Clinical, Autoimmune, and Genetic Characteristics of Very Young Children With Type 1 Diabetes

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OBJECTIVE — To study the characteristics of type 1 diabetes in very young children.

RESEARCH DESIGN AND METHODS — Clinical outcome, islet cell antibodies (ICA), insulin autoantibodies (IAA), antibodies against GAD (GADA), IA-2 antibodies (IA-2A), and HLA-DQB1–defined genetic risk were analyzed in 35 children diagnosed with type 1 diabetes before 2 years of age and compared with those in 146 children who were diagnosed between 2.0 and 4.9 years of age and with those in 620 children diagnosed between 5.0 and 14.9 years of age.

RESULTS — The youngest age-group had severer metabolic decompensation at clinical onset, and their serum C-peptide levels, compared with those of older children, were lower at the time of diagnosis and during the first 2 years after the diagnosis. The levels of ICA and IAA were highest in children <2 years of age, but there were no differences in GADA levels among the three age-groups. The youngest age-group had the lowest IA-2A levels. The HLA DQB1*02/*0302 genotype associated with strong genetic susceptibility was more frequent in children diagnosed <5 years of age, whereas the proportion of children carrying a genotype, which includes protective alleles, was higher among those diagnosed at ≥5 years of age.

CONCLUSIONS — The clinical presentation of type 1 diabetes at a very young age is associated with severe metabolic decompensation, poorly preserved residual β-cell function, strong humoral autoimmunity against islet cells and insulin, and strong HLA-defined disease susceptibility.

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The incidence of type 1 diabetes has been increasing in the last decades. Interestingly, the increase has been reported to be most remarkable in groups of children <2 years of age (1,2). However, the disease is still rarely manifested in children <2 years of age. As far as we know, there are no reports on the clinical, autoimmune, and genetic characteristics of the disease in such young patients.

There are a lot of data suggesting heterogeneity in the etiology and pathogenesis of type 1 diabetes between children and adults. The process of β-cell destruction is faster in affected children than in affected adults (3), and the metabolic deterioration at the diagnosis of diabetes is severer in young children than in older children (4). There are also well-documented differences in autoantibody frequency and levels among patients manifesting type 1 diabetes at various ages (5–9). HLA genes associated with disease susceptibility are more frequent in patients diagnosed during childhood than in those diagnosed during adulthood (10–12).

The objective of the present study was twofold: to evaluate clinical, autoimmune, and genetic characteristics at presentation and during the early course of clinical type 1 diabetes in subjects presenting with the disease before their second birthday; and to compare such characteristics with those of subjects diagnosed later in childhood.

RESEARCH DESIGN AND METHODS

Study population

As part of the Finnish nationwide Childhood Diabetes in Finland (DiMe) study, 801 probands <15 years of age, who were diagnosed as having diabetes during the recruitment period from 1 September 1986 to 30 April 1989, were invited to participate in the study. The study protocol has been described in detail previously (13), and it was approved by the ethical committees of all participating hospitals. The patients and/or their parents gave formal consent. At the time of diagnosis, capillary or venous blood pH levels, blood glycated hemoglobin levels, and serum C-peptide levels were measured. The probands were then followed in their own outpatient clinics (n = 31), and their data were collected at 6-month intervals for 2 years.

Methods

Autoantibody tests were performed in the Research Laboratory, Department of Pediatrics, University of Oulu, Finland. Islet cell antibodies (ICA) were determined by a standard indirect immunofluorescence assay performed on sections of frozen human blood group O pancreas (13). Rab-

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Abbreviations: DKA, diabetic ketoacidosis; GADA, GAD antibodies; IAA, insulin autoantibodies; IA-2A, IA-2 antibodies; ICA, islet cell antibodies; JDF U, Juvenile Diabetes Foundation units; RU, relative units.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Clinical data of the study population at time of diagnosis*

	Group 1 (<2 years)	Group 2 (2.0–4.9 years)	Group 3 (5.0–14.9 years)	*P*
pH <7.30	53.3	14.5	21.8	1 vs. 2 vs. 3: <0.001 1 vs. 2: 0.001 1 vs. 3: 0.003
Impaired consciousness	31.4	11.6	11.1	1 vs. 2 vs. 3: 0.002 1 vs. 2: 0.004 1 vs. 3: <0.001
C-peptide (nmol/l)	0.07 (0.05–0.13)	0.13 (0.09–0.20)	0.17 (0.12–0.26)	1 vs. 2 vs. 3: <0.001 1 vs. 2: 0.001 1 vs. 3: <0.001
Acute infection	54.3	48.6	23.2	1 vs. 2 vs. 3: <0.001 1 vs. 3: <0.001
Blood glucose (mmol/l)	22.9 (19.2–28.0)	18.8 (14.7–25.1)	20.3 (15.1–25.9)	1 vs. 2 vs. 3: 0.034 1 vs. 2: 0.008 1 vs. 3: 0.017
GHb	10.7 ± 6.3	10.2 ± 4.4	13.6 ± 6.1	1 vs. 2 vs. 3: <0.001 1 vs. 3: 0.027

Data are %, medians (interquartile range), or means ± SD.

bit anti–human IgG (Behringwerke, Marburg, Germany) was used to detect ICA. The results were expressed in Juvenile Diabetes Foundation units (JDF U) (15). The detection limit for ICA was 2.5 JDF U. The laboratory has participated in the international workshops on the standardization of the ICA assay with sensitivity, specificity, validity, and consistency of 100, 98, 98, and 98%, respectively, in the pertinent round.

Insulin autoantibodies (IAA) were measured by a modification of the liquid phase radioimmunoassay originally described by Palmer et al. (16). In this assay, the specific insulin binding in 105 nondiabetic children was 23.3 ± 11.2 nU/ml. The cutoff limit for antibody positivity was 57 nU/ml (mean + 3 SD in 105 nondiabetic subjects). The laboratory has participated in the international workshops on the standardization of insulin autoantibody assays and in the proficiency testing program for several years. Subsequently, the laboratory has achieved a sensitivity of 78%, a specificity of 100%, a validity of 92%, and a consistency of 100%.

Antibodies against GAD (GADA) were quantified with a radioligand assay as described by Petersen et al. (17). The results were expressed in relative units (RU), which represent the specific binding as a percentage of that obtained with a positive standard serum. The cutoff limit for antibody positivity was set at the 99th percentile of 372 nondiabetic children and adolescents (i.e., 6.6 RU). The intra-assay coefficient of variation was <5%, and the interassay coefficient of variation was <10%. The disease sensitivity of the present assay was 80%, and the specificity was 94% based on the 101 samples included in the Second International GAD Antibody Workshop (18).

IA-2 antibodies (IA-2A) were analyzed with a radio-binding assay previously described in detail (9). The results were expressed in RU. The limit for positivity (0.43 RU) was set at the 99th percentile for 374 nondiabetic Finnish children and adolescents. The interassay coefficient of variation was 12% at an IA-2A level of 0.63 RU, 10% at a level of 21.3 RU, and 8% at a level of 82.6 RU. The disease sensitivity of the assay was 62%, and the specificity was 97% based on 140 samples included in the 1995 Multiple Autoantibody Workshop (19).

The HLA-DQB1 typing method has been described in detail earlier (20). In brief, 158 bp of the second exon of the DQB1 gene were amplified by the polymerase chain reaction from each DNA sample using a primer pair with a biotinylated 3′-primer. The amplification product was bound to streptavidin-coated microtitration plates and denatured with NaOH. After washing, bound DNA was analyzed using two different hybridization mixtures: one containing a europium-labeled internal reporter probe for DQB1*0602 and *0603 alleles (0602-3) and one containing a terbium-labeled consensus probe. The second hybridization mixture included terbium-, samarium- and europium-labeled probes specific for DQB1*02, *0301, and *0302 alleles, respectively. To measure probe hybridization, microtiter plates were analyzed by time-resolved fluorescence (DELFIA Research Fluorometer; Wallac Oy, Turku, Finland). Different emission wavelengths and decay times were used to distinguish signals of each lanthanide label.

For the analysis, the subjects were divided into four groups according to their DQB1 alleles: the DQB1*02/*0302 genotype with both risk alleles; the DQB1*0302/x and DQB1*02/y genotypes with either of the two risk alleles in single or double dose but without any protective alleles (*0301, *0602, or *0603); and the DQB1z/z genotype, which represents those genotypes that include protective alleles.

Random serum C-peptide concentrations were analyzed with a radioimmunoassay, as described earlier (21), by using antiserum K6 (Novo Research Institute, Bagsvaerd, Denmark). The intra-assay coefficient of variation was 1.8%, and the interassay coefficient of variation was 10%. We have previously shown that there is a strong correlation between random serum C-peptide levels and serum C-peptide concentrations measured 120 min after a standardized meal and a 24-h urinary C-peptide secretion (6).

Standard methods for blood HbA_1 and HbA_{1c} analyses were used in the various hospitals participating in the study. To compare the results, data were expressed as the SD above the mean for nondiabetic subjects. Partial remission was defined as a period

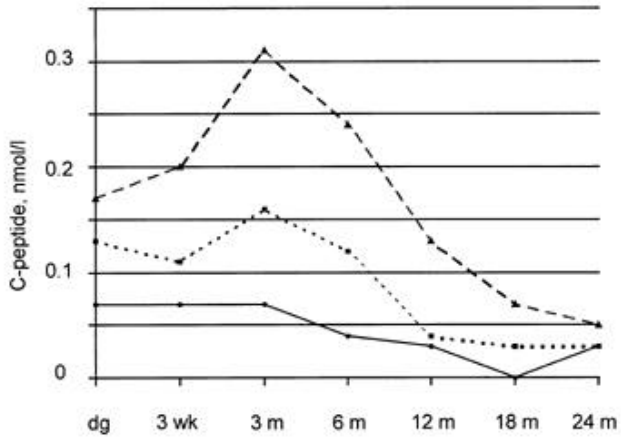

Figure 1—*Median serum C-peptide levels in different age-groups at time of diagnosis and during follow-up. —, <2 years; •••, 2.0–4.9 years; – – –, 5.0–14.9 years;* P < *0.001.*

in which the daily insulin dose was <0.5 IU · kg^{-1} · day^{-1} with standardized glycated hemoglobin (HbA_1 or HbA_{1c}) being <4 SD.

Diabetic ketoacidosis (DKA) was defined as a capillary or venous blood pH of <7.30. The degree of consciousness and dehydration was assessed by the clinician examining the patient at the time of hospital admission. Consciousness was estimated to be either normal or impaired. The recommended amount of insulin and the weight of the proband was registered at 3 weeks after diagnosis. Thereafter, the daily insulin dose was calculated every 6 months.

The data were evaluated statistically using cross-tabulation and χ^2 statistics, one-way analysis of variance, the Kruskal-Wallis test, and the Mann-Whitney *U* test (SPSS for Windows, SPSS, Chicago). The distribution of HLA-DQB1 genotypes was evaluated using the Mantel-Haenszel test for linear association.

RESULTS — A total of 35 (4.4%) patients were diagnosed with type 1 diabetes before the age of 2 years, 146 (18.2%) patients were diagnosed between 2.0 and 4.9 years of age, and 620 (77.4%) patients were diagnosed between 5.0 and 14.9 years of age. The majority of the patients were boys (440 vs. 361; $P < 0.005$). There were no significant differences in the proportion of boys among the three age-groups (<2 years, 51.4%; 2.0–4.9 years, 55.5%; 5.0–14.9 years, 55.0%). Furthermore, there were no differences in the frequency of family history of type 1 diabetes among the age-groups (<2 years, 6.7%; 2.0–4.9 years, 15.7%; 5.0–14.9 years, 13.2%).

The clinical findings at the diagnosis of type 1 diabetes are summarized in Table 1. The children in the youngest age-group presented more often with DKA, impaired consciousness, and signs of an acute infection than children in the other two age-groups. They also had lower serum C-peptide concentrations and higher blood glucose levels than the older children. Severe DKA (pH <7.10) was seen in 10.0% of the patients in the group <2 years of age, in 2.9% of the patients in the group 2.0–4.9 years of age, and in 4.7% of the patients in the group 5.0–14.9 years of age (NS).

Serum C-peptide levels were lowest in the youngest age-group at diagnosis and during follow-up (Fig. 1). At the 6-month follow-up visit, none of the children diagnosed before the age of 2 years but 27.4% of the children aged 2.0–4.9 years and 37.1% of those diagnosed between 5.0 and 14.9 years of age ($P < 0.001$) were in partial remission. At the same time, children in the youngest age-group had higher GHb levels than those in the older age-groups (<2 years, 8.8 SD; 2.0–4.9 years, 4.5 SD; 5.0–14.9 years, 3.8 SD above the mean for nondiabetic subjects; $P < 0.001$).

ICA were measured in 791 patients at the diagnosis of type 1 diabetes, while IAA were quantified in 789 patients, GADA in 769 patients, and IA-2A in 757 patients. Patients in the youngest age-group tested positive more often for IAA ($P < 0.001$) than those in the older age-groups (Fig. 2). There were no differences in ICA, GADA, or IA-2A positivity among the different age-groups.

All of the children in the youngest age-group (32 of 32, 100%) tested positive for at least one of the antibodies studied, while 130 of 131 (99.2%) and 568 of 583 (97.4%) of the children in the two older age-groups, respectively, were positive for at least one antibody (NS). The percentages of children testing positive for all four antibodies were 53.1 in the youngest age-group, 42.0 in the age-group between 2.0 and 4.9 years, and 26.9 in the oldest age-group ($P < 0.001$).

Serum levels of the different autoantibodies are shown in Table 2. Children in the youngest age-group had higher titers of ICA and IAA than those in the older age-groups. The levels of GADA were of the same magnitude in all three age-groups, whereas the IA-2A levels were lowest in children <2 years of age.

The HLA-DQB1 typing was successfully carried out in 647 patients. Children diagnosed before the age of 2 years carried the high-risk genotype DQB1*02/*0302 more often than the older children, whereas the proportion of those carrying protective genotypes (DQB1z/z) increased in the oldest age-group (Table 3).

CONCLUSIONS — In the present series, 35 of 801 (4.4%) patients diagnosed with type 1 diabetes before the age

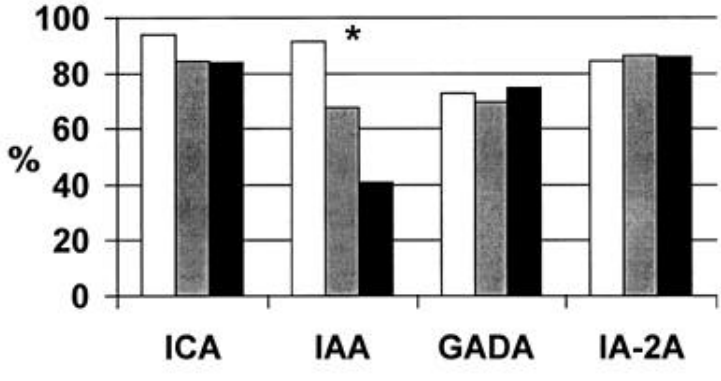

Figure 2—*Autoantibody positivity in three age-groups. □, <2 years; ■, 2.0–4.9 years; ■, 5.0–14.9 years;* *P < *0.001.*

Table 2—*Levels of autoantibodies according to age at diagnosis in subjects testing positive for autoantibodies*

	Group 1 (<2 years)	Group 2 (2.0–4.9 years)	Group 3 (5.0–14.9 years)	*P*
ICA (JDF U)	160 (113–320)	92 (34–169)	47 (18–141)	1 vs. 2 vs. 3: 0.001 1 vs. 2: 0.009 1 vs. 3: <0.001
IAA (nU/ml)	675 (364–1044)	197 (106–494)	125 (79–268)	1 vs. 2 vs. 3: <0.001 1 vs. 2: <0.001 1 vs. 3: <0.001
GADA (RU)	36.4 (10.6–64.0)	35.3 (12.8–87.0)	40.8 (16.3–88.5)	NS
IA-2A (RU)	6.0 (1.5–30.0)	52.1 (4.8–117.9)	71.8 (10.5–134.3)	1 vs. 2 vs. 3: <0.001 1 vs. 2: 0.01 1 vs. 3: <0.001

Data are medians (interquartile range).

of 15 years were <2 years of age at the time of clinical onset. The proportion of boys in the youngest age-group was 51.4%, and the proportion was somewhat higher, although not significantly, in the older age-groups. This is in agreement with a report from the Oxford region (22).

We have previously reported that the overall incidence of DKA in Finland is similar to that in many other countries (23). In the present study, we observed a conspicuously severe clinical decompensation at the diagnosis of type 1 diabetes in children <2 years of age. More than one-half of the youngest children (53.3%) presented with diabetic ketoacidosis, and close to one-third (31.4%) presented with impaired consciousness. However, similar proportions have reported elsewhere. Pinkney et al. (24) found that 19 of 43 (44%) children diagnosed with type 1 diabetes in the Oxford region presented with a blood pH level of 7.35 or lower. In their series, 12 of these children (28%) had severe DKA (pH <7.10) compared with 10% of the children <2 years of age in our survey.

The reasons for the poor clinical condition in very young children manifesting with diabetes are probably multifactorial. Their diabetic symptoms might have been difficult to recognize, especially as the disease rarely manifests at an early age. Acute infections, which in our study were seen in 54.3% of the youngest children at the presentation of type 1 diabetes, may also mask the diabetic symptoms and signs, thus delaying the clinical diagnosis of diabetes. On the other hand, the process of β-cell destruction leading to clinical type 1 diabetes may be faster early in childhood than later in childhood. This suggestion is supported by the finding that serum C-peptide levels at diagnosis were lowest in children diagnosed before 2 years of age, whereas glycated hemoglobin was highest in children diagnosed ≥5 years of age, indicating a longer duration of hyperglycemia in the latter age-group.

The age at onset of childhood diabetes has previously been reported to be positively correlated to the postprandial plasma C-peptide (25). It is also known that healthy young children have lower serum C-peptide levels than older ones (26). We found that children <2 years of age lose their endogenous insulin secretory capacity faster than older children, even after the clinical manifestation of type 1 diabetes. During the follow-up, the younger children had lower random serum C-peptide levels than the older children, and, actually, their endogenous insulin secretion did not recover at all after the diagnosis, while a substantial increase could be seen in the serum C-peptide concentrations over the first 3 months of overt disease in the oldest age-group. Thus, our finding of lower serum C-peptide levels in very young children versus those in older children probably cannot solely be explained by a low β-cell mass in very young children (27). In addition, none of the youngest children was in partial remission at 6 months after their diagnosis, while this phenomenon was observed in 27.4% of the children diagnosed with type 1 diabetes between 2 and 4.9 years and in 37.1% of those in the oldest age-group. These findings suggest that the loss of β-cell mass and function progressed faster in the very young children than in older children, even after clinical disease manifestation.

Metabolic control 6 months after diagnosis, as defined by serum glycated hemoglobin, was poorest in the youngest children. This is partly explained by their decreased endogenous insulin secretion. However, the differences in GHb levels among the age-groups remained even when only patients without partial remission were included (data not shown). Accordingly, we speculate that other factors, such as problems in cooperation, variable food intake and physical activity, and parental fears of hypoglycemia may have contributed to poor metabolic control in very young diabetic children.

It has previously been shown that young children are more often positive for some but not all diabetes-related autoantibodies at the time of diagnosis (5–9) and that the age at which antibodies are detected in the preclinical period is related to the pace of clinical diabetes progression (28). Our data showed that the very young children had the highest titers of IAA and ICA levels. In contrast, the titers of GADA levels were of the same magnitude, and the titers of IA-2 levels in very young children were lower than those of older children. On the

Table 3—*Distribution of the four HLA-DQB1 genotypes*

	<2 Years of age	2.0–4.9 Years of age	5.0–14.9 Years of age	*P*
*02/*0302	9 (31.0)	42 (35.6)	109 (21.8)	0.012
*0302/x	11 (37.9)	39 (33.1)	193 (38.7)	NS
*02/y	4 (13.8)	13 (11.0)	65 (13.0)	NS
z/z	5 (17.2)	24 (20.3)	133 (26.5)	0.039

Data are *n* (%).

other hand, children <2 years of age tested positive for IAA more often than older children, while there were no significant differences in the proportion of children testing positive for ICA, GADA, and IA-2A among the three age-groups. We have previously reported that children diagnosed at ≥10 years of age test positive for GADA more often than children <10 years of age (8). This difference cannot be seen in the present study because all children ≥5 years of age were grouped together. Contrary to GADA, we have not been able to show any significant association between age at diagnosis and the frequency of IA-2A in Finnish children and adolescents diagnosed before the age of 15 years (9).

More than half (53.1%) of the youngest children tested positive for all four antibodies, suggesting a strong autoimmune attack against β-cells in very young children with newly-diagnosed type 1 diabetes.

Patients diagnosed with type 1 diabetes in childhood are even more likely than adults to carry HLA genes associated with disease susceptibility (3,11,12). In our study, close to one-third of the children in the two youngest age-groups (31.0 and 35.6%, respectively) carried the high-risk genotype DQB1*02/*0302 compared with only 21.8% of the children in the oldest age-group. Correspondingly, approximately one-sixth (17.2%) of the children in the youngest age-group carried protective genotypes, which were seen in 20.3% of the children aged 2.0–4.9 years and 26.5% of the children in the oldest age-group.

It has recently been suggested that the age at diagnosis of type 1 diabetes would be genetically determined (29,30). If so, one could expect a strong genetic disease susceptibility in patients diagnosed very early in life. In the present study, although the DQB1*02/*0302 genotype associated with the highest risk was more common in children ≤5 years of age, close to one-fifth (17.2%) of the youngest children still carried protective genotypes. These findings support the idea that factors other than HLA-conferred genetic risk are important in determining the timing of the clinical manifestation of type 1 diabetes.

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APPENDIX

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