# Can Islet Cell Antibodies Predict IDDM in the General Population?

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**OBJECTIVE** — To evaluate the likely prognostic significance of ICAs in children with no family history of IDDM.

**RESEARCH DESIGN AND METHODS** — We examined the prevalence of ICAs in 2925 English schoolchildren aged 9–13 yr and in 274 age-matched siblings of children with diabetes from the same region, and we compared the estimated risk of progression to diabetes within 10 yr in the two groups.

**RESULTS** — ICAs were present at levels ≥4 JDF U in 2.8% of schoolchildren and 6.6% of siblings and at ≥20 JDF U in 0.8% of schoolchildren and 2.2% of siblings. Although ICAs are only 2–3 times more prevalent in siblings than schoolchildren, the estimated cumulative risk that siblings will progress to diabetes by age 21 is 13 times greater (2.8 vs. 0.21%).

**CONCLUSIONS** — ICAs are unexpectedly prevalent in English schoolchildren, but only a small minority, with this evidence of immune activation directed against islet cells, will progress to diabetes. Although ICAs alone have limited predictive value in the general population, combining two or more predictive tests in series could achieve a level of prediction equivalent to that now obtained in first-degree relatives.

Family studies have confirmed that IDDM develops on the basis of genetic susceptibility, is mediated by autoimmune processes, and typically has a long prodrome. ICAs, the best validated risk markers, are present at high titer (≥20 JDF U) in 2–2.5% of first-degree relatives, and some 35% of these are expected to require insulin treatment within 5 yr (1–3). Risk estimation can be improved if IAA levels and/or the first-phase insulin response to intravenous glucose also are taken into account (4). Prediction of diabetes is now considered sufficiently precise to justify intervention trials in high risk relatives (5,6).

Because 85–90% of all newly diagnosed diabetic children have no immediate family history of the disease (7,8), it is important to establish the prognostic significance of ICAs in the general population. Therefore, we measured the prevalence of ICAs and estimated the risk of progression to diabetes in a sample of schoolchildren aged 9–13 yr compared with age-matched siblings from the same region.

## RESEARCH DESIGN AND METHODS

— We recruited 2925 healthy schoolchildren 9–13 yr of age from 12 middle schools in Oxford and Windsor in southern England. Details of age and sex were obtained from the school and checked with the children, who also were asked whether they had a parent or sibling on insulin. Ethnicity (classified as Europid, Indian subcontinent, Afro-Caribbean, or other) was determined by observation and, where necessary, by asking the child where his/her parents originated.

We compared the schoolchildren with 274 nondiabetic siblings of children with IDDM resident in the same region and 9–13 yr of age at the time of entry into the Bart's-Windsor (105 children) or Bart's-Oxford Family Study (169 children). Recruitment into the Bart's-Windsor Family Study (9) ceased in 1984, and the prospective, population-based Bart's-Oxford Family Study was established in 1985 to identify all children with newly diagnosed IDDM resident in the Oxford Regional Health Authority area and to recruit their families for long-term follow-up (Table 1).

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RECEIVED FOR PUBLICATION 12 FEBRUARY 1992 AND ACCEPTED IN REVISED FORM 13 AUGUST 1992.

ICA, ISLET CELL ANTIBODY; IDDM, INSULIN-DEPENDENT DIABETES MELLITUS; IAA, INSULIN AUTOANTIBODY; JDF U, JUVENILE DIABETES FOUNDATION UNITS; IgG, IMMUNOGLOBULIN G; PBS, PHOSPHATE-BUFFERED SALINE; CV, COEFFICIENT OF VARIATION; CI, CONFIDENCE INTERVAL; MHC, MAJOR HISTOCOMPATIBILITY COMPLEX.

Table 1—*Clinical characteristics of study subjects*

| | Schoolchildren | Siblings of children with IDDM |
|---|---|---|
| *N* | 2925 | 274 |
| Age (yr)* | 11.35 (9.0–13.8) | 11.58 (9.0–14.0) |
| Boy/girl ratio | 1.07 | 1.03 |
| Ethnic origin (%) | | |
| Europid | 88 | 99 |
| Indian subcontinent | 8 | |
| Afro-Caribbean | 2 | |
| Other | 1 | |
| Affected first-degree relative (%) | 1.8 | 100 |
| Date of sampling (yr) | 1989–1990 | 1979–1990 |

*Values for age are means with range in parentheses.

### Sample collection

Venepuncture was performed on schoolchildren at the schools between April 1989 and November 1990 after the application of lignocaine/prilocaine cream (EMLA Cream, Astra, Kings Langley, England). Samples were taken between 0930 and 1530. Venous blood samples (5–10 ml) were collected into plain tubes and separated, and sera were frozen on dry ice within 60 min. Blood for random glucose estimation was collected into tubes containing fluoride oxalate, and plasma was separated and frozen on dry ice. Serum and plasma samples were stored at −20°C until assayed.

Venepuncture was performed on siblings at home visits. The results of the first sample taken from each child were used in this study. Serum samples were stored at −20°C. Initial screening for ICAs was performed within 6 mo of samples being taken, but ICAs in positive samples taken before 1987 were retrospectively quantified in JDF U.

### Follow-up

The parents of all schoolchildren with detectable ICAs were contacted by post, and the children were invited to give a second venous blood sample 7–14 mo after the initial screening and to remain under regular follow-up. Because all cases of newly diagnosed diabetes in children resident in the Oxford region are reported to the Bart's-Oxford Study as part of a continuing incidence survey (10), we are also in a position to identify children without detectable ICAs who develop IDDM.

### Ethical approval

Approval for the study was granted by the Central Oxford and East Berkshire Health Authority Research Ethics Committees, and the Oxfordshire and Berkshire County Council Education Departments. Written consent was obtained from the parents of all the children.

### ICA

Serum samples from schoolchildren and siblings were tested in the same assay. Undiluted sera were screened for conventional ICA-IgG by means of indirect immunofluorescence on 4-μm cryostat sections of blood group O human pancreas (11). Positive samples then were titered by doubling dilutions in PBS on tissue obtained from a single pancreas under standard incubation conditions (12). 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 (13). The 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.

### IAA

IAAs were assayed by using a modification of the methods described by Palmer and Kurtz (14,15). Sera were extracted by using acid-washed, Dextran-coated charcoal to remove endogenous insulin. Serum then was incubated at 4°C with 40 mM PBS and radiolabeled human insulin (Amersham, Buckinghamshire, England), with and without cold insulin (Humulin S, Lilly, Indianapolis, IN). The Ig fraction was precipitated by using polyethylene glycol 6000 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. IAA positivity was defined as >3 SD above the population mean.

### Plasma glucose

Plasma glucose was measured by the hexokinase/glucose-6-dehydrogenase method by using an autoanalyzer (CPA biochemical analyzer, Coulter, Hialeah, FL). The interassay CV was 4.6 and 4% at 2.5 and 15 mM, respectively.

### Estimation of risk of developing diabetes

The cumulative incidence of IDDM between ages 10 and 20 in the Oxford region was used to estimate the risk for the schoolchildren of developing diabetes before age 20. This was calculated from the age-specific rates of IDDM in the region in 1985–1986 (10) and the Registrar General's midyear estimates of population (Office of Population Censuses and Surveys, unpublished). The risk of developing diabetes between ages 10 and 20 in siblings was estimated from life tables derived from data from 1003 siblings in the Bart's-Windsor and Bart's-Oxford Family Studies (16).

Table 2—*Expected effect of differences in overall risk of disease*

| | SCHOOL-CHILDREN | SIBLINGS |
|---|---|---|
| RISK OF IDDM (%) | 0.21 | 2.8 |
| CALCULATED POSITIVE PREDICTIVE VALUE OF ICA ≥4 JDF U (%) | 9 | 57 |
| CALCULATED POSITIVE PREDICTIVE VALUE OF ICA ≥20 JDF U (%) | 34 | 88 |

### Statistical methods

CI were calculated for a Poisson distributed variable. Differences between recruited and nonrecruited children were assessed by $\chi^2$ testing. The expected effect of differences in disease prevalence on the positive predictive value of ICAs was based on the mathematical formula derived from Bayes' theorem of conditional probabilities (17).

$$\frac{\text{Positive predictive value} = \text{sensitivity} \times \text{prevalence}}{(\text{sensitivity} \times \text{prevalence}) + (1\text{-specificity})(1\text{-prevalence})}$$

This equation was applied to the published figures for sensitivity and specificity of ICAs at initial screening for the development of IDDM within 10 yr in siblings in the Bart's-Windsor Family Study (1). For ICAs ≥4 JDF U, sensitivity was 91% and specificity was 98%, and for ICAs ≥20 JDF U, sensitivity was 73% and specificity was 99.7%.

## RESULTS

### Recruitment

The overall recruitment rate in schoolchildren was 66%, varying between 57 and 72% in different schools. No differences in age and sex existed between children who did and did not volunteer for venepuncture in a sample of three randomly selected schools (total enrollment 1089).

### ICA

ICAs ≥4 JDF U were found in 82 of 2908 schoolchildren (2.8%, 95% CI 2.3–3.5%), and 24 had ICAs ≥20 JDF U (0.8%, CI 0.5–1.2%). Only 2 of 82 schoolchildren with ICAs ≥4 JDF U had an affected first-degree relative, and, if those were excluded, the prevalence of ICAs was 80 of 2856 (2.8%) ≥4 JDF U and 24 of 2856 (0.8%) ≥20 JDF U. In contrast, 18 of 274 siblings of children with diabetes had ICAs ≥4 JDF U on initial testing, and 6 of 274 had ≥20 JDF U (6.6%, CI 3.9–10.4% and 2.2%, CI 0.8–4.8%, respectively).

The prevalence of ICAs ≥4 JDF U did not differ significantly between male and female schoolchildren (2.2 vs. 3.4%). ICAs ≥20 JDF U were found in 1.1% boys and 0.6% girls (CI 0.6–1.7 and CI 0.3–1.1%, respectively, NS). Six of 334 non-Europid children had ICAs ≥4 JDF (1.8%, CI 0.7–3.9%).

### Follow-up

To date, second samples have been obtained from 55 of 82 children with ICAs ≥4 JDF U. Overall, 38 of 55 have detectable ICAs on repeat testing. Of the 17 children in whom ICAs were negative on repeat testing, 13 had low levels (<10 JDF U) on initial testing; the remaining 4 individuals had 15 and 23 JDF U. In contrast, 15 of 17 who had had ICAs ≥20 JDF U on initial testing had ICAs >15 JDF U on the second test.

Two schoolchildren, (a 13-yr-old girl with ICAs >80 JDF U and high levels of IAA, and an 11-yr-old boy with ICAs 10 JDF U) have subsequently developed IDDM, both within 3 mo of screening. The first child had no family history of diabetes; the father of the second had insulin-treated diabetes diagnosed at age 35.

### IAA

IAA levels were >3 SDs above the population mean in 2 of 78 (2.6%) schoolchildren and 2 of 15 (13.3%) siblings with ICAs ≥4 JDF U ($P = 0.1$).

### Random plasma glucose

The mean random plasma glucose from 2866 schoolchildren was 5.44 mM (SD 0.81).

### Risk of developing diabetes

The cumulative incidence of IDDM between ages 10 and 20 in the general population in the Oxford region is 0.21%. In life table analysis, the projected risk for a sibling of a child with IDDM of developing the disease between the same ages is 2.8%.

### Expected effect of differences in overall risk of disease

The calculated positive predictive value of ICAs ≥4 JDF U and ≥20 JDF U in the two populations is shown in Table 2. Observe, for example, that even at ICA levels ≥20 JDF U, the expected positive predictive value in schoolchildren is only 34%.

**CONCLUSIONS**— Our study revealed a high prevalence of ICAs in healthy schoolchildren 9–13 yr of age in the UK, 2.8% of whom had detectable antibody levels. Most other studies have reported much lower rates, ranging from 0.24% in The Netherlands (18), 0.3% in Spain (19), Japan (20), and Florida (21) to 1.6% in France (22). Only Sweden (3.0%) (23) and Finland (4.1%) (24) have reported higher rates, but these countries have an incidence of IDDM 1.5–2.5 times higher than that in England. These studies may not be strictly comparable, however, because of differences in age range and techniques for ICA measurement (25).

Previous studies have suggested that the prevalence of ICAs within a given population exceeds the anticipated cumulative incidence of IDDM (18,21, 24). We have confirmed this. Siblings of children with IDDM are 13 times more likely to develop IDDM between the ages of 10 and 20 than children in the general population, yet they have only twice the prevalence of ICAs, whether at low or

high titer. This implies that the predictive power of ICAs in the general population is about six times less than in family members. Because 89% of siblings with ICAs ≥20 JDF U in the Bart's-Windsor Family study developed IDDM within 10 yr (1), some 15% of children with no family history of diabetes and ICAs ≥20 JDF U could be expected to develop diabetes before age 20. Further, because a population-based study has suggested that only some 40% of cases of IDDM are diagnosed after that age (26), it seems likely that at least 70% of children reaching puberty with high titers of ICAs never will develop IDDM.

This empirical observation could have been predicted on theoretical grounds. The two groups have widely differing risks of diabetes, and the positive predictive value of a test depends on the prevalence of disease in the population tested. Markers with a high, positive predictive value in high-risk populations are less useful in populations with low disease prevalence, and even highly specific tests generate a high proportion of false positives when applied to a low-risk population. The effect of differences in disease prevalence can be calculated from the formula given above. In this study, the 13-fold difference in risk of IDDM between siblings and schoolchildren would be expected to result in a 6-fold reduction in the positive predictive value of ICAs ≥4 JDF U and at least a 2- to 3-fold fall in that of ICAs ≥20 JDF U between the two populations (Table 2). The limited prognostic value of ICA is intrinsic to any predictive test applied to a population with a low disease prevalence. This constraint cannot be overcome by improving the sensitivity and specificity of the test, unless the improbable goal of 100% specificity could be achieved. For example, even a test with 95% sensitivity and 99.8% specificity would have a positive predictive value of only 50% in a population with a predicted disease prevalence of 0.21%.

Our findings show that ICA alone can be only weakly predictive of diabetes in the general population. Fortunately, the effect of low disease prevalence can be overcome by using two or more tests, each insufficiently specific to be used in isolation, in series. This involves a two- (or more) step procedure whereby one test is used to identify a high-risk subpopulation to which the second test then can be applied. ICAs, measured in an assay with low detection threshold to maximize sensitivity, offer a cost-effective initial screening method to which, for example, analysis of genetic markers could be added. Genetic markers of susceptibility to IDDM are prevalent in the population as a whole and, therefore, have a limited predictive value if used in isolation. For example, possession of an aspartate residue at position 57 on either DQβ chain confers resistance against IDDM, but nonaspartate 57 homozygosity was found in 20% of control subjects in one North American population, limiting the absolute risk of this marker to only 2–3% (27). If, however, nonaspartate homozygosity was used in sequence with ICA screening, (the order in which these tests are performed does not matter) (17), prediction could be considerably enhanced (Fig. 1). Combinations of alleles could improve on this because DR3/DQ3.2,Dw4 or DR3/DQ3.2,Dw10 heterozygotes have an absolute risk of 6–7% (28,29). Use of such markers in sequence with ICAs in children with no family history of diabetes could achieve a positive predictive value at least as high as that of ICAs in siblings.

Prediction of progression to IDDM also could be improved by combined analysis of immune markers. IAAs enhance the predictive value of ICAs in family members (30), and other immune markers have recently been described (31). ICA positivity may itself be heterogeneous, with several distinct specificities, and ICAs that stain whole islets may carry a greater risk of progression than those that are selective for β-cells (32,33). Autoantibodies to a 64,000-$M_r$ islet protein, recently reported to be the enzyme glutamic acid decarboxylase

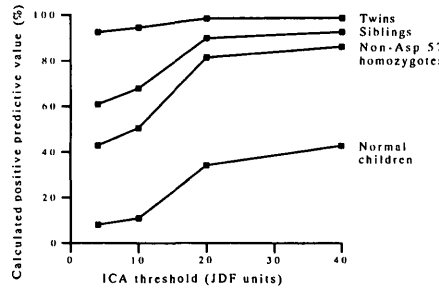


**Figure 1**—*The effect of differences in disease prevalence on calculated positive predictive value of ICAs. The positive predictive value for the development of IDDM within 10 yr has been calculated by using four thresholds of ICA for discordant monozygotic twins (lifetime risk of IDDM, 30%) (40), unaffected siblings (lifetime risk, 5%) (9), children with no family history of IDDM (lifetime risk, 0.3%) (10), and subjects homozygous for nonaspartate 57 (non-Asp$^{57}$) (lifetime risk, 2–3%) (27). The sensitivity and specificity of ICAs at initial screening for development of IDDM within 10 yr as found in the Bart's-Windsor Family Study have been used in the equation.*

(34), circulate in patients with IDDM (35) and have been detected in prediabetes (36), although the assays are still too cumbersome and expensive for population screening. Antibodies to this autoantigen may be heterogeneous, as revealed by analysis of antibody reactivity to tryptic fragments of the 64,000-$M_r$ protein (31), and antibodies binding the 37,000-$M_r$ fragment may prove better predictive markers than those binding the intact protein or 50,000-$M_r$ fragment (37). We may anticipate, therefore, that more discriminant assays of ICAs and other autoantigens will become available for combined use in population screening programs. Combined testing of genetic, immune, and metabolic markers (38) of risk therefore promises precise prediction of IDDM, even in the absence of a family history of the disease.

Incident cases of childhood diabetes represent the tip of an iceberg of susceptibility. Only a very small proportion of those carrying known genetic sus-

ceptibility alleles within the general population are likely to develop IDDM, and our study suggests that only a minority of those with high titers of ICAs, the best validated marker of risk, can be expected to progress to β-cell failure. This implies that the immune attack must pass through several stages, each perhaps involving multiple genetic or environmental determinants, before diabetes can develop. For example, the development of diabetes in the NOD mouse model of IDDM is influenced by genes in the MHC complex, but genes on other chromosomes have been shown to control the intensity and rate of progress of insulitis (39). The timing of exposure to environmental precipitants may be critical and is most commonly thought to occur in utero or the early postnatal period. Improved understanding of the natural history of ICA positivity, particularly in its early stages, may help direct the search for environmental agents, and indeed, it would seem logical to examine the role of such agents within the population that develops ICAs rather than within the subgroup that develops diabetes. Finally, as and when safe and effective forms of immune intervention become available, we can look forward to the day when population screening will form the basis for programs to eradicate childhood diabetes.

**Acknowledgments**— This study was supported by the British Diabetic Association, the Medical Research Council, and the Joint Research Board of St. Bartholomew's Hospital.

We thank the pupils and staff of Bayswater, Bishops Kirk, Donnington, Headington, Isis, Lawn Upton, Marston, Summerstown, and Temple Cowley middle schools in Oxford and Dedworth and St. Edwards Free Ecumenical and Trevelyan middle schools in Windsor for enthusiastic cooperation; Kate Isitt and colleagues from the Arts Education School for help with recruitment; all fieldworkers and others involved in sample collection; and Dr Aidan McFarlane, Community Pediatrician in Oxford.

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