Islet cell and other organ-specific autoantibodies in all children developing Type 1 (insulin-dependent) diabetes mellitus in Sweden during one year and in matched control children

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Summary. The majority (about 90%) of children developing Type 1 (insulin-dependent) diabetes mellitus do not have a first-degree relative with the disease. Nearly all (389/405, 96%) children (0–14 years) in Sweden, who developed diabetes during one year, were therefore studied to compare islet cell, thyroid peroxidase, thyroglobulin, and gastric H⁺, K⁺-ATPase antibodies with 321 age, sex, and geographically matched, but non-related, control children. Islet cell (cytoplasmic) antibodies were found in 81% (316/389) of the patients and in 3% (9/321) of the control children ($p < 0.001$). The median islet cell antibody levels were 70 (range 3–8200) Juvenile Diabetes Foundation (JDF) Units in the islet cell antibody positive patients, and 27 (range 17–1200) JDF Units in the control children (NS). Autoantibodies against thyroid peroxidase (8%), thyroglobulin (6%), and gastric H⁺, K⁺-ATPase (3%) were all increased in the patients compared with the control children, being 2% ($p < 0.001$), 2% ($p < 0.01$), and 0.3% ($p < 0.01$), respectively. During an observation time of 20–34 months, two of the nine islet cell antibody positive con-

trol children developed Type 1 diabetes, after 8 and 25 months respectively, while the others remained healthy and became islet cell antibody negative. None of the islet cell antibody negative control children developed diabetes during the same time of observation. This first investigation of an unselected population of diabetic children and matched control children shows: that islet cell antibodies are strongly associated with newly diagnosed childhood diabetes, that other autoantibodies are more frequent among diabetic children than control children, and that the frequency of islet cell antibodies in the background population of children is higher than previously documented, and could also be transient, underlining that factors additional to islet cell antibodies are necessary for the later development of Type 1 diabetes.

Key words: Type 1 (insulin-dependent) diabetes mellitus, islet cell antibodies, thyroid peroxidase antibodies, thyroglobulin antibodies, H⁺, K⁺-ATPase antibodies, case-control study.

Type 1 (insulin-dependent) diabetes mellitus is the most frequent chronic disease among children in developed countries [1–3] and is associated with a markedly increased mortality [4, 5]. Insulin treatment remains a substitution therapy, and a cure or prevention for Type 1 diabetes remains to be found, further stressed by the observation that the incidence of Type 1 diabetes in children seems to be increasing in several countries [6–9]. Geographical differences [6], seasonal variation [10, 11], and a low concordance rate in identical twins [12, 13] suggest that virus infections [14–17] or other environmental factors [18–19] may be of importance in the aetiology.

Several autoimmune phenomena related to the endocrine pancreas and of possible pathogenetic importance are detected at the clinical onset of Type 1

diabetes [20–22]. Insulinitis may be detected at, or shortly after, the clinical diagnosis [23–25] and there is a specific loss of islet B cells [26]. Moreover, islet cell (cytoplasmic) antibodies (ICA) [27, 28], islet cell surface antibodies [29], cytotoxic islet cell antibodies [30, 31], autoantibodies against an islet cell protein of M_r 64,000 [32, 33], and insulin autoantibodies [34] are common before, or at, the clinical onset [33, 35, 36]. In addition, Type 1 diabetic patients have an increased risk of developing other organ-specific autoimmune disorders perhaps representing a separate syndrome of polyendocrinopathy [37, 38]. The levels of ICA in newly diagnosed diabetic children [39–45] and the frequency of ICA in control children [39–49] have been variable and contradictory.

In the present study we had the unique opportunity to analyse ICA and other organ-specific autoantibodies in a nearly complete (389/405, 96%), one year population of newly diagnosed Type 1 diabetic children

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Table 1. Autoantibodies in a total annual population of newly diagnosed children with Type 1 (insulin-dependent) diabetes mellitus and matched control children

	Diabetic patients	Control children
Source	All 44 Swedish paediatric departments	<7 Years selected from the corresponding Paediatric department ≥7 Years selected from the Swedish Population Register
Number	389	321
Sex (male/female)	203/186	170/151
Age (years) median (range)	9 (0–14)	10 (0–15)
Delay median (quartile range)	1 (0–5) Days (days of insulin treatment before sampling)	5 (3–8) Weeks (weeks since sampling of the diabetic child)
Islet cell antibodies		
Frequency	81% (316/389) ^b	3% (9/321)
Median (range) (JDF Units) ^a	70 (3–8200)	27 (17–1200)
Thyroid peroxidase antibodies		
Frequency	8% (32/389) ^b	2% (7/321)
Median (range) (U/l) ^a	36 (16–318)	58 (22–318)
Thyroglobulin antibodies		
Frequency	6% (25/389) ^c	2% (5/321)
Median (range) (U/l) ^a	403 (246–11200)	260 (252–4760)
H ⁺ , K ⁺ -ATPase Antibodies		
Frequency	3% (12/389) ^c	0.3% (1/321)
Median (range) (U/l) ^a	7.3 (3.9–18.2)	3.8

^a Only positive values are taken into account; ^b $p < 0.001$ vs control children; ^c $p < 0.01$ vs control children

country wide, together with age, sex, and geographically matched control children. Standardized assays for autoantibodies against islet cell, thyroid peroxidase (thyroid microsomal antibodies), thyroglobulin, and gastric H⁺, K⁺-ATPase (parietal cell antibodies) were used to answer the following questions: What are the frequencies and levels of ICA and other organ-specific autoantibodies? Are there any associations between these antibodies? Are the autoantibodies influenced by age, sex, season, or geographic location at diagnosis of Type 1 diabetes? Are the autoantibodies associated with heredity of Type 1 diabetes or heredity of other diseases? What are the frequencies and levels of islet cell and other autoantibodies in control children matched for age, sex, and geographical location?

Subjects and methods

Patients

Sweden has 8.4 million inhabitants and 1.6 million of them are children 0–14 years of age. In accordance with the Swedish Health Care programme for diabetic children, all children who develop Type 1 diabetes are admitted to one of the 44 Paediatric departments. These departments are all members of the Swedish Childhood Diabetes Register

where all new cases of Type 1 diabetes in children up to 15 years of age are reported [6]. The present study comprised 96% (389/405) of all children 0–14 years of age in Sweden with Type 1 diabetes diagnosed between September 1, 1985–August 31, 1986 (Table 1). The missing samples were mainly due to technical reasons. The patients were 3 months–14 years of age (median 9 years), the sex ratio was 1.1 (203 boys and 186 girls), and the median time from insulin treatment to the serum sampling was 1 day (quartile range 0–5 days) (Table 1). Information of heredity was obtained from questionnaires completed on 85% (332/389) of the Type 1 diabetic children.

Control children

The Swedish Ethical Committee did not allow serum sampling exclusively for research purposes from children younger than 7 years of age, and because of this control children to patients younger than 7 years old ($n = 132$) were selected from children hospitalized for reasons other than diabetes in the same hospital as the corresponding diabetic patient. The hospital control children ($n = 98$) had been admitted for elective surgery (30%), elective investigation and X-ray (usually pyelography) (27%), observation (18%), trauma (3%), and unspecified or missing diagnosis (22%). They were selected as not having Type 1 diabetes or any indication of other endocrine diseases. For patients aged seven years or older ($n = 257$) two control children were selected from the Swedish Population Register, identified by their civic registration numbers, and matched to the Type 1 diabetic children with respect to age (born on the same day), sex, and residence (living in the same county) as described in detail [50]. Among the 514 invited control children, 223 (43%) agreed to participate and donate a serum sample. The total group of control children, therefore, consisted of 321 children (Table 1). The sex ratio was 1.1 (170 boys and 151 girls) and the median age at sampling was 10 years (range 3 months–15 years). The median time between serum sampling from the Type 1 diabetic patient to the control child was 5 weeks (quartile range 3–8 weeks) (Table 1). Information of heredity was available from the completed questionnaires of 98% (219/223) of the control children aged 7 years and older. For the younger control children information of heredity was obtained from 60% (59/98).

The local paediatrician examined the ICA positive control children and obtained additional fasting samples for determination of blood glucose, HbA_{1c}, and serum C-peptide [51]. The whole population of control children was later matched against the up-dated Diabetes Register to search for diabetes development among the control children, giving a follow-up time of 20–34 months.

Informed consent was obtained from all patients, control children, and their parents. The specific purpose of the study was not revealed. The study was approved by the Swedish Ethical Committee, and the Swedish Data Inspection Board.

Collection of sera

Venous blood samples were obtained from all children except two control children in whom capillary blood was taken. The blood samples were centrifuged, serum was transferred to a new tube at the local hospital, and sent unfrozen by express mail to the Department of Medicine, Malmö General Hospital. The mailing time was 1–3 days from all parts of Sweden. On arrival, sera were aliquoted and first stored for up to one week at -20°C and thereafter, at -80°C until analysis 1–6 months later.

Islet cell cytoplasmic antibodies

One unfixed, frozen pancreas from a human cadaver organ donor of blood group 0 was used in our indirect two-colour immunofluorescence assay [52, 53]. Briefly, 1–3 μm sections were allowed to dry on

slides. In a first incubation, 25 µl serum diluted in phosphate buffered saline (PBS) containing a monoclonal proinsulin antibody GS9A8 [54] and 0.5 mg/ml aprotinin (Trasylol, Bayer AG, Leverkusen, FRG) to prevent proteolysis [55] was added to each section. After incubation for 18 h in a dark, moist chamber, the slides were washed, and then incubated with 25 µl of a mixture of two fluorescent second antibodies; one being a Texas red-labelled sheep anti-mouse IgG (N 2031, Amersham, Buckinghamshire, UK) to visualize the mouse monoclonal proinsulin antibody, and the other fluoresceine isothiocyanate (FITC) conjugated rabbit anti-human IgG (Code F 202, Dako, Copenhagen, Denmark) to detect ICA. Coded slides were washed, mounted in Tris HC1 (pH 8.4) containing 30% glycerol and evaluated in a fluorescence microscope with epiillumination (Olympus BH 2) by at least two independent observers. The observers agreed in 93%.

Sera were diluted 1:2, 1:4, 1:8, etc., until end point titre. All sera were tested in at least two separate assays. Sera from ICA positive control children were tested repeatedly after the code was broken. The end point titres are expressed in Juvenile Diabetes Foundation (JDF) Units [56] obtained by the construction of a standard curve as a linear regression line between multiple end point titres of different dilutions of an international reference serum [56]. The two-colour assay allowed us to clearly distinguish between the ICA fluorescence reaction covering B cells and other islet cells. In each assay, one ICA positive internal standard sample in five different dilutions and one negative sample in three dilutions were included as assay quality controls. The interassay variation was 0.9 titration steps estimated from the standard deviation of the positive standard sera.

Thyroid antibodies

Antibodies against thyroid peroxidase (TPO) and thyroglobulin (TG) were determined in enzyme-linked immunosorbent assays (Pharmacia, Uppsala, Sweden). Purified human TPO [57] and purified human TG were used for coating. Sera were diluted 1:1000 in PBS containing 0.5% Tween-20 and incubated in duplicate wells. Bound antibodies were detected with β -galactosidase-conjugated rabbit anti-human IgG and substrate. Two different patient sera with high titres of TPO antibodies and TG antibodies were used as positive controls and for construction of standard curves. The absorbance values obtained with 1:10,000 dilution of the reference sera were defined as 100 U/l. The upper normal limits, determined as the means + 2 SD of the data obtained with the sera from the control children of the study, were 15 U/l for TPO antibodies and 245 U/l for TG antibodies, respectively.

Gastric H^+ , K^+ -ATPase antibodies

The autoantigen which detects parietal cell autoantibodies, H^+ , K^+ -ATPase [58], was isolated from porcine stomach and was used for coating wells in an enzyme-linked immunosorbent assay as recently described [59]. A patient serum with high titre of H^+ , K^+ -ATPase antibodies was used for constructing standard curves. The absorbance value of a 1:100 dilution was defined as 100 U/l. The upper normal limit, mean + 2 SD of values obtained with sera of the control children of this study, was 3.5 U/l.

Statistical analysis

Values are given as median and quartile range, or mean and standard deviation. Chi square and Fischer's exact test were used to compare frequencies of autoantibodies between patients and control children, with different age groups, sex, season at onset, geographical regions, and heredity of autoimmune diseases. The Sperman rank correlation test was applied when levels of autoantibodies were compared with age and when levels of thyroid autoantibodies were compared with

Fig. 1. Distribution of levels (JDF Units) of islet cell antibodies (ICA) in 316 ICA positive patients of a one year population of 389 incident cases of newly diagnosed Type 1 (insulin-dependent) diabetes in Sweden. The ICA levels of nine (9/321, 3%) positive control children are indicated by the dashed columns

Fig. 2. **a** Distribution of age at onset in 389 children (left part) who developed Type 1 (insulin-dependent) diabetes mellitus during one year in Sweden. The 321 matched control children are shown to the right. The number of individuals positive for islet cell antibodies are indicated by the dashed columns. **b** Seasonal distribution shown as month of sampling of 389 children who developed Type 1 (insulin-dependent) diabetes mellitus (left part) during one year, September 1985–August 1986, and of 321 age, sex, and geographically matched control children (right part). Individuals positive for islet cell antibodies (ICA) are indicated by dashed columns

Fig. 3. **a** Relation between the age at onset of Type 1 (insulin-dependent) diabetes mellitus and the individual level of islet cell antibodies (ICA) in 389 children developing Type 1 diabetes during one year in Sweden. **b** Levels of islet cell antibodies (ICA) in relation to age in the 9/321 (3%) control children who were ICA positive. All children were healthy at the time of sampling but those marked by the squares developed Type 1 (insulin-dependent) diabetes mellitus 8 and 25 months later

ICA positivity did not correlate with the presence of TPO, TG, or H^+ , K^+ -ATPase antibodies. A strong positive correlation was found between TPO and TG antibodies ($r_s = 0.87$, $p < 0.001$). H^+ , K^+ -ATPase antibodies occurred independently of the presence of the thyroid antibodies. Only three (0.8%) of the patients were positive for all four autoantibodies and 16 (4%) were positive for three of them. None of the control children were positive for more than two autoantibodies simultaneously.

each other. The Mann-Whitney test was used to evaluate differences between the age distribution in different groups. The logarithm of reciprocal 2 log titres of positive ICA were normally distributed as were the logarithms of TPO, TG, and H^+ , K^+ -ATPase antibodies. An analysis of variance was used to compare levels of antibodies with sex, geographical regions and season at onset. All tests were two-tailed. P -value less than 0.05 was considered as a significant difference.

Results

Frequencies and levels of autoantibodies

The frequency of ICA among the newly diagnosed Type 1 diabetic children was 81% (316/389), and the median level among ICA positive patient samples was 70 (range 3–8200) JDF Units. In the control group 3% (9/321) were ICA positive and the median level among those was 27 (range 17–1200) JDF Units (Fig. 1). The levels of ICA among the patients and control children positive for ICA did not differ significantly.

TPO and TG antibodies were detected in 8% (32/389) and 6% (25/389), respectively, of the Type 1 diabetic patients, demonstrating a significantly higher frequency as compared with the control children where it was 2% (7/321 and 5/321, respectively) for the antibodies ($p < 0.001$ and $p < 0.01$, respectively). In children with thyroid antibodies, the levels of these antibodies were not significantly different between the diabetic patients and control children. Antibodies to the gastric H^+ , K^+ -ATPase occurred significantly more often in the patient group, 3% (12/389) compared with 0.3% (1/321) in the control group ($p < 0.01$) (Table 1).

Effects of age, sex, season and geographic variation

Neither the frequency (Fig. 2a) nor the level of ICA (Fig. 3a) varied with the age of the patient, in fact, the frequency and levels were remarkably stable. However, if only ICA positive patients ($n = 316$) were taken into account the ICA levels tended to increase with increasing age of clinical onset ($r_s = 0.13$, $p < 0.02$). For ICA there was no difference between males and females, which was found for thyroid antibodies since TPO and TG antibodies were more frequent in female patients with a male/female ratio of 9/23 and 4/21, respectively ($p < 0.01$ and $p < 0.001$). The frequency of H^+ , K^+ -ATPase antibodies did not vary with age or sex. No difference in frequency (Fig. 2b) or levels of ICA (not shown) were observed when comparing results from patients diagnosed during winter (January–March), spring (April–June), summer (July–September), or autumn (October–December). When frequencies and levels of ICA, TPO, TG, and H^+ , K^+ -ATPase antibodies were compared in the 24 counties, in the six health care regions, or in three latitude regions, no significant geographical differences were observed. Multivariate analyses did not change the results.

Autoantibodies in relation to heredity for Type 1 diabetes and thyroid disease

Only 8% (28/332) of the newly diagnosed children had a first-degree relative with Type 1 diabetes compared with 1% (4/278) among the control children ($p < 0.001$).

The frequency and level of ICA among the 28 children with familial Type 1 diabetes (22/28, 79%, median 27 JDF Units), did not differ from that of diabetic children without familial diabetes (250/304, 82%, median 27 JDF Units). The frequency of thyroid disease among the first degree relatives to the Type 1 diabetic patients was 5% compared with diseased first degree relatives of the control children which was 3% (15/332 vs 6/223, NS). Among the thyroid autoantibody positive diabetic children only one patient had a first degree family member with thyroid disease.

ICA among the control children

All control children younger than 7 years of age (hospital control children) were ICA negative (Fig. 2a). Among the population based control children who were 7 years or older, there were no children selected by chance who already had Type 1 diabetes. All the nine (9/321) ICA positive control children were healthy at the time of sampling, the age range was 7–13 years (median 11 years) with a sex ratio of 2 (6 boys and 3 girls) (Table 2). Two of these children had autoantibodies against islet cells other than B cells, probably against A cells. The median level of ICA among the positive control children was 27 JDF Units (range 17–1200) which was not significantly different from that of the ICA positive Type 1 diabetic children. The presence of ICA positive control children occurred randomly throughout the study year (Fig. 2b) and without any geographical clustering. Two ICA positive control children had fathers with Type 1 diabetes, two had been adopted with unknown heredity, and the remaining five children had no heredity of Type 1 diabetes (Table 2).

Two of the ICA positive control children, a 7-year-old boy and a 9-year-old girl (Fig. 3b), developed Type 1 diabetes 8 and 25 months respectively, after their first ICA positive sample. In the samples obtained at diagnosis of Type 1 diabetes ICA were still present in both (Table 2, Fig. 4). None of the remaining ICA positive control children have developed diabetes so far, they have all turned ICA negative, and during 20–34 months of observation, fasting blood glucose, HbA_{1c}, and C-peptide values were normal.

All the initially ICA negative control children ($n=312$) were checked against the up-dated diabetes register, and during 20–34 months of observation none of these children had developed diabetes.

Discussion

Factors possibly involved in the aetiology and pathogenesis of Type 1 diabetes are difficult to identify, but will be required in future attempts to screen for children who may later develop diabetes. This investi-

Fig. 4. Levels of islet cell antibodies (ICA) in initial and follow-up samples from the nine control children found to be ICA positive. Three children were ICA negative in the second sample and four in the third sample. Two children indicated by squares developed Type 1 (insulin-dependent) diabetes mellitus during the time of observation

gation of a total population of incident cases of diabetic children was possible to carry out because Sweden has had a register of newly diagnosed Type 1 diabetic children since 1977 [6]. The population register gave us the unique opportunity to select non-related optimally matched control children. Despite the necessity of obtaining hospital control children from children younger than 7 years of age, our overall control group is clearly different from those in previous studies analysing ICA [39–49]. In addition, the new assays for thyroid peroxidase, thyroglobulin, and H⁺, K⁺-ATPase antibodies have recently become available [59] and have not previously been applied to sera from diabetic and control children.

Table 2. Characteristics of control children positive for islet cell antibodies (ICA)

Child	Age (years)	Sex	Heredity of Type 1 diabetes	ICA level (JDF Units) initial and follow-up	Fasting C-peptide (nmol/l) ^a	Time of follow-up (months)
1	13	M	Adopted	27, 43, Neg, neg	0.85, 0.27, 0.64	8, 21, 31
2	10	M	Father	27, 17, Neg	0.83, 0.31	12, 18
3 ^b	9	F	Father, cousin	1200, 760, 70, 70	0.60, 0.13, –	7, 16, 25
4	11	F	No	470, Neg, neg, neg	1.05, 0.32, 0.13	11, 18, 26
5	11	M	No	17, Neg	0.67	13
6 ^b	7	M	No	27, 43	–	8
7	9	F	No	17, Neg, neg	0.18, 1.0	9, 22
8 ^c	11	M	No	17, 6, Neg	0.60, 0.20	12, 16
9 ^c	11	M	Adopted	27, 17, Neg	0.48, 0.28	4, 17

^a The normal range of fasting C-peptide among children is 0.15–0.49 nmol/l [76]; ^b These children developed Type 1 (insulin-dependent) diabetes 8 and 25 months after the initial samples were obtained; ^c Autoantibodies against other islet cells than proinsulin containing, probably glucagon producing A cells

In this first case-control study of a total annual population of newly diagnosed diabetic children on a country-wide basis we establish that 81% of the patients are ICA positive, a frequency which is comparable to that in previous investigations [39–45]. The levels of ICA are difficult to compare since until now a standard has not been available [56, 60]. Interestingly, the ICA negative patient group did not differ with respect to age, sex, geographical location, or frequency of other autoantibodies compared with the ICA positive patients. In a subsample of all patients (40/389) we found antibodies against an M_r 64,000 islet protein in 73% (29/40) of the patients but in none of the control children (0/40) [61]. Based on these results only about 13% would be negative for both ICA and M_r 64,000 antibodies. In contrast to an earlier study [44], but confirming a recent report [45], the frequency of ICA in the children with diabetes in this study did not vary with age, although the levels of ICA among the ICA positive patients tended to increase with age. The occurrence of childhood diabetes is equal in both sexes [50] and we found no difference in ICA frequency or levels between the sexes either. Previous epidemiological studies [6] have shown geographical variations in the incidence of diabetes in Sweden but a corresponding geographical variation in frequency or level of ICA among the diabetic children was not found in our study. In addition, the frequency and level of ICA were not influenced by the season of clinical onset, despite the seasonal variation of diabetes incidence [6, 11]. Altogether, the uniformly distributed frequency and level of ICA in this complete population of newly diagnosed Type 1 diabetic children do not indicate any heterogeneity in the disease as claimed previously [62, 63].

The strong association between childhood Type 1 diabetes and HLA-DR 3 and/or 4 [64, 65] and even stronger to certain HLA-DQ alleles [66–68] and the low rate (8%) of familial cases [50] made it important to establish a control population consisting of individuals not genetically related but otherwise carefully matched. The control children who were invited but refused to donate a serum sample were unlikely to influence the results since the purpose of the study was not disclosed. No preceding selection was done in the group of control children but no control child had diabetes at first sampling. It is noteworthy, in this respect, that none of our control children younger than 7 years of age, and selected by a paediatrician, were found to be ICA positive.

The results of the control group suggest that the frequency of ICA in the background population might be as high as 3%. Most other studies have shown lower frequencies of ICA in control children [39–48], however, these children were not selected as matched control subjects to incident diabetic patients. Several investigators indicate that the frequency of ICA in the general population exceeds the prevalence of the disease itself [46–49], and in Finland with the highest known pre-

valence, the ICA frequency was 4% [49]. The 3% carrier frequency of ICA among the control children in this study should therefore be compared with the prevalence of diabetes in this age group in Sweden which is 0.15% [6]. A low detection limit in the ICA assay and the risk of false positive results seems unlikely, since the frequency of ICA in the diabetic patients was not higher than in previous studies, and the positive control sera had levels where most assays certainly would find them positive.

If the ICA test could be used to screen an unselected population of Swedish children with the purpose of finding prediabetic subjects, the present study would allow us to calculate the predictive value of the ICA test. The sensitivity (all diseased individuals who had a positive test) was 81% and the specificity (all non-diabetic individuals who had a negative test) was 98%. Since the prevalence of Type 1 diabetes is previously known to be 0.15%, the predictive value of a positive ICA test (the proportion of ICA positive children who will have, or within the near future will develop Type 1 diabetes) would be only 5%. Accordingly, the prevalence of ICA exceeds the prevalence of diabetes and ICA might, therefore, occur transiently without subsequent development of diabetes. In our study seven of initially nine ICA positive control children turned ICA negative without developing diabetes. Transiently occurring ICA has also previously been reported in children with mumps infection [69], in twins [70], as well as in Type 2 (non-insulin-dependent) patients [71].

Considering the 19% of the diabetic patients negative for ICA at diagnosis, these might have been recruited from subjects with previous transient ICA positivity. Anyhow, with this 3% frequency of ICA among control children it is unlikely that the mere presence of ICA will indicate a later development of Type 1 diabetes. To better define the prognostic value of ICA in childhood further investigations of larger numbers of HLA-DQ-typed [68] control children are in progress. Based on studies of first degree relatives it has been argued that ICA is a marker of a later Type 1 diabetes [35, 36, 72]. In our study, two of nine ICA positive control children developed diabetes 8 and 25 months after they entered as control children. Interestingly enough, one child had a father with Type 1 diabetes while the other had no family member with the disease (Table 2). The concordance of Type 1 diabetes among monozygotic twins is 25–50% [12, 13] and in this and other studies [6, 50] only 8–12% of children with diabetes have a first degree relative with Type 1 diabetes. Since almost all previous studies on ICA in non-diabetic individuals have focused on highly selected family members, we must therefore conclude that little is known about the natural history of Type 1 diabetes in non-familial subjects.

Increased frequency of other organ-specific autoantibodies or diseases such as thyroiditis, pernicious anaemia, and primary adrenal insufficiency have been reported in Type 1 diabetic patients [3, 20–22]. The rea-

son for the increased frequency is not understood but speculated to be due to a generally increased propensity to react strongly against certain antigens, or to a genetically poor ability to acquire tolerance to autoantigens, or perhaps to some common antigen shared in the tissues prone to autoimmune diseases [73–75]. In this study we have used novel and precise ELISA-tests to determine levels of TPO, TG, and gastric H^+ , K^+ -ATPase [59] antibodies. Our control children were used to define reference limits and apply these to the group of children with recent onset diabetes. In accordance with some [40, 73–75] but not all [42] studies we found significantly increased frequencies of these autoantibodies among the diabetic children. The girls were more often positive for TPO and TG antibodies than the boys, otherwise there was no association with age, sex, or geographical location for any of the autoantibodies. However, more important, only autoantibodies against TPO and TG showed a highly significant correlation but none of the other organ-specific autoantibodies were found to be associated with each other. In this study we could, therefore, not distinguish any subgroup of diabetic patients with several autoimmune antibodies. This could indicate, that diabetes in the 0–14 year age range develops independently of other organ-specific autoimmune disorders, also underlined by the observation that TPO and TG autoantibodies but not ICA occurred more frequently in girls. Our findings are, however, consistent with the hypothesis that an increased propensity to develop organ-specific autoantibodies is associated with an increased risk of developing Type 1 diabetes.

In conclusion, in this country-wide case-control study of childhood diabetes a high frequency (81%), and high levels of ICA among children with recent onset of Type 1 diabetes were found. Neither levels nor frequency of ICA were associated with age at onset, sex, geographical location, season of onset, or presence of other organ-specific autoantibodies. The fact that the majority of cases with childhood diabetes occurs in children without first degree relatives with Type 1 diabetes and that the frequency of ICA in the control population was as high as 3% suggests that markers other than familial occurrence of Type 1 diabetes and ICA will be necessary in the prediction of diabetes.

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