Risk of Type 1 Diabetes Development in Children With Incidental Hyperglycemia

A multicenter Italian study

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OBJECTIVE — The aim of our study was to determine whether children with incidental hyperglycemia are at an increased risk of developing type 1 diabetes.

RESEARCH DESIGN AND METHODS — A total of 748 subjects, 1–18 years of age (9.04 ± 3.62, mean ± SD), without family history of type 1 diabetes, without obesity, and not receiving drugs were studied and found to have incidental elevated glycemia defined as fasting plasma glucose >5.6 mmol/l confirmed on two occasions. Subjects were tested for immunological, metabolic, and immunogenetic markers.

RESULTS — Islet cell antibodies >5 Juvenile Diabetes Foundation units were found in 10% of subjects, elevated insulin autoantibody levels in 4.6%, GAD antibody in 4.9%, and anti-tyrosine phosphatase-like protein autoantibodies in 3.9%. First-phase insulin response (FPIR) was <1st centile in 25.6% of subjects. The HLA-DR3/DR3 and HLA-DR4/other alleles were more frequent in hyperglycemic children than in normal control subjects ($P = 0.012$ and $P = 0.005$, respectively), and the HLA-DR other/other allele was less frequent than in normal control subjects ($P = 0.000027$). After a median follow-up of 42 months (range 1 month to 7 years), 16 (2.1%) subjects (11 males and 5 females), 4.1–13.9 years of age, became insulin dependent. All had one or more islet autoantibodies, and the majority had impaired insulin response and genetic susceptibility to type 1 diabetes. Diabetes symptoms were recorded in 11 patients and ketonuria only in 4 patients. The cumulative risk of type 1 diabetes was similar in males and females, and it was also similar in subjects under or over 10 years, whereas the cumulative risk of type 1 diabetes was increased in subjects with one or more autoantibodies and in those with FPIR <1st centile.

CONCLUSIONS — Children with incidental hyperglycemia have a higher-than-normal frequency of immunological, metabolic, or genetic markers for type 1 diabetes and have an increased risk of developing type 1 diabetes.

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Type 1 diabetes results from cell-mediated autoimmune destruction of β-cells in the pancreas (1). Multiple genes contribute to the predisposition of the disease, and major histocompatibility complex (MHC) is the most important one (1). Moreover, evidence suggests that environmental factors, which are still poorly defined, may play a role in either precipitating the disease and/or dominantly shaping its course (1). Various autoantibodies against β-cell components are present in the serum of newly diagnosed patients with type 1 diabetes (1). More importantly, these circulating autoantibodies may be present for months to years preceding the onset of clinical diabetes (2). The rate of β-cell destruction is quite variable, as it is rapid in some individuals and slow in others (3). Some patients, particularly children and adolescents, may present with ketoacidosis at the first manifestation of the disease (4). Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stress (5).

Type 1 diabetes is one of the most serious and prevalent chronic diseases of children, with incidence rates increasing in many parts of the world (6). Therefore, the screening of individuals at risk for type 1 diabetes and the identification of a means to prevent type 1 diabetes are undeniably significant to public health (7).

With the availability of sensitive assays for measuring several autoantibodies, such as islet cell antibodies (ICAs), insulin autoantibodies (IAAs), anti-GAD65 antibodies (GADAs), and anti-tyrosine phosphatase-like protein autoantibodies (IA-2As), it is now possible to predict the disease in first-degree relatives of type 1 diabetic probands (2).

Among children who are not first-degree relatives of patients with type 1 diabetes, stress or incidental hyperglycemia, when associated with immunological markers and diminished early-phase insulin response, may identify a group at high risk of type 1 diabetes (5,8–12). However, these studies are biased because they are carried out only for a selected group of children.

The aim of our study was to determine whether children with incidental hyperglycemia, identified prospectively among the subjects enrolled in the Italian Registry, are at an increased risk of developing type 1 diabetes, as determined by the presence of prediabetes markers or

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Abbreviations: FPIR, first-phase insulin response; GADA, anti-GAD65 antibody; IA-2A, anti-tyrosine phosphatase-like protein autoantibody; IAA, insulin autoantibody; ICA, islet cell antibody; IVGTT, intravenous glucose tolerance test; JDF, Juvenile Diabetes Foundation; MHC, major histocompatibility complex; MODY, maturity-onset diabetes of the young; OGTT, oral glucose tolerance test; WHO, World Health Organization.

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

clinical disease within 1 month to 7 years of follow-up.

RESEARCH DESIGN AND METHODS

— In November 1991, the Italian Society of Pediatric Endocrinology and Diabetology (Parma, Italy) promoted the creation of the Screening and Treatment of Prediabetes Study Group. To date, three projects have been carried out: the first project set guidelines for the screening of prediabetes in children (13, 14); the second established normal values for first-phase insulin response (FPIR) in normal subjects (15); and the third kept a registry of Italian subjects at risk of type 1 diabetes (16).

According to the guidelines, there were four categories of individuals to be screened: *1*) first-degree relatives of type 1 diabetic probands (siblings or offspring); *2*) subjects with fasting plasma glucose >5.6 mmol/l (confirmed on two occasions), with no history of type 1 diabetes, without obesity, and not receiving drugs that cause hyperglycemia; *3*) subjects with autoimmune endocrinopathies; and *4*) subjects with congenital rubella.

A total of 748 of 2,467 subjects fitting into the four previously mentioned categories were found to have incidental elevated glycemia without any family history of type 1 diabetes (17). These subjects, 1–18 years of age (9.04 ± 3.62, mean ± SD) (480 males and 268 females) have been screened by 31 Italian pediatric centers. In the majority of cases, elevated glycemia was observed during a routine blood test. In a few subjects, high blood glucose levels were found during acute illnesses but needed to be confirmed after recovery, before enrollment into the study. Obese subjects (BMI >30 kg/m^2) were excluded.

Screening procedures, according to the flow chart established by the Italian Diabetes Study Group, included immunological, metabolic, and immunogenetic investigations. Moreover, to exclude the diagnosis of type 1 diabetes, an oral glucose tolerance test (OGTT) was recommended, according to World Health Organization (WHO) criteria (18). The following immunological markers were determined: ICAs, IAAs, and GADAs. Since 1996, IA-2As were also determined. FPIR to intravenous glucose tolerance testing (IVGTT) was used as a metabolic marker. The results were compared with centile values according to pubertal stage in 138 normal subjects (47 females and 91 males, 3–20 years of age), established by the Italian Prediabetes Study Group (15). Regarding immunogenetic markers, at least in the subjects with immunological and/or metabolic markers, serological HLA typing for class I and class II was done, and molecular analysis of HLA-DQA1 and -DQB1 genes was performed to identify the HLA-DQ genotypes that confer susceptibility to type 1 diabetes and to calculate the number of DQα and DQβ heterodimers.

Regarding the criteria for the follow-up, OGTT was performed almost every 6 or 12 months, according to the previous results (i.e., every 6 months in cases of impaired glucose tolerance and every 12 months in cases of normal glucose tolerance). A normal diet was allowed. Immunological markers and IVGTT were repeated in intervals of 6–12 months in the cases of abnormal results in initial studies. Insulin therapy was instituted according to WHO criteria.

Laboratory assays

Blood glucose concentration. Blood glucose concentration was measured in venous blood at the time of the venipuncture for routine evaluation. Each blood glucose value for hyperglycemic patients was confirmed by a second evaluation.

Autoantibody measurements

The presence of ICAs was determined by indirect immunofluorescence on unfixed snap-frozen human pancreas. A sample was considered to be positive if the undiluted serum staining was similar or more intense than the laboratory standard serum sample that had been calibrated to ~5 Juvenile Diabetes Foundation (JDF) units (19).

IAAs were measured by protein A/G microradiobinding assay, as previously described. Results for each assay were expressed as arbitrary units, and the threshold for positivity was 5 U, corresponding to the 99th centile of normal control subjects (20).

GADAs and IA-2As were measured by radiobinding assay, using in vitro transcribed and translated recombinant human protein, as previously described (21). The thresholds for positivity were 3 U (GADA) and 1 U (IA-2A). Using these thresholds, the assays gave a sensitivity, specificity, and reproducibility of 88, 98, and 100% for GADA and of 70, 99, and 100% for IA-2A in the combined autoantibody workshop (22).

IVGTT

A total of 409 subjects underwent IVGTT according to National Diabetes Data Group recommendations for OGTT, i.e., after 3 days of unrestricted diet (at least 150 g carbohydrates), normal physical activity, and absence of acute illness and administration of drugs that cause hyperglycemia (18). After an overnight fast for 12 ± 1 h, the test started between 7:30 A.M. and 10:00 A.M. The procedure followed the recommendations of the Italian Study Group (15). The sum of 1- and 3-min serum insulin values (FPIR) was calculated and expressed as microunits per milliliter. Blood samples for insulin assay were centrifuged at 4°C, and then plasma was kept at −20°C until analysis. All plasma samples were then sent to the Parma University Laboratory for insulinemia measurement. Insulin levels were measured by radioimmunoassay (Radim; Rome). The intra- and interassay coefficients of variation were 8.2 and 6.9% for low values and 7.7 and 6.0% for high values, respectively. The results were compared with the percentile established in healthy subjects (15).

OGTT

In all subjects, an OGTT was performed using a dose of 1.75 g glucose/kg body wt. Samples for blood glucose determination were obtained at baseline and 2 h after glucose administration, according to the criteria of the National Diabetes Data Group (18). Subjects underwent OGTT, as recommended by the National Diabetes Data Group, i.e., after 3 days of unrestricted diet (at least 150 g carbohydrates), normal physical activity, and absence of acute illness and administration of drugs that cause hyperglycemia (18). After an overnight fast of 12 ± 1 h, the test began between 7:30 A.M. and 10:00 A.M. The results were considered according to WHO criteria (18).

HLA typing and HLA-DQα/β gene polymorphism analysis

HLA typing was performed according to the microlymphocytotoxicity technique (23). The analysis of HLA-DQA1 and -DQB1 polymorphisms at genomic levels was performed by the polymerase chain reaction/sequence-specific primers technique (24). The results were compared with those of 40,071 Italian bone marrow

Table 1—*Characteristics of subjects with hyperglycemia who developed type 1 diabetes at enrollment*

Case no.	Sex	Age (years)	ICA (JDF U)	IAA	GADA	IA-2A	FPIR (centile)	2-h Glucose during OGTT (mmol/l)	HLA-DR	DQα-β heterodimers
1	M	4.1	<5	Neg	Pos	Pos	<1st	6.2	—	4
2	M	4.2	>5	—	—	—	<1st	9.2	—	4
3	F	4.9	>5	Pos	Pos	Pos	<1st	8.5	4, 5	1
4	M	6.7	15	Pos	Pos	Pos	<1st	10.1	3, 4	4
5	M	6.8	10	Neg	Neg	Pos	<1st	5.6	7, x	0
6	F	6.8	>5	Neg	—	—	<1st	7.9	3, x	—
7	M	7.9	80	Neg	Pos	—	<1st	5.8	3, 11	—
8	M	8.9	<5	Pos	Pos	Pos	10th	10.5	—	1
9	M	8.9	15	Pos	Pos	Neg	<1st	10.6	1, 4	2
10	M	9.8	20	—	—	—	<1st	9.9	—	4
11	F	10.1	40	Pos	—	—	25th	8.5	—	—
12	M	10.9	640	Pos	Pos	—	<1st	10.5	4, 8	2
13	F	11.9	80	Pos	—	—	<1st	8.7	—	—
14	M	12.4	>5	Neg	Neg	Pos	<1st	5.3	—	4
15	M	13.9	>5	Pos	Pos	Pos	<1st	10.1	4, 4	2
16	F	13.9	80	Pos	Pos	Neg	<1st	10.9	—	—

Neg, negative; Pos, positive.

donors enrolled in the Italian Bone Marrow Donor Registry and representative of the Italian population (25). Mean age of control subjects was 26 years and the male-to-female sex ratio was 0.98.

Statistical analysis

Descriptive statistics were computed for all variables: frequency distribution was reported for categorical variables, and mean and SD or median and quartiles were reported for continuous variables, if skewed. Cumulative event-free probability was computed by Kaplan-Meier estimates for the whole case series and after stratifying by risk factor. Event-free survival curves were drawn. The prognostic value for the occurrence of diabetes during follow-up of a series of covariates was assessed by univariate Cox models. Hazard ratios and 95% CIs were calculated for each tested variable. A χ^2 test was computed to compare HLA distribution between hyperglycemic patients and control subjects. Reported *P* values were not corrected for multiple test bias. Multivariate analysis could not be performed because of the low number of events. $P < 5\%$ was considered statistically significant. Stata 6 (StatCorp, College Station, TX) was used for computation.

RESULTS

Immunological markers

ICA levels >5 JDF U were present in 50 of 498 (10%) tested subjects with hyperglycemia, and IAA levels were elevated in 19 of 408 (4.6%) subjects, GADA in 23 of 465 (4.9%) subjects, and IA-2A in 16 of 410 (3.9%) subjects; 22 of 498 subjects (4.4%) had at least two positive markers (either ICA or IA-2A and GADA or IAA) for type 1 diabetes. Three autoantibodies were measured in 360 subjects, and 5 (1.4%) had three positive markers. Four autoantibodies were measured in 274 subjects, and 3 (1%) had four positive markers. No significant difference in the demographic characteristics between the tested and untested subjects was found.

Metabolic markers

OGTTs were normal in 70.5% of 748 subjects, impaired in 27% of subjects, and abnormal in 2.5% of subjects. Patients with abnormal OGTT were excluded from the follow-up study.

The FPIR was <1st centile, according

Table 2—*Characteristics at diagnosis of type 1 diabetes in the 16 children with hyperglycemia who progressed to clinical disease*

Case no.	Follow-up (months)	Age (years)	Symptoms	Diagnosis: Glucose (mmol/l)	Diagnosis: Ketonuria	HbA_{1c} (SD)	Insulin requirement ($U \cdot kg^{-1} \cdot day^{-1}$)*
1	17	5.6	Yes	13.1	No	>+2	0.5
2	4	4.6	Yes	11.5	No	>+2	1.2
3	1	4.9	Yes	11.7	No	+1 < +2	1.0
4	4	6.9	No	9.7	No	<+1	0.2
5	6	7.2	Yes	14.6	Yes	+1 < +2	0.3
6	14	7.9	No	11.5	No	>+2	1.0
7	3	8.1	Yes	11.8	No	<+1	0.8
8	7	9.4	No	9.2	No	+1 < +2	1.2
9	28	11.2	Yes	14.5	Yes	>+2	1.0
10	9	10.5	Yes	6.5	No	>+2	0.7
11	21	11.9	No	7.7	No	>+2	0.8
12	27	13.0	Yes	28.1	Yes	>+2	1.2
13	1	11.9	Yes	15.0	Yes	+1 < +2	0.8
14	11	13.3	Yes	7.2	No	>+2	0.9
15	13	14.9	Yes	25.0	No	>+2	0.5
16	6	14.4	No	6.1	No	<+1	1.1

*Mean value during the first month after diagNosis.

Table 3—*Univariate Cox models for occurrence of diabetes*

Variable	Hazard ratio	95% CI	*P*
Sex (F vs. M)	1.21	0.42–3.48	0.724
Age (≥10 vs. <10 years)	0.98	0.85–1.12	0.752
ICA (positive)	47.99	13.67–168.51	0.0001
IAA (positive)	46.04	15.40–137.61	0.0001
GADA (positive)	105.21	22.65–488.73	0.0001
IA-2A (positive)	63.01	15.58–254.86	0.0001
ICA or 1A-2A (positive)	164.02	21.66–1242.18	0.0001
Number of antibodies			0.0001
1 vs. 0	38.69	3.51–426.67	0.003
≥2 vs. 0	292.87	37.72–2274.21	0.0001
FPIR (<1st centile)	26.99	3.42–213.01	0.0001

to normal values for pubertal stages, in 105 of 409 (25.6%) subjects. No significant difference in the demographic characteristics between the tested and untested subjects was found.

Immunogenetic markers

Regarding HLA-DR typing, performed in 210 subjects, HLA-DR3/DR4, DR4/DR4, and DR3/DR3 genotypes were detected in 3.5%, HLA-DR3/other in 18.6%, HLA-DR4/other in 22.1%, and HLA-DR other/other in 48.8%. HLA-DR3/DR3 and HLA-DR4/other alleles were more frequent in hyperglycemic children than in control subjects ($P = 0.012$ and $P = 0.005$, respectively), whereas HLA-DR other/other genotypes were less frequent in hyperglycemic children than in control subjects ($P = 0.000027$).

Regarding DQA1 and DQB1 typing, four susceptible DQα-β heterodimers were present in 4.2% of subjects, two heterodimers in 25.5%, one heterodimer in 24.5%, and 0 heterodimers in 45.7%. Two or more heterodimers were more frequent in hyperglycemic children than in control subjects ($P = 0.025$).

Follow-up study

Subjects were followed for 1 month to 7 years (median 42 months) after enrollment into the study. The 3-year cumulative risk of developing type 1 diabetes was 2.5% (95% CI 1.5–4.0) in the whole population and 26.4% (16.7–40.4) in subjects with positive antibodies. This is comparable with 0.9% (0.5–1.5) and 9.7% (5.1–17.9), respectively, in 1,641 first-degree relatives in our family study (17). During the follow-up period, 16 (2.1%) subjects with incidental hyperglycemia became insulin-dependent. The

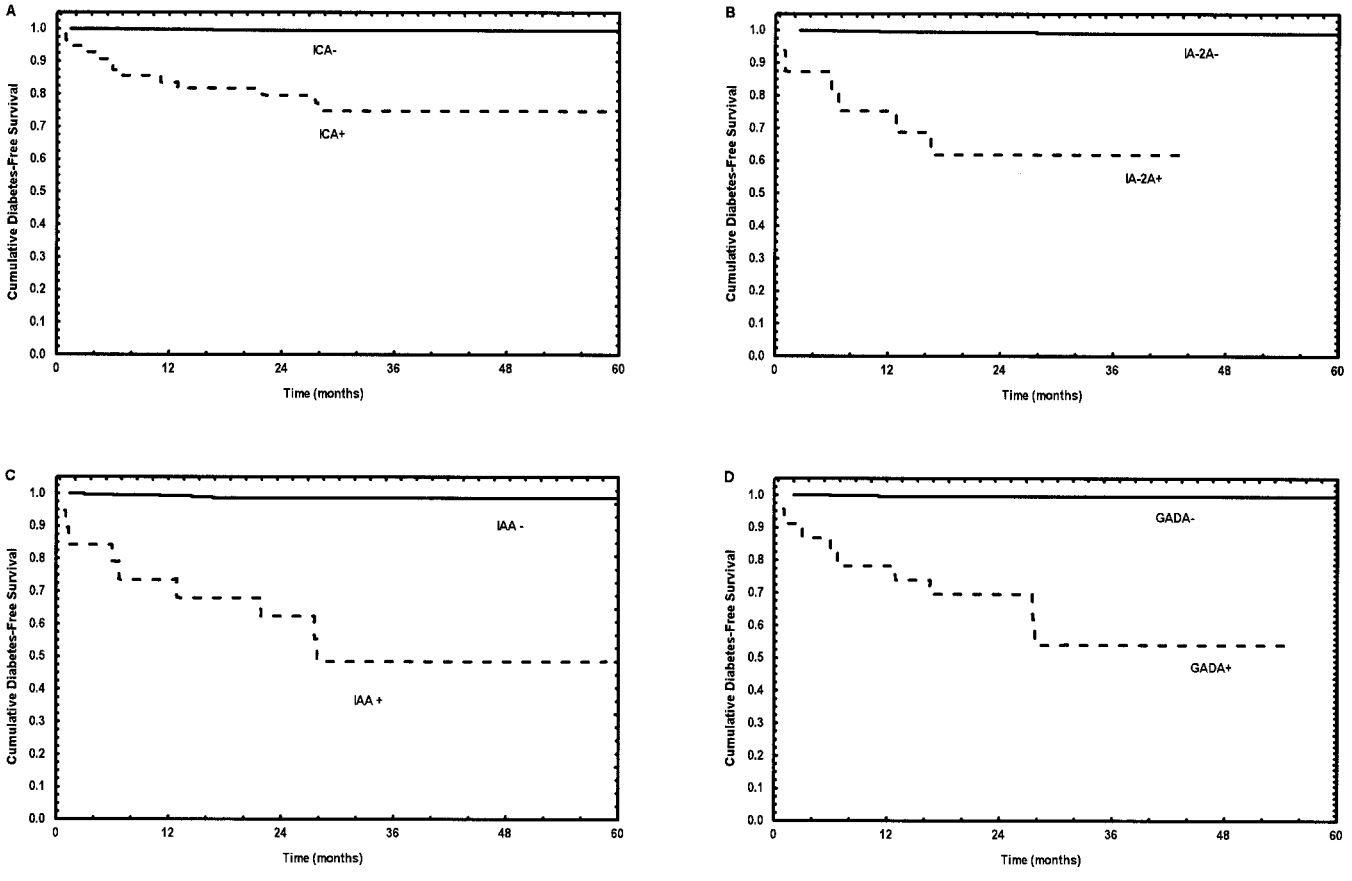
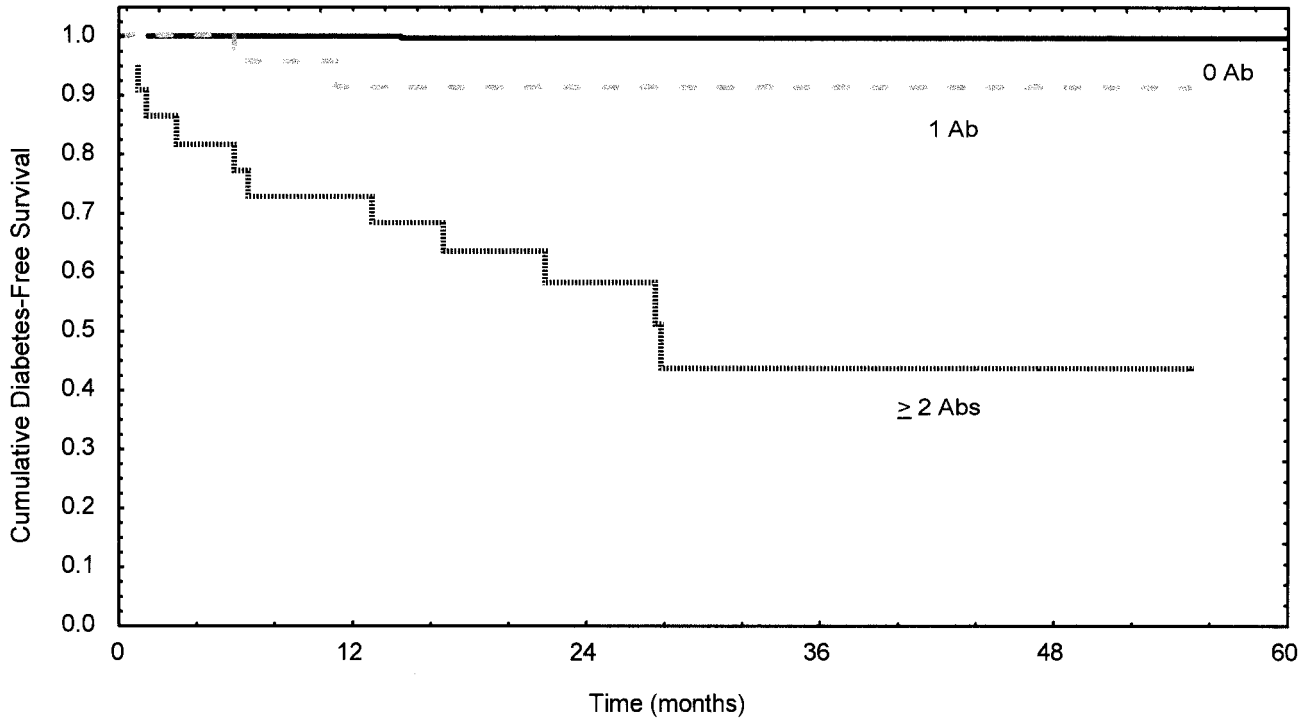


Figure 1—*Cumulative proportion event-free survival in hyperglycemic subjects by ICA positivity* (P = *0.0001*) (A), *IA-2A positivity* (P = *0.0001*) (B), *IAA positivity* (P = *0.0001*) (C), *and GADA positivity* (P = *0.0001*) (D).



0 Ab	452	416	340	218	145	87
1Ab	24	22	17	11	6	4
≥2Abs	22	17	10	7	5	4

Figure 2—*Cumulative proportion event-free survival in hyperglycemic subjects by number of antibodies (Abs); model:* P = *0.0001, 1 vs. 0:* P = *0.003, >2 vs. 0:* P = *0.0001, and ≥2 vs. 1:* P = *0.009. Subjects at risk at yearly intervals are reported under the figure for each of the three groups.*

characteristics of these 16 subjects at enrollment and diabetes diagnosis are reported in Tables 1 and 2. Of the 16 subjects, 11 were males and 5 were females, and the age at first evaluation ranged from 4.1 to 13.9 years. ICAs and/or IAAs, GADAs, and IA-2As were present in all of these subjects, and 13 subjects had two or more autoantibodies. FPIR was <1st centile in 14 subjects. OGTTs were impaired in 13 subjects. Seven of eight subjects with HLA-DR typing had a DR3- or DR4-containing genotype, and 10 of 11 subjects with DQ typing had at least one diabetes-susceptible DQα-β heterodimer. Type 1 diabetes developed at 1 to 28 months (median 10) from initial screening. A total of 11 patients had diabetic symptoms, and 4 had ketonuria. Diagnosis was done either during routine screening or because parents were informed to check urinalysis *1*) randomly and *2*) in case of symptoms compatible with diabetes.

To assess the association of risk factors with recurrence of diabetes during follow-up, Cox regression was applied, and results are summarized in Table 3. The cumulative risk of type 1 diabetes is similar in males and females and similar in subjects under or over 10 years of age, but it is increased in subjects with ICAs, IAAs, GADAs, and IA-2As (Fig. 1). The cumulative risk of type 1 diabetes is increased in subjects with two antibodies (Fig. 2) and in subjects with an FPIR ≤1st centile (Fig. 3).

CONCLUSIONS— At the time of type 1 diabetes diagnosis, >10% (4) of children are in a coma and ~1 of 200 children die in diabetic ketoacidosis (26). Morbidity and mortality would be significantly reduced by early diagnosis and institution of appropriate therapy before severe metabolic decompensation occurs (4).

First-degree relatives of type 1 diabetic patients carry a higher risk of acquiring diabetes than the general population (27); however, most children with type 1 diabetes do not have a family history of diabetes. Incidental hyperglycemia is a relatively common finding in the pediatric population (28). It has been reported that the risk of progression to type 1 diabetes is low when transient hyperglycemia occurs during a serious intercurrent illness (10–11). In contrast, the risk is high in children with transient hyperglycemia without a serious illness. We prospectively studied only children with incidental hyperglycemia without an intercurrent illness and found that these subjects had a risk of developing type 1 diabetes that was at least the same as that of first-degree relatives. These subjects had an increased prevalence of islet autoantibodies, and an elevated risk of type 1 diabetes was confined to those with this serological marker of prediabetes. Risk was also high in subjects with low FPIR. These findings are consistent with a previous study in children with transient hyperglycemia (10).

Therefore, our data suggest that children and adolescents found to have incidental hyperglycemia (or glycosuria) without intercurrent illness should be considered at risk of developing type 1

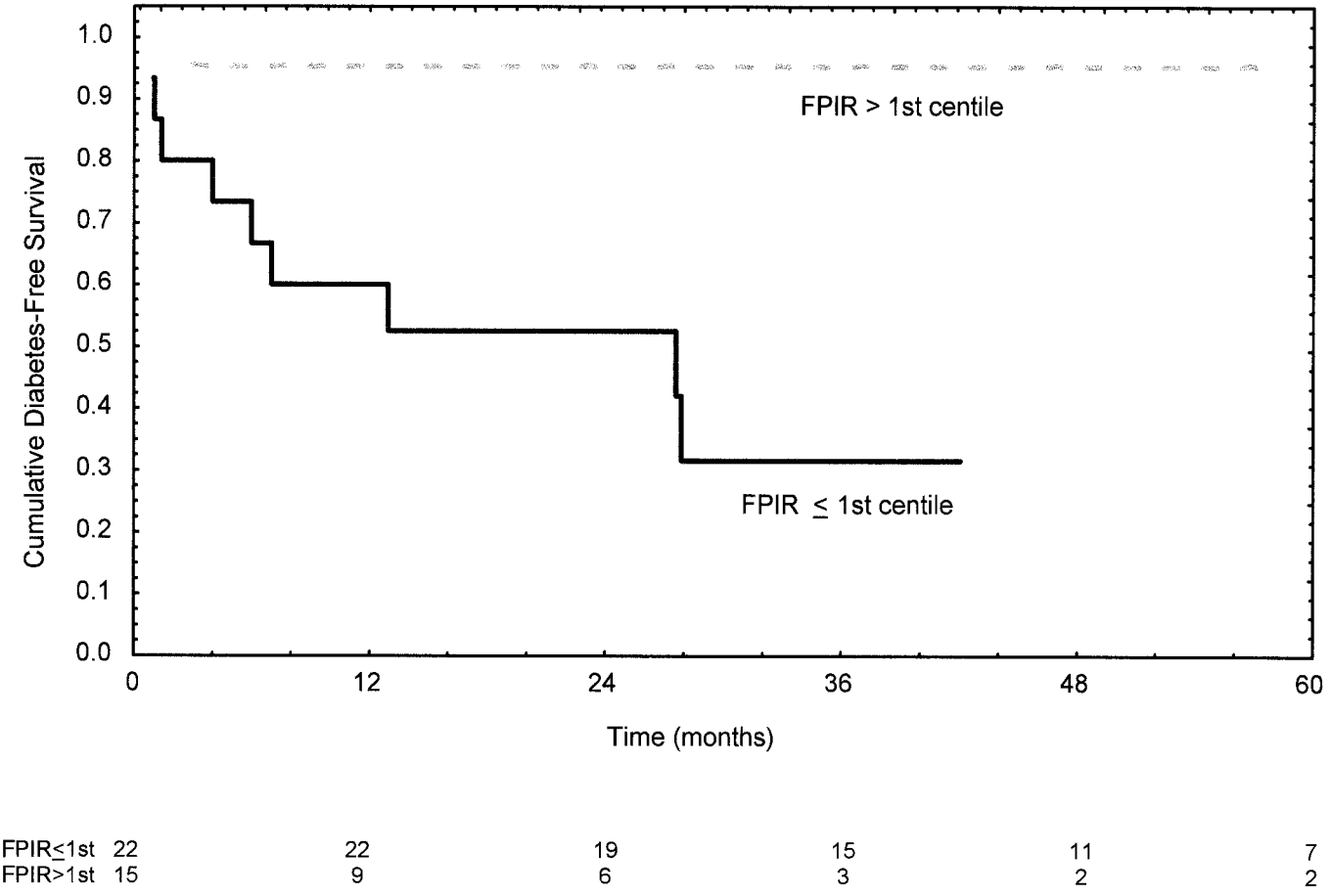


Figure 3—*Cumulative proportion event-free survival in antibody-positive hyperglycemic subjects by impaired FPIR;* P = *0.0001. Antibody-positive hyperglycemic subjects at yearly intervals are reported under the figure for each of the two groups.*

diabetes and should be screened for islet autoantibodies and metabolic markers.

In the absence of immunological and immunogenetic markers, incidental finding of hyperglycemia in children and adolescents appears unlikely to be associated with progression to insulin-dependent diabetes. Moreover, we would like to underline that in 30.5% of our subjects with incidental hyperglycemia without immunological, metabolic, or immunogenetic markers for type 1 diabetes and with a family history of type 2 diabetes, a clinical diagnosis of maturity-onset diabetes of the young (MODY) was made and confirmed by genetic analysis (29). This could explain the finding of an FPIR <1st centile in 25.6% of our hyperglycemic subjects, but clinical diabetes developed only in 2.5% of them.

In conclusion, our observations confirm that the finding of transient hyperglycemia without serious intercurrent illness is a risk factor for diabetes. We suggest that all pediatricians should look for evidence of markers for type 1 diabetes and MODY in children and adolescents with incidental hyperglycemia and closely follow subjects with these markers.

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