Progression to Diabetes in Relatives With Islet Autoantibodies

Is it inevitable?

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OBJECTIVE — A large cohort of family members with islet cell antibodies (ICA) ≥20 Juvenile Diabetes Foundation units (JDF U) was examined to determine whether there was a subgroup at low risk of progression to diabetes; whether risk of progression changed over time; and whether rate of progression to diabetes varied according to age, islet autoantibodies, and genetic markers of susceptibility.

RESEARCH DESIGN AND METHODS — Individuals with ICA ≥20 JDF U were identified from 4,423 family members recruited to prospective family studies in the U.K. Subjects were followed for up to 18 years. Antibodies to insulin, GAD, and IA-2 were measured in the first sample, and HLA class II typing was performed.

RESULTS — Of 147 family members with ICA ≥20 JDF U on at least one occasion, 29 developed type 1 diabetes after a median of 3.2 years (maximum 18.1). The cumulative risk of developing diabetes within 15 years was 47% (95% CI 28–67) for all family members with ICA ≥20 JDF U, 2.8% (0–8.2) for those with ICA alone, and 66% (44–87) for those with at least one additional autoantibody marker. There were no differences in age, HLA class II type, or levels of ICA, insulin autoantibodies, or IA-2 antibodies between those who developed diabetes within 5 years of testing and those who developed diabetes after this time. GAD antibody levels were, however, higher in those who progressed more slowly.

CONCLUSIONS — Family members with ICA alone are at low risk of progression to diabetes. Rapid development of disease after ICA detection could not be distinguished from delayed development on the basis of autoantibodies or markers of genetic susceptibility, and those with multiple antibodies remained at high risk throughout long-term follow-up. This suggests that all family members with multiple islet autoantibodies are destined to develop autoimmune diabetes.

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Islet cell antibodies (ICA) and other islet autoantibodies indicate a high risk of progression to type 1 diabetes in unaffected first-degree relatives, and ICA ≥20 Juvenile Diabetes Foundation units (JDF U) are associated with a 35–40% cumulative risk of diabetes within 5 years (1–4). High rates of progression over this period are associated with loss of first-phase insulin secretion, multiple antibodies, high autoantibody titers, and young age (4–6); IA-2 antibodies and high-risk HLA genotype are also reported to be markers of rapid progression (5,7,8). However, relatively few high-risk individuals have been followed beyond 5 years, and it is not known how many high-risk individuals will subsequently develop diabetes or whether they will continue to do so at the same rate.

We therefore set out to examine progression to diabetes in a large cohort of family members with ICA ≥20 JDF U who were followed up to 18 years and to compare those who developed diabetes within 5 years of first detection of ICA ≥20 JDF U with those who developed diabetes after 5 years. Our goals were to determine if there was a subgroup of the cohort at low-risk of progression to diabetes, to establish whether the rate of progression in those with multiple antibodies remained constant or showed signs of reaching a plateau over long-term follow-up, and to test whether rapid and delayed development of disease after ICA detection could be differentiated on the basis of age, autoantibodies, and genetic markers of susceptibility.

RESEARCH DESIGN AND METHODS

Subjects

The Bart's-Windsor (1) and Bart's-Oxford (9) prospective family studies recruited parents and siblings of patients with type 1 diabetes who were diagnosed before 21 years of age from the Oxford Regional Health Authority area in England. Recruitment for the Bart's-Windsor Study took place between 1978 and 1984, and recruitment for the Bart's-Oxford Study took place from 1985 onward. By 31 December 1995, 4,423 nondiabetic first-degree relatives had been tested on a median of three occasions (range 1–44), and 147 (75 parents and 72 siblings) had been found to have ICA ≥20 JDF U in at least one sample. One of these subjects, a parent with common varied immunodeficiency, was excluded from the analysis. The remaining 146 family members comprised the study cohort. At the time of the analysis, the subjects had been followed for up to 18.1 years (median 4.0) with repeated sam-

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Abbreviations: FPIR, first-phase insulin response; IAA, insulin autoantibodies; ICA, islet cell antibodies; IDS, Immunology of Diabetes Society; JDF U, Juvenile Diabetes Foundation units.

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

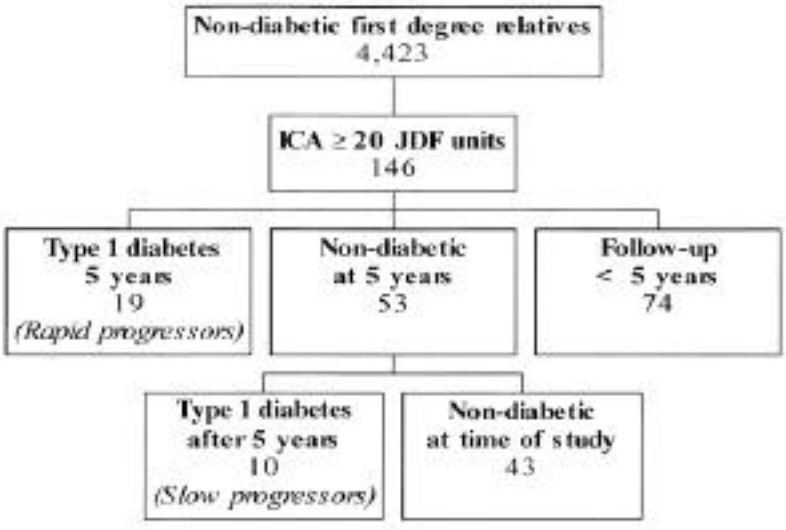

Figure 1—*The study cohort.*

pling for ICA and other autoantibodies, and 29 had developed type 1 diabetes after a median of 3.2 years. The study cohort is described in Fig. 1.

Insulin autoantibodies (IAA) and antibodies to GAD and protein tyrosine phosphatase IA-2 were measured on the first eligible sample from 25 of the 29 individuals who developed diabetes. For the remaining four individuals, no prediagnosis serum samples were available for further autoantibody analysis. The first eligible samples from all 117 family members who remained nondiabetic at the time of last contact at entry were also tested for these markers. Samples for genetic analysis were available from 19 of 29 people who developed diabetes and 11 of 16 people with multiple antibodies who remained nondiabetic after at least 5 years.

Assays

GAD and IA-2 antibodies. Antibodies to in vitro translated [^{35}S]GAD65 and [^{35}S]PTP-IA-2$_{ic}$ were measured by immunoassay as previously described (10). Immune complexes were isolated on Protein A Sepharose (Pharmacia, Biotech AB, Uppsala, Sweden). After washing, bound counts per minute were expressed as arbitrary units derived from a standard curve that was constructed using eight doubling dilutions of positive sera with an arbitrary value of 100 U. Values above 100 U were beyond the steepest gradient of the standard curve and were reported as >100 U. The interassay coefficient of variation of the GAD antibody assay was 17% for samples with 1.5 U and 9% for samples with 17 U of antibody. The interassay coefficient of variation of the IA-2 antibody assay was 15% for samples with 1 U and 21% for samples with 9 U of antibody. The GAD antibody assay achieved 91% sensitivity with 99% specificity, and the IA-2 antibody assay achieved 74.4% sensitivity with 99% specificity in the First Immunology of Diabetes Society (IDS) Combined Antibody Workshop (11).

Insulin autoantibodies. Antibodies to ^{125}I-labeled insulin were measured using a format similar to the one used to measure GAD and IA-2 antibodies (12). Immune complexes were isolated with Protein A Sepharose (Pharmacia). Bound counts for each sample were calculated after subtraction of background counts, and results were expressed in arbitrary units derived from a standard curve constructed from nine doubling dilutions of serum from a patient with long-standing type 1 diabetes in normal human serum; results ranged from 0.39 to 100 U. Sera with insulin binding >0.4 U were tested in a competition assay in which further duplicate wells of each sample were incubated with 15,000 cpm of ^{125}I-insulin that was diluted in buffer that contained unlabeled human insulin (Humulin, Lilly, Basingstoke, Hants, U.K.). Specific bound counts were calculated for each sample by subtracting the counts of the tubes with excess unlabelled insulin from those with label alone and were converted into arbitrary units as described above, including an additional standard at 0.2 U. The interassay coefficient of variation of the screening assay was 17% at 0.5 U and 13% at 1.4 U; the interassay coefficient of variation of the competition assay was 31% at 0.6 U and 16% at 1.4 U. The IAA assay achieved 58% sensitivity with 99% specificity on the samples included in the First IDS Combined Antibody Workshop.

Islet cell antibodies. ICA were measured in undiluted sera by indirect immunofluorescence as previously described (13). Endpoint titers of test samples were converted to JDF U by comparison with a standard curve of $\log_2$ JDF U vs. $\log_2$ of endpoint titer of the standard sera. The threshold of detection was 4 JDF U. The interassay coefficient of variation was 10% at 13 JDF U and 4.3% at 80 JDF U. The assay achieved 78.4% sensitivity with 98% specificity in the First IDS Combined Antibody Workshop.

Genotyping. DNA was extracted from 5 ml frozen EDTA blood by proteinase K digestion of sodium dodecyl sulfate–treated cells and subsequent salt precipitation (14). HLA-DQ typing was performed using a polymerase chain reaction and sequence-specific primers technique. A low resolution method as described by Bunce et al. (15) was used as the initial screen. This method positively identified the HLA-DQB1 alleles corresponding to the serologically defined series HLA-DQ2, -DQ4, -DQ5, -DQ6, -DQ7, -DQ8, and -DQ9, but could neither distinguish between the protective allele DQB1*0602 and the neutral DQB1*0611 nor between the susceptibility allele DQB1*0201 and the neutral DQB1*0202. If DQ2 or DQ6 were identified, high resolution HLA-DQB1 typing (16) and HLA-DR typing were performed to distinguish between these alleles.

Results of previous HLA-DR serotyping by two-color immunofluorescence were used to assign genetic risk in 10 participants in the Bart's-Windsor Family Study, from whom we were unable to obtain further samples for genetic analysis (17).

Statistical analysis

Kaplan-Meier survival curves were used to estimate the cumulative risk of development of type 1 diabetes (18). The follow-up period for each family member was calculated from the date of the first sample in which ICA ≥20 JDF U were detected, and the end of follow-up was

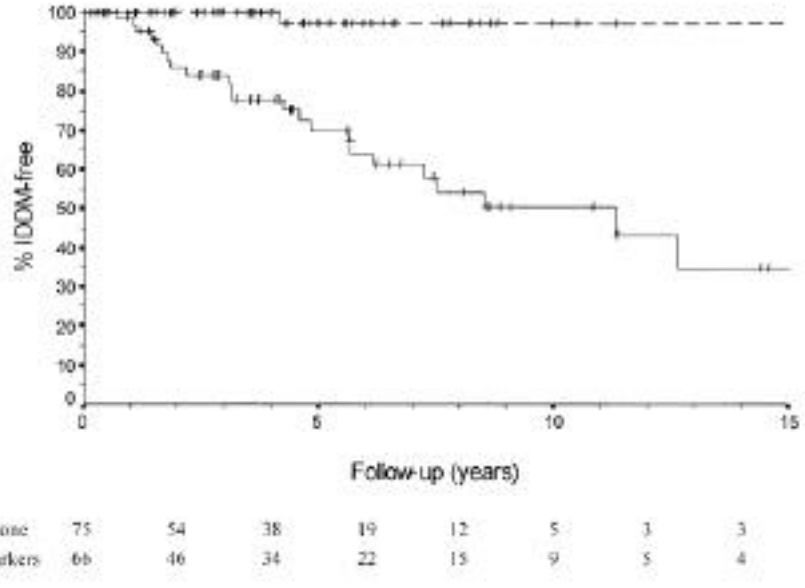

Figure 2— *The cumulative risk for development of diabetes in all family members with ICA ≥20 JDF U alone (– – –) and with ICA and at least one additional autoantibody marker above the 97.5th centile of schoolchildren control subjects (——). The number of subjects in the two groups included in each year of follow-up is shown below the figure.*

defined as date of last contact, date of diagnosis, or date of entry into an intervention trial. Diabetes was defined according to World Health Organization criteria (19). Baseline characteristics of the groups were compared using the nonparametric Mann-Whitney *U* test or χ^2 testing as appropriate.

Antibody prevalences were expressed in relation to centiles derived from a population of 2,860 schoolchildren from the Oxford Region (10). The threshold selected was the 97.5th centile, which corresponded to 1.6 U for GAD antibodies, 0.9 U for IA-2 antibodies, and 0.2 U for IAA. Distributions of autoantibody levels were compared using the Mann-Whitney *U* test and Spearman's rank correlation coefficient. *P* values were corrected for the number of antibodies compared ($n = 4$).

RESULTS

Subject characteristics

The median age of the patients at study entry was 28.3 years (range 1.6–58.6); 66 of the subjects were female and 80 were male. All four antibody markers were measured in the first sample with ICA ≥20 JDF U from 141 subjects. Of those subjects, 23 (16%) had IA-2 antibodies, 62 (44%) had GAD antibodies, and 29 (21%) had IAA above the 97.5th centile. ICA alone were detected in 75 (53%), 30 (21%) had one additional marker, 27 (19%) had two additional markers, and 9 (6%) had three additional markers above the 97.5th centile.

Cumulative risk of diabetes

The cumulative risk of developing diabetes for all family members with ICA ≥20 JDF U was 18% (95% CI 11–26) within 5 years, 34% (21– 46) within 10 years, and 47% (28– 67) within 15 years. The person with the longest follow-up developed diabetes 18.1 years after ICA ≥20 JDF U were first detected.

The Kaplan-Meier survival curve for development of diabetes in family members with ICA ≥20 JDF U is shown in Fig. 2. The cohort has been divided according to the number of autoantibodies above the 97.5th centile in the first sample in which ICA ≥20 JDF U were detected. Risk was highest in family members with at least one autoantibody marker above the 97.5th centile in addition to ICA. These family members had a 30% cumulative risk of developing diabetes within 5 years (17–43), a 50% risk within 10 years (33–66), and a 66% risk within 15 years (44–87). Only one family member with ICA alone developed diabetes, which indicates a 5-year cumulative risk of 2.8% (0–8.2).

Characteristics of family members who progressed to diabetes

The characteristics of the 29 family members who developed diabetes are shown in Table 1. The median period of follow-up before diagnosis was 3.2 years (range 0.5–18.1). The age at the time of the first eligible sample did not differ between family members who developed diabetes within 5 years (median age 18.3 years), and those who developed diabetes after >5 years (median age 12.3 years, $P = 0.54$). ICA and IAA levels were also similar ($P = 0.12$ and $P = 0.67$, respectively). GAD antibody levels were higher in those who progressed more slowly (median 12 vs. 82 U, $P_{corrected} = 0.013$) and were correlated with time to diabetes ($R^2 = 0.22$, $P = 0.02$). IA-2 antibody levels did not differ between the groups ($P = 0.28$), but there appeared to be two populations among the individuals who developed diabetes within 5 years. There were eight family members who had IA-2 antibodies >100 U with only moderate elevation of GAD antibodies (maximum 16 U), and the remaining eight individuals who were tested all had levels of IA-2 antibodies below the 97.5th centile (0.9 U) with elevation of GAD antibodies (range 33–89 U). Those with high levels of IA-2 antibodies and those with low levels did not differ in median time to diabetes (1.8 years, range 1.6–3.7 vs. 3.1, range 1.0–4.0, $P = 0.92$) or age at the time the sample was taken (10.9, range 1.6–44.6 vs. 21.3, range 2.1–45.5, $P = 0.53$). There was an inverse correlation between GAD and IA-2 antibody levels (Spearman's rank correlation coefficient −0.62, $P = 0.001$).

Of the 25 individuals tested for all markers at entry, 24 had at least one antibody above the 97.5th centile in addition to ICA, and the number of additional antibodies did not differ between the groups ($P = 0.855$) (Fig. 3). Of 12 family members in whom genotyping was performed and who developed diabetes within 5 years of first detection of ICA, four had the highest risk DR3/DR4 or DR3-DQ2/DQ8 genotype compared with two of eight slow progressors. One person (case 21) had neither DR3 nor DR4 haplotypes. Of those who developed diabetes, none had the protective DR2 or DQB1*0602 alleles.

Characteristics of family members who remained nondiabetic after more than 5 years

After at least 5 years of follow-up from the date of the first sample in which ICA ≥20 JDF U were detected, 43 family mem-

Table 1—*Family members who developed diabetes*

Case	Age at testing (years)	Sex	Time to diabetes (years)	ICA	IA-2 antibodies	GAD antibodies	IAA	Autoantibody markers	HLA class II	
1	17.2	F	0.5	80	—	—	—	*	DR3-DQ2	DQ8
2	6.22	F	0.7	>80	—	—	—	*	—	—
3	11.01	M	0.7	>80	0.3	32.5	0.0	2	—	—
4	1.58	M	1.0	30	0.8	80.3	16.3	3	—	—
5	2.13	M	1.2	>80	>100	1.1	27.9	3	DR3-DQ2	DQ8
6	4.23	M	1.4	>80	>100	2.5	1.2	4	DR4	DR4
7	41.53	M	1.6	32	0.4	76.8	0.0	2	DR3-DQ2	DQ2 (not DR3)
8	37.27	F	1.7	23	>100	4.8	0.4	4	—	—
9	45.51	F	1.8	>80	>100	15.6	0.0	3	DQ8	DQ5
10	6.88	M	1.8	>80	>100	7.4	2.7	4	DQ5	DQ8
11	18.31	M	1.9	30	—	—	—	—	—	—
12	30.51	M	2.2	52	>100	8.6	0.0	3	DQ8	DQ8
13	44.61	M	3.1	32	0.3	89.3	0.8	3	DR6	DR4
14	10.08	M	3.2	>80	>100	0.2	1.1	3	DR3-DQ2	DQ8
15	28.05	F	3.2	80	0.8	70.8	0.5	3	DQ8	DQ2 (not DR3)
16	22.89	F	4.2	50	0.4	0.7	0.0	1	DR3-DQ2	DR3-DQ2
17	19.70	M	4.3	21	0.3	81.3	0.0	2	—	—
18	19.27	M	4.6	74	0.5	69.2	0.0	2	DR3-DQ2	DQ5
19	11.80	M	4.8	>80	>100	6.3	0.3	3	DR3-DQ2	DQ8
20	3.06	F	5.7	80	0.6	94.3	0.0	2	DR3-DQ2	DQ8
21	10.60	F	5.7	45	0.4	80.4	0.9	3	DQ5	DQ6 (not *0602)
22	9.46	M	6.2	69	26.5	81.7	0.5	4	DR3-DQ2	DQ6 (not *0602)
23	5.54	F	6.7	>80	—	—	—	—	—	—
24	5.60	M	7.3	30	29.0	14.0	0.9	4	DR1	DR4
25	14.07	M	7.5	20	0.3	>100	0.0	2	DR3-DQ2	DR3-DQ2
26	16.07	M	8.5	40	0.6	80.7	>100	3	—	—
27	42.03	M	11.3	>80	10.5	>100	0.0	3	DQ2 (not DR3)	DQ8
28	22.72	F	12.6	30	0.3	89.0	0.0	2	DQ8	DQ8
29	26.03	M	18.1	28	>100	63.4	0.0	3	DR3	DR5

*These samples have previously been shown to have IAA and antibodies to GAD and 37/40K antigens in immunoprecipitation assays (5).

bers remained nondiabetic. ICA alone were detected in 26 individuals (60%), and 16 family members (37%) had at least one antibody above the 97.5th centile in addition to ICA. A serum sample was not available from one subject. The median age of the individuals with multiple antibodies was 20.2 years (range 9.2–58.6) when ICA ≥20 JDF U were first detected. Of the subjects with multiple antibodies, nine (56%) were female. The median duration of follow-up was 8.8 years (maximum 18). Autoantibodies to GAD above the 97.5th centile were found in 15 (94%) of the subjects; autoantibodies to IA-2 were found in 4 (25%) of the subjects; and autoantibodies to insulin were also found in 4 (25%) of the subjects. Only one antibody marker in addition to ICA was detected in 10 (62%) of these subjects, 5 (31%) had two additional markers, and in one individual, all four markers tested were above the 97.5th centile. IA-2 and insulin autoantibody levels did not differ from those in family members who had developed diabetes either before or after 5 years since the first detection of ICA ≥20 JDF U. GAD antibody levels were higher in family members with multiple antibodies who remained nondiabetic after >5 years from first detection of ICA ≥20 JDF U than in those who developed diabetes within 5 years, but were similar to those in individuals who progressed more slowly (median 58 vs. 82 U, $P_{corrected} = 0.23$). Of 11 individuals in whom genotyping was performed, 4 were heterozygous for DR3/DR4 or DR3-DQ2/DQ8.

CONCLUSIONS — The Seattle Family Study (20) has suggested that there is a "survivor" subgroup of first-degree relatives with ICA with a nonprogressive form of β-cell autoimmunity, and subsequent studies have shown that ICA in the absence of other islet autoantibodies are only weakly associated with diabetes (5,6,21). This finding was confirmed in our study: only 1 of 75 individuals with ICA ≥20 JDF U in the absence of other autoantibodies developed diabetes. The main focus of our analysis was on the remaining 66 individuals who had at least one other autoantibody marker in addition to ICA and in whom the increase in cumulative risk was linear during a follow-up period of up to 18 years (Fig. 2).

We proceeded to investigate determinants of individual rate of progression to diabetes within this group. It is well-established that loss of first-phase insulin response (FPIR) to the intravenous glucose tolerance test provides the best indication of rate of progression to diabetes, at least in the latter stages of the disease after FPIR has fallen below 100 mU/l (4). Metabolic testing is, however, labor-intensive, invasive, and difficult to standardize. Several studies have suggested that rate of progression to diabetes in antibody-positive relatives is

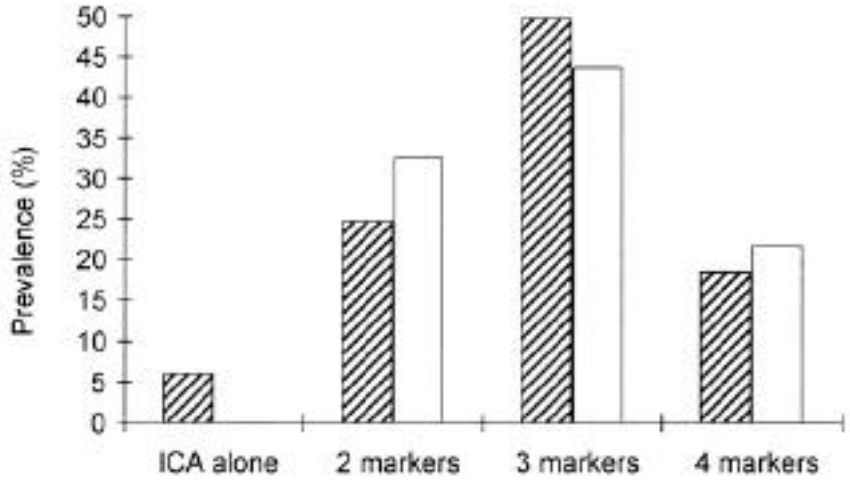

Figure 3— *The prevalence of islet autoantibody markers above the 97.5th centile in family members who developed diabetes within 5 years of first detection of ICA ≥20 JDF U (▨) compared with family members who developed diabetes after >5 years (□).*

related to the number of antibodies detected, autoantibody titers, and age (4–6), while IA-2 antibodies and high-risk HLA genotype are also reported to be markers of rapid progression (5,7,8). To examine this question, we compared those who developed diabetes within 5 years of first detection of ICA ≥20 JDF U with those who developed diabetes after 5 years of first detection of ICA ≥20 JDF U.

There were no baseline differences in level of ICA or IAA, but we did find a weak negative correlation between high GAD antibody levels and duration to diabetes development. This finding is consistent with the observations of shorter duration studies that high levels of GAD antibodies appear protective (22), but it differs from the latest findings from the Seattle Family Study (23). The prevalence of antibodies to IA-2 above the 97.5th centile was similar in the two groups, and there was no difference in the distribution of antibody levels (Fig. 3). Very high levels of IA-2 antibodies were, however, generally associated with rapid progression to diabetes as previously described (5,8). There were however exceptions to these general rules. Case 29, for example, had very high levels of IA-2 for 18 years before he developed diabetes, while cases 4, 5, and 7, who progressed rapidly, had high levels of antibodies to GAD but not IA-2.

Multiple antibody positivity was strongly associated with disease (5), but the number of elevated antibody markers was similar in the groups of relatives who developed diabetes before and after 5 years of first being tested. It has been suggested that diabetes might develop more rapidly in those with the highest load of genetic susceptibility (4,7), but there was little evidence of skewing toward the highest-risk HLA-DR/DQ genotype in those who developed diabetes soon after study entry, although there were few subjects in each group. The inference is that markers of high risk do not necessarily imply rapid progression to disease, with the probable exception of FPIR.

The lack of clear differentiation between relatives who developed diabetes soon after first detection of ICA and those in whom clinical onset was delayed was entirely consistent with the observation that progression to diabetes was linear over time in those with multiple autoantibodies over a follow-up period of up to 18 years (Fig. 2). This suggests that the risk associated with humoral autoimmunity against multiple islet autoantigens does not diminish with time and that ultimate progression to diabetes is to be expected within this category.

Our findings, therefore, confirm that high levels of multiple antibody markers at baseline provide the best means of distinguishing those family members with ICA ≥20 JDF U who will and will not progress to diabetes. This excludes people with transient or spurious elevation of ICA and those with ICA alone who are at low risk. Our population was identified on the basis of initial screening for ICA, but we anticipate that similar findings will emerge if GAD or IA-2 antibodies are used for first-line testing because elevated levels of any one marker in isolation are likely to be associated with low risk (10,24). Individuals with multiple autoantibodies continued to develop diabetes throughout the period of follow-up, and many who remained nondiabetic had evidence of impaired β-cell function (data not shown). We conclude that family members with persistent markers of islet autoimmunity directed against more than one antigen remain at high risk of developing diabetes over long-term follow-up. We have found no evidence of a separate survivor population within the group with multiple antibodies and would postulate that all of these family members are destined to develop autoimmune diabetes.

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