Combined Analysis of Autoantibodies Improves Prediction of IDDM in Islet Cell Antibody-Positive Relatives

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Prediction of insulin-dependent diabetes mellitus (IDDM) is still largely based on islet cell antibodies (ICAs), but it may be improved by combined analysis with other humoral markers. We examined autoantibodies to insulin (IAAs), glutamic acid decarboxylase (GAD), and M_r 37,000 and M_r 40,000 fragments of islet antigens (37 and 40 kDa) together with ICA subtypes in 101 family members with ICAs $\geq$10 Juvenile Diabetes Foundation units (JDF U) followed for up to 14 years, of whom 18 have developed IDDM. Life-table analysis showed a 43% risk of IDDM within 10 years for those with ICAs $\geq$10 JDF U, rising to 53% for those with ICAs $\geq$20 JDF U. The risk for ICAs $\geq$10 JDF U was 62% in the family members in the youngest age quartile (<13.2 years) and fell with increasing age to 4% in those >40.7 years of age ($P = 0.03$). ICAs $\geq$10 JDF U combined with IAAs gave a risk of 84% ($P = 0.03$ compared with IAA$^-$), and ICAs $\geq$10 JDF U combined with GAD antibodies gave a risk of 61% ($P = 0.018$). The risk for ICAs $\geq$10 JDF U with antibodies to 37-kDa antigen was 76% ($P < 0.0001$). Risk increased with the number of autoantibodies, from 8% for ICAs alone to 88% with $\geq$3 autoantibodies (14 cases detected) ($P < 0.0001$). The increased risk associated with multiple antibodies was observed independent of age. The median time to diagnosis in those with antibodies to 37- and/or 40-kDa antigen was 1.5 years, compared with 7.2 years in those with IAAs and GAD antibodies in the absence of antibodies to 37/40 kDa. The intensity and range of the autoantibody response offers better overall prediction of diabetes than any single autoantibody specificity, although antibodies to 37-/40-kDa antigens may prove to be useful markers of early clinical onset. We found that 78% of future cases of IDDM in ICA$^+$ relatives came from the 27% with multiple autoantibodies and estimate that 88% of individuals within this category will need insulin treatment within 10 years. We propose a simple predictive strategy based on these observations. *Diabetes* 43:1304–1310, 1994

Risk of progression to insulin-dependent diabetes mellitus (IDDM) can be assessed with reasonable accuracy in unaffected first-degree relatives of a child with the disease, and such estimates have been used to design clinical trials of agents that may delay or prevent the onset of diabetes (1). Islet cell antibodies (ICAs) offer highly sensitive prediction when measured by an assay with a low threshold of detection. We found that 82% of those who developed diabetes within 10 years had ICAs $\geq$10 Juvenile Diabetes Foundation units (JDF U) on entry into the Bart's-Windsor family study (2). ICAs do, however, offer less specific prediction, because only 40% of relatives with ICAs $\geq$10 JDF U would be expected to develop IDDM within 10 years (2,3). In contrast, loss of the first-phase insulin response to intravenous glucose is a highly specific marker of progression, giving a 90% risk over 4 years (4), but it is relatively insensitive, identifying around 20% of those family members who will develop diabetes within 5 years (1). Other potential autoantibody markers include antibodies to insulin (IAAs) (5), to glutamic acid decarboxylase (GAD) (6), and to other islet proteins detectable as M_r 37,000 and M_r 40,000 proteolytic fragments (37- and 40-kDa antigens) (7). We set out to examine the predictive value of these additional humoral markers in a large population of ICA$^+$ relatives, with the aim of developing an approach to screening that would combine the sensitivity of ICA with improved specificity.

RESEARCH DESIGN AND METHODS

The Bart's-Windsor and Bart's-Oxford prospective family studies have recruited parents and siblings of patients with IDDM diagnosed before age 21 from within the Oxford Regional Health Authority area in England. By 1 March 1992, 2,722 nondiabetic first-degree relatives had been screened, of whom 109 had been found to have ICAs $\geq$10 JDF U with detectable ICAs (<4 JDF U) on at least one other occasion. In this analysis, we have included 101 relatives (48 parents and 53 siblings) who fulfilled these criteria. Serum from the remaining eight was no longer available for study. The median age at entry to the study was 19.8 years (range 2–56 years). Subjects were followed for up to 14.3 years (median 3.8 years) with repeated sampling for ICAs and other autoantibodies. Eighteen (4 parents and 14 siblings) developed IDDM. The cumulative risk for the development of IDDM for the whole group and the duration of follow-up are summarized in Fig. 1.

Autoantibody assays. Autoantibodies were all determined on the earliest available sample in which ICAs $\geq$10 JDF U were detected. Sequential samples taken at approximately annual intervals between study entry and diagnosis of IDDM were also tested where available. All assays were performed blind on coded samples.
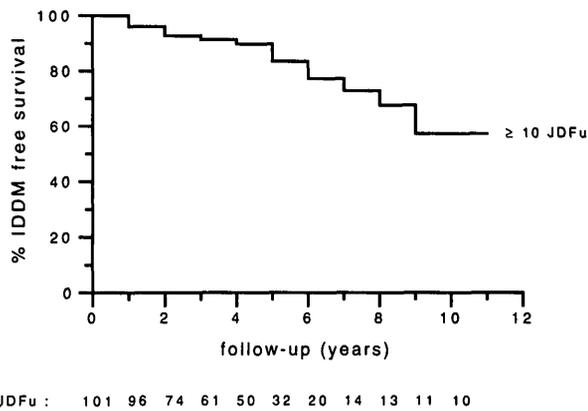
FIG. 1. The cumulative risk of IDDM for the whole study population. The number of subjects included in each year of follow-up is shown below the figure.

FIG. 2. Antibody combinations grouped according to level of ICAs, showing the number of individuals within each category.

ICAs. Undiluted sera were screened for conventional ICA-IgG by means of indirect immunofluorescence on 4-μm cryostat sections of blood group O human pancreas (8). Positive samples were then titered by doubling dilutions in phosphate-buffered saline on tissue obtained from a single pancreas under standard incubation conditions (9). Local standard sera calibrated to 2, 4, 8, 16, 32, and 80 JDF U were included in each assay. End-point titers were converted to JDF U (10). The coefficients of variation (CVs) between assays for control sera with 8, 32, and 80 JDF U tested in 13 consecutive assays were 11, 7, and 6%, respectively, when expressed geometrically (SD $\log_2$ JDF U/mean $\log_2$ JDF U). The threshold of ICA detection was 4 JDF U.

IAAs. IAAs were assayed using a modification of the methods described by Palmer et al. (5) and Kurtz et al. (11). Sera were extracted using acid-washed, dextran-coated charcoal to remove endogenous insulin; 80 μl of serum was then incubated for 48 h at 4°C with 80 μl of 40 mmol/l phosphate buffer and 5.3×10^{-3} pmol radiolabeled human insulin (specific activity 2,000 Ci/mmol; Amersham, Amersham, U.K.), with and without excess (2.55 pmol/tube) cold insulin (Actrapid, Novo Nordisk, Bagsvaerd, Denmark). The immunoglobulin fraction was precipitated using polyethylene glycol 6000 (12.9% wt/vol) and washed. The specific binding was calculated by subtracting the counts in the presence of cold insulin from the counts without the cold insulin. Results were expressed as percentage displaced binding. The CVs between assays for control sera with percentage displaced binding of 1, 10, and 50 were 25, 26, and 9%, respectively. Individuals were classified as IAA^+ if the corrected binding was >3 SD above the mean of 172 adult blood donors (mean ± SD; $-0.04 \pm 0.26\%$ displaced binding).

GAD antibodies. Antibodies to GAD in sera were measured by determining the enzyme activity immunoprecipitated by sera from a soluble extract of rat brain, as previously described (12). GAD activity immunoprecipitated was calculated relative to a standard positive serum included in each assay. The intra-assay CV was 15.4%. Sera were regarded as positive for anti-GAD antibodies if the relative antibody activity exceeded 2 SD of the activity in sera from a group of 30 healthy control individuals (mean ± SD; 6.2 ± 3.4% of positive control subjects). Using this assay, antibodies to GAD were found in 16 of 25 newly diagnosed patients with IDDM (12) and 1 of 28 control subjects (13).

Antibodies to 37- and 40-kDa islet antigens. Antibodies to 37- and 40-kDa islet antigens were measured by immunoprecipitation of [^{35}S]methionine-labeled proteins from RIN 5AH cells extracted in Triton X-114 detergent as described previously (7). Immunoprecipitates were treated with trypsin (0.1 mg/ml) before sodium dodecyl sulfate-polyacrylamide electrophoresis and autoradiography. Serum samples were regarded as positive for antibodies to tryptic fragments of islet 64-kDa antigens if a band corresponding to the appropriate polypeptide could be detected on the autoradiogram. Antibody activities on positive samples were quantified by densitometric scanning of bands on autoradiograms expressing band density relative to that in a standard antibody-positive serum used in previous studies. Using this assay, antibodies to 37- and/or 40-kDa antigens were detected in 21 of 27 patients with newly diagnosed IDDM and in 0 of 26 control subjects (14).

ICA subtypes. ICAs have been subclassified on the basis of the ability of rat brain homogenate to inhibit islet staining in the ICA assay. This has been shown to correlate with staining pattern; those ICAs inhibited by rat brain homogenate give a β-cell selective or restrictive pattern, while those not inhibited give a whole islet or unrestricted pattern (15).
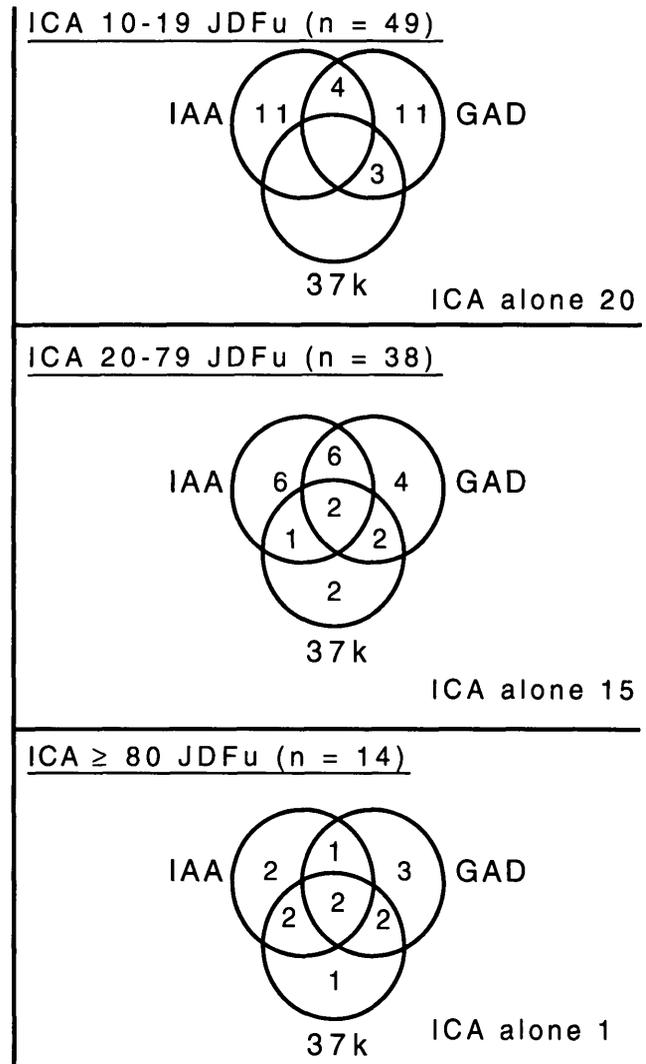
Inhibition experiments were performed on samples with ICAs ≥20 JDF U using Wistar-Furth rat brain homogenate. Sera were preincubated overnight with either rat brain homogenate or homogenate buffer. Each was titered to end point in phosphate-buffered saline and tested in the ICA assay (15). Sera were classified as inhibited if the end-point titer in the sample preincubated with rat brain homogenate was two or more doubling dilutions less than that preincubated with homogenization buffer only. A control serum in which ICA staining was completely inhibited by rat brain homogenate and one in which staining was not inhibited were included in each assay.

Statistical analysis. Life tables were used to estimate the time to development of IDDM. Follow-up time for each subject was calculated from the date when ICAs ≥10 JDF U were first detected. The start of insulin treatment was used as the date of diagnosis of IDDM. χ^2 testing was used to assess associations between antibodies and with age. Survival experience was compared using the Lee-Desu statistic in SPSS-PC. Point estimates of risk are quoted as cumulative risk (95% confidence interval [CI]).

RESULTS

Prevalence of autoantibodies. The combinations of antibodies detected in individuals grouped according to level of ICAs are shown in Fig. 2. Thirty-six individuals had ICAs alone, 38 had ICAs and one other antibody, 16 had two others, 7 had three others, and 4 had all the antibodies tested. ICAs ≥80 JDF U were most frequent in children in the lowest age quartile, <13.2 years of age ($P = 0.03$). Antibodies to 37-

TABLE 1
Characteristics of subjects who developed IDDM during follow-up

	Sex	Age at entry	Years before diagnosis	ICAs (JDF U)	IAAs (SD score)	GAD (% positive control serum)	37 kDa (% positive control serum)	40 kDa (% positive control serum)
Siblings								
1	M	3.0	0.2	37	5.8	47	–	–
2	F	6.2	0.7	>80	10.6	15	6	4
3	F	17.2	0.7	80	20.7	379	50	100
4	M	2.1	1.1	>80	50.2	2	0	31
5	M	3.6	1.5	>80	0.9	3	105	93
6	M	6.6	1.9	30	4.6	2	86	93
7	M	9.6	3.2	49	5.7	90	82	104
8	M	12.2	4.3	80	3.3	8	43	44
9	F	18.9	4.4	50	1.8	12	–	–
10	F	10.3	5.2	13	−0.8	56	–	–
11	F	2.7	6.0	13	3.8	103	–	–
12	M	17.6	6.7	16	0.7	124	–	–
13	M	5.6	7.3	30	20.8	30	–	–
14	M	15.6	8.5	30	6.6	30	–	–
Parents								
15	F	45.5	0.4	80	−2.4	16	80	78
16	M	30.5	2.2	52	2.0	8	7	75
17	F	40.4	4.7	15	0.7	90	–	–
18	M	36.1	8.1	10	3.4	85	–	–

−, negative.

and/or 40-kDa antigens were more frequent in those with ICAs ≥80 JDF U ($P < 0.0001$) and in children in the lowest age quartile ($P = 0.04$), but IAAs and GAD antibodies were not significantly associated with ICA titer or age. There were no significant associations between IAAs, antibodies to GAD, and antibodies to 37- and/or 40-kDa antigens. Multiple antibodies were detected more frequently in individuals with ICAs ≥80 JDF U ($P < 0.05$).

Family members who developed IDDM during follow-up. The characteristics of the 18 family members who developed IDDM and the autoantibodies detected are shown in Table 1. The median time to diagnosis of IDDM was 2.7 years.

Effect of age. Of the 25 individuals who were <13.2 years of age at entry into the study, 10 developed IDDM, compared with 4 of 25 family members in the second age quartile (aged between 13.2 and 19.5 years), 3 of those in the third quartile (aged 19.5 to 40.7 years), and 1 in the highest age quartile. The cumulative risk of diabetes within 10 years was 62% (CI 33–92%) in the youngest age-group, 40% (CI 6–75%) in the second quartile, 35% (CI 0–74%) in the third quartile, and 4% (CI 0–22%) in those >40.7 years of age ($P = 0.02$).

Quantitative measurement of ICAs. Of the 49 individuals with ICAs between 10 and 19 JDF U, 5 developed IDDM; the cumulative risk of IDDM within 10 years in this group was 34% (CI 8–60%). Of the 38 with ICAs of 20–79 JDF U, 7 became diabetic, giving a 10-year cumulative risk of 52% (CI 16–88%). Of the 14 with ICAs ≥80 JDF U, 6 developed IDDM. The cumulative risk of IDDM within 5 years was 63% (CI 33–100%). Only one individual in this group remained nondiabetic after 5 years of follow-up. The survival curves were significantly different in the three groups ($P < 0.0001$) (Fig. 3A).

ICA subtype. Inhibition of ICA staining with rat brain homogenate could be examined in 44 individuals with high titer ICAs (≥20 JDF U); this method could not be applied to those with lower titers. ICAs were inhibited in seven; one of these developed IDDM after 5 years. Rat brain homogenate did not inhibit ICA staining in 29, and in 8 samples, the

results were equivocal (combined risk 61% after 10 years, CI 31–90%). The risk of diabetes was not significantly increased by the exclusion of individuals in whom ICA staining was not inhibited.

IAAs. IAAs were positive in 37 family members, of whom 11 developed IDDM after a median follow-up of 3.2 years. The cumulative risk after 10 years was 84% (CI 55–100%) in IAA⁺ and 23% (CI 6–41%) in IAA⁻ individuals, and the survival curves were significantly different ($P = 0.03$). Five family members developed diabetes between 4 and 8.5 years after entry, so that after only 5 years of follow-up, there was no significant difference in survival curves of IAA⁺ and IAA⁻ groups (cumulative risk at 5 years 25% [CI 7–42%] in IAA⁺ vs. 12% [CI 2–22%] in IAA⁻) (Fig. 3B).

GAD antibodies. Antibodies to GAD were detected in 40 family members overall and in 12 of those who developed diabetes. The difference in the survival curves for groups with and without GAD antibodies after 10 years was not statistically significant ($P = 0.18$) (Fig. 3C).

Antibodies to 37- and 40-kDa antigens. Antibodies to the 37- and 40-kDa islet antigens were found in 13 individuals. An additional four had antibodies to the 40-kDa antigen only. Nine of the patients who developed IDDM had antibodies to one or both antigens. All of these developed diabetes within 5 years of study entry. The median time to diabetes was 1.5 years. The cumulative risk of IDDM within 5 years was 76% (CI 46–100%) in those with 37-kDa antibodies compared with 7% (CI 0–15%) in those without ($P < 0.0001$). After 10 years, a total of nine cases had been diagnosed in 37/40 kDa antibody-negative family members, and the risk in this group was 38% (CI 17–60%) (Fig. 3D). The cumulative risk of IDDM within 5 years in those with antibodies to 40-kDa antigen was 61% (CI 33–85%).

Combining autoantibodies. The risk of diabetes increased with the number of antibody specificities detected. Of 36 individuals (36% of the cohort) with ICA alone, only 1 developed IDDM. The cumulative risk of IDDM within 10 years in this group was 6% (CI 0–18%). Of the 38 individuals with ICA and one other autoantibody, 3 developed IDDM; the
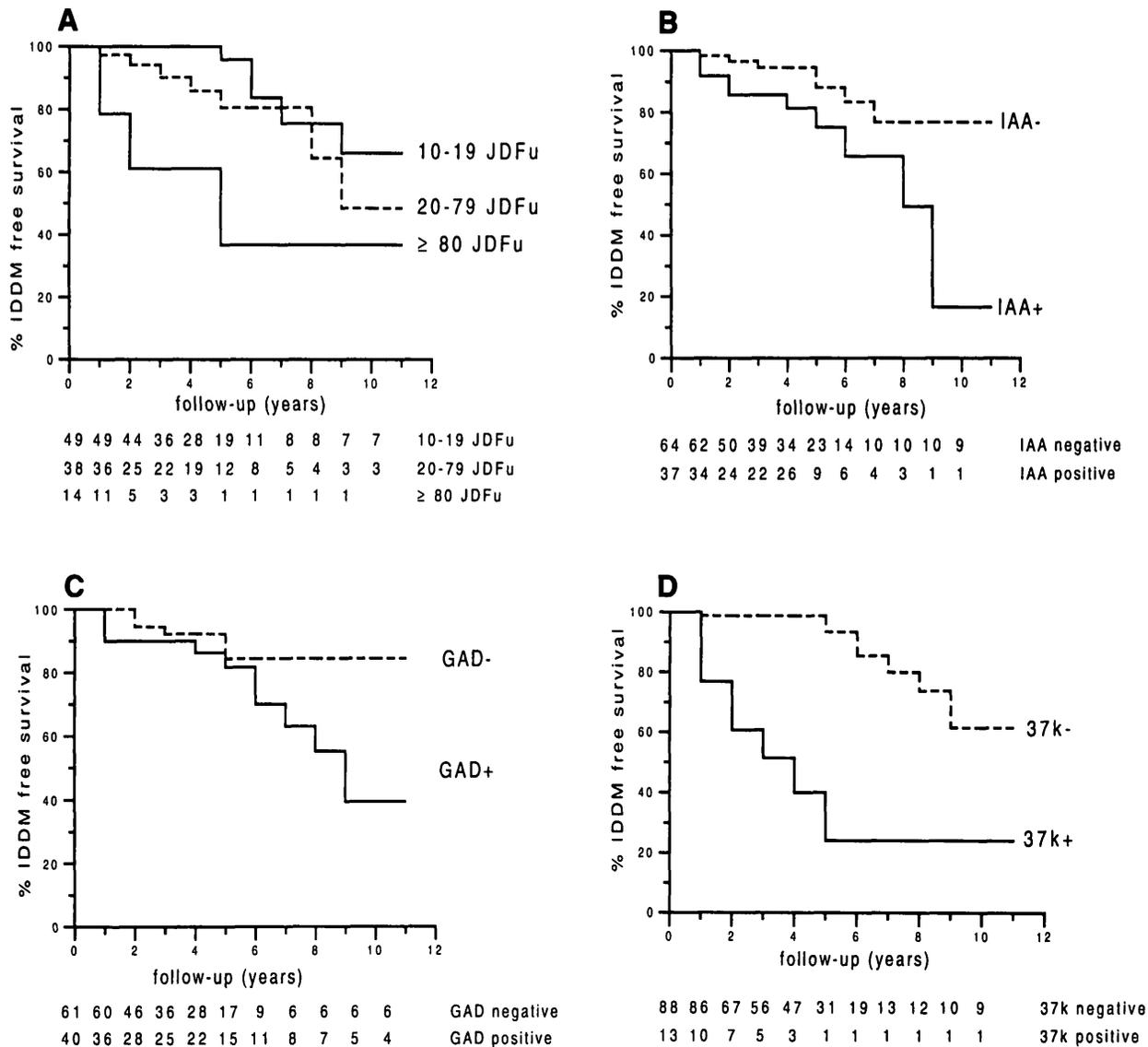
A

% IDDM free survival

follow-up (years)

49	49	44	36	28	19	11	8	8	7	7	10-19 JDFu
38	36	25	22	19	12	8	5	4	3	3	20-79 JDFu
14	11	5	3	3	1	1	1	1	1		≥ 80 JDFu

10-19 JDFu
20-79 JDFu
≥ 80 JDFu

B

% IDDM free survival

follow-up (years)

IAA-
IAA+

| 64 | 62 | 50 | 39 | 34 | 23 | 14 | 10 | 10 | 10 | 9 | IAA negative |
| 37 | 34 | 24 | 22 | 26 | 9 | 6 | 4 | 3 | 1 | 1 | IAA positive |

C

% IDDM free survival

follow-up (years)

GAD-
GAD+

| 61 | 60 | 46 | 36 | 28 | 17 | 9 | 6 | 6 | 6 | 6 | GAD negative |
| 40 | 36 | 28 | 25 | 22 | 15 | 11 | 8 | 7 | 5 | 4 | GAD positive |

D

% IDDM free survival

follow-up (years)

37k-
37k+

| 88 | 86 | 67 | 56 | 47 | 31 | 19 | 13 | 12 | 10 | 9 | 37k negative |
| 13 | 10 | 7 | 5 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 37k positive |

FIG. 3. The cumulative risk of IDDM at 10 years by level of ICAs (*A*), IAAs at entry (*B*), autoantibodies to GAD at entry (*C*), and autoantibodies to 37-kDa antigen (*D*).

cumulative risk of IDDM by 10 years was 27% (CI 0–54%). The group of 27 family members who had at least three of the five autoantibodies tested contributed 14 of the 18 cases in the study (median time to diabetes 2.0 years, range 0.2–8.5 years), and the cumulative risk of IDDM within 10 years was 88% (CI 66–100%) (Fig. 4).

This effect was also seen in family members with moderate levels of ICAs. Of the cohort, 87 had ICAs between 10 and 79 JDF U and 35 had ICAs alone; one of these developed IDDM, giving a cumulative risk of IDDM by 10 years of 6%. An additional 38 had this level of ICA and a single additional antibody; 3 of these developed IDDM, giving a cumulative risk of IDDM within 10 years of 30%. Of the 19 individuals with this level of ICA in association with two or more additional antibodies, 8 developed IDDM, and the cumulative risk of IDDM within 10 years was 84% (CI 56–100%).

The relationship between risk of diabetes and the number of antibodies appeared to be independent of age. The risk increased with the number of antibodies within each age quartile. In the youngest quartile, the cumulative risk of IDDM within 10 years associated with ICAs alone was 0%, with two antibodies was 12%, and with three or more

antibodies was 88% (*P* = 0.003). In the second quartile, the risks were 15, 50, and 100% (NS); in the third quartile, risks were 0, 20, and 63% (NS), and in the oldest quartile, they were 0, 0, and 67%, respectively (*P* = 0.03).

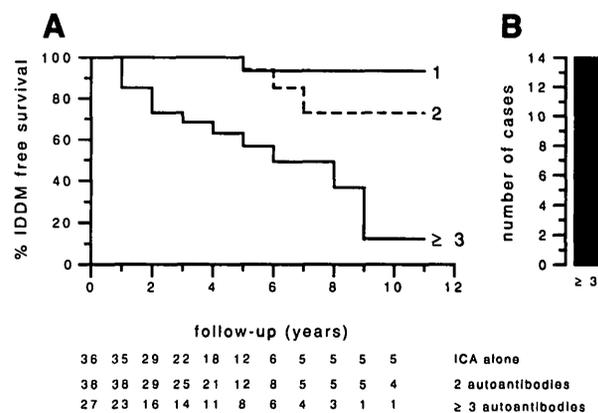Family members with three or more autoantibodies, who

A

% IDDM free survival

follow-up (years)

1
2
≥ 3

B

number of cases

≥ 3 2 1

36	35	29	22	18	12	6	5	5	5	5	ICA alone
38	38	29	25	21	12	8	5	5	5	4	2 autoantibodies
27	23	16	14	11	8	6	4	3	1	1	≥ 3 autoantibodies

FIG. 4. *A*: the cumulative risk of IDDM after 10 years by the number of autoantibodies detected. *B*: the number of individuals who developed IDDM with one, two, or three or more autoantibodies.

were 37- and/or 40-kDa antibody positive, had a median time to diagnosis of 1.5 years compared with 7.2 years in those with ICAs, IAAs, and GAD antibodies in the absence of 37- or 40-kDa antibodies. The cumulative risks of IDDM within 5 years were 64% (CI 37–91%) in the former group and 9% (CI 0–25%) in the latter. By 9 years, however, the cumulative risk of IDDM in those with ICAs, IAAs, and antibodies to GAD in the absence of 37- or 40-kDa antibodies was 100%, and the survival curves over the whole study period were not significantly different.

Changes in autoantibodies before diagnosis. Serial samples were examined from 14 of the family members who developed diabetes. Up to four samples per case were tested. Most individuals showed the same combination of antibody specificities throughout follow-up. The only changes in IAAs and antibodies to GAD were that one child (case 5) was IAA⁻ at study entry but had high levels of the autoantibodies 4 months before diagnosis, and one parent (case 18) had GAD binding of 85% at study entry 8.1 years before diagnosis, but this was within the normal range in later samples. Sequential samples were tested from eight of nine individuals who were initially 37- and 40-kDa antibody negative and who later developed diabetes. These latest samples were taken between 0.3 and 2.7 years before insulin was started. Only one individual (case 10) appeared to develop 37- or 40-kDa antibodies between entry into the study and onset of diabetes. The antibodies were weakly positive for the first time in the last sample taken 2.7 years before diagnosis, having been negative in samples taken 30 and 18 months earlier.

DISCUSSION

All screening procedures aim for high sensitivity, to avoid missing future cases, combined with sufficient specificity to avoid false-positive results and unnecessary treatment. These aims inevitably conflict, because the specificity of a screening method is reciprocally related to its sensitivity. The more confident we become that individuals in a certain category will develop diabetes, the greater the proportion of those at risk we thereby exclude from the possible benefits of intervention. Highly sensitive prediction of IDDM in family members can be achieved with ICAs, provided an assay with a low detection threshold is used (2,3). In the Gainesville study, 13 of 40 future cases had ICAs <10 JDF U at entry into the study (3), while current analysis of the Bart's-Windsor and Bart's-Oxford family studies shows that 7 of 28 future cases were ICA⁻ (<4 JDF U) at entry, and that 3 of these had detectable ICAs during follow-up. We set out to develop an approach that would retain the sensitivity of ICAs as an initial screening procedure, while enhancing their predictive value by combined analysis with other diabetes-associated autoantibodies.

High-titer ICAs offer more specific prediction (2), but raising the ICA threshold from 10 to 20 JDF U only increased the cumulative risk from 43 to 53% after 10 years, while excluding 5 of 18 cases. Some ICAs are inhibited by rat brain homogenate, stain predominantly β-cells (β-cell-selective or restricted ICAs), and carry a lower risk of progression than ICAs that are not inhibited by rat brain homogenate (15,16). These differences in ICA can currently only be assessed in sera with higher titers of ICA; only 44 could be evaluated, of which 7 were inhibited. Although this subtype was associated with a relatively low risk of IDDM, elimination from the

analysis of those with ICAs ≥20 JDF U only raised the cumulative risk from 53 to 61% (not significant).

IAAs and antibodies to GAD and to 37- and 40-kDa islet antigens are all strongly associated with IDDM. IAAs and antibodies to 37- and 40-kDa antigens achieved a significant increase in cumulative risk in ICA⁺ individuals, but in each patient, this was associated with loss of sensitivity. IAAs were the most useful marker in combination with ICAs, increasing the risk at 10 years to 84%, but seven cases were IAA⁻ at initial testing. In contrast with previous studies (17), IAAs did not distinguish those with rapid onset of disease. Antibodies to GAD were present in 66% of those who progressed to diabetes but did not significantly increase the level of risk. Antibodies to 37-kDa antigen were, in contrast, highly specific markers for IDDM, associated with a 76% risk of IDDM within 5 years, but were found in only 50% of future cases. This confirms observations in monozygotic twins discordant for IDDM (12) and patients with polyendocrine autoimmunity (18).

Clear advantages emerged when all the markers were analyzed in combination. We found that 27 of the original cohort and 14 of 18 of those ICA⁺ relatives who progressed to diabetes had two or more additional autoantibodies (Fig. 4). In other words, 78% of future cases came from 27% of the ICA⁺ population. Life-table analysis shows that individuals in this category have an estimated 88% risk of developing diabetes within 10 years, irrespective of the presence or absence of metabolic abnormalities.

ICAs ≥80 JDF U are associated with a very high risk of progression to IDDM, and excluding those with ICA staining inhibited by rat brain homogenate from the analysis increased the cumulative risk to 100% within 5 years. There is, therefore, little scope for improving prediction in this group. Only 6 of 18 individuals who progressed to IDDM fell into this category, however. Since 12 cases came from those with ICAs from 10 to 79 JDF U, conferring a cumulative risk of only 17% within 5 years and 40% within 10 years, this is the category within which we need to improve prediction. Our analysis suggests that this can be achieved by considering antibody markers in combination. Overall, 87 individuals had ICAs from 10 to 79 JDF U, and identification of those with at least two other autoantibody species enabled us to characterize a subgroup of 19 with an 84% risk of diabetes within 10 years. The approach is useful even with ICAs of 10–19 JDF U, because those with two other autoantibodies had a 63% risk of IDDM within 10 years. IAAs and antibodies to GAD proved particularly useful in those with ICAs between 10 and 79 JDF U. The detection of IAAs and/or antibodies to GAD identified over half the cases and were associated with risk at 10 years of 81 and 63%, respectively. Antibodies to 37- and 40-kDa antigens were associated with high titer ICAs and did not improve prediction in those with lower titers.

Because the risk of progression to diabetes is inversely related to age, and because children are more likely to have high levels of ICAs, antibodies to 37 kDa, and multiple antibodies, our findings might simply reflect these associations. This does not appear to be the case. A gradient of risk existed even within the youngest quartile, such that no children with ICAs alone developed IDDM, those with one other antibody had a cumulative risk within 10 years of 12%, and all those with two or more other antibodies developed diabetes. A similar pattern of risk is found at all ages. ICAs alone confer a low risk of IDDM in any age-group, while

individuals with three or more antibodies had a cumulative risk of IDDM of 100% if aged <20 years and of 66% if older than this. Analysis of the risk associated with different levels of ICAs, with IAAs, and with antibodies to GAD showed that the gradient of cumulative risk was similar within each age-group.

These findings imply that the intensity and range of the humoral autoimmune response determines overall risk. Our approach can therefore readily be extended to other candidates on the ever-growing list of anti-islet antibodies (19). Antibody type may, however, influence time to diabetes; antibodies to the 37- and 40-kDa antigens were detected when clinical onset of diabetes was imminent, while the combination of ICAs, IAAs, and antibodies to GAD was associated with a similar risk of diabetes over the 10 years, but was also found in those in whom clinical onset of diabetes was delayed. Antibodies to 37 or 40 kDa therefore appear to be associated with both high-titer ICAs and rapid progression to diabetes. Examination of sequential samples suggests that levels of antibodies to 37- and 40-kDa antigens change little during the disease prodrome, rather than appearing toward the time of diagnosis. This, in turn, suggests that they reflect true differences in the underlying disease process and not just a stage in its development.

An individual's probability of developing diabetes can be set out as a decision tree. Family history is the first major risk determinant, and the sibling of a child with IDDM within our region has an ~3% risk of becoming diabetic within 10 years (1), rising to 43% in those with ICAs ≥10 JDF U, and to 88% with the screening strategy we propose (Fig. 5). Even higher specificity can be achieved in those with loss of the first-phase insulin response in the intravenous glucose tolerance test (4), but metabolic screening of ICA⁺ individuals will miss the majority of the at-risk population, at least from a single screening point (1). Further, those it identifies are likely to have advanced β-cell damage, with a correspondingly reduced margin of benefit from therapy. We have estimated that ICAs are much less predictive of diabetes in those with no family history of the disease (20) and have proposed a screening strategy based on sequential analysis of genetic and immune markers for the general population. In family members, however, a logical approach would be to use ICA as the initial screen, using a low threshold assay, followed by testing for other antibodies in those who screen positive. Metabolic testing could then be used to its best effect within the high-risk category to identify those with and without end-stage prediabetes. The decision tree approach is sufficiently flexible to allow other characteristics, such as age (3), to be taken into account, and could allow precise targeting of those individuals who might benefit most from therapy.

In conclusion, ICAs, despite technical limitations, remain sensitive markers of future IDDM. Prognosis is strongly related to the number of antibody specificities detected. The practical implication is that a single screening test promises to identify >75% of future cases of IDDM in ICA⁺ relatives, including those with relatively low levels of ICAs. The presence of antibodies to the 37- and 40-kDa antigens may supplement metabolic testing as an indication of the rate of progression to diabetes. This approach offers a simple potential means to identify candidates for intensive follow-up and for secondary prevention.
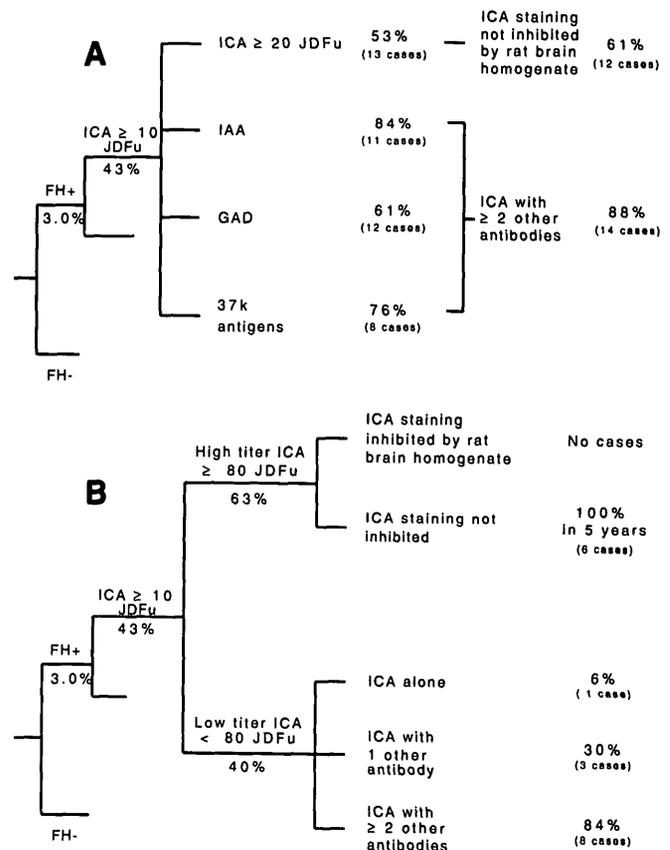

FIG. 5. Two complementary approaches to decision tree analysis of risk in family members with ICAs ≥10 JDF U. The percentages shown represent the cumulative risk of developing IDDM within 10 years based on autoantibodies detected at entry into the study. A: a summary of the results presented in this study. B: a demonstration that the value of combined marker analysis lies mainly in those with lower titers of ICAs.

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