1 diabetes

1.7.1 Prediction

The prediction of T1D (through a combination of genetic, immunological and metabolic markers) has been a major target of diabetes research over recent decades. The long subclinical prodromal period offers the opportunity to identify individuals at risk before clinical manifestation of the disease, and these children
a represent a target group for prevention studies. There is substantial individual variation in the duration of preclinical T1D, however, and during that period there are fluctuations in antibody levels and in the disease process as it affects a given individual (Knip 2002b). Unaffected family members of patients with T1D are a congenial target group for the prediction of T1D. Since the index cases need regular follow-up, the identification and monitoring of siblings is relatively simple to implement (Bonifacio et al. 2004, Tuomilehto et al. 1992). A more comprehensive way of predicting T1D is nevertheless to identify subjects at risk in the general population, starting from those with a genetic susceptibility to T1D.

Regular autoantibody testing of such children from birth can theoretically identify 65-70% of future childhood T1D. (Kupila et al. 2001), In general, multiple islet autoantibody seroconversion seems to predict progression to type 1 diabetes. An analysis of data pooled from prospective cohort studies performed in Colorado, Finland and Germany examining children genetically at risk for type 1 diabetes (Ziegler et al. 2013) showed the majority (84.2%) out of 585 children with multiple islet autoantibody seroconversion to have progressed to type 1 diabetes within the next 15 years, thus indicating that type 1 diabetes is currently not preventable in these children.

Dysglycaemia detected in an OGTT or based on random plasma glucose or an increase in HbA1C levels can be useful markers for predicting the time to the onset of type 1 diabetes in children with an HLA-conferred risk involving two or more autoantibodies (Helminen et al. 2015a, Helminen et al. 2015b, Sosenko et al. 2015). Additionally, an acceleration of decline in first-phase insulin response (FPIR) has been found during the progression of T1D (Sosenko et al. 2013).
Table 1. The prediction of Type 1 diabetes by family history of T1D, genetic susceptibility, beta-cell autoimmunity and metabolic markers. HR1 is reflecting the hazard ratio of islet autoimmunity and HR2 the hazard ratio of progression from islet autoimmunity to T1D. Risk % is reflecting the percentage of children progressing to T1D.

<table>
<thead>
<tr>
<th>Predictive values</th>
<th>Significance in Prediction (HR1/ HR2 or risk %)</th>
<th>Key References</th>
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<tbody>
<tr>
<td>Family history</td>
<td>Mother with T1D (3%)</td>
<td>(Bonifacio et al. 2004)</td>
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<td></td>
<td>Father with T1D (5%)</td>
<td>(Hemminki et al. 2009)</td>
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<td></td>
<td>Sibling with T1D (8%)</td>
<td>(Tuomilehto et al. 1992)</td>
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<td></td>
<td>Multiple FDR with T1D (20%)</td>
<td>(Redondo et al. 2008)</td>
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<td></td>
<td>Identical twin (30%)</td>
<td>(Kaprio et al. 1992)</td>
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<tr>
<td>Genetic susceptibility</td>
<td>Background risk (0.9%)</td>
<td>(Harjutsalo et al. 2013)</td>
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<td></td>
<td>HLA Class II (3.96 / 1.063)</td>
<td>(Lambert et al. 2004, Lipponen et al. 2010)</td>
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<tr>
<td></td>
<td>-HLA DR4-DQ8/DR4-DQ8 (3%)</td>
<td>(Lemmark et al. 2011a)</td>
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<tr>
<td></td>
<td>-HLA DR3/DR4-DQ8 (5%)</td>
<td>(Ziegler &amp; Nepom 2010)</td>
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<td></td>
<td>NON-HLA</td>
<td>(Bell et al. 1984)</td>
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<td>-INS (1.29/1.65)</td>
<td>(Nisticco et al. 1996)</td>
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<td></td>
<td>-PTPN22 (1.87/1.59)</td>
<td>(Todd et al. 2007)</td>
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<td></td>
<td>-CTLA4 (1.20/0.95)</td>
<td>(Bottini et al. 2004)</td>
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<td></td>
<td>-UBASH3A (1.55/1.44)</td>
<td>(Steck et al. 2014)</td>
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<td></td>
<td>-IL2RA (0.61/1.15)</td>
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<td></td>
<td>-IFIH1 (1.07/1.47)</td>
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<tr>
<td>Beta-cell autoimmunity</td>
<td>ICA</td>
<td>(Kulmala et al. 1998)</td>
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<td></td>
<td>IAA</td>
<td>(Bingley et al. 1999)</td>
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<tr>
<td></td>
<td>GADA</td>
<td>(Siljander et al. 2007)</td>
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<td></td>
<td>IA-2A</td>
<td>(Steck et al. 2015)</td>
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<tr>
<td></td>
<td>1 out of IAA, GADA or IA-2A (11%/5 yrs)</td>
<td>(Ziegler et al. 2013)</td>
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<tr>
<td></td>
<td>2 out of IAA, GADA or IA-2A (36%/5 yrs)</td>
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<td></td>
<td>&gt;2 out of IAA, GADA or IA-2A (47%/6 yrs)</td>
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<td></td>
<td>≥2 out of IAA, GADA or IA-2A (70%/10 yrs)</td>
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<td></td>
<td>≥2 out of IAA, GADA or IA-2A (84%/15 yrs)</td>
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<td>Metabolic markers</td>
<td>OGTT</td>
<td>(Sosenko et al. 2015)</td>
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<td>FPIR</td>
<td>(Sosenko et al. 2013)</td>
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<td>IFG (~3.2)</td>
<td>(Helminen et al. 2015b)</td>
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<td>IGT (~8.3)</td>
<td>(Helminen et al. 2015a)</td>
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<td>HbA1C (~5.7, if 10% increase in HbA1C)</td>
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1.7.2 Prevention and preventive trials

Efforts for the prevention of T1D may be targeted at subjects representing different stages in the pathogenic disease process. The activity may focus on cases with no signs of beta-cell autoimmunity and aim at averting the initiation of the disease process (primary prevention), protecting individuals at risk from the putative environmental triggers, for example.

Prevention strategies may also focus on the preclinical phase of T1D, with the aim of delaying or preventing the progression of the disease process in subjects who already have signs of beta-cell autoimmunity (secondary prevention).

Prevention may also be targeted at subjects who have already been diagnosed with T1D if significant numbers of beta cells remain (tertiary prevention). The preventive studies and the results are represented in Table 2.
Table 2. The preventive trials, the results and key references of the studies. In the primary prevention study, the objective was to reduce the risk of beta-cell autoimmunity and T1D in children with increased genetic risk. In the secondary prevention studies, the aim was to prevent or delay T1D after the detection of islet autoantibodies in children with genetic risk or first degree relatives of T1D patients. In the tertiary prevention studies the aim was to preserve C-peptide production of newly diagnosed children.

<table>
<thead>
<tr>
<th>Prevention Study</th>
<th>Intervention</th>
<th>Result</th>
<th>Key references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary prevention</td>
<td><strong>TRIGR</strong> Casein hydrolysate formula</td>
<td>No benefit</td>
<td>(Åkerblom <em>et al.</em> 2011)</td>
</tr>
<tr>
<td></td>
<td><strong>FINDIA</strong> Bovine insulin-free cow’s milk formula</td>
<td>Benefit during first 3 follow-up years</td>
<td>(Vaarala <em>et al.</em> 2012)</td>
</tr>
<tr>
<td></td>
<td><strong>BABYDIET</strong> Gluten-free diet in infants</td>
<td>No benefit</td>
<td>(Hummel <em>et al.</em> 2011)</td>
</tr>
<tr>
<td></td>
<td><strong>D-VITAMIN</strong> Vitamin D3 supplementation in infancy (2000 IU/d vs. 400 IU/d)</td>
<td>Pilot Study</td>
<td>(Wicklow &amp; Taback 2006)</td>
</tr>
<tr>
<td>Secondary prevention</td>
<td><strong>ENDIT</strong> Nicotinamide</td>
<td>No benefit</td>
<td>(Gale <em>et al.</em> 2004)</td>
</tr>
<tr>
<td></td>
<td><strong>DPT-1</strong> Parental or oral insulin</td>
<td>No benefit</td>
<td>(Skyler <em>et al.</em> 2005)</td>
</tr>
<tr>
<td></td>
<td><strong>DIPP</strong> Nasal insulin</td>
<td>No benefit</td>
<td>(Näntö-Salonen <em>et al.</em> 2008)</td>
</tr>
<tr>
<td></td>
<td><strong>PRODIA</strong> Probiotics during the first 6 months of life</td>
<td>No benefit</td>
<td>(Ljungberg <em>et al.</em> 2006)</td>
</tr>
<tr>
<td>Tertiary prevention</td>
<td><strong>Cyclosporine</strong></td>
<td>Benefit, renal toxicity</td>
<td>(Stiller <em>et al.</em> 1984)</td>
</tr>
<tr>
<td></td>
<td><strong>Horse antithymocyte globulin and prednisone</strong></td>
<td>Benefit, bone marrow toxicity</td>
<td>(Eisenbarth <em>et al.</em> 1985)</td>
</tr>
<tr>
<td></td>
<td><strong>Monoclonal anti-CD20 antibody</strong></td>
<td>No benefit</td>
<td>(Pescovitz <em>et al.</em> 2009)</td>
</tr>
<tr>
<td></td>
<td><strong>Cyclophosphamide and ATG with autologous BMT</strong></td>
<td>Benefit</td>
<td>(Couri <em>et al.</em> 2009)</td>
</tr>
</tbody>
</table>
1.8  Diabetic ketoacidosis

1.8.1 Pathophysiology

The pathophysiology of DKA is summarized in Figure 2. Progressive beta-cell destruction leads to insulin deficiency, inducing impaired glucose utilization and an excess of counterregulatory hormones. Alternatively, children with established T1D may omit their insulin treatment or its action may be antagonized by physiological stress, e.g. sepsis. Additionally, technical problems in respect of insulin pump therapy may induce DKA rapidly. Together, these hormonal changes stimulate glucose production through glycogenolysis and gluconeogenesis, resulting in hyperglycaemia, osmotic diuresis, electrolyte loss, dehydration, decreased glomerular filtration and hyperosmolarity. Concomitantly, lipolysis produces increased free fatty acids and the oxidation of these acids facilitates gluconeogenesis and generates ketones (acetoacetic acid and \( \beta \)-hydroxybutyric acids). The buffering capacity of the body is thereby overwhelmed, resulting in metabolic acidosis combined with lactic acidosis due to poor tissue perfusion. A self-perpetuating cycle of progressive metabolic decompensation results from increased stress hormone secretion arising from progressive dehydration, hyperosmolarity, acidosis and electrolyte disturbances (Wolfsdorf et al. 2009).
Fig. 2. The pathophysiology of DKA. Figure modified from Wolfsdorf et al (Wolfsdorf et al. 2014).
1.8.2 Definition

The definition of diabetic ketoacidosis (DKA) varies considerably in the literature, which complicates comparisons of the findings reported. According to the ESPE/LWPES Consensus Statement on DKA in children and adolescents published in 2004, the biochemical criteria for its diagnosis include hyperglycaemia with a venous pH <7.30 and/or bicarbonate <15 mmol/l, with associated glucosuria, ketonuria and ketonaemia. DKA is generally categorized by the severity of the acidosis, varying from mild (pH <7.30 and/or bicarbonate <15mmol/l) to moderate (pH <7.20 and/or bicarbonate <10 mmol/l) or severe (pH <7.10 and/or bicarbonate <5 mmol/l) (Dunger et al. 2004a). The definitions and categories of DKA have remained unchanged in the more recent ISPAD guidelines (Craig et al. 2009).

1.8.3 Frequency

The frequency of DKA in children at the diagnosis of T1D varies widely in geographical terms and correlates inversely with the regional incidence of T1D (Levy-Marchal et al. 2001, Usher-Smith et al. 2012). In a study of over 29 000 children in 31 countries its frequency ranged from 12.8% to 80%, with the highest figures reported in the United Arab Emirates, Saudi Arabia and Romania and the lowest in Sweden and Canada. Multivariable modelling showed the frequency of DKA was inversely associated with gross domestic product (GDP) and latitude (Usher-Smith et al. 2012). There are only few studies of the frequency of DKA at diagnosis of T1D in developing countries, in one centre in Karachi, Pakistan the frequency was even 94% (Lone et al. 2010). There are some indications that the frequency of DKA in children at clinical presentation with T1D may be decreasing in many countries (Jackson et al. 2001, Pozzilli & Andreani 1990, Samuelsson & Stenhammar 2005, Stipancic et al. 2011), although contradictory findings have also been reported in some studies (Bui et al. 2002, Dabelea et al. 2014a, Pinkney et al. 1994, Rosenbauer et al. 2012). Rewers et al. have even reported an increasing frequency of DKA in a recent study performed in Colorado (Rewers et al. 2015). The frequencies of DKA in the different countries are presented in Table 3.
Table 3. The frequency of DKA at diagnosis of type 1 diabetes in the different countries classified on the basis of the gross domestic product (GDP) of the country during the time of data collected. Low GDP is defined as <10 000 US$/capita, median GDP as 10 000–20 000 US$/capita and high GDP as >20 000 US$/capita. Modified from Usher-Smith et al 2012.

<table>
<thead>
<tr>
<th>Country</th>
<th>Annual incidence of T1D (cases/100 000)</th>
<th>DKA (%)</th>
<th>Key references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low GDP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turkey</td>
<td>3.2</td>
<td>29</td>
<td>(Bober et al. 2001)</td>
</tr>
<tr>
<td>Poland</td>
<td>13</td>
<td>38-54.7</td>
<td>(Charemska et al. 2003, Levy-Marchal et al. 2001)</td>
</tr>
<tr>
<td>Slovenia</td>
<td>8.5</td>
<td>28.6</td>
<td>(Levy-Marchal et al. 2001)</td>
</tr>
<tr>
<td>Slovak Republic</td>
<td>9.2</td>
<td>35.6</td>
<td>(Levy-Marchal et al. 2001)</td>
</tr>
<tr>
<td>Romania</td>
<td>4.8</td>
<td>67</td>
<td>(Levy-Marchal et al. 2001)</td>
</tr>
<tr>
<td>Median GDP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finland</td>
<td>35.3</td>
<td>21.7</td>
<td>(Veijola et al. 1996)</td>
</tr>
<tr>
<td>New Zealand</td>
<td>17.9</td>
<td>29</td>
<td>(Campbell-Stokes &amp; Taylor 2005)</td>
</tr>
<tr>
<td>England</td>
<td>17.7</td>
<td>27</td>
<td>(Sundaram et al. 2009)</td>
</tr>
<tr>
<td>Iceland</td>
<td>13.9</td>
<td>30</td>
<td>(Levy-Marchal et al. 2001)</td>
</tr>
<tr>
<td>High GDP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>31.7</td>
<td>14.5-16</td>
<td>(Hanas et al. 2007, Sadauskaite-Kuehne et al. 2002)</td>
</tr>
<tr>
<td>Austria</td>
<td>10.3</td>
<td>37.2</td>
<td>(Schober et al. 2010)</td>
</tr>
<tr>
<td>USA</td>
<td>23.9</td>
<td>27-38.9</td>
<td>(Mallare et al. 2003, Rewers et al. 2015, Vehik et al. 2009)</td>
</tr>
<tr>
<td>Kuwait</td>
<td>22.3</td>
<td>37.7</td>
<td>(Abdul-Rasoul et al. 2010)</td>
</tr>
<tr>
<td>Canada</td>
<td>29.7</td>
<td>18.6</td>
<td>(Bui et al. 2010)</td>
</tr>
</tbody>
</table>

The risk of DKA in patients with established T1D has been observed to be up to 10 episodes per 100 patient years, with 20% of the patients accounting for 80% of the episodes (Rewers et al. 2002). In a recent multinational study including 49859 children and adolescents diagnosed with T1D more than 1 year ago in Austria, Germany, England and Wales the frequency of DKA during the preceding year was 5.0-7.1% (Maahs et al. 2015).

1.8.4 Predisposing factors and prevention

The risk of DKA varies according to the age of the child at the diagnosis of T1D. In Western countries DKA risk has been observed to be more common in children <5 years of age at diagnosis [OR 1.59 (95% CI 1.38-1.84)] (Komulainen et al. 1999, Pinkney et al. 1994, Quinn et al. 2006, Rewers et al. 2008), and children <2
years of age have a particularly high risk of DKA, with frequencies as high as 53-85% having been reported [OR 3.41 (95%CI 2.54-4.59)] (Dabelea et al. 2014a, Komulainen et al. 1999, Pawlowicz et al. 2009, Szypowska & Skorka 2011). Although young children have been reported to have a shorter duration of symptoms before diagnosis (Levy-Marchal et al. 2001, Samuelsson & Stenhammar 2005), a delay in diagnosis may also occur in these cases, because the symptoms may be difficult to recognize, especially as the disease is rare under the age of 1 year. A concomitant infectious illness has often been observed in young children at the diagnosis of T1D, and these infections may precipitate metabolic decompensation in a child with limited beta-cell reserves and also possibly mask the symptoms of T1D (Neu et al. 2001). Some studies have reported girls to be overrepresented in the group with ketoacidosis at presentation with T1D (Neu et al. 2009, Neu et al. 2003), although others have found no gender difference (Komulainen et al. 1996, Rewers et al. 2008).

DKA has been reported in most of the published research to occur less frequently in families with at least one member affected by T1D (de Vries L. et al. 2013, Rewers et al. 2008, Sadauskaite-Kuehne et al. 2002, Usher-Smith et al. 2011), but in some cases a positive family history has had no significant effect on the frequency of DKA in children with newly diagnosed T1D (Pawlowicz et al. 2009, Quinn et al. 2006, Rosenbauer et al. 2002). Similarly, the frequency of DKA has been associated with lower family income in some countries (Rewers et al. 2008), but no such association has been observed in Finland, although children from families with a low parental educational level in Finland had DKA at diagnosis more often than those from families with a higher education (Komulainen et al. 1996).

It has been reported in prospective studies that children diagnosed through screening for genetic predisposition to T1D and a subsequent follow-up have a less severe onset of the disease, probably due to their earlier diagnosis. This was reported in the DAISY study (Barker et al. 2004), for instance. Similarly, the children screened and followed up in the TEDDY study and diagnosed with T1D at <2 or <5 years of age had DKA less frequently at diagnosis than the population-based incidence of DKA would predict (Elding et al. 2011). In the BABYDIAB study (Winkler et al. 2011), screening for islet autoantibodies in children led to an earlier diagnosis, resulting in fewer complications at diagnosis. The children who were followed up after screening and were positive for islet autoantibodies had a lower prevalence of ketoacidosis at diagnosis (3.3% vs. 29.1%). Moreover, the children monitored in the DiPiS (Diabetes Prediction in
Skåne) study and diagnosed with T1D at 2–12 years of age had DKA at diagnosis less frequently (2% vs. 18%) (Lundgren et al. 2014).

Children with DKA at the diagnosis of T1D had a medical encounter before diagnosis as much as 55-68% more frequently than did children without DKA (Mallare et al. 2003, Szypowska & Skorka 2011). Bui et al. 2010 reported that during the week before diagnosis, 38.8% of children with DKA and 34.4% of children with diabetes without DKA had at least 1 medical visit. The three most commonly recorded diagnosis in the diabetic patients <3 years of age were upper respiratory tract infection, diarrhea/gastroenteritis, and serous otitis media. For patients over 3 years the most common diagnosis were upper respiratory tract infection, cystitis and other disorders of the urinary tract or diarrhea/gastroenteritis (Bui et al. 2010). This emphasizes the importance of enhancing both public and medical awareness of diabetes in children (Lokulo-Sodipe et al. 2014), especially as a higher frequency of DKA has been observed to be associated with a diagnostic delay [OR 3.35 (95% CI 2.25-4.79)], even though the mean duration of symptoms was similar in the children presenting with and without DKA (Usher-Smith et al. 2011). When an information programme on DKA was provided for teachers, students, parents and paediatricians in the Parma region of Italy for 8 years the frequency of DKA in children with newly diagnosed T1D decreased from 78% in 1987–1991 to 12.5% in 1991–1997, thus confirming the possibility for reducing the frequency of DKA in children by increasing the availability of information about the disease (Vanelli et al. 1999). An inverse correlation has been reported between the frequency of DKA and the background incidence of T1D, reflecting improved knowledge of the symptoms of T1D in countries with higher incidence figures (EURODIAB ACE Study Group 2000, Sadauskaite-Kuehne et al. 2002).

Little is known about the association between HLA class II genotypes and the risk of DKA at the diagnosis of T1D. The high-risk HLA-DQB1*02/*0302 genotype has been reported to be more frequent in children <2 years of age at diagnosis, who often have DKA upon presentation (Komulainen et al. 1999). High-risk HLA genotypes were associated with an increased risk of presenting with DKA at the diagnosis of T1D in an Italian study (Marigliano et al. 2013), while in the East Asia predominantly in Japan, China and Korea, a novel clinical entity called fulminant T1D has been reported in the adult patients which is characterized by an abrupt onset of hyperglycaemia, rapid progression to ketosis or ketoacidosis (within 1 week), high levels of serum amylase and lipase, markedly reduced pancreatic beta-cell secretory capacity, but near-normal
haemoglobin A1c (HbA1c) (Imagawa et al. 2000). There is a clear association of this fulminant disease with certain specific HLA-DR and HLA-DQ haplotypes, and it may be mediated by multiple factors that include viral infections, pregnancy and the drug sensitivity syndrome (Imagawa et al. 2005, Zheng et al. 2011).

The risk factors for recurrent DKA in children with established T1D are poor metabolic control, female gender (in adolescence), psychiatric disorders including eating disorders, difficult or unstable family circumstances, limited access to medical services, a migration background and insulin pump therapy T1D (Fritsch et al. 2011, Maniatis et al. 2005). Only rapid-acting or short-acting insulin is used in pumps, and as a result the interruption of insulin delivery for any reason will quickly lead to insulin deficiency. In some studies, however, the frequency of DKA in patients with insulin pump therapy has been observed to be lower than in children with multiple daily insulin injections (Jakisch et al. 2008). Ethnic minority status and an HbA1c value above the target have also been reported to be associated with an increased risk of recurrent DKA (Maahs et al. 2015). An intercurrent infection is seldom the cause of recurrent DKA provided that the patient or family is properly educated in diabetes management and is receiving appropriate care from a diabetes team with a 24-hour telephone service (Flood & Chiang 2001, Grey et al. 2000).

1.8.5 Treatment

The treatment of DKA includes an appropriate assessment of the clinical condition of the patient, monitoring, fluid and electrolyte therapy and insulin therapy.

Assessment

The clinical severity of the dehydration can be assessed based on the patient’s weight loss and clinical findings, including skin turgor, humidity of the mucous membranes, capillary refill time and signs of sunken eyes, tachycardia, weak or impalpable peripheral pulses, hypotension or shock. The Glasgow coma scale can be used to assess the level of consciousness. The precipitating factors like infections and alcohol or medicament abuse have to take into account in the assessment of the patient (Reilly et al. 1988, Wolfsdorf et al. 2009).
**Monitoring**

The documentation of clinical observations, intravenous and oral medication, fluids and laboratory results should be maintained during the entire treatment period. Monitoring should include heart and respiratory rate, blood pressure, fluid input and output, electrocardiograms in the case of severe DKA, and hourly or more frequent neurological observations for warning signs and symptoms of cerebral oedema. Laboratory tests including blood gases, plasma glucose, serum electrolytes, osmolality, calcium, magnesium and phosphorus should be repeated every 2–4 hours or more frequently as clinically indicated. Plasma glucose should be measured hourly. Other laboratory tests such as haematocrit, urea and creatinine should be repeated at 6–8-hour intervals until the results are normal (Dunger *et al.* 2004b, Wolfsdorf *et al.* 2009).

**Fluid and electrolyte therapy**

DKA is characterized by severe depletion of water and electrolytes from both the intracellular and extracellular fluid compartments. Specific deficits in fluids and electrolytes at presentation with DKA can vary in magnitude in an individual patient depending upon the duration and severity of the illness and the intake of fluids and food prior to diagnosis. It is often difficult to estimate the magnitude of dehydration accurately, but despite dehydration, urine output will continue until extreme volume depletion leads to a critical decrease in renal blood flow and glomerular filtration. Children with DKA have approximately a 5–10% deficit in extracellular fluid volume at diagnosis, but shock is a rare phenomenon in these patients. On the other hand, the magnitude of dehydration in DKA has not been observed to be reflected in either clinical or biochemical parameters in all studies. The objectives of fluid and electrolyte replacement are restoration of the circulating fluid volume and acidosis, replacement of sodium and extracellular and intracellular fluid deficits in terms of water, restoration of glomerular filtration and avoidance of excessive rates of fluid administration because of the possible risk of cerebral oedema (Sottosanti *et al.* 2012, Ugale *et al.* 2011, Wolfsdorf *et al.* 2009).

The fluid therapy in children consists of fluid resuscitation, repair of dehydration and maintenance fluid therapy. Volume expansion in order to restore the peripheral circulation (resuscitation) should begin immediately with an isotonic solution (0.9% saline or balanced solution such as Ringer’s lactate). The
volume and rate of initial fluid replacement will depend on the circulatory status, typically being 10–20ml/kg over the first 1–2 hours, possibly repeated if necessary (Wolford et al. 2009). The repair of dehydration and maintenance therapy are performed with glucose-containing fluids together with insulin therapy (Dunger et al. 2004a).

The optimal protocol for intravenous fluid administration is an area of particular controversy. Although there have been few systematic comparisons of fluid regimens, many studies recommend low-speed fluid deficit correction with isotonic or near-isotonic solutions for DKA treatment (24–48 hours). Rapid fluid replacement with hypotonic fluid carries with it an increased risk of cerebral oedema. Nevertheless, depending on the number of initial bolus infusions and their size, the estimated degree of dehydration, and the maintenance and fluid replacement rates chosen, it is possible to exceed the suggested safe limit of 4 litres/m²/24 hours (Dunger et al. 2004b, Edge & Dunger 1994, Felner & White 2001). A prospective 13-centre randomized trial to study fluid regimens for paediatric DKA (FLUID, Fluid Therapies Under Investigation in DKA) has been going on to evaluate the effects of the rehydration rate and fluid sodium content on neurological status during DKA, the frequency of clinically overt cerebral oedema and long-term neurocognitive outcomes following DKA (Glaser et al. 2013).

The serum sodium concentration is an unreliable measure of the degree of extracellular fluid concentration, because glucose restricted to the extracellular space will cause osmotic movement of water into that space, inducing dilutional hyponatraemia (Katz 1973) and the elevated lipid fraction of the serum in DKA will have a low sodium content. Accordingly, it is recommended to monitor corrected sodium levels (= measured Na + 2 x [(glucose mmol/l–5.6) ÷5.6]) throughout the course of therapy (Wolford et al. 2009). Calculation of the effective osmolality [2 x (Na +K) + glucose mmol/l] may be a valuable guide for fluid and electrolyte therapy, as maintenance of a hyperosmolar state will stimulate the cellular production of substrates with intracellular osmotic activity in order to preserve the intracellular water (Piva et al. 2007).

Children with DKA suffer from a potassium deficit of around 3–6 mmol/kg. The major loss of potassium is from the intracellular pool and is caused by vomiting, urinary ketoanion excretion and osmotic diuresis. Although total-body depletion of potassium occurs, serum potassium levels may be normal, increased or decreased (Adrogue et al. 1986). Potassium replacement therapy is thus required regardless of the serum potassium concentration and should continue
throughout the period of intravenous fluid therapy together with monitoring of serum potassium levels. In patients with hypokalaemia potassium replacement should start immediately after the initial volume expansion and before starting insulin therapy because insulin is driving potassium into the cells. If serum potassium concentrations decrease abruptly, cardiac arrhythmias may follow. Also, the depletion of intracellular phosphate occurs due to phosphate loss as a result of osmotic diuresis and by insulin administration as phosphate re-enters cells. Thus, potassium phosphate could be used together with potassium chloride, the maximum recommended rate of intravenous potassium replacement being 0.5 mmol / kg / h (Wolfsdorf et al. 2006).

Since experimental studies suggest that metabolic acidaemia can impair myocardial contractility, reduce cardiac output, affect oxyhaemoglobin dissociation and tissue oxygen delivery, inhibit intracellular enzymes, alter cellular metabolism and result in vital organ dysfunction, the therapeutic target in DKA has historically placed importance on the rapid reversal of acidaemia in addition to fluid and insulin therapy. The correction of severe acute acidaemia with intravenous bicarbonate is debatable. A systematic review of 44 papers on the topic found no evidence of improved glycaemic control or clinical efficacy due to intravenous bicarbonate, whereas there was retrospective evidence of an increased risk of cerebral oedema and prolonged hospitalization in children who received bicarbonate and weak evidence of a transient paradoxical worsening of ketosis and an increased need for potassium supplementation. Thus the evidence available to date does not justify the administration of bicarbonate for the emergent treatment of DKA, especially not in a paediatric population (Chua et al. 2011).

**Insulin therapy**

Although rehydration alone causes some decrease in plasma glucose concentration, insulin therapy is needed to normalize plasma glucose and to suppress lipolysis and ketogenesis. Various routes (subcutaneous, intramuscular and intravenous) and doses have been used for insulin therapy in cases of DKA, but there is extensive evidence to indicate that “low-dose” intravenous insulin administration should be the standard (Kitabchi 1989). It has also been suggested, however, that the use of subcutaneous insulin every 1 or 2 hours represents a safe and effective alternative for treating uncomplicated DKA in adult patients (Umpierrez et al. 2004). In the pediatric trial, 60 children with DKA (venous pH
<7.3 and/or bicarbonate <15 mmol/l) were randomized to subcutaneous insulin treatment (0.15 units/kg) given every 2 hours or intravenous insulin treatment (0.1 unit/kg/h). Capillary glucose levels decreased by 2.9 and 2.6 mmol/hour in the subcutaneous and intravenous groups, respectively, but blood glucose fluctuated at different time intervals. Metabolic acidosis and ketosis resolved earlier in the intravenous insulin group; however no complications were seen in any group (Della et al. 2005)

Based on physiological studies, it has been recommended that intravenous insulin should be used at a dose of 0.1 unit/kg/hour, which will achieve steady-state plasma insulin levels of 100-200 µmol/l within 60 minutes. Such plasma levels are able to offset insulin resistance and inhibit lipolysis and ketogenesis (Dunger et al. 2004b). On the other hand, it has been found that very low doses of insulin (1 unit/hour) may constitute a safer approach in the case of adults with severe DKA (Wagner et al. 1999). In one recent published study low-dose insulin infusion (0.05U/kg per hour) was found to be “not inferior to” the standard dose (0.1U/kg per hour) with respect to the rate of blood glucose decrease and the resolution of acidosis in children with diabetic ketoacidosis (Nallasamy et al. 2014).

1.8.6 Complications

DKA is the leading cause of hospitalization, death and morbidity in children with newly diagnosed or established T1D, the mortality rate being from 0.15% to 0.30% in developed countries (Curtis et al. 2002, Edge et al. 1999). However, in the developing countries the mortality rate of DKA is still higher; the frequencies of 7.1–13.2% have been published (Jayashree & Singhi 2004, Zargar et al. 2009). Most DKA mortality and morbidity arises as a consequence of the development of cerebral oedema, a rare and incompletely understood complication of DKA and/or its management. Cerebral oedema occurs in 0.5–1.0% of cases with DKA in developed countries and is associated with 20–30% mortality, accounting for 70–80% of all diabetes-related deaths. On the other hand, survival from an episode may lead to significant neurological sequelae, the frequency of morbidity being approximately 10–35% (Glaser et al. 2001, Lawrence et al. 2005, Zucchini et al. 2016). Only a minority of deaths in cases of DKA are attributable to other causes, such as sepsis, other infections (including mucormycosis), aspiration pneumonia, pulmonary oedema, acute respiratory distress syndrome, pneumomediastinum, hypo- or hyperkalaemia, hypoglycaemia, cardiac arrhythmias, central nervous
system (CNS) haematoma or thrombosis and rhabdomyolysis. Significant cerebral oedema will usually develop within 4–12 hours after starting treatment for DKA, but it can also occur before the beginning of the treatment (Glaser et al. 2001, Lawrence et al. 2005). The symptoms of this complication are variable, including headache and slowing heart rate, changes in neurological status (restlessness, irritability, increased drowsiness, incontinence and cranial nerve palsy), rising blood pressure and lowered oxygen saturation. The pathogenesis of both its initiation and its progression is unclear and incompletely understood, but evidence for disruption of the blood-brain barrier has been found in cases of death from cerebral oedema (Hoffman et al. 2009). There are many epidemiological studies identifying potential risk factors for cerebral oedema, including greater hypocapnia, increased serum urea nitrogen and more severe acidosis at presentation with DKA, bicarbonate treatment for correcting acidosis, an attenuated rise in measured serum sodium concentration during therapy, greater volumes of fluid given during the first 4 hours or administration of insulin in the first hour of fluid treatment (Wolfsdorf et al. 2009). More recent study, however, has shown the degree of oedema formation during DKA to correlate with the degree of dehydration and hyperventilation at presentation but not with factors related to initial osmolality or osmotic changes during treatment. This data indicate that cerebral oedema is related to cerebral hypoperfusion during DKA, and that osmotic fluctuations during treatment do not play a primary causal role (Glaser et al. 2008).

In the developing countries, the frequency of cerebral edema is still higher. In a centre of India, 26% of children with diabetic ketoacidosis had cerebral edema; the frequency of mortality was 25%. On the multiple regression analysis, fluid refractory shock and presence of azotaemia at admission were the predictors for developing of cerebral edema (Tiwari et al. 2012). It is nowadays thought, that the higher frequency of cerebral edema and resultant mortality rate in developing countries may be more caused by the initial factors of the patient, such as the severity of dehydration, acidosis, serum osmolality and hypocapnia than the used fluid protocol or insulin treatment (Glaser et al. 2001, Jayashree & Singhi 2004, Levin 2008).

1.8.7 Long-term outcome

It is a common belief that early diagnosis of T1D and subsequent early commencement of insulin treatment are desirable for achieving not only an
obviously better clinical condition in the patient, but also a positive long-term outcome. One of the most important consequences would be better preservation of endogenous insulin secretion as assessed through the measurement of serum C-peptide levels, which is in turn associated with better metabolic control. It has been shown that early diagnosis of T1D and early initiation of insulin treatment are related to better metabolic control in the first years after diagnosis (Barker et al. 2004, Bonfanti et al. 1998, Montanya et al. 1997), although it has also been claimed that the severity of clinical presentation at diagnosis had no significant influence on residual beta-cell function and long-term metabolic control (Salardi et al. 2003).

It has been reported that children with T1D are typically of average overall intellectual ability (Kovacs et al. 1990), but exhibit deficits in neuropsychological measures of memory. The origin of these deficits is not fully understood, but complications such as hypoglycaemia and DKA have been implicated (Ghetti et al. 2010, Hershey et al. 2003, Perantie et al. 2008, Strudwick et al. 2005). DKA has been reported to disrupt memory function in a case-control study, thus emphasising the importance of its prevention (Ghetti et al. 2010).
2 Aims of the research

The aims of the current work were

1. to assess the frequency of DKA in children in Finland at clinical presentation with T1D with the hypothesis that the frequency of DKA has decreased over time,
2. to evaluate age-related differences and possible age-related temporal changes in the frequency of DKA at diagnosis, with the hypothesis that DKA has decreased in young children,
3. to assess the prevalence of T1D and T2D among first-degree relatives and grandparents of children with newly diagnosed T1D and to investigate whether family history of diabetes decreases the frequency of DKA at diagnosis,
4. to explore possible associations between HLA risk genotypes in children with newly diagnosed T1D and the frequency of DKA with a hypothesis that high risk HLA genotype is associated with increased DKA risk, and
5. to define whether the frequency of DKA at diagnosis of childhood T1D had decreased concurrently with the ongoing prospective T1D follow-up studies in Finland.
3 Subjects and methods

The work for this thesis was carried out at the Department of Paediatrics, Faculty of Medicine, University of Oulu, Finland, and Oulu University Hospital over the years 2002–2015. The subjects and primary outcomes in involved in papers I–IV are summarized in Table 4.

Table 4. Numbers, specifications and mean ages (range) of participants in the studies reported in papers I-IV and primary outcomes of the research.

<table>
<thead>
<tr>
<th>Paper</th>
<th>Subjects</th>
<th>N</th>
<th>Mean age (range)</th>
<th>Primary outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Children diagnosed with T1D at Oulu University Hospital in 1982–2001</td>
<td>585</td>
<td>8.30 (0.2–14.9)</td>
<td>Frequency of DKA at diagnosis in Oulu University Hospital District, temporal changes</td>
</tr>
<tr>
<td>II</td>
<td>Children diagnosed with T1D throughout Finland in 2002–2005</td>
<td>1656</td>
<td>8.00 (0.28–14.99)</td>
<td>Age-related differences in the frequency of DKA at diagnosis</td>
</tr>
<tr>
<td>III</td>
<td>Children diagnosed with T1D and recorded in the Finnish Paediatric Diabetes Register in 2002–2005</td>
<td>1518</td>
<td>7.90 (0.28–14.99)</td>
<td>Effect of a family history of diabetes and class II HLA genotypes on the frequency of DKA at diagnosis</td>
</tr>
<tr>
<td>IV</td>
<td>a) Children born since September 1995 and diagnosed with T1D at Oulu University Hospital by December 2014</td>
<td>517</td>
<td>6.30 (0.52–14.87)</td>
<td>Effect of screening for class II HLA genotypes from cord blood and follow-up of children with HLA-conferred susceptibility to T1D on the frequency of DKA at diagnosis</td>
</tr>
<tr>
<td>IV</td>
<td>b) Children diagnosed with T1D at Oulu University Hospital in 2002–2014</td>
<td>579</td>
<td>7.53 (0.52–14.99)</td>
<td>Frequency of DKA at diagnosis in Oulu University Hospital District, age-related differences, temporal changes</td>
</tr>
</tbody>
</table>

Ketoacidosis at the diagnosis of type 1 diabetes in children in Oulu University Hospital District (papers I and IV)

The cohort studied in paper I included children who had been diagnosed with T1D at the Department of Paediatrics, Oulu University Hospital, during a period of 20 years (1982–2001) and that in paper IV children diagnosed in 2002–2014.
All the children were <15 years of age at the diagnosis of T1D. The patients were identified from the hospital register and the register kept at the Diabetes Clinic in the Department of Paediatrics. The research was approved by the Ethics Committee of Oulu University Hospital.

The clinical data (age at diagnosis, duration of symptoms, growth data and insulin treatment) and laboratory results (acid-base balance, plasma glucose, plasma β-hydroxybutyrate, serum osmolality, serum creatinine, serum sodium and serum potassium) were collected retrospectively from the patients’ medical records. Height and weight at diagnosis were measured and the estimated decrease in relative weight was calculated by means of the Finnish growth charts. The data of the patients are represented in Table 5. One child, an 8–year-old girl, died of cardiac arrest induced by severe hyperkalaemia (6.6mmol/l) soon after the diagnosis during 1982–2001.

Table 5. The number of the patients, gender, mean age (range) and numbers (%) of the patients in different age groups of the papers I, II, III and IVb.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Paper I</th>
<th>Paper II</th>
<th>Paper III</th>
<th>Paper IVb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of children</td>
<td>585</td>
<td>1656</td>
<td>1518</td>
<td>579</td>
</tr>
<tr>
<td>Male/Female</td>
<td>328/257</td>
<td>936/700</td>
<td>851/667</td>
<td>341/238</td>
</tr>
<tr>
<td>Mean age (range)</td>
<td>8.30(0.2–14.9)</td>
<td>8.0 (0.28–14.9)</td>
<td>7.9 (0.28–14.99)</td>
<td>7.53 (0.52–14.9)</td>
</tr>
<tr>
<td>&lt;2 years, N (%)</td>
<td>48 (8.2%)</td>
<td>103 (6.2%)</td>
<td>94 (6.2%)</td>
<td>41 (7.1%)</td>
</tr>
<tr>
<td>0–4 years, N (%)</td>
<td>137 (23.4%)</td>
<td>436 (26.3%)</td>
<td>398 (26.4%)</td>
<td>185 (32.0%)</td>
</tr>
<tr>
<td>5–9 years, N (%)</td>
<td>234 (40%)</td>
<td>629 (38.0%)</td>
<td>583 (38.6%)</td>
<td>205 (35.4%)</td>
</tr>
<tr>
<td>10–14 years, N (%)</td>
<td>289 (32.6%)</td>
<td>591 (35.7%)</td>
<td>527 (35.0%)</td>
<td>289 (32.6%)</td>
</tr>
</tbody>
</table>

The patient data in paper I were analysed in four 5–year periods (1982–1986, 1987–1991, 1992–1996 and 1997–2001) or two 10-year periods (1982–1991 and 1992–2001) and those for paper IV in three equal periods. In addition, the various age groups were compared, i.e. children aged <2 years vs. ≥2 years at diagnosis, children aged <5 years vs. ≥5 years and children aged 0–4 years vs. 5–9 years vs. 10–14 years.

3.1 Ketoacidosis at diagnosis of type 1 diabetes in children over the whole of Finland (paper II)

The cohort in paper II comprised children under the age of 15 years who had been diagnosed with type 1 diabetes between June 1, 2002, and May 31, 2005, in all
paediatric centres in Finland, including university hospitals, central hospitals and a few smaller hospitals. Data were obtained from the Finnish Paediatric Diabetes Register, the nationwide register established in 2002, and information was gathered on newly diagnosed cases of childhood diabetes, including clinical and laboratory data supplied to the register by paediatricians and diabetes nurses after informed consent from the families concerned.

Since not all the families have participated in the register, we asked the centres to re-check their hospital records and report the gender, age and blood pH at diagnosis for all additional cases diagnosed with T1D within the given period. In this way we ensured that the data encompassed all children <15 years of age diagnosed with T1D in Finland during the 3-year period. The register protocol was approved by the Ethical Committee of the Hospital District of Helsinki and Uusimaa, and our analysis was approved by the local Ethics Committee and the register’s Steering Committee.

The final cohort included 1656 children (936 boys, 56.5%), the majority of whom were recorded in the Finnish Paediatric Diabetes Register (1518, 91.7%). The data of the patients is represented in Table 5. The children were treated at 27 centres, five of which were university hospitals (Helsinki, Kuopio, Oulu, Tampere and Turku). The prospective DIPP Study has been running at three of these university hospitals (Oulu, Tampere and Turku) since the mid-1990s. One 8-year-old girl died of cerebral oedema soon after diagnosis.

Height and weight were measured at diagnosis and the BMI was calculated (kg/m²). The mean duration of symptoms before diagnosis was assessed on the basis of information provided by the index case and/or his/her parents/guardians.

The subjects grouped for analysis on the basis of their age at diagnosis (aged 0–4 years, 5–9 years and 10–14 years, under vs. ≥2 years).

3.2 Associations of a family history of diabetes and class II HLA genotypes with diabetic ketoacidosis at the diagnosis of diabetes in the children (paper III)

The subjects in paper III comprised children diagnosed with T1D in one of the 27 paediatric clinics in Finland between June 1, 2002, and May 31, 2005, and recorded in the Finnish Paediatric Diabetes Register and the associated sample repository. The register protocol was approved by the Ethical Committee of the Hospital District of Helsinki and Uusimaa, the local Ethics Committee and the register’s Steering Committee. Family histories with regard to type 1 and type 2
diabetes was obtained by interviewing the parents/guardians at diagnosis, and clinical information and local laboratory values were supplied for the register by the relevant nurses and doctors. First-degree relatives (FDR), including the mother, father and full siblings of the affected child, were classified as having type 1 diabetes, type 2 diabetes or no diabetes. In addition, the diabetes history of the grandparents was recorded. The impact of a family history of diabetes on the frequency of DKA at diagnosis of T1D in the child was evaluated. The patients are represented in Table 5.

DNA was isolated from peripheral blood using a salting-out method (Miller et al. 1988) and HLA DR-DQ genotypes were determined centrally by a method based on PCR and time-resolved fluorometry with allele-specific chelate-labelled probes (Hermann et al. 2003b). The subjects were divided into five risk groups according to the various HLA DRB1-DQA1-DQB1 haplotype combinations: children with markedly increased, moderately increased, slightly increased, neutral or decreased HLA-conferred susceptibility to type 1 diabetes. The occurrence of DKA was analysed in these HLA-associated risk groups and also in two larger categories (subjects with markedly or moderately increased risk vs. the subjects with slightly increased, neutral or decreased risk), according to the eligibility criteria employed in the DIPP study.

3.3 The effect of prospective type 1 diabetes follow-up studies on the frequency of diabetic ketoacidosis at diagnosis (paper IVa)

The DIPP study in Finland (Kupila et al. 2001) is based on the screening of HLA-DQB1-associated genetic susceptibility to T1D in infants born at the university hospitals in Turku, Oulu and Tampere. A baby having markedly or moderately increased risk HLA genotypes are invited to regular clinical, immunological and metabolic follow-up visits (Table 7.) The aims are to predict type 1 diabetes and to search for possibilities for delaying or preventing the disease in a large population-based cohort. Meanwhile, the TEDDY study (Lernmark et al. 2011a) is an international, longitudinal investigation with the aim of identifying environmental triggers of T1D in children with HLA-conferred genetic susceptibility. The DIPP study was initiated at Oulu University Hospital in September 1995 and is still recruiting infants, whereas the TEDDY study screened children born at Oulu University Hospital during the period from September 1, 2004, to February 28, 2010. TEDDY Study screened newborns during the same time period also at Turku and Tampere University Hospitals.
Information on T1D derived from both of these studies and screening for the disease-associated risk genes have been offered to all families with a newborn baby, and those with a baby having markedly increased risk HLA genotypes are invited to regular follow-up in order to detect possible signs of autoimmunity against pancreatic beta cells, a condition that is known to be associated with a considerable increase in the risk of developing clinical T1D and to identify the precipitating exposures behind the process (Kupila et al. 2001, Lernmark et al. 2011b). The children were followed up either one prospective diabetes study (DIPP or TEDDY).

The paper IVb cohort included all the children <15 years who had been born since September 1995, when the DIPP started at Oulu University Hospital, and had been diagnosed with T1D at the same hospital by December 2014. The data for these children were compiled from the material used in papers I and IVa. Altogether 517 children (mean age 6.30 years, range 0.52-14.87 years) were included in this cohort, comprising 227 (43.9%) aged <5 years at the diagnosis of T1D, 194 (37.5%) aged 5–9 years and 96 (18.6%) aged 10–14 years. Of these 517 children, 229 (44.3%) had not been screened within the prospective T1D studies, because they had not been born in Oulu University Hospital or the families had not been willing to take part in the screening, while out of the 288 children who had been screened, 81 (15.7%) had no risk genotype and had thus not been monitored within the DIPP or TEDDY protocol, 48 (9.3%) carried a risk genotype but had not been monitored and 159 (30.7%) had a risk genotype and had been followed up in one or other of the prospective follow-up studies.

### 3.4 Definition of diabetic ketoacidosis

DKA was defined in two ways in paper I: DKA(i) as a blood pH<7.30 and DKA(ii) as a blood pH<7.30 and/or a bicarbonate concentration of <15mmol/l, according to the ESPE/LWPES statement (Dunger et al. 2004b). Severe DKA was defined as pH<7.10.

In papers II, III and IVa and IVb DKA was defined as a blood pH <7.30 and considered severe if the pH was <7.10.

Only the definitions of DKA as a pH <7.30 and severe DKA as a pH <7.10 are used in this thesis.
3.5 Statistical methods

Continuous data were described in terms of the mean and standard deviation and categorial variables in percentages. The statistical analyses were carried out using Student’s two-tailed t-test for independent samples when comparing variables with a normal distribution between two groups (I-IV). The Mann-Whitney U-test was used for unequally distributed variables (I-IV), and distributions were analysed by cross-tabulation and Chi-square statistics (I-IV). The Pearson correlation analysis, Kruskal-Wallis test and one-way ANOVA were employed in paper II. A logistic regression model was used in paper III and the results were reported as odd ratios (OR) with 95% confidence intervals (CI). A two-tailed uncorrected *P*-value of <0.05 was considered to indicate statistical significance.

The data analyses were performed with the SPSS for Windows® statistical software, versions 12.0 in papers I and III, 15.0 in paper II and 19.0 in paper IV (SPSS, Chicago, IL, USA). The regression lines illustrating time trends presented in paper I were drawn using Origin®, version 7 (OriginLab Corp., Northampton, MA, USA).
4 Results

4.1 Frequencies of diabetic ketoacidosis at diagnosis of type 1 diabetes in children in Oulu University Hospital District and the whole of Finland, temporal changes (papers I, II and IV)

It was found in paper I that 18.1% of the patients examined in Northern Finland during a period of 20 years (1982–2001) had ketoacidosis (pH<7.30) at the diagnosis of T1D, and that this situation was more common during the first 10 years (1982–1991) than during the latter 10 years (1992–2001) (22.4% vs. 15.2%, \( p=0.028 \)). Severe DKA occurred infrequently in both periods (4.6% vs. 2.2%, \( p=0.11 \)). The frequency of DKA, severe DKA and the proportion of children <5 years are represented in Figures 3. and 4. In spite of the decrease in the overall frequency of DKA in the figure, the frequency of DKA did not decline any further in the more recent 12–year period (2002–2014) examined in paper IV. The results for the total span of 32 years (1982–1991, 1992–2001 and 2002–2014) are presented in Table 4.

![Fig. 3. The annual proportion of children <5 years at diagnosis of T1D in Oulu University Hospital in 1982–2014. The linear regression line represent time trend.](image-url)
Fig. 4. The annual proportion of children with DKA (pH<7.30) and severe DKA (pH<7.10) at diagnosis of T1D in Oulu University Hospital in 1982–2014. The linear regression line represent time trend.
Table 6. DKA (pH<7.30) and severe DKA (pH<7.10) at the diagnosis of T1D in children in Oulu University Hospital District in three time periods (1982–1991, 1992–2001, 2002–2014). The overall frequencies of DKA (pH<7.30) and severe DKA (pH<7.10) and the frequencies of DKA in different age groups are compared between the different time periods. The results are shown as percentage (%) of children having DKA/severe DKA in concerned group and as 95% CI. The uncorrected $P$-value of <0.05 was considered to indicate statistical significance.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of newly diagnosed children</td>
<td>268</td>
<td>317</td>
<td>579</td>
<td></td>
</tr>
<tr>
<td>Male/Female</td>
<td>154/114</td>
<td>174/143</td>
<td>341/238</td>
<td>0.511</td>
</tr>
<tr>
<td>Mean age at diagnosis, years,</td>
<td>8.3</td>
<td>8.3</td>
<td>7.53</td>
<td>0.067</td>
</tr>
<tr>
<td>DKA</td>
<td>22.4% (17.3–27.5)</td>
<td>15.2% (11.2–19.2)</td>
<td>18.5% (15.3–21.7)</td>
<td>0.090</td>
</tr>
<tr>
<td>Severe DKA</td>
<td>4.6% (2.1–7.1)</td>
<td>2.2% (0.6–3.9)</td>
<td>3.5% (2.0–5.0)</td>
<td>0.286</td>
</tr>
<tr>
<td>DKA in children &lt;2 years</td>
<td>50.0% (30.8–69.2)</td>
<td>39.1% (19.2–59.0)</td>
<td>17.1% (5.6–28.6)</td>
<td>0.021</td>
</tr>
<tr>
<td>DKA in children 5–9 years</td>
<td>32.1% (19.9–44.3)</td>
<td>17.7% (9.3–26.1)</td>
<td>13.0% (8.1–17.8)</td>
<td>0.007</td>
</tr>
<tr>
<td>DKA in children 10–14 years</td>
<td>13.0% (6.7–19.3)</td>
<td>9.8% (4.6–15.1)</td>
<td>14.0% (9.2–18.2)</td>
<td>0.526</td>
</tr>
<tr>
<td>The overall frequency of DKA at diagnosis in children &lt;15 years over the whole country in 2002–2005 was 19.4%. Severe DKA was present in 4.4% of the cases.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.2 Age-specific risk groups for diabetic ketoacidosis at the diagnosis of type 1 diabetes (papers I, II and IVa)

4.2.1 Children <2 years

The frequency of DKA (pH<7.30) decreased significantly during the time in children <2 years at diagnosis of T1D (50% of children <2 years in 1982–1991, 39.1% of children <2 years in 1992–2001 and 17.1% of children <2 years at diagnosis in 2002–2014, $p=0.021$) (Table 6.). In 1982–2001 the children aged <2 years at the diagnosis of T1D in Northern Finland had DKA more often than did the older children (44.7 vs. 16.1%, $p<0.001$), and the duration of symptoms in these very young children was shorter (10.2 vs. 20.9 days, $p=0.001$) (paper I). Also, the frequency of severe DKA was significantly higher in these younger children (10.4 vs. 2.7%, $p=0.004$).
The frequency of DKA in children aged < 2 years at the diagnosis of T1D over the whole country was still higher than in the older children in 2002–2005 as well (30.1% vs. 18.6% respectively, p<0.001) (paper II).

4.2.2 Children aged 0–4 years, 4–9 years and 10–14 years

When the data of children diagnosed T1D in Oulu University Hospital in 1982–2001 were analysed in three age groups (0–4 years, 5–9 years and 10–14 years), the frequency of DKA (pH<7.30) was seen to be most common in the children aged <5 years (23.7%), followed by those aged 10–14 years (23.1%) and finally those aged 5–9 years (11.3%) (p=0.001). The duration of the symptoms before diagnosis was longer in the children aged ≥5 years than in the younger ones (22.1 vs. 13.5 days, p<0.001).

During the period of 1982–2014 (papers I and IV b) the frequency of DKA among children aged <5 years decreased markedly in the two later periods (32.1% in 1982–1991, 17.7% in 1992–2001 and 13.0% in 2002–2014, respectively, p=0.007) (Table 4.). In the most recent period, 2002–2014 (paper IVb) the frequency of DKA was observed to be the lowest (13.0%) in the children aged <5 years at diagnosis, second lowest (14.0%) in those aged 5–9 years and highest (28.6%) at 10–14 years of age. Correspondingly, severe DKA was seen most frequently in the children aged 10–14 years (6.9%) vs. 2.2% in those aged 0–4 years and 1.5% in those aged 5–9 years (p=0.008).

In the whole of Finland in 2002–2005 (paper II) the frequency of DKA was seen to be lowest at 5–9 years of age (14.6%), second lowest at 0–4 years (16.5%) and highest in the oldest age group (26.4%; p<0.001). The older children had also more often had symptoms for longer than 2 weeks before diagnosis than had those aged <5 years at diagnosis (p=0.006). Severe DKA occurred in 3.7% of the cases aged 0–4 years, 3.1% of the 5–9-year-old cases and 5.9% of those aged 10–14 years p=0.048).

4.3 Gender and diabetic ketoacidosis at the diagnosis of type 1 diabetes

There was a preponderance of boys among the patients in all the cohorts (56.1% in paper I, 56.5% in paper II, 56.1% in paper III and 58.9% in paper IVa), but there were no significant differences between the boys and girls in the frequency of DKA or severe DKA in any of the cohorts. It was the case, however, that the
girls had more often had symptoms for longer than 2 weeks before diagnosis than the boys (39.4 vs. 33.2%, \(p=0.021\)) (paper II).

4.4 Family histories of diabetes in children with newly diagnosed type 1 diabetes and their effect on the frequency of diabetic ketoacidosis at diagnosis (paper III)

A total of 174/1384 children (12.6%) had at least one FDR affected by T1D at the times of its diagnosis in the child. In 154 cases (88.5%), there was only one family member with T1D, whereas in 20 cases (11.5%) there were two or even more affected family members. A total of 81/1396 (5.8%) of the index cases had an affected father, whereas 40/1414 (2.8%) had a mother with T1D. Close to 5% of the children (69/1418; 4.9%) had a sibling with T1D. The proportion of children having at least one grandparent with T1D was 6.6%, and a total of 19.7% of the children had at least one FDR and/or a grandparent with T1D at the time of diagnosis.

The children aged <2 years at the onset of T1D more often had their father affected by T1D than did the older children (11.2% vs. 5.4%, \(p=0.024\)), and these younger children more frequently had at least one FDR with T1D (19.1% vs. 12.1%, \(p=0.055\)) and an FDR or grandparent by T1D than did the older children (31.1% vs. 19.0%, \(p=0.011\)). No differences in the proportion of cases with affected family members were observed between the age groups 0−4, 5−9 and 10−14 years.

The children having at least one FDR with T1D had DKA less frequently at diagnosis than did the other children (7.4% vs. 20.5%, \(p<0.001\)), and the same applied to severe DKA (1.2% vs. 3.9%, \(p=0.082\)). The frequency of DKA tended to be lower in children with a grandparent with T1D (11.3% vs. 20.5%, \(p=0.076\)), but type 2 diabetes in the parents or grandparents had no effect on the frequency of DKA in children with newly diagnosed T1D.

4.5 HLA-conferred susceptibility to type 1 diabetes and the frequency of diabetic ketoacidosis at diagnosis (paper III)

HLA-associated genetic disease susceptibility was analysed in 1064 (70.1%) children with newly diagnosed T1D over the whole of Finland in 2002−2005 (paper III). The risk groups were classified as shown in Table 7.
Table 7. Classification and frequencies of HLA-associated genetic disease susceptibility in children with newly diagnosed T1D.

<table>
<thead>
<tr>
<th>Risk group, frequency (%)</th>
<th>HLA genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Markedly increased risk, 23.2%</td>
<td>Heterozygosity for the two risk haplotypes DRB1<em>0401/2/4/5/8-DQA1</em>03-DQB1<em>0302/4 and [DRB1</em>03]-DQA1<em>05-DQB1</em>02</td>
</tr>
<tr>
<td>Modestly increased risk, 45.2%</td>
<td>Homozygosity for the above two risk haplotypes or DRB1<em>0401/2/4/5/8-DQA1</em>03-DQB1<em>0302/4 combined with a neutral haplotype, or the [DRB1</em>03]-DQA1<em>05-DQB1</em>02/[DRB1<em>09]-DQA1</em>03-DQB1*03 genotype</td>
</tr>
<tr>
<td>Slightly increased risk, 12.8%</td>
<td>[DRB1<em>03]-DQA1</em>05-DQB1<em>02 with a neutral haplotype or the DRB1</em>0401/2/5/8-DQA1<em>03-DQB1</em>0302/4/[DRB1<em>1301]-[DQA1</em>01]-DQB1*0603 genotype</td>
</tr>
<tr>
<td>Neutral risk, 15.2%</td>
<td>Genotypes in which a risk haplotype is combined with a protective one (DRB1<em>15-[DQA1</em>01]-DQB1<em>0602,[DRB1</em>11/12/13]-DQA1<em>05-DQB1</em>0301,[DRB1<em>14]-[DQA1</em>01]-DQB1<em>0503, DRB1</em>07-DQA1<em>0201-DQB1</em>0303,DRB1<em>0403-[DQA1</em>03]-DQB1<em>0302/4 and [DRB1</em>1301]-[DQA1<em>01]-DQB1</em>0603 (except the combination in group 3)) or combinations of two neutral haplotypes</td>
</tr>
<tr>
<td>Decreased risk, 3.6%</td>
<td>A combination of two protective haplotypes or a protective haplotype associated with a neutral one</td>
</tr>
</tbody>
</table>

When the distributions of HLA risk genotypes were analysed by age groups those aged 10–14 years tended to have markedly or moderately increased risk genotypes less frequently than the younger children (70.8% among those aged 0–4 years, 70.9% of those aged 5–10 years and 64.3% of those aged 10–15 years, \( p=0.086 \)).

DKA occurred less frequently in the children with higher-risk HLA genotypes \( (p=0.024) \), and severe DKA was also less frequent in these cases \( (p=0.031) \). When comparing the children with HLA genotypes conferring a markedly or moderately increased risk of T1D with the other children we found that DKA was less frequent in the former group than in the latter one (17.4% vs. 23%, respectively, \( p=0.032 \)). In paper IVa, when comparing children who had markedly or moderately increased risk of T1D and not followed up in the prospective studies with children in lower risk groups and thus not followed up, we found that 23.4% children in first mentioned group and 26.3% of children in later group had DKA at diagnosis \( (p=0.721) \).

The logistic regression model was applied to explain DKA including age at diagnosis as a continuous variable and several categorized variables (sex, HLA DQB1 risk groups, and presence of T1D or T2D in FDR). Significant association
were observed between DKA and absence of T1D in FDR ($p=0.001$, OR=3.23 (95%CI 1.6-6.52)), low risk HLA-genotype ($p=0.029$, OR=1.45 (95%CI 1.04-2.03)) and older age at diagnosis ($p=0.02$, OR 1.07 (95%CI =1.03-1.12)).

4.6 The effect of prospective follow-up studies on diabetic ketoacidosis (paper IVb)

The analysis of the effect of the screening and follow-up provided in the prospective T1D studies on the frequency of DKA undertaken in paper IVa. The data of metabolic decompensation at diagnosis of T1D in children who were born in September 1995 or later, during which time period the prospective type 1 diabetes studies, DIPP and TEDDY have recruited newborn infants in Oulu University Hospital, and who were diagnosed with T1D by December 2014 in Oulu University Hospital was analysed. In the comparison of four groups based on screening and follow-up in the prospective studies, we found that the frequency of DKA at the diagnosis of T1D was lowest, 5.0%, in children who had been screened and followed up as compared with a frequency of 22.7% in those not screened, 26.3% in those screened who had no risk genotype and accordingly were not followed up, and 23.4% in those screened and found to carry a risk genotype for T1D but were still not followed up ($p<0.001$). Similarly, while comparing the frequency of severe DKA in the four groups, it was found only in 1/159 children (0.6%) observed prospectively vs. 4.4% of the children not screened, 6.3% in those screened but having no risk genotype and 4.3% in those screened and found to have a risk genotype but not followed up ($p=0.098$).
5 Discussion

5.1 Temporal changes in the frequency of diabetic ketoacidosis at the diagnosis of type 1 diabetes in children under the age of 15 years

5.1.1 Overall frequency of DKA (papers I, II, IV)

The frequency of DKA at the diagnosis of T1D varies considerably, between 15% and 80% in developed countries, and correlates inversely with the background incidence of T1D. In parts of the world where the prevalence of T1D is low, the symptoms of the disease may be less familiar to health care personnel and the public, resulting in a higher DKA frequency at diagnosis (Levy-Marchal et al. 2001, Usher-Smith et al. 2011). The incidence of T1D in Finland, on the other hand, is the highest in the world and an accelerating increase has been reported up to 2005, when the incidence in children younger than 15 years was 64.2 per 100,000 person-years (Harjutsalo et al. 2005, Harjutsalo et al. 2008). Fortunately, the incidence has levelled off in the recent years, with no further increase after 2006, when the current peak incidence of 64.9 was observed (Harjutsalo et al. 2013). Because of the high incidence in Finland, however, the disease and its symptoms are quite familiar to health care staff and the general public, facilitating an earlier diagnosis. We thus observed in the present work that the frequency of DKA both at Oulu University Hospital and throughout Finland had remained at a moderate level during the last few decades as compared with most other European countries. In fact, we found the overall frequency of DKA in Northern Finland to have been 22.4% in 1982–1991 and 15.2% in 1992–2001, representing a decrease in frequency with time, although our most recent analysis, covering the period 2002–2014, showed no further decrease, with DKA in 18.5% of cases. Even lower frequencies of DKA at T1D diagnosis, 12.8–16%, have been reported from Sweden (Hanas et al. 2007, Samuelsson & Stenhammar 2005), the rate of ketoacidosis, which should be possible to achieve also in Finland because of the almost similar incidences of T1D and health care systems. However, the latest published data from Sweden included the children diagnosed in 2000-2004, thus the more recent frequency of DKA and possible variations are unknown.

The mean age-specific incidences of T1D in Finland in time period of 1980–2005 were 31.0 per 100,000 person-years for children aged 0–4 years, 50.5 per
100 000 person-years for children 5–9 years, and 50.6 per 100 000 person-years for children 10–14 years (Harjutsalo et al. 2008). In 2006–2011, the age specific rates per 100 000 per year were 55.0, 71.5 and 60.6 at ages 0–4 years, 5–9 years and 10–14 years, respectively (Harjutsalo et al. 2013). Although the increase in incidence of T1D was the highest in children 0–4 years of age, we suppose that the increasing incidence of DKA in the latest time period (2002–2014) is rather resulted from teenagers’ undesirable frequency of DKA at diagnosis of T1D.

The data suggesting decreasing rates of DKA at diagnosis are somewhat controversial, as most studies have suggested a stable or even increasing frequency (Dabelea et al. 2014a, Jefferies et al. 2015, Rewers et al. 2015) although decreasing frequencies of DKA have been observed in some countries (de Vries L. et al. 2013, Samuelsson & Stenhammar 2005, Ucar et al. 2013). The economic reasons or lack of awareness of T1D may be found on the ground of increasing/stable frequency of DKA. In Colorado, the increasing incidence of DKA correlated temporally with an increase in Colorado child poverty prevalence from 10% in 2000 to 18% in 2010 (Rewers et al. 2015). Similarly, in larger geographical area in United States, the frequency of DKA in youth with type 1 diabetes, although stable, remains high, indicating a persistent need for increased awareness of signs and symptoms of diabetes and better access to health care (Dabelea et al. 2014b). In New Zealand, there is a social security system that provides medical care free of charge, thus factors beyond ‘awareness’ are important contributors to risk of DKA (Jefferies et al. 2015).

The most important factor pointed out in earlier studies as affecting the risk of developing DKA at the diagnosis of T1D has been the awareness of T1D symptoms among parents and primary care physicians (de Vries L. et al. 2013). The decreasing frequency of DKA may be associated with better resources of health care, like it was thought in the study of Israel (de Vries L. et al. 2013) or increasing awareness for example by national programs like was thought to decrease DKA rate in Turkey (Ucar et al. 2013). A 8-year campaign of medical information on the early symptoms of diabetes in the Parma area of Italy is reported to have led to a dramatic reduction in the frequency of DKA in children with newly diagnosed T1D (Vanelli et al. 1999), and similarly a 2-year intervention period with T1D education offered to child care centres, schools and doctor’s surgeries resulted in a 64% decrease in the frequency of DKA at the diagnosis of T1D in the intervention region in Australia (King et al. 2012). Likewise, the prospective T1D follow-up studies that have been going on at Oulu University Hospital since September 1995 have increased knowledge of the
disease among both the health care personnel and the families concerned. Although, the overall frequency of DKA in Oulu University Hospital has not been decreasing markedly during the prospective diabetes studies, the effect on the DKA rate in young children <5 years of age has been very important. In addition, modern electronic information technology has offered better possibilities for disseminating information about health issues to the public.

5.1.2 Age at diagnosis and diabetic ketoacidosis (papers I, II, IV)

Young age has been an important risk factor predisposing children to DKA at the diagnosis of T1D in most studies (Jefferies et al. 2015, Rewers et al. 2015), and young children aged 0–4 years, and especially <2 years, certainly have a high risk of DKA at diagnosis. Its frequency in the youngest age group (<2 years) varies from 53% to 69% in the literature (Dabelea et al. 2014a, de Vries L. et al. 2013, Jefferies et al. 2015, Quinn et al. 2006, Rewers et al. 2008), while in the Finnish DiMe Study of 745 children and adolescents diagnosed with T1D in 1986–1989 the children aged <2 years at diagnosis were observed to be more susceptible to DKA than those aged 2–10 years (53.3% vs. 16.9%, p<0.001).

The frequency of DKA in these very young children (<2 years) was evaluated in the present work over a prolonged period of time (more than 33 years) at Oulu University Hospital, and a definite decrease in the frequency of DKA in this age group was found, from 50% in 1982–1991 to 39.1% in 1992–2001 and only 17.1% in 2002–2014. This is really a delightful observation, which has not been in evidence in previous studies. A delay in diagnosis has often been seen in this age group for various reasons, e.g. concurrent infections and misdiagnosis (Neu et al. 2001), but conversely, we found a shorter symptomatic period before diagnosis among these children than in older ones. The ongoing DIPP and TEDDY prospective T1D studies are most likely to have contributed to the decreasing rate of DKA in our hospital. (Kupila et al. 2001, Lernmark et al. 2011a), as it has also been observed by others that participation in prospective follow-up studies for children with increased risks can lead to earlier diagnosis of diabetes and result in fewer complications at diagnosis (Barker et al. 2004, Lundgren et al. 2014, Winkler et al. 2011). In the cohort of paper II, encompassing all the children in Finland with newly diagnosed T1D in 2002–2005, the frequency of DKA was 30.1% in those aged <2 years at diagnosis, which represents the overall frequency of DKA in Finland at that time. In the same period in Oulu University Hospital,
the frequency of DKA in this age group was little higher, 36% (4/11 children <2 years had ketoacidosis).

The DKA frequency also decreased in patients aged 0–4 years at T1D diagnosis during the 33-year observation period at Oulu University Hospital, probably because of the same reasons as among those aged <2 years, the frequency of DKA in this age group being 32.1% in 1982–1991, 17.0% in 1992–2001 and 14.0% in 2002–2014. The frequency of DKA in the whole of Finland in 2002–2005 was similarly remarkably low, 16.5%, thus documenting a favourable overall change in this age group. The rate of DKA in the middle age group (5–9 years) remained low throughout the period studied here.

Teenagers had an alarmingly high frequency of DKA at diagnosis in the present data as a whole, the frequency of DKA at this age in the Oulu University Hospital data being 27.4% in 1982–1991, 19.5% in 1992–2001 and 28.6% once more in 2002–2014 and that in the whole of Finland in 2002–2005 26.4%. More attention should be paid to this age group in the future in order to reduce the frequency of DKA at presentation with T1D. The reasons behind this trend may be multidimensional. The school health care system has changed substantially in recent decades, and the children do not meet a school health nurse as often as earlier because of reduced access (Kivimäki H et al. 2007). Thus, the possible weight loss or other symptoms of T1D may not be identified. In addition, many parents have split up, so that children may live in more than one family. Additionally, parents may not supervise their adolescent children as carefully as earlier, because adolescents can usually take care of themselves quite well, families spend less time together nowadays and have fewer meals together than previously (Ojala et al. 2006). The adolescents may also find it difficult to speak with their parents or other adults about their personal health problems.

5.2 The effect of familial T1D on the risk of DKA (paper III)

The frequency of DKA at the diagnosis of T1D has been reported to be reduced in children with at least one affected FDR (Sadauskaite-Kuehne et al. 2002). We similarly found a reduced frequency of DKA in children with at least one FDR having T1D (7.4% vs. 20.5%). Additionally, in the meta-analysis of 46 studies involving more than 24 000 children in 31 countries, the protective factor against DKA was a FDR with T1D at the time of diagnosis [OR 0.33 (0.08 to 1.26)] (Usher-Smith et al. 2012). This finding is also supported by a relatively recent Italian report quoting a DKA frequency of 13.0% among children with an affected
FDR as compared with 37.4% among those without any FDR with T1D (Marigliano et al. 2013). The finding is not very surprising, because these families know the symptoms of diabetes very well and they all have glucometers at home and can check capillary glucose levels easily. It is actually somewhat surprising that the frequency of DKA was not even lower among these children with a positive family history. On the other hand, some families may not want to accept such symptoms marking a chronic disease affecting the quality of life, thus leading to a delayed diagnosis. In any case there are some reports of the rate of DKA at the diagnosis of T1D being equal in children with or without an affected FDR (Quinn et al. 2006, Rosenbauer et al. 2002).

In our study, the children <2 years at diagnosis had more often at least one FDR with T1D at diagnosis than older children (19.1% vs. 12.1%), the finding which supports the genetic predisposition of the disease and may be influencing on the frequency of DKA at diagnosis in this age group.

5.3 The effect of the distribution of II HLA genotypes on diabetes ketoacidosis at diagnosis (paper III)

Although the specific HLA class II gene alleles at the HLA-DRB1, DQA1 and DQB1 loci are known to be closely associated with T1D (Hermann et al. 2003b), little is known about the associations between the various HLA class II genotypes and the rate of DKA at the diagnosis of T1D. In our third paper we found the frequency of DKA to be higher in children with HLA genotypes conferring a lower risk of T1D. This observation is somewhat surprising. It is possible that the prospective follow-up studies (DIPP, TEDDY) may have reduced the DKA frequency among participating children carrying high or moderate-risk HLA genotypes, resulting in a seemingly higher DKA frequency among carriers of the lower-risk HLA genotypes, but it must be remembered that the children taking part in the prospective studies represent only a small minority (<5%) of all the newly diagnosed cases in Finland during the period 2002–2005. It is more likely, that the oldest children with higher DKA risk at diagnosis together with less frequent high or moderate-risk HLA genotypes affect on the current result. In Italy a contradictory finding; has actually been reported in which HLA high-risk genotypes were associated with an increased risk of presenting with DKA at the diagnosis of T1D (Marigliano et al. 2013).
5.4 The effect of prospective type 1 diabetes studies on the frequency of DKA at diagnosis (paper IV)

We reported in papers I and IV that the DKA frequency in younger children aged 0–4 years and <2 years decreased with time. Furthermore, we observed in paper III that children carrying high-risk HLA genotypes for T1D had a reduced risk of DKA at its diagnosis. We think that these observations are at least partly a consequence of the prospective T1D follow-up studies going on in Finland. In paper IV we assessed more closely the effect of participation in these studies in the case of Oulu University Hospital. The DIPP study was initiated in Oulu in September 1995 and is still recruiting infants and families for screening of the HLA-conferred T1D risk, while the TEDDY study screened children born in Oulu during the years 2004–2010 for HLA eligibility. In both protocols information about screening for T1D-associated risk genes is provided for all families with a baby born in that hospital. The infants having a high DQB1-conferred risk of T1D are invited for regular clinical, immunological and metabolic follow-up consultations in order to detect possible signs of autoimmunity against pancreatic beta-cells. All the families with an eligible child are given information about T1D and early symptoms of the disease at their first visit to the clinic, and those families taking part in the prospective follow-up receive further information about the disease during their subsequent visits. Moreover, if the child seroconverts to positivity for two or more islet autoantibodies the follow-up protocol becomes more intensive, including HbA1c and plasma glucose measurements at each visit and sequential oral and intravenous glucose tolerance tests. As a consequence, T1D may often be diagnosed even before the appearance of symptoms or clinical signs of the disease.

In paper IV the frequency of DKA at the diagnosis of T1D was found to be lowest (5.0%) among the children screened for genetic risk and followed up prospectively, while it was 22.7% among the children not screened, 26.3% among those screened but, having no risk genotype, were not followed up, and 23.4% among those screened and found to be carrying a risk genotype for T1D but not followed up. Accordingly, we concluded that screening alone without follow-up did not prevent DKA at diagnosis. It should also be noted that the children with no increased risk genotype upon screening had the highest frequency of DKA at diagnosis. This is worrying, because such families may mistakenly think that their children will never present with T1D and are therefore likely to miss the early
symptoms of the disease. In the future, this point has to consider in the information given to the parents.

Earlier observations regarding the effect of participation in prospective studies on the frequency of DKA are unambiguous. In the Diabetes Autoimmunity Study (DAISY) children screened for genetic T1D risk and followed-up regularly had a less severe onset of the disease, characterized by less frequent hospitalization and lower mean HbA1C at onset (Barker et al. 2004), while in the German BABYDIAB-study it was found that the children with an affected first degree relative and followed up regularly had lower HbA1C and a lower prevalence of DKA (pH<7.30) at diagnosis (3.3% vs. 29.1%, p<0.001) (Winkler et al. 2011). In the TEDDY study follow-up the young children (<2 years and 0–4 years) had a significantly lower prevalence of DKA at the diagnosis of type 1 diabetes than in the total data collected during similar periods from studies and registers in all the TEDDY participant countries (Elding et al. 2011). Similarly, a recent report has shown that the first 100 children followed up in the TEDDY study and diagnosed with type 1 diabetes (at ages of 0.69-6.27 years) had a low risk of DKA (8%). Additionally, 36% of these children had no symptoms of diabetes before diagnosis (Elding et al. 2014).

5.5 Strengths and limitations of the research

This study includes data of all children <15 years diagnosed with T1D and treated in Oulu University Hospital during the long period of 33 years (1982–2014). In Finland, all children diagnosed with T1D are treated in paediatric units, thus, our data represents well those children of Oulu University District area. This study images reliably the frequencies of DKA in total and in the groups of different characteristics in the hospital area. Additionally, it produces information of the groups needing more resources to prevent ketoacidosis at T1D diagnosis. Furthermore, the changes in the frequencies of DKA at diagnosis have been observed inclusively, because of the long study period.

In the paper II, the frequency of DKA was evaluated in whole Finland, including Oulu University Hospital. It presents interesting data of the country with the highest T1D incidence of the world, considering the early finding of the inverse correlation between incidence if T1D and the frequency of DKA at diagnosis (Levy-Marchal et al. 2001). The results can be compared with findings of published studies from other countries. Additionally, it makes it possible to compare the frequency of DKA in Oulu University Hospital District with the
other areas of the country. We found, that the frequency of DKA in Oulu University Hospital does not differ from whole Finland. This is not a surprise, because there is a municipal healthcare system with similar resources and policies in the whole country.

During the data period, the type 1 diabetes prospective follow-up studies have been going on in Oulu University Hospital. Our study produces the important information of the effect of these ongoing studies on the frequency of DKA at T1D presentation on the population level, considering the earlier published date of the prospective studies (Elding et al. 2011, Winkler et al. 2011).

Because of the incidences of type 1 diabetes vary a lot between the countries; the findings of the frequencies of DKA of our country are not generalizable in the other countries. Additionally, the comparison of our results with findings of other countries is a little inaccurate, because of different health care systems, coverage of the material and possible different definitions of DKA used.

5.6 Future challenges

Although the frequency of DKA at the diagnosis of T1D is moderate in most age groups in Finland, the prevention of this potentially life-threatening condition in Finnish children is still an important goal. DKA is the most common cause of death in children with T1D (Edge et al. 1999), and its treatment is also expensive. In a German study, paediatric patients with DKA at diagnosis had up to 3.6-fold higher diabetes-related costs in the initial treatment than those without DKA (Icks et al. 2013).

The most important tool for preventing DKA at diagnosis in the future will be increased public awareness. Parental awareness of the symptoms characteristic of emerging T1D and increased knowledge of T1D among health care professionals and the public will be essential for this. Although several studies have documented a reduced incidence of DKA among children carrying a high genetic risk of developing T1D and followed up in prospective studies, genetic screening for the sole purpose of reducing the incidence and costs of DKA at disease onset has not been shown to be economically viable (Meehan et al. 2015). The challenge for the future will be to effectively reduce the rate of DKA at the diagnosis of T1D, especially in children aged 10 years or over at diagnosis. One potential strategy could be to increase the information on type 1 diabetes provided at schools in the form of a health education effort and to improve the awareness of the symptoms of T1D among parents and health care professionals.
6 Conclusions

The following main conclusions can be drawn from the data presented here:

- The overall frequency of diabetic ketoacidosis at the diagnosis of T1D in children <15 years of age both at Oulu University Hospital and in Finland as a whole is low and has stabilized at a level of less than 20.0% with no indications of any further decrease. However, there are still challenges and it is surely possible to decrease the frequency of DKA for instance by increasing the public knowledge of the disease (child health centres, health care personnel and schools).

- The frequency of DKA at diagnosis in children aged <2 years or 0–4 years at diagnosis has decreased markedly at Oulu University Hospital over a time interval of 33 years (1982–2014). Probably the most important influential effect is the ongoing prospective follow-up studies with increased information to the families with newborn babies.

- Children older than 10 years at the diagnosis of T1D have an increased risk of DKA, and this group would need more attention in the future in order to identify possible means of reducing their DKA frequency. Especially the decrease of severe DKA is important. The possible way could be to increase the information of the disease for the teenagers at schools and free-time events. Also the personnel of school health care have to be aware of the symptoms of T1D.

- Children with a first-degree relative affected by T1D had a reduced risk of DKA at diagnosis.

- The frequency of DKA was higher in children with a weaker HLA-conferred risk of T1D. This may be partially result from the finding that teenagers had infrequently higher risk HLA-genotype for T1D but more often DKA at diagnosis.

- Children taking part in prospective T1D studies that included screening for HLA-DQB1-associated genetic susceptibility to T1D in cord blood and subsequent clinical, immunological and metabolic follow-up had a reduced frequency of DKA at diagnosis. Genetic screening with no follow-up did not reduce the frequency of DKA. In the future, the families of children with no T1D associated risk genotypes should be informed more clearly about the low but possible chance of the disease.
References


Original articles


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Anne Hekkala

KETOACIDOSIS AT DIAGNOSIS OF TYPE 1 DIABETES IN CHILDREN UNDER 15 YEARS OF AGE