EPIDEMIOLOGY OF TYPE 1 DIABETES IN CHILDREN IN HUNGARY

PhD Thesis

Éva K Gyürüs, MD

Department of Paediatrics

Faculty of Medicine

University of Pécs

Program leader: Gyula Soltész MD, PhD, DSc

2011

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Abbreviations:

BMI-SDS	Body mass index Standard deviation score (z score)
DKA	Diabetic ketoacidosis
GAD	glutamic acid decarboxylase
GDM	gestational diabetes mellitus
HbA1c	glycated haemoglobin
HLA	human leucocyte antigen
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
MODY	maturity onset diabetes of the young
OGTT	oral glucose tolerance test
T1DM	type 1 diabetes
T2DM	type 2 diabetes mellitus

THE EPIDEMIOLOGY AND INCIDENCE OF TYPE 1 DIABETES

I. INTRODUCTION

Diabetes mellitus is a complex multifactorial and heterogeneous syndrome characterized by hyperglycaemia resulting from inadequate insulin secretion and/or insulin action. Several pathogenic processes ranging from autoimmune destruction of the β-cells of pancreas to abnormalities that result in resistance to insulin are involved in the development of diabetes.

The chronic hyperglycaemia of diabetes is associated with long-term complications including cardiovascular disease, renal disease, peripheral neuropathy, visual abnormality and dermatologic problems. Diabetes mellitus represents a huge burden to the individual, the family and to the society. Furthermore, as its rate has reached an alarming proportion worldwide, diabetes is a disease of great public health importance.

1. Classification of diabetes

The new classification proposes that hyperglycaemia, regardless of the underlying cause, can be subcategorized as follows: insulin-requiring for survival, insulin-requiring for control (i.e., for metabolic control, not for survival), not insulin-requiring (i.e. with treatment by non-pharmacological methods or drugs other than insulin). IGT is now categorized as a stage in the natural history of disordered carbohydrate metabolism. IGT is coupled with impaired fasting glucose (IFG) (6.1-7.0 mmol/l).

The classification of diabetes includes four clinical classes: type 1 diabetes, type 2 diabetes, other specific types of diabetes and gestational diabetes.

Table 1 - Etiologic classification of diabetes mellitus based on the report of the Expert Committee on the Diagnosis and Classification of Diabetes (1)

I. Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency)
   A. Immune mediated
   B. Idiopathic
II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance)
III. Other specific types
   A. Genetic defects of β -cell function (MODY[a], mitochondrial diabetes…)
   B. Genetic defects in insulin action
   C. Diseases of the exocrine pancreas (pancreatitis, cystic fibrosis, hemochromatosis...)
   D. Endocrinopathies (acromegaly, Cushing's syndrome, pheochromocytoma...)
   E. Drug- or chemical-induced (glucocorticoids, thyroid hormone, thiazides…)
   F. Infections (congenital rubella, cytomegalovirus...)
   G. Uncommon forms of immune-mediated diabetes (anti–insulin receptor antibodies…)
   H. Other genetic syndromes sometimes associated with diabetes (Down syndrome...)
IV. Gestational diabetes mellitus (GDM)

[a]MODY: maturity onset diabetes of the young

1.1 Type 1 diabetes

Nowadays, we may subdivide T1DM in three groups from the etiological point of view: autoimmune, idiopathic and double. The autoimmune group is represented by: type 1A, which is polygenic and it is the most frequent type of this disease, corresponding to approximately 80-90% of all T1DM cases. LADA (latent autoimmune diabetes in the adult), the other subtype of this group is a slowly progressive form of type 1 diabetes and is characterised by GAD antibody (anti-GAD) positivity, older age at onset, less ketosis-proneness and no immediate (for at least 6 months) insulin needs (2). Type 1B, also called idiopathic, has all the clinical features of type 1A, but the autoimmune component is not detected (3).

Finally, the denomination of mixed, 1.5 or double (type 1 plus type 2) diabetes has been proposed when we have the type 1A (autoimmunity) plus type 2 (obesity, insulin resistance, dyslipidemia) diabetes characteristics in the same individual (4).

Type 1 diabetes is broadly seen as a form of diabetes requiring insulin therapy. But the severity of metabolic features, both before and at the diagnosis of type 1 diabetes, is wide ranging. Diabetes-associated autoantibodies are not sufficient to define a categorical disease phenotype; patients who progress towards insulin requirement are characterized not only by autoantibodies but also by younger age at diagnosis, lower endogenous insulin secretion, leaner body mass and high HbA1c at the time of diagnosis (Figure 1). It is the juvenile-onset classic type 1 diabetes which is occupying one end of a spectrum as the most genetically determined and severe form of the disease.

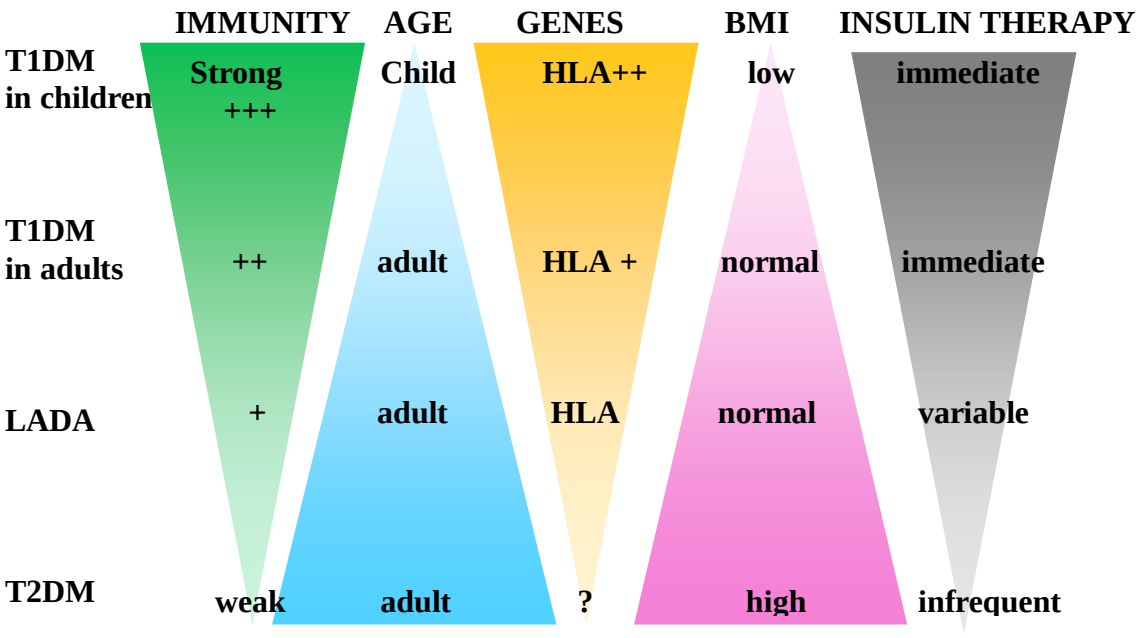

Figure 1 **-** The spectrum of diabetes encompasses variable risk according to type of diabetes for immune changes, age at presentation, HLA genetic susceptibility, obesity (as body mass index), and insulin therapy (5)

Type 1 diabetes (T1DM) is perceived as a chronic immune-mediated disease characterized by selective loss of insulin-producing β-cells in the pancreatic islets in genetically susceptible individuals. Based on histological studies of pancreas specimens from patients with new-onset type 1 diabetes, beta cell mass is reduced by ~ 80-90% at the time of clinical manifestation of the disease (6, 7).

The appearance of autoantibodies in the prediabetic phase prior to the onset of immune-mediated type 1 diabetes is the first detectable sign of emerging β-cell autoimmunity. There are five disease-related autoantibodies that have been shown to predict clinical T1DM (8, 9). These include classical islet cell antibodies (ICA), insulin autoantibodies (IAA), autoantibodies to the 65 kD isoform of glutamic acid decarboxylase (GADA), the protein tyrosine

phosphatase-related IA-2 molecule (IA-2A) and the zink transporter autoantibodies (ZnT8A). These autoimmunity markers can be used to identify at-risk subjects during the prodromic phase when autoimmune intervention is most likely to be effective and may also be therapeutic agents in their own right. The duration – months, years or even decades of the pre-clinical phase may vary according to the intensity of the pathologic process and beta cell repair (9, 10).

Several genes involved in the aetiology of type 1 diabetes have already been identified, but we still have little idea of the etiologic mechanisms that trigger autoimmunity and promote progression to disease. Type 1 diabetes may develop at any age but displays a marked age-dependent heterogeneity in the severity of clinical presentation (11, 12). Past descriptions depict type 1 diabetes as a disease of childhood and early adulthood, however, more recent data suggest that only about 50–60% of those with type 1 diabetes are younger than 16–18 years at presentation and that such disease occurs at a low incidence level throughout adulthood (11-13).

1.2 Type 2 diabetes

Type 2 diabetes, which accounts for 90–95% of those with diabetes encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency. The disease is heterogeneous in terms of genetic, metabolic and clinical characteristics.

Since the 1990s the incidence of type 2 diabetes in children and adolescents has risen dramatically in some countries and ethnic groups, which – apart from the special genetic predisposition of some ethnic groups - mirrors growth in urbanization, economic development, and the associated increase in overweight and obesity.

A Hungarian study (14) from 1989 to 2001 also reported rising incidence rates over time, with 57% of T2DM cases and 77% of IGT cases being diagnosed in the last six years of the study

(14). It is important to note, however, that this was a clinic-based and not a population-based study. A more recent study using risk-stratified screening and OGTT in Hungarian children and adolescents (15) has described a 2.5 % prevalence of IGT, and a 0.35% prevalence of type 2 diabetes in high risk children (being overweight and having additional risk factors according to the ADA criteria).

Overall, type 2 diabetes is characterised by an older age of onset, is frequently associated with obesity (with predominantly abdominal fat distribution) and lack of physical activity.

1.3 Maturity-onset diabetes of the young

The clinical characterisation of MODY established that diabetes could develop on a familial basis without the requirement of insulin resistance (16). Six MODY genes (HNF4A, GCK, TCF1, PDX1, TCF2) which, overall, are involved in ~85% of MODY cases (17). All six MODY genes are expressed in the beta cell, with glucokinase (encoded by GCK) serving as the glucose sensor that controls the set point for insulin secretion, and the rest acting as transcription factors regulating pancreatic beta cell development and final beta cell mass.

2. Clinical presentation of diabetes

The manifestation of diabetes may differ from patient to patient, according to the type of diabetes and age at onset. Affected patients generally have a preceding history of the classic symptoms of diabetes. The period of time from when abnormalities in glucose control can be identified until the development of symptoms generally is relatively brief. The classic symptoms of diabetes are polydipsia, polyuria, polyphagia, and weight loss. Re-emergence of bedwetting, nocturia, and a need to leave classes in school to use the bathroom are complaints

that suggest polyuria. The other typical presentation for children who have type 1 diabetes is metabolic deterioration into diabetic ketoacidosis (DKA), presenting with nausea, vomiting, dehydration, and lethargy. In certain situations, the diagnosis of diabetes should be considered in the absence of the classic symptoms (infant who presents with an acute febrile illness in whom a plasma glucose value obtained as part of a chemistry panel is elevated). Chronic hyperglycaemia in girls and in infants and toddlers of both genders commonly leads to perineal candidiasis.

The rate of beta-cell loss postdiagnosis is highly variable and may depend in part on the aggressiveness of the type 1 diabetes disease process. This aggressiveness may be determined by several factors including underlying genetic predisposition, age of the patient, metabolic control, and may vary within individuals over the course of their diabetes.

3. Immune markers of type 1 diabetes

The initial evidence for autoimmunity in patients with type 1 diabetes came from immunofluorescence studies, which showed that a high percentage of sera from newly diagnosed type 1 diabetic patients reacted with pancreatic islet cells (islet cell cytoplasmic antibodies or ICA) (18, 19). Since then, a series of autoantigens have been identified in T1DM including insulin, glutamic acid decarboxylase (GAD65) (20), the protein tyrosine phosphatase-related islet antigen 2 (IA-2) (21), and most recently the zinc transporter Slc30A8 residing in the insulin secretory granule of the β-cell (22).

Several studies have shown that β-cell autoimmunity may be induced early in life (23). Around 4% of offspring of parents with type 1 diabetes in the BABYDIAB study and around 6% of genetically at-risk infants from the general population in the Finnish Diabetes Prediction and

Prevention (DIPP) study have developed islet autoantibodies by age 2 years (24, 25). Children who develop autoantibodies within the first 2 years of life are those who most often develop multiple islet autoantibodies and progress to type 1 diabetes in childhood (24). Autoantibodies do not exclusively develop before age 2 years, but children who develop autoantibodies later have a slower progression to multiple antibodies and type 1 diabetes (24).

There is no consensus whether there is any primary autoantigen in T1DM. According to the suggestion of a recent article (26) in the beginning of the autoimmune process against pancreatic beta cells, we may have three or more antigens, but at the end, there are endless antigens which are activating the process, i.e., the greater the beta cell lesion, the more antigens are expressed, which will reactivate the process. This proposal covers a new concept for the natural history of T1A diabetes mellitus which, in its preclinical stage, would be characterized by a succession of relapses and remissions with interrelation between regulatory T cells and effectors cells, and regeneration of beta cells up to the moment when the percentage of beta cell destruction would no longer allow a proper insulin secretion, resulting in the expression of hyperglycaemia. Within this context, it becomes important to mention the low capacity of regeneration/neogenesis of beta cells mainly when they are exposed to hyperglycaemia, which is a stimulus metabolic factor to the insulin secretion, but it is also glycotoxic. When proper glycaemic control is instituted at the beginning of the disease, these cells have acquiescence and may to keep the levels of C peptide secretion for a additional period of time.

Type 1B, also called idiopathic, has all the clinical features of type 1A, but the autoimmune component is not detected (3).

4. Genetics

The importance of genetic components in the pathogenic process leading to type 1 diabetes is supported by the observed higher concordance rate of monozygotic twins compared to dizygotic twins, by the familial clustering of the disease and by ethnical differences in the incidence of type 1 diabetes (27, 28). The vast majority of individuals who develop type 1A diabetes do not have a first degree relative with the disorder (>85%). Extending the ability to identify extreme risk to the general population without a relative with type 1A diabetes is thus an important goal.

The risk of complex diseases such as type 1 diabetes is generally thought to be influenced by multiple genetic and non-genetic factors, and it has been hypothesised that interactions between genes, or epistasis, are very common for such diseases (29). There are now at least four genetic loci that are established as causally involved in the aetiology of type 1 diabetes. The major genetic susceptibility is encoded by specific allelic combinations of DRB1, DQA1 and DQB1 in the human leucocyte antigen (HLA) complex. Variants in the insulin gene (INS), the cytotoxic T lymphocyte antigen-4 gene (CTLA4) and the protein tyrosine phosphatase, non-receptor type 22 gene (PTPN22) have been repeatedly associated with type 1 diabetes susceptibility using different approaches. All established loci are thought to be involved somehow in immune regulation, but details of the mechanisms relating the polymorphisms to risk of type 1 diabetes are in most cases poorly understood.

5. Environmental factors

Environmental factors have been implicated in the pathogenesis of type 1 diabetes both as triggers and potentiators of beta-cell destruction (30-32). Studies in monozygotic twins suggest that only 13–33% are pairwise concordant for the disease (33, 34), which implies that there is either acquired postconceptional genetic discordance or differential exposure to putative environmental factors. The tenfold difference in incidence of children under the age of 15 years reported in Europe with the highest incidence rate occurring in Finland (40.2/100000) and the lowest in Macedonia (3.2/100000) can hardly be explained by genetic factors (35). A considerable increase in the incidence of type 1 diabetes has been documented globally over the last decades, particularly in Europe (36). The steep increase cannot be exclusively due to an enhanced genetic disease susceptibility in the population but must mostly reflect changes in lifestyle and environment. Available data of migrant studies indicate that the incidence of type 1 diabetes has increased in population groups who have moved from a low-incidence region to a high-incidence area, emphasizing the influence of environmental conditions (31). Studies on HLA genotypes demonstrated that the proportion of subjects with high-risk DR and DQ alleles has decreased over the last decades among patients with newly diagnosed type 1 diabetes, whereas the proportion of people with low-risk or even protective HLA genotypes has increased (37, 38) suggesting the increase of the environmental pressure.

Various exogenous triggers, such as certain dietary factors and viruses (39, 40), are thought to induce the immune-mediated process leading to extensive beta cell destruction and ultimately to the clinical manifestation of type 1 diabetes. Changes have occurred in multiple environmental conditions over the last half-century. These include perinatal factors (41, 42), increased weight gain in infancy (43, 44), sunlight exposure and vitamin D sufficiency (45),

dietary factors (46-48), use of pharmaceutical products (eg antibiotics), socioeconomic factors (49) and psychological factors (50), (51).

6. Incidence of childhood type 1 diabetes

During the last decades large international collaborative studies, using standardized ascertainment schemes have offered significant contributions to our knowledge of the global epidemiology of the disease.

In 1983 an international meeting was held in Philadelphia, where the participating epidemiologists discussed the importance of establishing standardised registries to facilitate comparisons between countries. This meeting led to the birth of the Diabetes Epidemiology Research International (DERI) group. The DERI group played a key role in collecting standardised incidence data of T1DM between the late 1970s up to mid 1980s. Since then a lot of registries have been established all over the world.

In 1988 the European Economic Community launched a study called EURODIAB ACE (Europe and Diabetes: Aetiology of Childhood Diabetes on an epidemiological basis) with the participation of 44 European centres to asses incidence of childhood T1DM in Europe, to gather information to determine the causes and pathogenesis of the disease (52) in children under the age of 15 years. The WHO Multinational Project for Childhood Diabetes (DIAMOND) was started in 1990. The DIAMOND network includes 112 centres from 57 countries from around the world, representing about 84 million children with the data set of 43013 children diagnosed between the years 1990 and 1999 (53). Wide variation in incidence of type 1 diabetes in children younger than 15 years has been well characterised by registry

reports from the EURODIAB study group within Europe and the DIAMOND project group worldwide.

All of these registries have employed standardised protocols so that incidence data all over the world can be compared more effectively. Epidemiological data on type 1 diabetes are still lacking for the major part of the global population of children, especially in Africa, Asia, and South America (53).

6.1 Type 1 diabetes worldwide

It is estimated that on an annual basis some 76,000 children aged 14 years and under develop type 1 diabetes worldwide. In 2010, some 480000 children worldwide were estimated to have type 1 diabetes.

24% of these newly diagnosed children come from South-East Asia and 23% come from Europe.

Table 3 - *Source: Diabetes Atlas 4th edition International Diabetes Federation, 2009*

Type 1 diabetes (0-14 ys)	**2010**
Child population	1.9 billion
Number of children with type 1 diabetes	479000
Type 1 diabetes prevalence	0.02%
Annual increase of incidence	3.0%
Estimated number of newly-diagnosed cases per year	75800

One of the most striking characteristics of childhood-onset T1DM is its huge geographical variation in incidence. According to the data of the DiaMond study the incidence level shows a

more than 350-fold variability across the populations studied between the years 1990 and 1999 (53). Finland has the highest incidence of type 1 diabetes worldwide, reaching about 40 new cases per 100000 children at risk per year in the 1990s, whereas the Zunyi region in China was reported to have the lowest incidence with a rate of 0.1 cases per 100000 children at risk. Large intercontinental variation in incidence rates was also described (53); Figure 2).

In most *Asian populations* the incidence was very low (less than 1/100000/year); except Kuwait, with a very high incidence of 22/100000/year.

Among *African populations*, incidence was low or intermediate (1-9/100000/year). The incidence among *South American populations* varied between very low to high (1-10/100000/year). In *Central America and the West Indies*, the range of variation was from 2 to 17 /100000/year. In *Oceania*, the incidence of Type 1 diabetes was high or very high (14-22/ 100000/year), reflecting difference in the ethnicity of populations within this region. The highest incidence rates were among European and *North American populations* varying from low to very high in Europe, (4-41 /100000/year) and from high to very high in North America (11- 25/100000/year).

Europe has by far the most informative and reliable data. In *Europe*, a north–south gradient has been described (35, 52) in incidence, varying from the highest in Finland (43.9/100000/year) and other Scandinavian countries to the lowest in Macedonia (3.6/100000/year). Overall, the incidence rates were high in Northern and North Western Europe and low in Central, Southern and Eastern Europe (35), (Figure 3). Sardinia as an outlier being 3000 km south of Finland was an exception to this pattern with a five to seven times higher incidence rate than continental Italy (54).

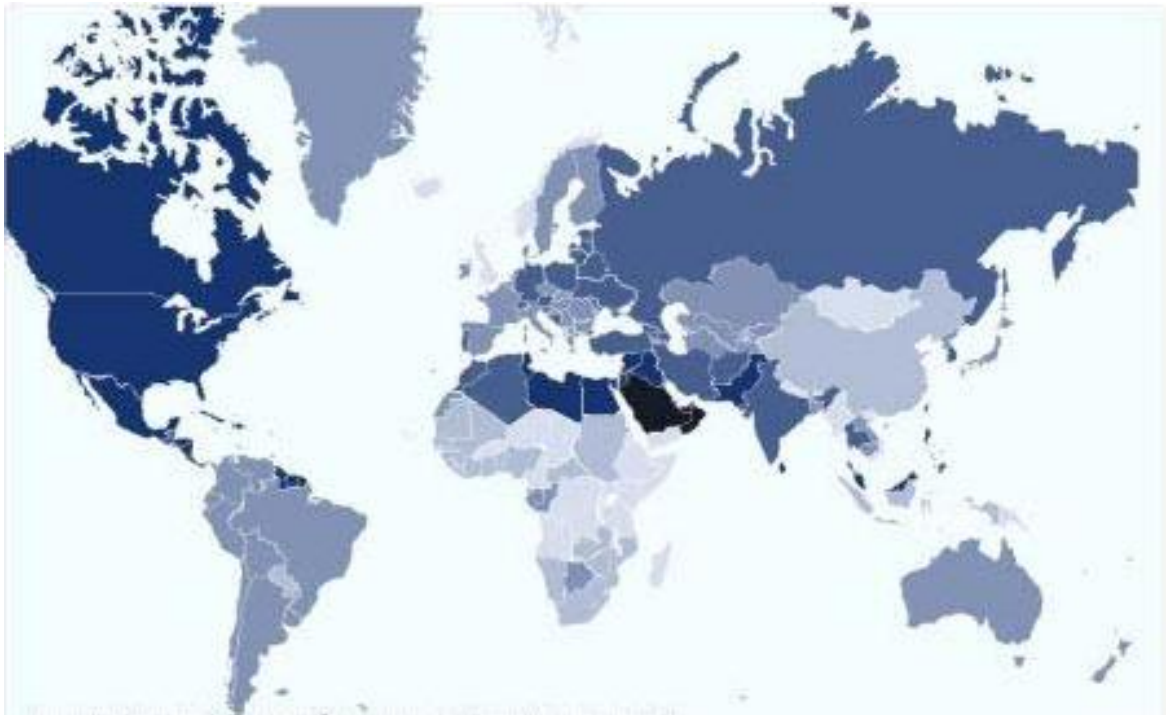

Figure 2 **–** Incidence rates of type 1 diabetes in children 0-14 years (cases/ 100000 population/ year) *Source: Diabetes Atlas 4th edition International Diabetes Federation, 2009*

Countries in Central Europe such as Austria, Hungary seem to be medium-risk countries between the high-risk populations of the Nordic countries and the low-risk South-European countries. The incidence of type 1 diabetes diagnosed under 15 years in Hungary (as determined in 18 counties) is intermediate in Europe. The Hungarian incidence is similar to the values observed in our neighbouring countries Austria, Slovakia and Slovenia.

Sharp contrast in incidence can also be observed among neighbouring countries. Even within Scandinavia, with genetically homogenous populations, equally developed societies and at the same latitude, there are differences in incidence rates varying between Finland (42.9/100000/year, 1980-2005) (55), Denmark (22/100000/year, 1996–2000) (56), Iceland (19/100000/year, 2001–2005) (57), and Sweden (28.9/100000/year, 1983-2000) (58).

Finland and Sweden lie on the northern and eastern side of the Baltic Sea. Estonia lies to the east of the Baltic sea and despite of its similar language and genetic background to that in Finland (37.7/100000/year) Estonia has a considerably lower incidence (11.2/100000/year) of type 1 diabetes (59). Latvia (6.9/100000/year) and Lithuania (7.2/100000/year) nearby Estonia have about five times lower incidence compared with Finland (59). In marked contrast Poland south of the Baltic Sea has a comparable incidence rate (7.6/100000/year) to Lithuania in the age group 0-14 during 1987-1999.

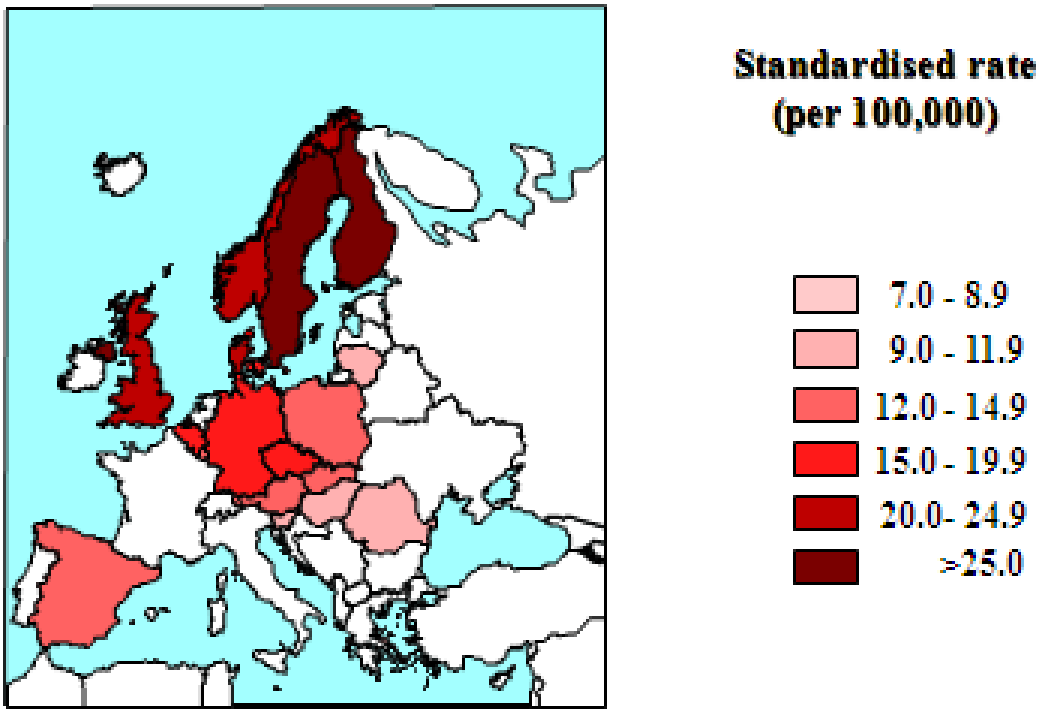

Figure 3 - Variation in incidence of type 1 diabetes in children aged 0-14 years in Europe (60)

6.2 Within-country variation

Within-country variation in incidence rate was observed in the Scandinavian countries such as in Sweden (61), Finland (62), in England (63), Italy (64) and Sardinia (65). Furthermore, studies in Finland (66), Sweden (67), Scotland (68) and Northern Ireland (49) have shown a higher incidence in rural than in urban areas. In contrast, reports from Lithuania (69) and Italy (64) have shown the opposite.

Geographical variations in incidence rates of T1DM in children may be associated with the different distribution of socioeconomic factors between urban and rural area, population density and ethnic differences.

6.3 Ethnicity

The incidence of T1DM shows remarkable variations between races and ethnic groups, the risk being much greater among Caucasians, less in Blacks and extremely low in Asians and Pacific Islanders and correlates strongly with the variability in incidence across countries.

According to the results of the largest population-based study in the US (SEARCH) the incidence of T1DM was highest among non-Hispanic white children (18.6 for 0-4 years; 28.1 for 5-9 years; 32.9 for 10-14 years), followed by African American (9.7 for 0-4 years; 16.2 for 5-9 years; 19.2 for 10-14 years) and Hispanic youth (9.1 for 0-4 years; 15.7 for 5-9 years; 17.6 for 10-14 years), and lowest among American Indian (4.1 for 0-4 years; 5.5 for 5-9 years; 7.1 for 10-14 years) and Asian/Pacific Islander youth (6.1 for 0-4 years; 8.0 for 5-9 years; 8.3 for 10-14 years) (70).

A recent migration study, carried out among German residents, comparing the incidence of German children versus Italian children originating from a very high-risk region (Sardinia) and from medium-risk areas (continental Italy) showed that children from Italy had incidence rates of T1DM that are closer to those of their native regions than to those of German children (71).

This finding indicates that genetic factors play a predominant role in the pathogenesis of T1DM.

Muntoni et al. found that Sardinian-heritage children living in a region with low incidence (Lazio, Lombardy) still maintain a much higher incidence of the disease relative to non-Sardinian children in the host region which is consistent with a stronger genetic susceptibility predominating over environmental factors (72).

The incidence of T1DM in Asian and Pacific Islanders is very low. Monozygotic twins and first degree relatives of patients with T1DM in Japan had a similar risk of diabetes to twins and relatives of patients in the United States, suggesting that most of the between-country variation in diabetes risk might relate to genetic differences rather than environmental factors (73).

6.4 Seasonality

6.4.1 Seasonality at diabetes onset

The seasonality at type 1 diabetes onset with a peak incidence in colder months and a nadir in warmer months has been extensively studied previously. Although many studies have found evidence for seasonality (74, 75), others have not (76) and some studies only found seasonality in population subgroups (77). The EURODIAB Study has shown heterogeneity in the seasonal pattern at type 1 diabetes onset according to age and region: seasonality was less pronounced in children diagnosed under 5 years and in Scandinavian countries.

The incidence of type 1 diabetes is generally highest in older age groups which may explain that the seasonal pattern is more often found in older children. A recent study (78) with the participation of 105 centres also demonstrated a global seasonal pattern at diagnosis of type 1 diabetes. This seasonality pattern appears to be dependent on the geographical position, at least as far as the northern／southern hemisphere dichotomy is concerned.

This seasonal variation has been taken as an indirect argument in favour of the role of environmental factors – such as viral infection, average daily ultraviolet B (UVB) radiation – in the development of the disease. One must remember, however, that the autoimmunity, which leads to clinical diabetes is a long process, therefore, the environmental factors associated with seasonality can only be considered as disease precipitators acting at a late stage of prediabetes.

6.4.2 Seasonality of birth

Several epidemiological studies (79, 80) have reported a seasonal pattern of birth, with a generally higher incidence for those born in spring and summer, than for those born in autumn and winter (81, 82), but little evidence have been found to support the hypothesis that seasonal environmental factors operating during fetal neonatal life have any influence on the development of type 1 diabetes in future life (83).

6.5 Age

T1DM is the major type of diabetes in youth, accounting for 85% or more of all diabetes cases in youth less than 20 years of age worldwide. The incidence increases from birth and peaks during puberty. The increasing incidence of T1DM throughout the world is especially marked in youngest children (0-4 years). Incidence rates decline after puberty especially in women but remains relatively high in young adult males up to the 29–35 yr of age (13, 84).

6.6 Gender

Although data from various regions suggest a slight male excess in populations of high (23/100000/year<) incidence and a minor female excess in low-incidence populations, on average both genders carry similar risks. With an increasing incidence of T1DM the sex ratio appears to shift from a female excess to a male excess (85). Male excess has been observed in populations of European origin while slight female predominance has been reported in African or Asian populations (13, 84).

6.7 Temporal trends in the incidence of T1DM

The incidence of childhood-onset type 1 diabetes exhibits remarkable temporal variation concerning secular trends. Reports from the cumulative data from the large international collaborative incidence registration systems have consistently found that since about 1990 the incidence of childhood-onset type 1 diabetes has been rising globally (35, 53, 60), with the possible exemption of Central America and West Indies (53).

According to the latest report from EURODIAB (60), in Europe the overall annual increase in incidence is 3.9% (ranging from 0.6% to 9.3%) during 1989-2003. The most striking changes with the steepest increase are observed in Central and Eastern Europe where the incidence is relatively low (Figure 4). These increases may result in a tendency for regional differences in European incidence rates to become less pronounced.

The rise in incidence is most pronounced in youngest children aged 0-4 years with an annual rate of 5.4% compared to 4.3% in the age group 5-9 years and 2.9% in 10-14 years old children (60).

In 2005 in Europe, the estimated number of newly diagnosed children with type 1 diabetes aged 0-14 years was 15000 and expected to increase to 24400 in 2020. Due to the sharp increase observed in youngest children, it is predicted that the percentage distribution of new

cases across the three age-groups will be more uniform at 29% (0–4 years), 37% (5–9 years), and 34% (10–14 years) compared to the ratios of 24% (0-4 years), 35% (5-9 years) and 41% (10-14 years) observed in 2005 (60).

The lifetime incidence is probably stable with incidence increasing in children and decreasing in adults (86).

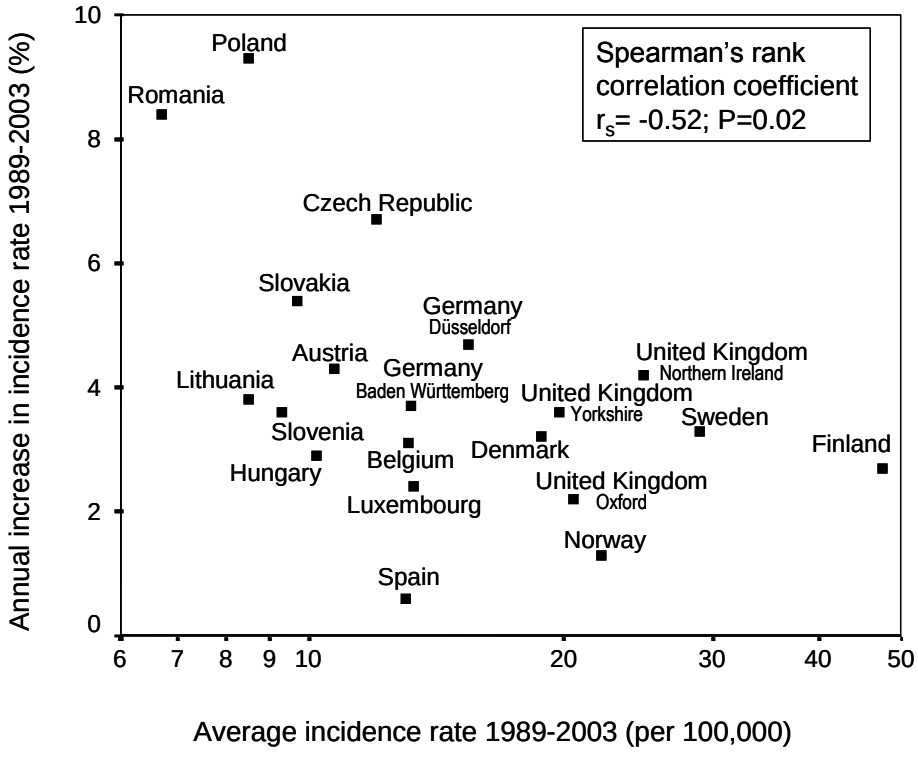

Figure 4 - Inverse association between rate of incidence increase and average incidence *Incidence rate on horizontal axis, plotted on a logarithmic scale. Spearman rank correlation coefficient rs=–0.52, p=0.02* (60)

II. AIMS

Our study had two main aims. First to analyse the incidence of type 1 diabetes in the age group 0–14 years in Hungary for the period 1989–2009, second to ascertain the spectrum of severity of presentation, the prevalence of diabetic ketoacidosis at diagnosis of diabetes, and the potential predictors of DKA.

INCIDENCE OF TYPE 1 DIABETES IN CHILDREN (0-14 YEARS) IN HUNGARY

1. Incidence trends over time

1.1 To evaluate whether the previously described increase in incidence observed between 1978 and 1998 has continued.

1.2. To predict future trends

2. Gender, age-group specific incidence

2.1 To analyze gender difference

2.2 To analyze age-group specific incidence

3. Seasonality

3.1 To investigate whether the seasonal pattern of clinical onset of type 1 diabetes is a general characteristic or it is restricted to gender and/or age at diagnosis subgroups.

4. Regional differences in incidence

4.1 To investigate any regional differences among counties in Hungary

4.2 To examine if population density was associated with incidence of type 1 diabetes

CLINICAL CHARACTERISTICS OF TYPE 1 DIABETES AT TIME OF DIAGNOSIS IN HUNGARIAN CHILDREN (2002-2009)

1. Clinical and biochemical characteristics at time of diagnosis during 2002-2009

1.1 To determine clinical characteristics at onset of type 1 diabetes in children aged 0-14 years

1.2 To investigate gender and age group specific differences in clinical and biochemical characteristics

1.3 To separately evaluate disease onset in subjects presenting with type 1 diabetes before their second birthday during the period from 2002 to 2009

2. Diabetic ketoacidosis

2.1 To investigate the frequency of diabetic ketoacidosis at type 1 diabetes onset, to analyze temporal trends and any regional differences in the occurrence of DKA

2.2 To compare clinical characteristics of children presenting with and without DKA

2.3 To identify predictors of diabetic ketoacidosis in newly diagnosed children under 15 years of age in Hungary

III. INCIDENCE OF TYPE 1 DIABETES IN CHILDREN (0-14 YEARS) IN HUNGARY

Study design

The Hungarian Childhood Diabetes Register has been collecting data of all newly diagnosed children with type 1 diabetes under the age of 15 years since 1978. Data for the first 11 years (1978–1988) were collected retrospectively. Prospective registration as part of the EURODIAB Study started in 1989 (52). I have been the data manager of the database from the beginning of the prospective data collection, and I have performed the statistical analyses. The study included the data of 18 counties. Budapest, the capital, and county of Pest were excluded from our study because historically we have found that the ascertainment level was low (87). There are several paediatric departments in the capital, and some of them - particularly in the first years of the registry - were unable to provide reliable case data. The ascertainment level has considerably improved in recent years and incidence data for the last year are now available for the capital, as well (vide infra). The children's population of Budapest is about one fourth of national total. The Hungarian population aged 0–14 years in 18 counties decreased by one third from 1579450 (1989) to 1052184 (2009) during the study period.

Type 1 diabetes mellitus was defined on the basis of a clinical diagnosis of idiopathic diabetes by a physician. Cases secondary to other conditions (eg. having cystic fibrosis or high-dose steroid treatment or MODY diabetes) were excluded. Date of onset was defined as the date of the first insulin injection.

All cases on the register were included in the analysis if they were diagnosed between the 1 January 1989 and 31 December 2009. The capture–recapture methodology, which assumes

that independent primary and secondary sources of ascertainment are available, was used to estimate the completeness of registration. In Hungary, children with new-onset diabetes aged 0–14 year are hospitalized in paediatric departments at the time of diagnosis. Hospital case records served as primary source of ascertainment. The list of children who attended diabetes summer camps was used as an independent secondary source of ascertainment. These camps, organized by trade unions and later by charity organizations, were open to all diabetic children. Ascertainment was above >95% throughout the 21-year period with a uniform pattern over time: 1989-1993, 97.9%; 1994-1998, 95.9%; 1999-2003, 97.5%; 2004-2009, 100%.

All denominators for incidence calculations were provided annually by the Central Bureau of Statistics.

Statistical methods

Annual end-year population estimates were used as denominators for calculation of rates. Age- and sex-standardized incidence rates were obtained using the direct method with a standard population consisting of equal numbers of children in each of the six subgroups defined by age group (0–4y, 5–9y and 10–14y) and gender. Confidence intervals for rates were calculated assuming that the observed number of cases followed a Poisson distribution. Poisson regression models were used to assess time trends in incidence. Models with terms for age group, gender and calendar year were fitted. Interactions were fitted to assess if the trends differed significantly between age groups or genders. Cyclic behaviour in the incidence trend was estimated by a Poisson regression model as described by Edwards JH (88). Heterogeneity between counties was assessed using Poisson regression. Population density was calculated by dividing the population by the geographical area (in square kilometres).

Predictions for future new cases until 2030 were made by extrapolating rates using the annual percentage increase in incidence in each age/sex subgroup estimated from the Poisson regression model.

Statistical analyses were performed using STATA release 8.0 (Stata Corporation, College Station, TX, USA).

Results

THE AVERAGE STANDARDIZED INCIDENCE RATE

A total of 3432 patients (1777 boys; 1655 girls) under the age of 15 years were registered during the observation period.

The average standardised incidence rate was 12.5 (95%CI 12.0-12.9) per 100000 per annum over the 21 years, 12.6 (95% CI 12.0-13.2) in boys and 12.3 (95% CI 11.72-12.9) in girls. The age-specific incidence rates were 8.8 (95% CI 8.2-9.5), 13.5 (95% CI 12.7-14.3) and 15.1 (95% CI 14.3-15.8) per 100000 person years in the 0-4, 5-9 and 10-14 year age groups, respectively.

INCIDENCE TREND

The incidence increased from 7.7 (95%CI 6.3-9.0) per 100000 person years in 1989 to 18.2 (95%CI 15.7-20.9) in 2009 (Figure 5).

Table 4 shows the mean age- and sex-specific incidence rates by three 5 year and one 6 year periods. The increase in incidence rate for the entire cohort (and in both sexes and in all three age groups) was highest between the periods 1999-2003 and 2004-2009.

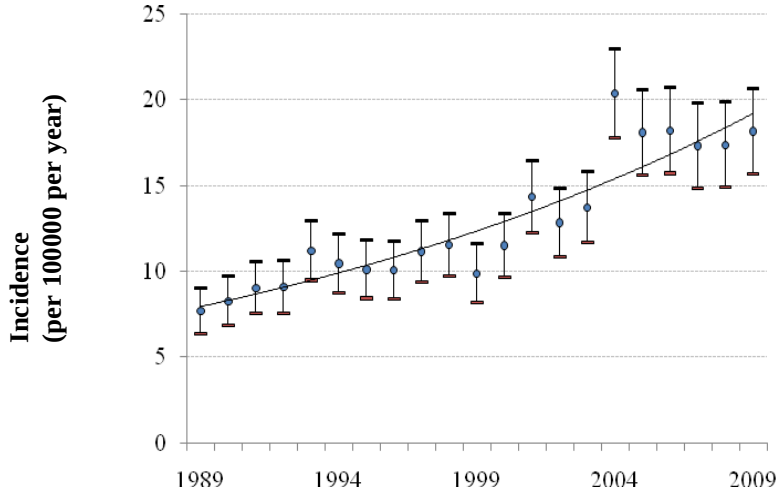

Figure 5 - Age-standardised incidence rate (with 95% confidence intervals) for type 1 diabetes in children aged 0-14 years in Hungary for each year in the period 1989-2009 with fitted log-linear trend.

Table 4 - Age- and sex-specific incidence rates of type 1 diabetes in Hungary during 1989-2009; incidence per 100 000 per year (95% CI) by three 5-year and a 6-year periods

		1989-1993	**1994-1998**	**1999-2003**	**2004-2009**
Boys	0-4	5.1 *(3.8-6.4)*	8.0 *(6.3-9.7)*	8.3 *(6.4-10.1)*	14.3 *(12.0-16.6)*
	5-9	9.7 *(7.9-11.4)*	10.4 *(8.5-12.2)*	12.7 *(10.6-14.9)*	21.1 *(18.4-23.8)*
	10-14	11.5 *(9.8-13.3)*	12.4 *(10.4-14.4*	16.6 *(14.3-18.9)*	22.4 *(19.8-25.0)*
Girls	0-4	5.4 *(4.0-6.7)*	7.2 *(5.5-8.8)*	9.5 *(7.5-11.6)*	13.8 *(11.5-16.1)*
	5-9	9.4 *(7.6-11.2)*	12.1 *(10.1-14.2*	13.8 *(11.6-16.1)*	19.9 *(17.2-22.6)*
	10-14	13.0 *(11.1-14.9)*	13.9 *(11.7-16.0*	13.5 *(11.4-15.7)*	17.7 *(15.5-20.1)*
Boys&Girls	0-4	5.2 *(4.3-6.3)*	7.6 *(6.5-8.9)*	8.9 *(7.5-10.3)*	14.1 *(12.6-15.9)*
	5-9	9.5 *(8.3-10.8)*	11.3 *(9.9-12.7)*	13.3 *(11.8-14.9)*	20.5 *(18.6-22.5)*
	10-14	12.3 *(11.0-13.6)*	13.1 *(11.7-14.6*	15.1 *(13.6-16.8)*	20.1 *(18.3-21.8)*
Stand incidence		9.0 *(8.4-9.7)*	10.6 *(9.9-11.5)*	12.4 *(11.6-13.3)*	18.3 *(17.3-19.4)*

Results of the Poisson regression analysis are summarized in Table 5. This analysis demonstrated a highly significant linear trend in incidence over the 21-year period (line 1, Table 5). Fitting of a quadratic term in year showed no evidence of significant departure from linearity in the secular trend (line 2, Table 5). Interestingly, the year 2004 is an outlier with its unusually high incidence rate and 95%CIs above the estimated trendline, although rates for the years 2005 and 2006 are also above the linear prediction (Figure 5). There was also evidence of a significant difference in the trends between age groups (line 3, Table 5) and between genders (line 4, Table 5) with rates increasing faster in the younger age-groups and in boys. However, the test for interaction (line 5, Table 5) did not detect any significant difference between boys and girls in the patterns of increase by age-group.

Table 5 - Summary of Poisson regression analyses fitted to data in 3 age groups, 2 sexes and 21 years

	Model		**Likelihood ratio test on last model term**			**Goodness-of-fit test**		
		-2log L	χ^2	***df***	**P**	χ^2	***df***	**P**
0	A+S+A•S	1020.37				381.97	120	<0.001
1	A+S+A•S+Y	784.34	236.04	1	<0.001	145.93	119	0.05
2	A+S+A•S+Y+Y^2	784.23	0.11	1	0.74	145.82	118	0.04
3	A+S+A•S+Y+Y•A	769.19	15.14	2	0.001	130.79	115	0.15
4	A+S+A•S+Y+Y•A+Y•S	763.37	5.82	1	0.016	124.97	116	0.27
5	A+S+A•S+Y+Y•A+Y•S+Y•A•S	760.36	3.01	2	0.22	121.96	114	0.29

A - Age, S - Sex,
Y - linear term in year,
Y^2 - quadratic term in year,
Y•A - Interaction between Y and A

Table 6 shows that there was an average annual increase of 4.4% (95%CI 3.9-5.0; p<0.001) after adjustment for age and sex.

Table 6 - Summary of Poisson regression analyses showing age-and sex-specific annual increases (95% CI) expressed as % during 1989-2009

	Boys	**Girls**	**Boys & Girls**
0-4 y	6.4 (4.6-8.2)	6.0 (4.2-7.9)	6.2 (4.9-7.5)
5-9 y	5.2 (3.8-6.6)	4.6 (3.2-6.0)	4.9 (3.9-5.9)
10-14 y	4.5 (3.3-5.7)	1.9 (0.7-3.2)	3.3 (2.4-4.1)
All ages	5.1 (4.3-5.9)	3.7 (2.9-4.6)	4.4 (3.9-5.0)

Figure 6 shows that the increase was evident in both genders with higher rates for boys (5.1%, 95% CI 4.3-5.9; p<0.001) compared to girls (3.7%; 95% CI 2.9-4.6; p<0.001). Boys showed faster rates of increase in incidence of type 1 diabetes in all three age groups than did girls, but only in the oldest age-group was this difference significant (p=0.004), (Table 6).

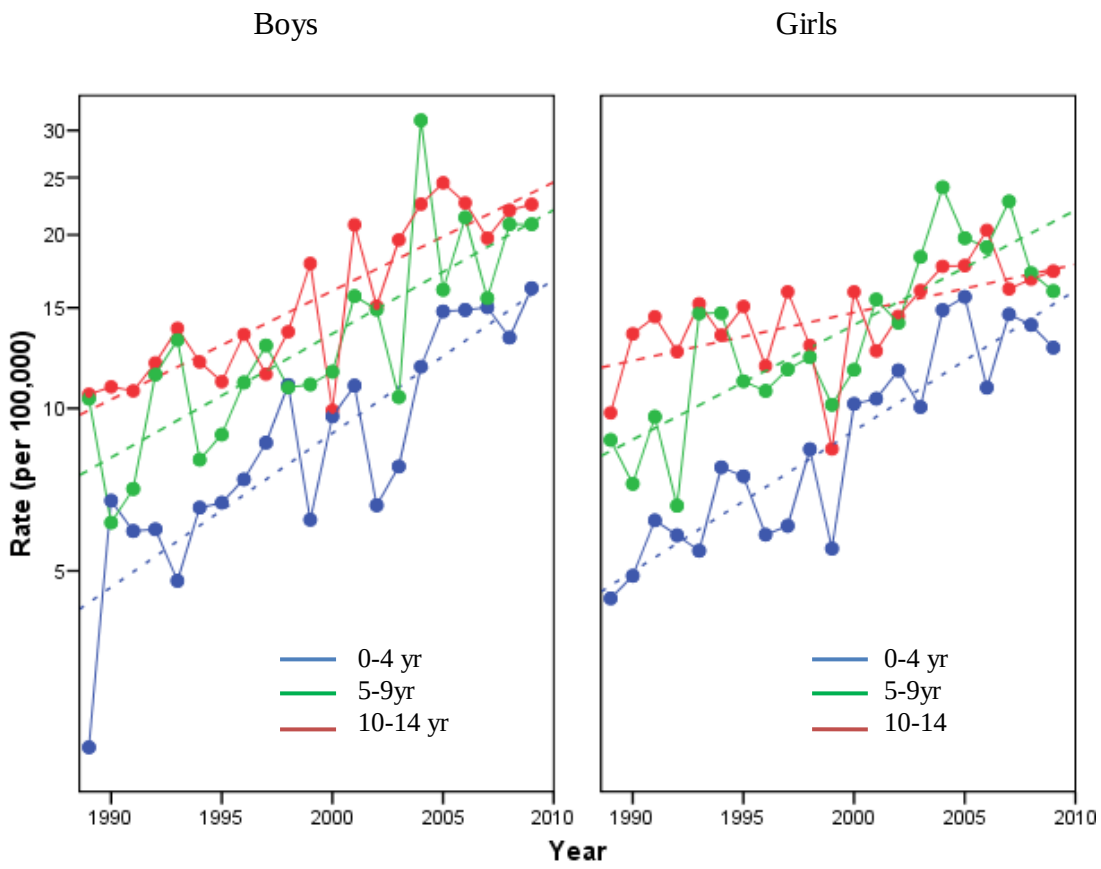

Figure 6 - Time trends in age-specific incidence rates in boys and girls plotted on a logarithmic scale for each year in the period 1989-2009.

AGE- AND SEX SPECIFIC INCIDENCE RATES

Figure 7 shows the age distribution of the incidence for girls and boys. The incidence peak for girls occurred 2 years earlier than the peak for boys. The incidence rate was similar in boys and girls at or before the age of 10 years, but thereafter it started to diverge (Figure 8). The overall boy-to-girl ratio in incidence was 1.02, 1.0, 0.97, 1.08 for the age groups 0-4 year, 5-9 years and 10-14 years, respectively.

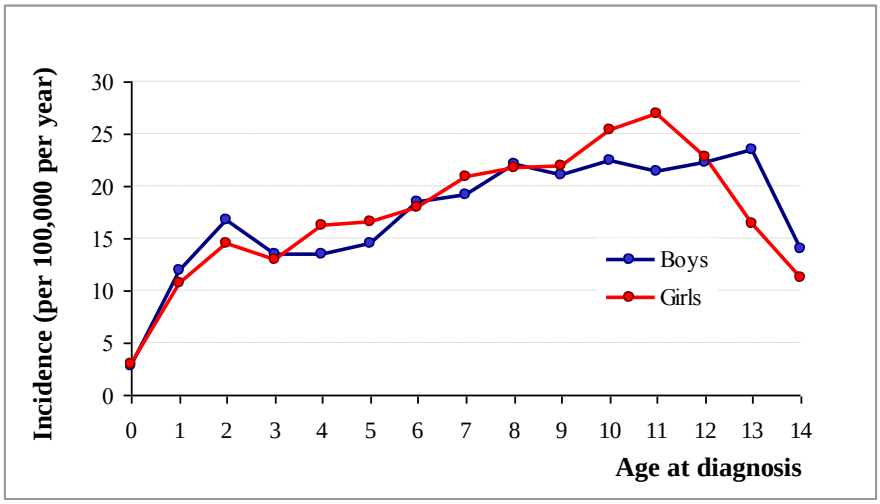

Figure 7 – Age specific and sex specific incidence rates per 100000 per year in Hungarian children

INCIDENCE PREDICTION

Assuming that the rates of increase observed in 1989-2008 continue, by 2030 the number of newly diagnosed children aged 0-14 years in Hungary is predicted to be 2460 with a distribution of 31% being in the 0-4 year, 38% in the 5-9 year and 31% in the 10-14 year age groups. The predicted age-specific incidence rates are 56 per 100000, 59 per 100000, and 43 per 100000, respectively for the three age groups (Figure 8).

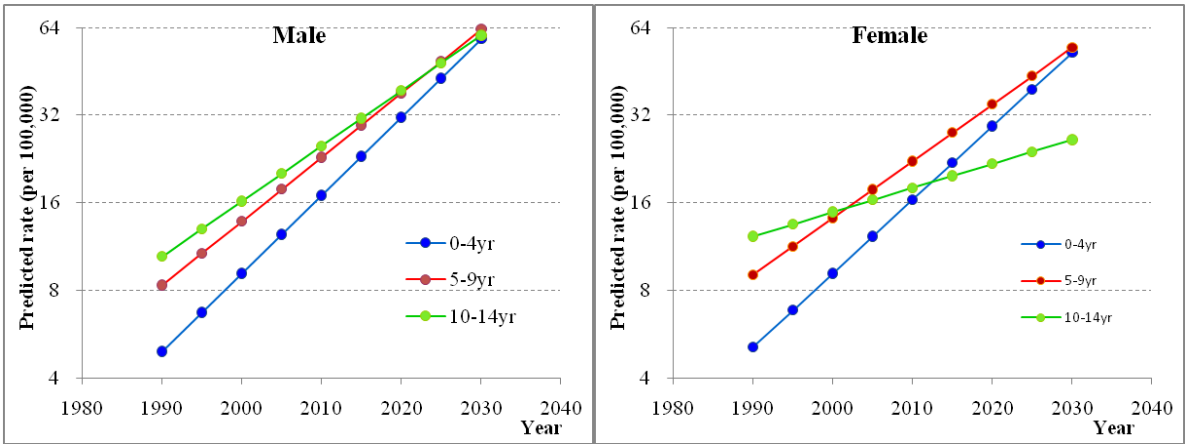

Figure 8 - Estimated and predicted incidence of newly diagnosed type 1 diabetes

SEASONALITY AT DIAGNOSIS

There was a clear seasonal variation over the year ($\chi 2=72.8$; $p<0.001$), with a peak in the winter months and a trough in the summer months (Figure 9).

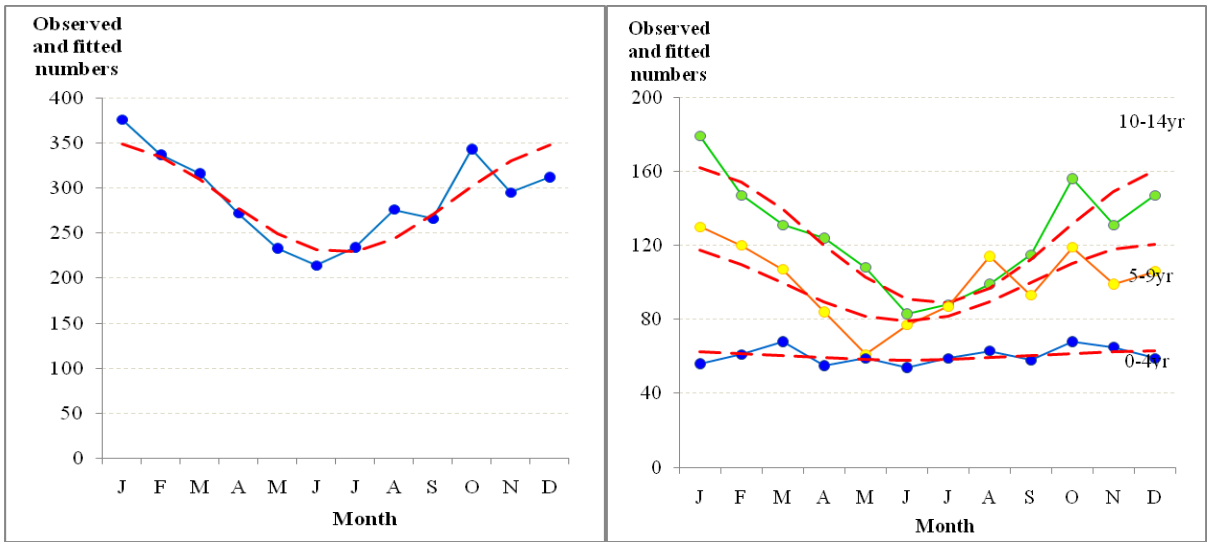

Figure 9 - **a.** Seasonality at first insulin injection in Hungary

b. Seasonality of first insulin injection by age groups during the period 1989 and 2009

The *solid* lines represent the observed values transformed into results for months of equal length. The *dotted* lines represent the fitted sinusoidal trend. **a** 0–4 years: cyclic trend: $\chi 2=0.56$, $p=0.76$, **b** 5-9 years: cyclic trend: $\chi 2=26.1$, $p<0.001$. **c** 10–14 years: cyclic trend: $\chi 2=66.21$, $p<0.001$

When the data were further analyzed by age groups, the seasonal variation was observed in the 10-14 years age group ($\chi 2=66.2$; $p<0.001$) and in the 5-9 years old children ($\chi 2=26.1$; $p<0.001$) but not in the youngest children. January had the highest peak whereas June showed the lowest number of newly diagnosed children. The corresponding amplitudes of oscillation were + / - 3.9%, + / - 20.9% and + / - 29.6% for the age groups 0-4yr, 5-9yr and 10-14yr, respectively.

REGIONAL DIFFERENCES

There was evidence of a difference in age-standardised incidence rates among the counties ($\chi 2=109.4$; $p<0.001$).

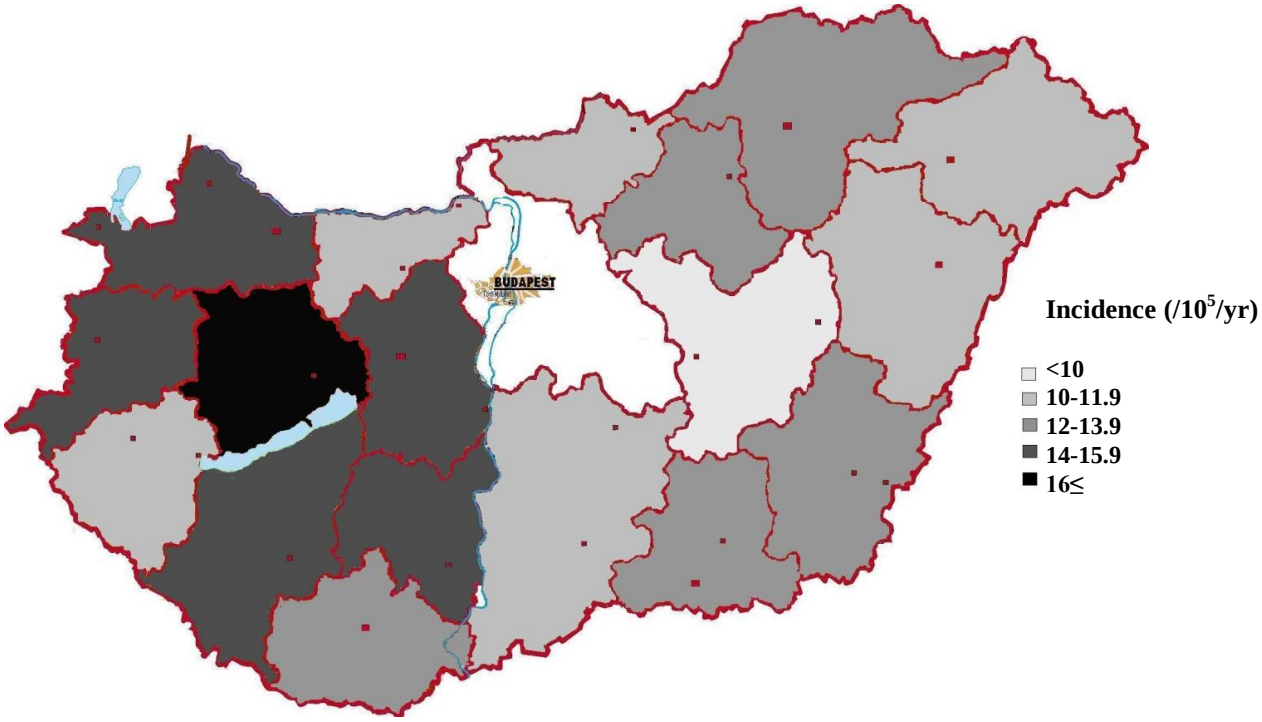

Figure 10 – Regional differences in incidence of type 1 diabetes in children aged 0-14 years in Hungary (1989-2009)

The addition of a year by region interaction term was not significant, indicating that the rate of increase did not differ among the counties. Counties in the western part of Hungary tend to have higher incidence rates than the overall average while the eastern part show lower incidence rates (Figure 10). Population density failed to show any significant association with incidence (r=0.02; p=0.94).

Discussion

The present study has reported the 21-year data of the Hungarian Childhood Diabetes Registry during 1989-2009 in a genetically stable, homogenous population using standard diagnostic criteria and epidemiological methods, with an ascertainment level of >95% over the years. With the overall incidence rate of 12.5 per 100000 per year, which is comparable to the incidence in the surrounding countries (89-92), Hungary belongs to the medium incidence geographical regions. During the observation period the background population of children aged below 15 years has dropped by a third, and at the same time the number of newly diagnosed children increased considerably by around 40 new cases per year in boys and 20 new cases per year in girls. These changes have resulted in the 2.4 fold increase in incidence of type 1 diabetes in Hungarian children during 1989-2009 exceeding the earlier prediction – based on the observed trend between 1989-1998 - of a doubling in incidence rates in 15 years (93). The incidence of type 1 diabetes increases with age, the peak is around puberty with the associated gender effect as at the beginning of the study period but because of the rapid increase in younger children the difference in incidence among the age groups seems to be diminishing; the incidence in the 5 to 9 year age group has now reached that of the 10 to 14 year age group, particularly in females. Although our study has been restricted to children younger than 15 years of age, more data are becoming available about the incidence of type 1 diabetes in young adults. According to these results the incidence rate decreases substantially

after the age of 15 years and seems to stabilize in young adults (15-29 years), although there are some indications of an increasing trend up to the age of 39 years.

In more recent years a male excess in the age standardised rate has become evident compared to a female excess noted previously, and the Poisson regression analysis detected this difference in rates of increase between the sexes (93).

The statistical analysis showed an overall increasing trend with little evidence of deviation from linearity. However, the year 2004 was an outlier with higher incidence than predicted compared to previous years and the subsequent periods, mainly because of the unexpectedly high number of new cases observed in the 5-9-year old males. The "peak" incidence in the year 2004 was followed by stable rates for a number of years. We observed similar transient stabilization of incidence (at a lower level) between 1980 and 1984 (87). Some of the high risk countries have also shown no increase in incidence for brief periods over the last 20 years (94, 95) . The latest report from Finland (55) shows strikingly stable rates between 1980 and 1987 with subsequent steep increase. Recent publications from neighbouring Austria (91) and Croatia (90) have also reported transient periods of stabilization (1979 to 1989 and 1995 to 2000, respectively), although the general trend remained clearly upward. When we compared the first 10-year period with the second 11 years we found that the increase was larger during 1999-2009 (5.2% vs. 3.6% during 1989-1998) although the difference was not significant. Publications from the neighbouring countries of Hungary as well as other European populations reported similar increasing trends in incidence with accelerated increase in recent years (90, 91, 96-98). Although we found no evidence of systematic departures from log linear trends, in countries with high incidence where the rate of increase is less steep suggesting a levelling off compared to medium or low-incidence regions, a log linear trend may not always be appropriate (94, 99). Reports of stabilization of rates should therefore be interpreted with caution, particularly if they are based solely on visual impression of short-term changes in rates

or on the fitting of smoothed non-linear relationships that do not offer any significant improvement in model fit. The prevailing pattern seems to be one of continuing increase.

Many attempts have been made to explain the rising trend in incidence over the past decades and several studies indicated the role of life-style-related risk factors (environmental exposures) such as high calorie intake, rapid early growth and rapid early weight gain (100-103). It is of considerable significance that a recent publication from Sweden reported a levelling off of incidence in birth cohorts from the year 2000 and thereafter (99) paralleling a decline in the prevalence of overweight and obesity in 4-year-old children. It is at present too early to know whether or not this new observation is an isolated phenomenon unique to Sweden or if it will generalize to other populations in due course. However, the claim that this represents a shift back to older age at onset (99) seems premature until data are available on the risks in this young cohort at older age-groups.

Consistent with previous reports, we found a significant seasonal pattern at clinical diagnosis of type 1 diabetes, with peak values between January and March. The seasonal variation was comparable with a sinusoidal curve, and the observed amplitude of oscillation averaged ±20.6%. Although the seasonal variation in the 10-14 age-group is well described as a sinusoidal pattern in Hungarian children this is not the case for the 5-9 age-group. In contrast to EURODIAB, our study failed to observe seasonal variation in the youngest age group, possibly due to a lower number of cases in this age group. It is interesting to note, that all age-groups show a larger than expected number of cases diagnosed in October possibly due to return to school.

Regional differences between continents, countries and areas within countries in incidence of type 1 diabetes have widely been observed. The finding of a higher incidence in rural areas compared to urban areas has been linked to a theory called “hygiene hypothesis”. According to this hypothesis, high-dense population urban areas with crowded households and higher

infectious exposure in early life are expected to have lower incidence rates due to the protective effect of infections (104). A number of studies have supported this finding; the results are however, controversial. Although, the current analysis showed some evidence of difference in incidence among counties, no clear pattern has been identified. Furthermore, no association with population density could be confirmed. There were some indications that countries in the western part of Hungary have higher incidence rates compared to those lying in the Eastern part. More detailed analysis using small area units and including socioeconomic factors are needed to investigate any possible differences among areas.

One possible limitation of our study is that Budapest and county of Pest covering about 25% of the children's population were excluded for their low level of ascertainment. In 2010 we managed to collect more accurate data, which show that the incidence in Budapest (25.6/100000/year; 95% CI 19.3-33.3) and in county of Pest (18.6/100000/year; 95% CI 13.1-25.5) was comparable with the national average incidence and we assume that it was similar in previous years.

IV. CLINICAL CHARACTERISTICS OF TYPE 1 DIABETES AT ONSET IN CHILDREN AGED 0-14 YEARS IN HUNGARY

Introduction

Ketoacidotic episodes are still the most common causes of hospitalisation and deaths in children with type 1 diabetes and also seem to influence the longer term clinical course of type 1 diabetes. Children with diabetic ketoacidosis at diagnosis have poorer glycaemic control, less residual β cell function up to two years after diagnosis (105, 106) and a lower frequency of remission (106, 107).

Diabetic ketoacidosis at onset has been the subject of many investigations and reviews during the last decade. Although some studies have found that the clinical presentation at onset has become less severe with time (108, 109); there is no clear evidence that the prevalence of diabetic ketoacidosis at time of diagnosis has decreased over the past years (110, 111); in fact with increasing incidence of type 1 diabetes reported to be most remarkable in youngest children, an actual increase is anticipated.

There were only isolated reports based on hospital records on the clinical characteristics of the disease in children (112-114), but no population-based study has been initiated so far to assess the frequency of diabetic ketoacidosis at time of diagnosis in Hungarian children.

Study design

Prospective registration of laboratory and clinical data at diagnosis as part of the Hungarian Childhood Diabetes Register has started in January 1, 2002. All children with new-onset type 1 diabetes aged 0-14 years diagnosed between 1 January 2002 and 31 December 2009 were involved in our study.

In order to investigate clinical characteristics at type 1 diabetes onset and to provide reliable information about the prevalence of diabetic ketoacidosis in Hungary I prepared a standard data sheet for prospective registration of clinical and laboratory data. Data collection using this new form (by e-mail and by post) from all paediatric diabetes centres started on 1st January, 2002. Information regarding the clinical presentation, duration (given as number of days) of symptoms prior to diagnosis observed by a parent (or reported by a child) and preceding infection were recorded. Level of consciousness (classified into three categories of normal, impaired consciousness and coma), hydration status (normal or dehydrated) were evaluated by the clinician examining the patient at the time of hospital admission. Weight and height at diagnosis was documented. BMI (kg/m2) was expressed as body mass index

standard deviation score (BMI-SDS) based on the national standard data of the longitudinal growth study by Joubert et al (115). Biochemical data were obtained before administering intravenous fluids or insulin at the hospital. Data were submitted continuously to the Central Coordinating Office, Department of Paediatrics, University of Pécs, Pécs, Hungary. Individual data were entered into a Microsoft Access database (set up and maintained by myself).

Standard laboratory methods were used to measure serum glucose, HbA1c, electrolytes, blood urea nitrogen, venous blood gases and urinalysis. HbA1c was measured by HPLC (high-performance liquid chromatography method) with a reference range of 4 to 6%.

Ketoacidosis was defined as a blood pH less than 7.3. Ketoacidosis was categorized by severity of the acidosis, varying from mild with $7.3>pH\geq7.2$, to moderate with $7.2>pH\geq7.1$ l and severe with $pH\leq7.1$.

Positive family history was defined as having a first-degree relative with type 1 diabetes.

Statistical methods

Statistical analyses were carried out using SPSS for Windows (version 15.0, Chicago, IL). For normally distributed data results data were summarized as mean values ± standard deviations (SD). Unequally distributed results expressed as median and interquartile range (IQR) were analyzed by Kruskal-Wallis and Mann-Whitney U tests. Continuous variables were compared by the independent sample Student`s t- or one-way analysis of variance tests; categorical variables, using cross tabulation calculation. Univariate analyses were followed by a dichotomous (DKA yes/no) logistic regression model with forward, stepwise elimination methods, including all variables that showed $p<0.10$ on univariate analysis. Main effects and interaction effects were estimated. The Hosmer-Lemeshow method was used to assess goodness of fit.

Results

Between 1 January, 2002 and 31 December, 2009, a total of 1558 newly diagnosed children (828 boys; 730 girls) were identified with type 1 diabetes. The mean age at diabetes diagnosis was 8.6±3.9 years (range 0.4-14.9); girls were significantly younger (8.3±3.8 years; p=0.02) as compared to boys (8.8±4.0 years). The mean blood glucose concentration was 26.5±11.9 mmol/l (range 6.9-140mmol/l), the mean glycated haemoglobin (HbA1c) was 11.9±2.6 % (range 5.6-25.8%) at the time of diagnosis.

CLINICAL CHARACTERISTICS AT TIME OF DIAGNOSIS

3% of the children had no symptoms before diagnosis. The median duration of symptoms before diagnosis was 14 days (range 0-364). 514 (33%) children had duration of symptoms ≤7 days, 4.6% of the children had symptoms over 2 months. Polyuria and polydipsia, the most frequent symptoms in all age groups were present in 89.2% of all cases (Figure 11). 7.9% of the children were reported to have impaired consciousness at diagnosis and 0.2% was considered unconscious. 296 children (19%) had an infection at diagnosis. Girls had significantly lower BMI-SDS compared to boys (-0.73±1.1 vs -0.55±1.1; p=0.002). Other clinical and biochemical characteristics at onset did not differ significantly between boys and girls.

Blood pH ranged from 6.76 to 7.60 (mean±SD; 7.30±0.14). DKA (pH<7.3) was identified in 32% of the children (n = 498), of which 44% had mild, 26% moderate, and 30% severe DKA. The highest prevalence of ketoacidosis occurred in more underweight children with a BMI-SDS ≤ -1 (42% vs 32% with -1< BMI-SDS ≤ 0 vs 29% with 0< BMI-SDS ≤ 1 vs 19% with a 1 < BMI-SDS; p<0.001).

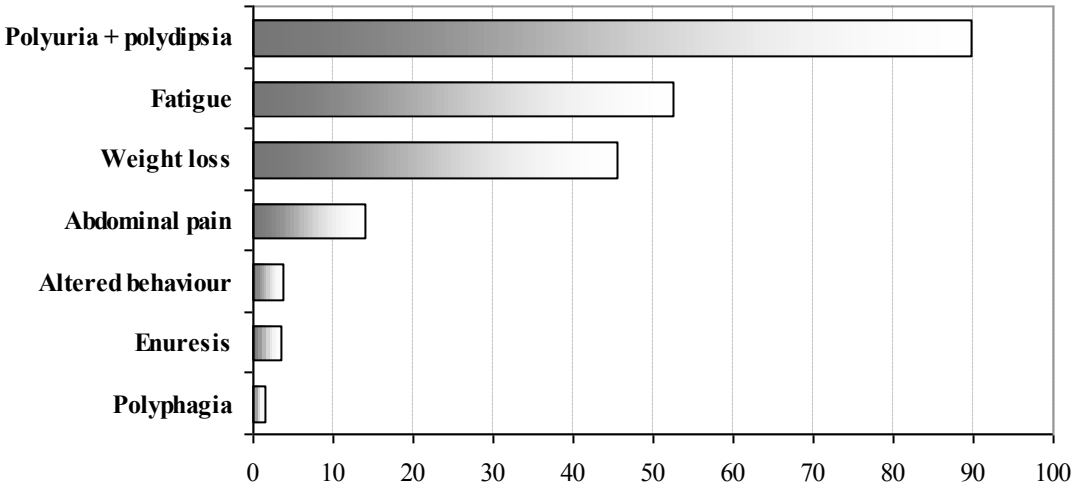

Figure 11 – Clinical symptoms

The frequency of DKA was similar over the years from 2002 to 2009 (p=0.29), ranging from 30.2% in 2003 to 40.5% in 2008 (Figure 12).

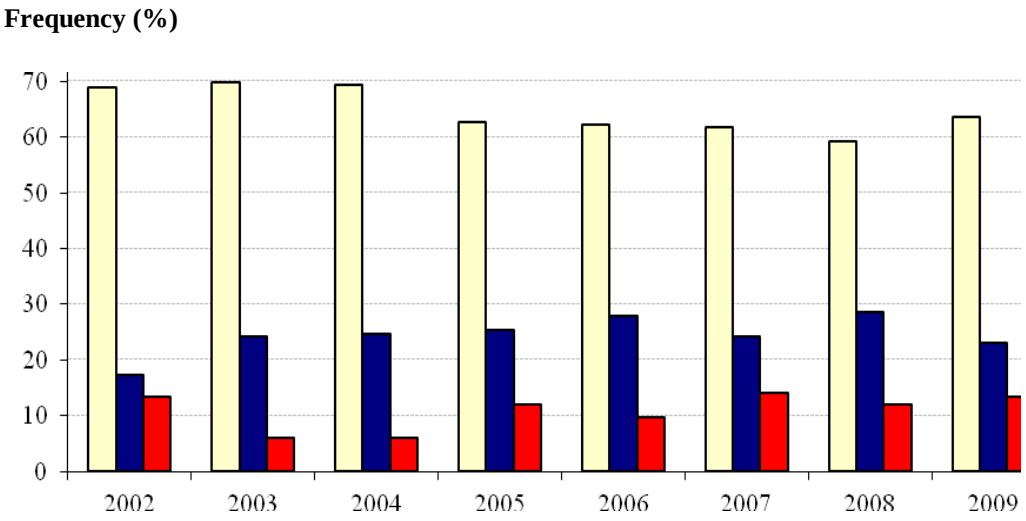

Figure 12 – Temporal trends in DKA rates of children aged 0-14 years with type 1 diabetes onset in Hungary (2002-2009) *white columns:* No acidosis, *blue columns:* mild DKA 7.1≤pH<7.3, *red columns:* severe acidosis pH<7.1 (p=0.14)

Comparison of clinical and biochemical characteristics of patients with DKA and those without DKA are shown in Table 7. Patients with ketoacidosis at onset were younger, had a significantly lower BMI-SDS, higher mean blood glucose and mean HbA1c level and one-quarter of them had an acute infection at presentation. The occurrence of DKA was less frequent in children with a first degree relative of type 1 diabetes.

Table 7 - Comparison of clinical and laboratory data of children according to metabolic status

Clinical characteristics	**DKA**	**No DKA**	**p**
Male (%)	263 (53)	497 (57)	NS[1]
Age [year]	7.4±4.1	8.4±3.8	<0.001[2]
Duration of symptoms days [(IQR)]	14 (7-22)	14 (7-28)	0.07[3]
BMI-SDS	-0.9±1.1	-0.5±1.0	<0.001[2]
Infection [%]	27.3	15.8	<0.001[1]
Dehydration [%]	88.3	43.8	<0.001[1]
Positive family history [%]	11.0	16.0	0.012[1]
Blood glucose [mmol/l]	30.8±13.1	24.5±10.6	<0.001[2]
HbA1c [%]	12.5±2.4	11.6±2.6	<0.001[2]

Data are mean±SD or frequency or median (interquartile range).

[1] Crosstabulations, [2] T-test, [3] Mann-Whitney U test

COMPARISON OF CLINICAL CHARACTERISTICS ACCORDING TO AGE GROUPS

351 children (22.5%) were aged 0-4 years, 553 children (35.5%) were aged 5-9 years and 654 children (42%) were 10-14 years at diagnosis (χ^2=91.7; p<0.001). Gender distribution of the

two younger age groups was similar; however in oldest children more boys compared to girls have been diagnosed (369 boys vs 285 girls; χ^2=10.3; p=0.001).

The median duration of symptoms before diagnosis was 14 days (range 7-2.5 days) and it was significantly shorter in youngest children [9 days (IQR 7-19) in 0-4yr vs 14 days (IQR 7-21) in the 5-9 yr vs 21 days (IQR 7-28) in 10-14yr; (χ2=60.9; p<0.001)]. Symptoms at presentation were similar in all age groups. Consciousness was more often impaired in the very young children (16.4% in 0-4 yr vs. 6.3% in 5-9 yr vs. 5.2% in 10-14 yr; p<0.001). A concomitant infection was more frequently observed in the youngest age group (31.5% in 0-4 yr vs. 19% in 5-9 yr-, vs. 13.3% in 10-14 yr; χ2=40.7; p<0.001).

Lower BMI SDSs compared with age matched reference data were found in all age groups (Table 8). Youngest children had gross hyperglycaemia with mean blood glucose close to 30 mmol/l, while HbA1c was lowest in children aged 0-4 years, indicating a shorter duration of hyperglycaemia in this age group. The youngest age group had significantly lower BMI-SDS compared to older children. Patients in the age group 0-4-years had more severe acidosis compared to older children and about half of them had DKA (Table 8).

Although, a decreasing frequency of DKA was observed with increasing age in both genders, there was an indication of the lowest frequency of DKA to be found around 5-6 years (Figure 13).

Table 8 - Clinical and biochemical characteristics of children with newly diagnosed type 1 diabetes according to age groups

	0-4 years	**5-9 years**	**10-14 years**	**p**
n (%)	351 (22.5)	553 (35.5)	654 (42)	<0.001
Male (%)[§]	176 (50)	283 (51)	369 (56)	NS[1]
Duration of symptoms (days)[*]	9 (7-19)	14 (7-21)	21 (7-28)	<0.001[1]
				<0.001[2]
				0.02[3]
BMI-SDS	-1.04±1.3	-0.54±1.1	-0.51±1.0	<0.001[1]
				<0.001[2]
				NS[3]
Blood glucose (mmol/l)[**]	29.4±13.1	26.0±11.0	25.4±11.6	<0.001[1]
				<0.001[2]
				NS[3]
HbA1c (%)[**]	10.9±2.2	12.0±2.6	12.3±2.6	<0.001[1]
				<0.001[2]
				NS[3]
pH[**]	7.26±0.16	7.31±0.13	7.31±0.13	<0.001[1]
				<0.001[2]
				NS[3]
DKA, pH<7.30 (%)[§]	46.4	33.2	30.7	<0.001[1]
				<0.001[2]
				NS[3]
Severe DKA, pH<7.10 (%)[§]	17.2	9.8	7.9	<0.001[1]
				<0.001[2]
				NS[3]

Data are mean±SD or median (interquartile range) or frequency

[*]Mann-Whitney, [**] T-test, [§] Crosstabulations

[1]0-4yr vs 5-9 yr; [2]0-4yr vs 10-14 yr; [3]5-9yr vs 10-14 yr

Figure 13 – DKA (pH<7.3) frequency in children with type 1 diabetes aged 0-14 years at diabetes onset in Hungary (2002-2009).

CLINICAL CHARACTERISTICS IN CHILDREN AGED <2 YEARS

From 2002 to 2009, a total of 94 (6.0%) patients were diagnosed before the age of 2 years and 257 (16.5%) patients were diagnosed between 2.0 and 4.99 years of age. Comparison of children aged <2 years at diagnosis with children aged 2.0–4.9 years is displayed in Table 9. There were no significant differences in the duration of symptoms and the presenting symptoms before diagnosis. Furthermore, there were no differences in the frequency of a preceding infection (37% children <2 yr vs 29.5% in 2.0-4.9 yr; NS) and a family history of type 1 diabetes between the age-groups.

Table 9 - Clinical and biochemical characteristics of children with newly diagnosed type 1 diabetes according to age groups

	<2 years	**2.0-4.9 years**	**p**
Male (%)	49 (52)	127 (49)	NS[1]
BMI-SDS	-1.14±1.1	-1.0±1.4	NS[2]
Blood glucose (mmol/l)	32.1±16.2	28.3±11.7	0.04[2]
HbA1c	10.3±2.1	11.2±2.2	0.006[2]
pH	7.22±0.17	7.28±0.16.	0.007[2]
DKA, pH<7.30	59.3	41.2	0.003[1]
Severe DKA, pH<7.10	25.3	14.0	0.01[1]

Data are mean±SD or frequency

[1] Crosstabulations, [2] T-test

The proportion of children with impaired consciousness tended to be higher in children aged less than 2 years (25% in children <2 yr vs 13.1% in 2.0-4.9 yr; χ^2=8.53, p=0.01). Children aged <2 years had a significantly higher rate of DKA at presentation.

RISK FACTORS OF DIABETIC KETOACIDOSIS

The results of the univariate analysis and multiple logistic regression models used to examine the risk factors for DKA at diagnosis are shown in Table 9. Using logistic regression analysis, we found that children <2 years of age had two times the risk of presenting in diabetic ketoacidosis as children 10-14 years of age (Table 10). Children with lower BMI-SDS are more likely to present in diabetic ketoacidosis. Higher blood glucose and a history of infection were associated with an increased risk of diabetic ketoacidosis. Our study failed to show a significant association with a family history of type 1 diabetes.

Table 10 - Results of univariate and multivariate logistic regression analyses related to factors associated with diabetic ketoacidosis (pH<7.30) The analysis was performed with a binary logistic regression model using the reference levels as age group: 10–14 yr, infection status: no and family history: no.

Variable	**Univariate analysis**	**Logistic regression analysis**	
		Adjusted Odds Ratio (95% CI)	p value
Age groups	0.02[1]		
<2 years		2.24 (1.3-3.7)	0.002
2-4.9 years		1.2 (0.8-1.7)	0.3
5-9.9 years		1.0 (0.8-1.4)	0.7
Infection	<0.001[1]	1.7 (0.9-2.1)	0.001
Blood glucose [mmol/l]	<0.001[2]	1.04 (1.03-1.05)	<0.001
Duration of symptoms [weeks]	0.07[3]	0.95 (1.2-2.4)	0.06
BMI-SDS	<0.001[2]	0.8 (0.7-0.9)	<0.001
Positive family history	0.012[1]	0.7 (0.5-1.1)	0.1
HbA1c [%]	<0.001[2]		
Ketonuria	<0.001[1]		
Dehydration	<0.001[1]		

[1] Crosstabulations;[2] T-test; [3] Mann-Whitney U test

Discussion

Our study showed that 30% of Hungarian children had DKA (pH<7.3) at diagnosis of type 1 diabetes and 9.6% presented with severe DKA (pH<7.1) during the study period of 8 years, with no significant time trend over the years.

This observed prevalence is somewhat higher than published in Sweden (116) and Finland (117) and comparable with recent and earlier results in other European countries (118-122) although the wide range of definition for diabetic ketoacidosis used in different studies makes the comparison difficult.

The clinical pattern has not changed in the past few years; polydipsia and polyuria, the classic symptoms of diabetes as well as fatigue and weight loss were the most commonly reported symptoms. Although one-third of the children was reported to have duration of symptoms before diagnosis ≤ 7 days, the higher mean HbA1c level suggested that symptoms might have been present for a longer period without being recognized. Some studies found clear evidence that at least some children with diabetic ketoacidosis experienced diagnostic or treatment delays as up to a third of the children had at least one medical consultation in the week before diagnosis, and misdiagnosis was associated with a threefold increase in diabetic ketoacidosis. We failed to observe higher frequency/risk of DKA at onset in older children as recently reported by studies in Austria (120), Finland (117) and Germany (121). In agreement with other studies (120), younger age was associated with an increased risk of diabetic ketoacidosis at diagnosis. This increased risk was most noticeable in children less than 2 years old and disappeared by the age of 5 years. The reasons for this are probably multifactorial. DKA can easily be overlooked in younger children, since the classic symptoms of diabetes in this age group may be subtle and decompensation usually develops quicker as the mechanisms of metabolic compensation are less developed (108). On the other hand, β cell destruction may be more aggressive in young children: serum levels of proinsulin C peptide are lower in

children under 2 years old at time of diagnosis and they continue to lose their endogenous insulin secretory capacity faster than older children after diagnosis (122).

Multiple factors affect the risk of developing diabetic ketoacidosis at the onset of type 1 diabetes in children. In our study, younger age at diagnosis, history of preceding infection, lower BMI-SDS and higher blood glucose were the factors associated with increased risk of presenting with diabetic ketoacidosis at time of diagnosis.

It is unclear why some children present in diabetic ketoacidosis whereas others do not and whether the development of diabetic ketoacidosis is a consequence of delayed diagnosis or whether it reflects a particularly aggressive form of diabetes. Understanding which factors are associated with diabetic ketoacidosis at diagnosis and the relative importance of delayed diagnosis and treatment is, therefore, important.

V. THESIS

I. *INCIDENCE OF TYPE 1 DIABETES IN CHILDREN (0-14 YEARS) IN HUNGARY*

Using the large database of a prospective registry, including 3432 incident cases, covering about 75% of the total 0-14 year-old population in Hungary we were able to study incidence trends with precise statistical methods over two decades. Crucial for time-trend analysis is a sustained high level of ascertainment over time. Ascertainment procedures show a consistently high ascertainment rate above 95%. The variability over time is thus assessed to be a maximum of 4%, which should not affect the time trend analysis in this age group.

1. Incidence of type 1 diabetes in children aged 0-14 years in Hungary

1.1 With the overall incidence rate of 12.5 per 100000 per year, Hungary belongs to the medium incidence geographical regions. During 1989-2009 the background population of children aged below 15 years has dropped by a third, and at the same time the number of newly diagnosed children increased considerably by around 40 new cases per year in boys and 20 new cases per year in girls, resulting a 2.4 fold increase in incidence of type 1 diabetes in Hungarian children. The most important finding of the present study was that the annual increase (3.6%) of type 1 diabetes during 1979-1998 has continued further at an even higher rate (5.3%) in the last 11 years with no evidence of levelling off. The increase follows a linear trend with random fluctuations

1.2 Extrapolation of incidence rates would suggest that by 2030 the predicted number of new cases is 2460 but this change is not shared evenly between the age groups with incidence of type 1 diabetes in the youngest age group expected to increase by 3.5 times in both sexes compared with a factor of 2.5 in boys and 1.5 in girls in the oldest age group. Our model suggests that in 2030 the percentage distribution of new cases across the three age-groups will

be more uniform at 31% (0–4 years), 38% (5–9 years), and 31% (10–14 years), with the excess of new cases in the 5–9 year age range being most apparent in boys.

2. Gender, age-group specific incidence

2.1. Most populations worldwide have shown that males and females have a similar risk for developing the disease, concurring with our results. Although a slight female excess has previously been noted (1989-1998), in more recent years a male excess (but not significant) in the age standardised rate has become evident. The increases in incidence seen in the present study were evident in both genders, although with a difference between the sexes primarily explained by the lower rate of increase in the 10-14 yr old girls compared to boys. Boys showed faster rates of increase in incidence in all three age groups than did girls, but only in the oldest age-group was this difference significant.

2.2. From the beginning of the study period our results demonstrated a greater relative increase in incidence in the youngest children (6.2%), particularly in boys, compared to the increase in 5-9 years old (4.9%) and 10-14 years old children (3.3%). In spite of the rapid increase of the 0-4 year-old age group, the incidence rates (total and sex-specific) remain lowest in the youngest children and highest in 10-14 year-old children. The difference in incidence among the age groups, however, seems to be diminishing; at the end of the study period the incidence in the 5 to 9 year age group has now reached that of the 10 to 14 year age group, particularly in females.

3. Seasonality

3.1 Our study confirms the well-known seasonality of type 1 diabetes at diagnosis with a peak during winter (January) and a trough during summer (June). Seasonal variation was most apparent in 10-14 year-old children and absent in youngest children, possibly due to a lower number of cases in this age group.

4. Regional differences in incidence

4.1 Disease maps demonstrated marked geographical variation in incidence within Hungary. There were indications that counties in the Western part of Hungary tend to have higher incidence rates, while counties in the Eastern part had lower rates than the overall average, however the rate of increase failed to show any significant difference.

4.2 Our observation could not confirm any association between population density and the incidence of type 1 diabetes.

Continued registration of incidence is of paramount importance to recognize long term tendencies, which may provide clues to the environmental exposures behind these trends. Furthermore, appropriate planning of services and resources are necessary to meet the need for the increased number of children diagnosed with diabetes. The rising incidence has far reaching effects beyond the diabetes teams: in schools, the community and other components of the healthcare system that these children use currently and in the future.

II. CLINICAL CHARACTERISTICS OF TYPE 1 DIABETES AT TIME OF DIAGNOSIS IN HUNGARIAN CHILDREN

1. Clinical and biochemical characteristics at time of diagnosis during 2002-2009

1.1 The duration of clinical symptoms in type 1 diabetes is short, about 14 days and one third of the children were reported to have symptoms for less than 7 days. Polyuria and polydipsia remain the most common symptoms noted in 89% of the children. The majority of children have no family history of type 1 diabetes. Biochemical characteristics at diagnosis were in agreement with those from other studies: the mean blood glucose level was 26.5±11.9 mmol/l, mean HbA1c was 11.9±2.6 % and mean pH 7.30±0.14.

1.2 The median duration of symptoms before diagnosis was longer in older children. The clinical symptoms at onset did not differ significantly between groups with regard to age and gender. A concomitant infection was more frequently observed in the youngest age group. Youngest children had gross hyperglycaemia with mean blood glucose close to 30 mmol/l, while HbA1c was lowest in children aged 0-4 years, indicating a shorter duration of hyperglycaemia in this age group.

1.3 The clinical picture at diagnosis was comparable between the two youngest age groups and similar to the presenting symptoms in older children. The higher blood glucose and lower HbA1c was even more pronounced in children under 2 years of age.

2. Diabetic ketoacidosis

2.1 Overall 32% of children aged <15 years presented with ketoacidosis at the time of T1DM onset. In Hungary, the frequency of DKA is high and has not changed over the years despite the increase in incidence of T1DM. We have not found any regional differences in the prevalence of DKA among the counties.

2.2 Our study showed an increased frequency and severity of DKA at presentation in the younger age groups and a reduction with increasing age. Nearly half of the young children 0-4 years of age presented in DKA at diagnosis compared to about one third in children aged 10-14 years. We observed a conspicuously severe clinical decompensation at onset of type 1 diabetes in children less than 2 years of age; about 60% of them presented with diabetic ketoacidosis and one fourth presented with impaired consciousness. Patients with ketoacidosis at onset had a significantly lower BMI-SDS, higher mean blood glucose and mean HbA1c level and one-quarter of them had an acute infection at presentation. The occurrence of DKA was less frequent in children with a first degree relative of type 1 diabetes.

2.3 We also sought to investigate factors that could be associated with a higher prevalence of DKA, such as age at onset, duration of symptoms, history of a preceding infection, history of

T1DM among first degree relatives, BMI-SDS and blood glucose at time of diagnosis. As expected, DKA was more prevalent in younger age groups. Our study showed that children <2 years old had two times the risk of presenting in diabetic ketoacidosis as children aged 10-14 years. This increased risk was not present at 5years of age. Furthermore, higher blood glucose, lower BMI-SDS and a history of infection were significantly associated with an increased risk of DKA. Neither a history of T1DM in a first-degree relative nor the duration of symptoms predicted a diagnosis of new-onset diabetes before progression to DKA.

In Hungary despite considerable improvements in diabetes therapy, no significant decline was observed in the prevalence of onset DKA over the past 8 years. This finding suggests that the general public is still not familiar with the signs and symptoms of type 1 diabetes, parents seek medical attention late, and that can lead to more profound metabolic decompensation, especially in younger children. The unacceptably large proportion of diabetic children with a high blood glucose, glycated haemoglobin, dehydration and ketonuria indicate a relatively severe metabolic decompensation at the time of diagnosis and call for further improvement in the diagnostic acumen of the paediatric community, as well. The very young children, in whom clinical presentation is more acute and early diagnosis may be difficult are particularly vulnerable.

From an epidemiological perspective, further improvements should be made in the educational programs to increase the sensitivity of parents, child carers and physicians to the early signs of hyperglycaemia. Only a higher level of public awareness can reduce the frequency and severity of DKA in children as it has already been proven by information campaigns promoted in Italian schools (123, 124). A similar campaign has started in Hungary with an article recently published (13th April 2011) in NŐK LAPJA, the widely read weekly women's magazine.

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2. **Gyürüs E**, Soltesz Gy and The Hungarian Childhood Diabetes Epidemiology Group: Changing pattern in incidence of type 1 diabetes in Hungarian children over 1989-2008. 36th Annual Meeting of the International Society of Pediatric and Adolescent Diabetes (ISPAD), 27-30 October 2010, Buenos Aires, Argentina

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4. **Gyürüs E**, Soltesz Gy and The Hungarian Childhood Diabetes Epidemiology Group: Clinical characteristics at time of diagnosis – prospective analysis of 1444 cases in incidence of type 1 diabetes in Hungarian children (2002-2008). XX. Scientific Meeting of the Hungarian Diabetes Association, 22-25 April 2010, Tihany, Hungary

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ACKNOWLEDGEMENTS

This PhD thesis would not have been possible without the guidance and the help of several individuals who in one way or another contributed and extended their valuable assistance in the preparation and completion of this study.

I would like to express my sincere gratitude to Professor Gyula Soltesz, my mentor, who has introduced me to the field of diabetes as a medical student and later supported me throughout my clinical studies. I am grateful for his detailed and constructive comments, and for his important support throughout this work. This thesis would not have been possible without his guidance, kind support, inspiration and persistent encouragement.

I would like to express my warm and sincere thanks to Professor Anders Green, who has introduced me to the field of epidemiology and statistics. His perpetual energy and enthusiasm in research has always motivated me. I also thank him for his support and the opportunity of spending some time in the Department of Epidemiology and Public Health, Aarhus, Denmark. I am grateful to his wife Bodil, for the friendly atmosphere during my stay in Aarhus and for both of their friendship.

I would like to thank my co-author, Dr Chris Patterson for his guidance in statistical analysis and his invaluable input.

The realisation of my work would not have been possible without the continuous collaboration of the members of the Hungarian Childhood Diabetes Epidemiology Group. I also thank Mrs

Györgyné Molnár Senior Counsellor of The Hungarian Central Statistical Office, who provided me with the important population data throughout the years.

I also wish to thank my colleagues in the Endocrinology and Diabetes Unit for their contribution to my work and the friendly atmosphere.

Most of all, I wish to thank the love and continuous support of my husband and my wonderful children, not only on this thesis, but also throughout the years. I would like to thank my parents and my brother, whose love and guidance are with me in whatever I pursue. I could not have managed without their encouragement.