Successful Prospective Prediction of Type 1 Diabetes in Schoolchildren Through Multiple Defined Autoantibodies

An 8-year follow-up of the Washington State Diabetes Prediction Study

James M. LaGasse, ms[1]
Michael S. Brantley[1]
Nicola J. Leech, md[2]
Rachel E. Rowe, md[2]
Stephanie Monks, phd[3]

Jerry P. Palmer, md[3]
Gerald T. Nepom, md, phd[2]
David K. McCulloch, md[3,4]
William A. Hagopian, md, phd[1,3]

OBJECTIVE — Almost 90% of type 1 diabetes appears in individuals without a close family history. We sought to evaluate the best current predictive strategy, multiple defined autoantibodies, in a long-term prospective study in the general population.

RESEARCH DESIGN AND METHODS — Autoantibodies to pancreatic islets (islet cell antibodies [ICAs]) and defined autoantibodies (d-aab) to human GAD, IA2/ICA512, and insulin were tested in 4,505 Washington schoolchildren. Eight years later, 3,000 (67%) subjects were recontacted, including 97% of subjects with any test >99th percentile.

RESULTS — Six subjects developed diabetes (median interval 2.8 years), all from among the 12 individuals with multiple d-aab, representing 50% positive predictive value (95% CI 25–75%) and 100% sensitivity (58–100%). Among the others, diabetes occurred in 0 of 6 with one d-aab plus ICA, 0 of 26 with ICA only, 0 of 7 with one d-aab equaling the 99th percentile and another d-aab equaling the 97.5th percentile, 0 of 86 with one d-aab, and 0 of 2,863 with no d-aab or ICA. Adjusted for verification bias, multiple d-aab were 99.9% specific (99.86–99.93%). At this age, new d-aab seldom appeared. Once present, d-aab usually persisted regardless of disease progression, although less so for insulin autoantibodies. Insulin secretion by sequential glucose tolerance testing remained normal in four multiple d-aab subjects not developing diabetes. Of children developing diabetes, five of six (83%) would be included if HLA-DQ genotyping preceded antibody testing, but HLA-DQ did not explain outcomes among high-risk subjects, even when considered along with other genetic markers.

CONCLUSIONS — Multiple d-aab were established by age 14 years and prospectively identified all schoolchildren who developed type 1 diabetes within 8 years.

Type 1 diabetes is an organ-specific autoimmune disease with aberrant immune responses to specific β-cell autoantigens. Worldwide incidence appears to be increasing (1). Prevention is important because diabetes interrupts normal development in children and carries the threat of severe complications in the most active period of life (2).

Both environmental and genetic factors play etiological roles. The most informative genetic locus, HLA class II, confers about half of the total genetic risk (3) but has low positive predictive value (PPV) when used alone in the general population (4,5). Autoantibodies provide a practical readout of β-cell autoimmunity, are easily sampled in venous blood, and have become a mainstay of type 1 diabetes prediction efforts. Initially described in terms of the islet cell antibody (ICA) immunofluorescence assay on pancreatic sections, autoantibodies are now often described in terms of defined ICA target antigens, such as insulin (6,7), GAD (8), and the tyrosine phosphatase homologue IA2/ICA512 (9–11).

Autoantibodies are useful to detect developing type 1 diabetes in close relatives of diabetic patients, whose risk is ~3%. However, most cases are sporadic rather than familial (12), necessitating general population screening. This has been difficult in part because the observed autoantibody prevalence greatly exceeds the low disease prevalence in nonrelatives (13–16), leading to high false-positive rates.

Single defined autoantibody (d-aab) positivity may result from persistent memory B-cells in lymph nodes or bone marrow after brief transient insulitis not resulting in clinical diabetes. Because different d-aab appear sequentially over time during prediabetes (17), the presence of multiple d-aab may mark more persistent insulitis and greater diabetes risk. In fact, a strategy based on multiple positive d-aab works well in first-degree relatives, including newborns (18,19). Population-based cross-sectional data (20) are consistent with this strategy being reasonably predictive in the general population. This outcome forms the rationale for our prospective general population study (21) and those underway elsewhere (5,22–24). The overall goal is to efficiently detect prediabetes while minimizing both family

anxiety and unnecessary exposures to preventive therapy (2).

Combined use of the three most informative d-aab tests (GADA, IA2/ICA512A, and IAA) in a large long-term prospective study of the general population has previously not been reported. Here we report initial screening and median 8-year follow-up data from the Washington State Diabetes Prediction Study (21), in which 4,505 children without a type 1 diabetic relative were tested using standardized high-throughput autoantibody assays for all three major d-aab as well as immunoflorescent ICAs. The highest-risk subjects were also genotyped at four loci of known type 1 diabetes risk (3). Fully 67% of subjects were successfully recontacted. The results confirm high type 1 diabetes risk among individuals with multiple antibodies to the three major diabetes autoantigens (GAD, IA2/ICA512, and insulin) and show that this strategy can successfully predict type 1 diabetes among schoolchildren.

RESEARCH DESIGN AND METHODS

Patient and sera
In 1990–1993, blood was drawn with informed consent from 4,505 healthy schoolchildren (median age 14 years [range 12–18], 58% female, 82% Caucasian, 5% Hispanic, 3% black, 6% Asian, and 3% Native American), recruited as described (21). The prevalence of antibodies and of diabetes, as well as sensitivity and PPV of multiple d-aab, were unchanged by including the 62 schoolchildren with a first-degree type 1 diabetic relative ascertained during standard recruitment. Therefore, consistent with the original study design (21), these subjects were not reported. Under continuing Institutional Review Board approval, subjects were recontacted after a median of 8 years (range 6–11) to ascertain disease status. All subjects initially having any autoantibody and a 331-subject random subset among antibody-negative subjects (21) were invited for repeat sampling. Subjects with multiple antibodies were sampled more frequently. Overall, 58% of invited subjects provided additional sera, averaging 2.8 samples per subject.

Radiobinding autoantibody assays
Human $GAD65_{1-585}$ (complete protein) and human $IA2/ICA512_{603-979}$ (cytoplasmic portion) were labeled with ^{35}S-methionine as described (25). High-performance liquid chromatography–purified human ^{125}I-monoiodo-Tyr_{A14}-insulin was purchased. Each serum was mixed with labeled antigen and incubated in triplicate at 4°C using antigen-specific protocols. Precipitation of bound antigen and counting proceeded in 96-well Millipore Multiscreen membrane plates. Five standard sera included on each plate were used to calculate both antibody indexes and extensive quality control parameters as detailed elsewhere (25). Values from the 4,505 healthy schoolchildren sera were used to define 99th and 97.5th percentile threshold cutoffs for each assay (25). Only 99th percentile thresholds were used unless otherwise noted. The World Health Organization/Juvenile Diabetes Foundation (JDF) world standard serum for ICA, GADA (GAD index 1.00, equivalent to 800 GADA units), and IA2/ICA512A (ICA512 index 1.00, equivalent to 500 IA2/ICA512A units) (26) was used in every GADA and IA2/ICA512A assay (25). Because the 99th percentile threshold for IA2/ICA512A was very low (index 0.007), all samples with IA2/ICA512A indexes between the threshold and the low positive standard were retested before final designation. This ultimately resulted in somewhat fewer positive samples for IA2/ICA512A than for the other antigens, where similar retesting in all cases confirmed antibody positivity. The IAA index positive serum used throughout the study was chosen as a moderately positive sample from a newly diagnosed child. These assays achieved sensitivities of 78, 58, and 21% and specificities of 94, 99, and 100% (97.5th percentile thresholds) for GADA, IA2/ICA512A, and IAA assays, respectively, in the 2001 Centers for Disease Control–sponsored Diabetes Autoantibody Standardization Program Workshop (27).

ICA
ICAs were measured by indirect immunofluorescence on sections from one blood group O frozen human pancreas used throughout the study. Coded slides were evaluated by two independent observers. Each assay included internal standards. Samples were diluted sequentially to determine end point titer for conversion to

JDF units (JDFU). Assay standard deviation was 1.0 titration steps, corresponding to a coefficient of variation of 15%. The laboratory had a consistency and specificity rating of 100% and a sensitivity rating of 81% in ICA proficiency workshops (28). The lower limit of detection was 1 JDFU, and our 99th percentile threshold cutoff was 4 JDFU, consistent with general population percentile levels observed in Germany and Florida (22,29).

Genotyping
HLA-DQB1 and -DQA1 were each typed by direct sequencing of exon 2 after PCR using published primers (5,30). MIC-A was typed by PCR (31) followed by direct sequencing across a coding region $(GTC)_n$ polymorphism. Insulin gene 5′ variable number of tandem repeats (VNTR) typing used PCR/restriction fragment–length polymorphism at a polymorphic -23 *Hph*I site (3). CTLA-4 genotyping at the 3′ untranslated region $(AT)_n$ polymorphism used PCR (32) followed by electrophoresis on LICOR sequencing gels (33).

β-Cell function testing
Consenting subjects underwent repeated evaluation of insulin secretion by intravenous glucose tolerance testing, reported as acute insulin response to glucose (AIR_g) during the first 10 min after an intravenous glucose bolus. The first percentile for insulin secretion among 93 normal young adults (mean age 28 years) was ~100 pmol/l (34).

Statistical analysis
Significance of categorical variable differences between small groups was estimated by Fisher's exact test. Because of the small sample sizes of subjects with multiple d-aab or developing diabetes, 95% CIs for sensitivity, PPV, and disease prevalence in antibody-negative individuals were calculated as exact intervals based on the binomial distribution (35). Ascertainment of diabetes status was achieved for nearly all children with at least one antibody >99th percentile but only 67% of antibody-negative subjects. Hence, the estimate for specificity and its 95% CI were adjusted for verification bias using the procedure of Begg and Greenes (36). This procedure could not be used to adjust for verification bias for the CI for sensitivity because all subjects developing

Table 1—*Twelve subjects positive for two or more d-aab at initial sampling*

Subject	Sex	Ethnicity	Age screened	Years to dx	GADA	ICA512A	IAA	ICA	HLA-DQ haplotypes	ins5′ VNTR	CTLA-4	MIC-A
7197	M	Caucasian	14.4	6.1	**0.09**	**1.52**	0.00	158	A0501-B0301; A0301-B0302	**C/C**	**102** 120	**5.0** 6.0
7664	F	Caucasian	16.9	3.0	**0.57**	0.00	**0.08**	19	**A0501-B0201; A0501-B0201**	**C/C**	104 106	**5.1 5.1**
10211	F	Hispanic	13.1	1.3	**0.09**	**1.06**	*0.04*	39	**A0501-B0201; A0301-B0302**	C/N	084 084	4.0 4.0
10262	F	Caucasian	15.3	2.4	**0.25**	**0.59**	**0.68**	19	A0401-B0402; **A0301-B0302**	**C/C**	084 **102**	**5.0** 9.0
21038	F	Caucasian	14.5	3.2	**0.35**	**0.25**	**0.05**	21	A0401-B0402; **A0301-B0302**	**C/C**	084 **102**	**5.0 5.1**
20703	M	Caucasian	12.7	0.1	*0.05*	**0.36**	**0.05**	6	**A0501-B0201; A0301-B0201**	**C/C**	084 **102**	**5.0 5.1**
7035	M	Caucasian	17.8	Healthy	**0.75**	0.00	**0.25**	19	**A0501-B0201; A0201-B0303**	**C/C**	**102 102**	4.0 9.0
9347	M	Caucasian	13.7	Healthy	**0.41**	0.00	**0.09**	11	**A0501-B0201; A0101-B0501**	C/N	084 **102**	**5.1 5.1**
7147	F	Caucasian	16.3	Healthy	**0.27**	0.00	**0.11**	4	A0401-B0402; **A0301-B0302**	**C/C**	084 084	**5.0** 9.0
11215	F	Caucasian	12.6	Healthy	**0.97**	**0.02**	**0.18**	158	**A0501-B0201; A0301-B0302**	**C/C**	084 084	**5.0 5.1**
9270	F	Caucasian	14.5	Healthy	**0.09**	**0.10**	0.00	4	A0101-B0603; **A0301-B0302**	**C/C**	122 122	**5.1** 6.0
11320	F	Caucasian	13.5	Healthy	**0.13**	**0.21**	**0.01**	70	**A0301-B0302; A0301-B0302**	**C/C**	124 **102**	4.0 6.0

Detailed immunogenetic analysis of 12 subjects with two or more defined autoantibodies at initial sampling. Autoantibody indices above the 99th percentile are in bold, and those above only the 97.5th percentile are in italics. For all genotypes, bolding denotes diabetes risk. Overall HLA DQ genotypes were assigned as susceptible or resistant based on published relative risks. The insulin gene promoter was typed by PCR/restriction fragment–length polymorphism. C, cutter (susceptible); N, noncutter (resistant) (41). For CTLA-4, 3′ untranslated region microsatellite allele 102 is in bold to indicate susceptibility, as shown in other autoimmune diseases (35). MHC Class I Chain Associated (MIC-A) coding region microsatellite alleles 5.0 and 5.1 are in bold to denote susceptibility (34). Ins, insulin; dx, diagnosis.

diabetes were positive for multiple d-aab. However, because 0 of 2,863 antibody-negative subjects developed diabetes, it is unlikely that any cases would have been ascertained, even given complete follow-up of the additional 1,502 antibody-negative subjects, leading to the same estimate and 95% CI that we have provided. This result is further supported by the incidence of cases in our sample (6/4,505 = 0.13% within 8 years from age 14–21 years), identical to published Swedish incidence data (37). The underlying genetic risk of type 1 diabetes in Washington State is similar to that in Sweden (21).

RESULTS

Patterns of diabetes-related defined autoantibodies in the general population
GADA, IA2/ICA512A, and IAA were tested in 4,505 schoolchildren (median age 14 years) using the high-throughput microtiter plate assay. Of the 114 subjects with any d-aab at first sampling, 89% had only one d-aab, and 11% had multiple d-aab. Of those with only one d-aab, 41 had GADA only, 21 had IA2/ICA512A only, and 40 had IAA only. Of those with more than one d-aab, four had GADA and IA2/ICA512A, four had GADA and IAA, one had IA2/ICA512A and IAA, and three had all three d-aab. Overall, 12 (0.3%) of the entire cohort had multiple d-aab, 102

(2.3%) had a single d-aab, and 4,391 (97.5%) were negative for all d-aab.

Immunoflourescent ICA testing
ICAs were measured in 4,186 of 4,505 sera (93%). Levels equaling 1 JDFU were detected in 75 of 4,186 (1.8%). The 99th percentile threshold for ICA was 4 JDFU, present in 47 of 4,186 subjects (1.1%). Overall, like subjects with one d-aab, most subjects with ICAs had no other autoantibodies. Of the 47 sera with ICA ≥ 4 JDFU, 3 had triple d-aab, 9 had double d-aab, 8 had single d-aab, and 27 had no d-aab. A total of 5 of the 27 subjects with ICA but no d-aab had ICA ≥19 JDFU. Subjects with multiple d-aab in all cases had detectable ICAs, which is not surprising because ICA component reactivities include GAD, IA2/ICA512, and insulin (7,38,39). Of the 4,505 schoolchildren cohort, a total of 141 (3.1%) had ICAs and/or d-aab.

Correlation between autoantibodies and diabetes during a long-term follow-up
We recontacted 3,000 of the 4,505 (67%) subjects to ascertain diabetes status with median follow-up interval of 8 years. Fully 137 of 141 (97%) subjects with any autoantibody and 2,863 of 4,364 (67%) of all antibody-negative subjects were included. Only six (6 of 3,000, 0.20%) had developed clinical type 1 diabetes. Remarkably, only subjects with multiple d-

aab at initial sampling had developed diabetes, representing 100% sensitivity (95% CI 58–100%). Multiple d-aab had a 50% PPV (95% CI 25–75%) for clinical diabetes within 8 years. Among other risk groups, based on combinations of d-aab and/or ICA, diabetes occurred in 0 of 6 with one d-aab plus ICA, 0 of 26 with ICA but no d-aab, 0 of 7 with one d-aab equaling the 99th percentile and another d-aab equaling the 97.5th percentile, and 0 of 86 with only one d-aab. Of those with no d-aab or ICA, 0 of 2,863 had diabetes, representing 99.9% specificity (95% CI 99.86–99.93%). ICA (≥4 JDFU, 99th percentile) was equally sensitive (100%) but much less predictive (13%) than multiple d-aab, similar to findings in family studies (40). Requiring higher ICA titers (≥9 JDFU, 99.5th percentile) increased predictive value somewhat, but decreased sensitivity (5 of 21 developed diabetes, sensitivity 83%, PPV 24%).

One subject positive for two d-aab developed gestational diabetes that resolved after delivery. She has now been normoglycemic for 18 months. Another subject positive for a single d-aab developed gestational diabetes during each of two pregnancies but is currently nonpregnant and normoglycemic. All four of her grandparents have been diagnosed with type 2 diabetes, and her BMI before the current pregnancy was 32.3. Both factors carry a high risk of type 2 diabetes. Nevertheless, we cannot absolutely rule out autoimmu-

nity as the cause of gestational diabetes in these subjects.

Use of genetic markers to further define risk in subjects with multiple d-aab

For all 12 subjects with multiple d-aab, genotypes were determined at several known diabetes risk loci, including HLA-DQ, the insulin promoter (41), CTLA-4 (32), and MIC-A in the HLA class I region (31). The first six subjects displayed in Table 1 developed clinical diabetes, whereas the last six remained normoglycemic. After assigning allelic risk based on published studies, there were no significant associations (in this small number of subjects) with disease outcome at single loci such as HLA-DQ (5/6 vs. 5/6), the insulin promoter (5/6 vs. 5/6), MIC-A (3/6 vs. 2/6), or CTLA-4 (4/6 vs. 3/6). HLA-DQ genotype could not predict disease among subjects with multiple d-aab, and presence of diabetes-associated alleles at the other typed loci could not explain the observed exceptions to DQ prediction patterns.

Persistence of autoantibodies among subjects sampled repeatedly

A total of 286 teenage subjects underwent repeated sampling over an average period of 3.2 years (maximum 8.9 years). Included were 47 subjects initially with ≥1 antibody and 3 who later developed diabetes. More than 95% of subjects did not change antibody status for each of GADA, IA2/ICA512A, and IAA. A new d-aab appeared in five subjects (three GADA, one IA2/ICA512A, and one IAA). The one subject who developed IA2/ICA512A later developed diabetes. d-aab disappeared in seven subjects (one GADA, one IA2/ICA512A, and five IAA). None developed diabetes. d-aab fluctuated (changed status more than once) in five subjects (one GADA, one IA2/ICA512A, and three IAA). None developed diabetes. Overall, IAA changed status the most, in 9 of 12 subjects versus only 5 of 14 for GADA ($P = 0.05$ vs. IAA) and only 3 of 10 for IA2/ICA512A ($P = 0.05$ vs. IAA). Subjects initially with ≥1 antibody were sampled more frequently (median of five times) than subjects initially without antibodies (median of two times). Therefore, observed antibody changes in 14 of 17 cases reflected evolving status for one antibody in subjects already positive for another antibody rather than initial sero-

conversions among the general population. Results suggest that established autoantibodies are for the most part stable but that some changes occur within this age-group, especially for IAA.

Individual cases are plotted in Fig. 1, which shows evolution of sequential d-aab levels in the nine subjects who agreed to frequent testing. Of the subjects shown, five initially with multiple d-aab do not yet have diabetes, two with ICA but no d-aab do not yet have diabetes, one with a single d-aab does not yet have diabetes, and one with three d-aab developed diabetes after 3 years. Other subjects developing diabetes provided only a single sample before diagnosis and are therefore not shown. Sequential data for each antibody in a given subject were measured within the same assay. Antibody levels were generally quite stable over time, although several panels illustrate the aforementioned IAA fluctuations. Use of normalized autoantibody levels (the observed index divided by the 99th percentile threshold index) and a logarithmic scale allowed all three d-aab and insulin secretion to be displayed on the same panel. Overall patterns are similar to those of prospective family studies (40). Four subjects who initially had multiple d-aab and one with only ICA agreed to sequential β-cell function testing. As shown in Fig. 1, AIR_g was stable over several years of study, even in those with multiple d-aab, consistent with the observed lack of clinical progression to diabetes in these subjects.

CONCLUSIONS — Several studies have reported variable results in long-term follow-up of general populations screened with autoantibodies. Of 2,808 Dutch children (42), 4 of 8 initially with ICA and 3 without ICA had diabetes 11.5 years later (sensitivity 50%, PPV 57%). Of 3,854 Florida children (22), 10 of 57 initially with ICA and none without ICA had diabetes after 2.0–8.2 years (sensitivity 100%, PPV 18%). Of 1,212 Finnish children (13), 3 of 50 with ICA and none without ICA had diabetes 8 years later (sensitivity 100%, PPV 6%). Of 198 French children initially with ICA (43), only 1 developed diabetes within 2.5–4.0 years (PPV 0.5%). Two very recent prospective studies screened for antibody combinations. In one, GADA, IA2/ICA512A, and ICA (but not IAA) were tested in 1,031 children (23). Six children

developed diabetes within 10 years. All three tests were positive in two children (sensitivity 33%, PPV 80%), two children had ICA and GADA, one had only GADA (sensitivity 83%, PPV 21%), and one initially had no antibodies. Another brief report described 3,652 children screened for GADA, IA2/ICA512A, IAA, and ICA. Of 21 with more than one antibody, four developed diabetes within 5.3 years (sensitivity 100%, PPV 19%), although the exact distribution of d-aab and ICA is not specified (44).

Importantly, our study is the first to simultaneously measure the three most informative d-aab (GADA, IA2/ICA512A, and IAA) in a large schoolchildren cohort and then follow a large number prospectively for 8 years. Given low disease prevalence, all studies testing antibody prediction strategies directly in the general population, ranging from ~1,000 to 13,000 subjects, have been statistically "underpowered." Our study is no exception. However, 95% CIs allow estimation of the effect of sample size on our results. We believe that 95% CIs for sensitivity (58–100%) and PPV (25–75%) support the use of this strategy in the general population. These strong results are likely due to standardized high-throughput autoantibody assays with built-in quality control standards and objective cutoff thresholds (25) as well as repeated samplings to verify antibody positivity (5). Substantial proportions (although not all) of the ICA signal are proven to be attributable to reactivity against each of the three d-aab we tested (7,38,39). Therefore, ICA may not be a truly independent marker in subjects with d-aab. We feel that defining subjects with ICA and one d-aab as doubly positive risks decreases the high specificity required for successful diabetes prediction in low-prevalence general populations, and we did not do so here. In support of our view, we observed no greater diabetes risk among our subjects with ICA and one d-aab than in those with one d-aab alone.

This study includes subjects in the teenage years just after the peak incidence. It confirms that new d-aab are less likely to appear at this age than earlier in life (17), allowing their detection with infrequent sampling. More frequent sampling as well as the greater prevalence of IAA (17) should allow the multiple d-aab strategy to succeed in young children as in teenagers, but this requires prospective testing.
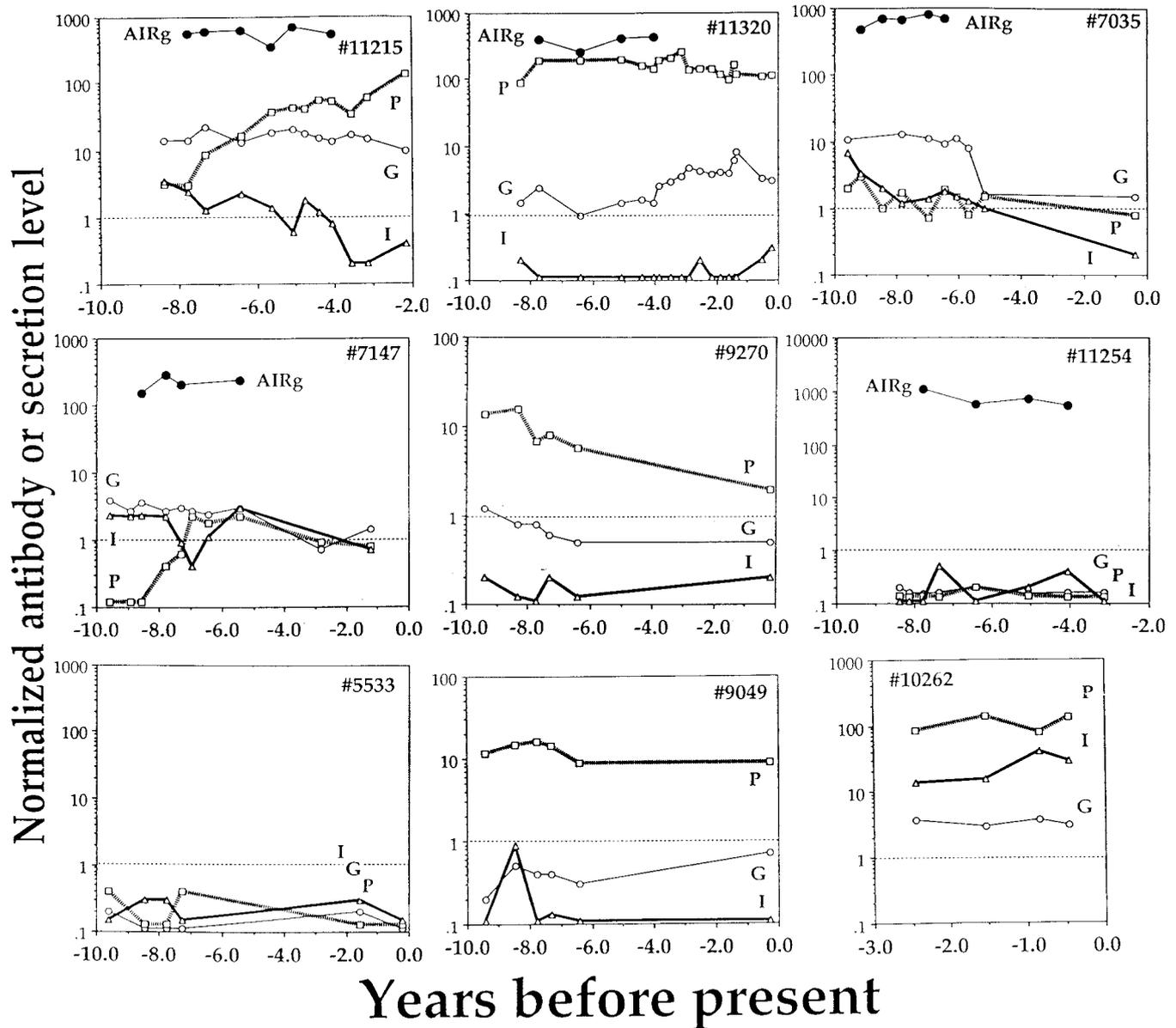
Figure 1—*Sequential autoantibody and insulin secretion profiles. Results from eight subjects not progressing to clinical diabetes over up to 8 years of prospective follow-up and one subject (#10262) progressing to diabetes after 3 years are shown. Of those not yet with diabetes, five initially had multiple d-aab (#11215, #11320, #7035, #7147, and #9270), two had persistent ICA without d-aab (#11254 and #5533), and one had a single d-aab (#9049). d-aab levels are plotted as the logarithm of the ratio of the sample index to the threshold index. ICA titers are not shown. GAD antibodies are shown by open circles and thin solid lines and denoted by a bolded "G," IA2/ICA512A by open squares and stippled lines and denoted by a bolded "P," and IAA by open triangles and thick solid lines and denoted by a bolded "I." Five subjects underwent multiple intravenous glucose tolerance tests to measure AIR_g, which is plotted as the logarithm of summed insulin secretion.*

Our cross-sectional study of 492 Swedish 0- to 15-year-old type 1 diabetic patients (4) found that only 73% had multiple d-aab at diagnosis. In contrast, our prospective study now finds that all subjects developing diabetes had multiple d-aab. Whereas 73% is well within the 95% CI for sensitivity in the current study, other explanations are also possible. Assay technology available at the time of those studies (recombinant human GAD expression in rodent cells and PEG precipitation for IAA assays) may have led to lower sensitivities in those assays versus current methods. Further, multiple prospective samplings before diagnosis may be more sensitive than a single sampling at diagnosis.

Accurate prediction of future diabetes in healthy children remains an important goal for type 1 diabetes prevention. Prevention therapy may be more effective early in the pathogenesis because of some or all of the following factors: 1) less clonal expansion of cytotoxic effector populations resulting in less cells to inhibit, 2) less inter- and intramolecular antigen spreading resulting in a less diverse immune response to interrupt, 3) β-cells not being stressed to maximal insulin out-

put where they may be more susceptible to immune killing, *4*) a greater proportion of noninflamed islets that may escape immune infiltration after therapy, and *5*) greater β-cell neogenesis/replication in the absence of hyperglycemia and inflammation. These theoretical advantages may result in early effectiveness for certain therapies unable to reverse diabetes when given after diagnosis.

Promising intervention therapies are on the horizon. For example, humanized monoclonal antibodies that modulate T-cell function have already led to major advances in control of islet transplantation alloimmunity and may have similar potential to control autoimmunity (45). However, any therapy must be carefully examined to ensure it is effective at delaying or preventing disease, whereas its toxicity must not be worse than the disease itself under current optimal therapy. Use of sufficiently specific criteria in low prevalence populations to achieve a high PPV is essential to minimize exposure of false-positives to therapeutic risk.

The potential for greater effectiveness with early treatment offsets costly population screening to detect preclinical diabetes. Practical screening will likely require efficient sampling through the public health infrastructure as well as inexpensive assay tests in scalable formats, only recently possible for IAA (46) and difficult for ICA assays using pancreas sections. Finally, because no prevention therapy is yet proven effective, general population prediction remains within the research arena, with attendant ethical requirements for informed consent and education of large numbers of families. HLA typing improved PPV only slightly among our children with multiple antibodies. Nevertheless, HLA prescreening, as is currently being tested in Washington State and in several other studies worldwide, would include most of the highest risk subjects while greatly decreasing the cost and invasiveness of antibody testing.

Because of the difficulty of large long-term prospective studies in children without familial diabetes, the effectiveness of the strategy of multiple defined autoantibodies for general population screening for type 1 diabetes risk has not previously been proven. We now confirm a high risk of progression to diabetes among schoolchildren with multiple defined antibodies and suggest that this approach is highly successful for prediction of type 1 diabetes among teenage schoolchildren.

References
1. Onkamo P, Vaananen S, Karvonen M, Tuomilehto J: Worldwide increase in incidence of type I diabetes: analysis of the data on published incidence trends. *Diabetologia* 42:1395–1403, 1999
2. Dahlquist GG: Primary and secondary prevention strategies of pre-type 1 diabetes: potentials and pitfalls. *Diabetes Care* 22 (Suppl. 2):B4–B6, 1999
3. Pugliese A: Unraveling the genetics of insulin-dependent diabetes: the search must go on. *Diabetes Reviews* 7:39–54, 1999
4. Hagopian W, Sanjeevi C, Kockum I, Landin-Olsson M, Karlsen A, Sundkvist G, Dahlquist G, Palmer J, Lernmark Å: GAD-, insulin-, and islet cell-antibodies and HLA typing to detect diabetes in a general population-based study of Swedish children. *J Clin Invest* 95:1505–1511, 1995
5. Rewers M, Bugawan T, Norris J, Blair A, Beaty B, Hoffman M, McDuffie R Jr, Hamman R, Klingensmith G, Eisenbarth G, Erlich H: Newborn screening for HLA markers associated with IDDM: diabetes autoimmunity study in the young (DAISY). *Diabetologia* 39:807–812, 1996
6. Palmer JP, Asplin CM, Clemons P, Lyen K, Tatpati O, Raghu PK, Paquette TL: Insulin antibodies in insulin-dependent diabetics before insulin treatment. *Science* 222:1337–1339, 1983
7. Scott-Morgan L, Keller U, Heitz P, Mirza I, Diaz J, Casey C, Wilkin T: Insulin autoantibodies may cause false positive ICA results in the indirect immunofluorescence test. *Diabetes Res* 8:45–48, 1988
8. Baekkeskov S, Aanstoot H, Christgau S, Reetz A, Solimena M, Cascalho M, Folli F, Richter-Olesen H, De Camilli P: Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature* 347:151–156, 1990
9. Rabin D, Pleasic S, Palmer-Crocker R, Shapiro J: Cloning and expression of IDDM-specific human autoantigens. *Diabetes* 41:183–186, 1992
10. Rabin D, Pleasic S, Shapiro J, Yoo-Warren H, Oles J, Hicks J, Goldstein D, Rae P: Islet cell antigen 512 is a diabetes-specific islet autoantigen related to protein tyrosine phosphatases. *J Immunol* 152:3183–3188, 1994
11. Lan M, Lu J, Goto Y, Notkins A: Molecular cloning and identification of a receptor-type protein tyrosine phosphatase, IA-2, from human insulinoma. *DNA Cell Biol* 13:505–514, 1994
12. Dahlquist G, Blom L, Holmgren G, Hägglöf B, Larsson Y, Sterky G, Wall S: The epidemiology of diabetes in Swedish children 0–14 years old: a 6-year prospective study. *Diabetologia* 28:802–808, 1985
13. Karjalainen J: Islet cell antibodies as predictive markers for IDDM in children with high background incidence of disease. *Diabetes* 39:1144–1150, 1990
14. Levy-Marchal C, Tichet J, Fajardy I, Gu XF, Dubois F, Czernichow P: Islet cell antibodies in normal French school-children. *Diabetologia* 35:577–582, 1992
15. Landin-Olsson M, Palmer J, Lernmark Å, Blom L, Sundkvist G, Nystrom L, Dahlquist G: Predictive value of ICA and IAA for IDDM in a population-based study of newly diagnosed diabetic and matched control children. *Diabetologia* 35:1068–1073, 1992
16. Aanstoot H, Sigurdsson E, Jaffe M, Shi Y, Christgau S, Grobbee D, Bruining G, Molenaar J, Hofman A, Baekkeskov S: Value of antibodies to GAD65 combined with islet cell cytoplasmic antibodies for predicting IDDM in a childhood population. *Diabetologia* 37:917–924, 1994
17. Yu L, Rewers M, Gianani R, Kawasaki E, Zhang Y, Verge C, Chase P, Klingensmith G, Erlich H, Norris J, Eisenbarth G: Anti-islet autoantibodies usually develop sequentially rather than simultaneously. *J Clin Endocrinol Metab* 81:4264–4267, 1996
18. Roll U, Christie M, Fuchtenbusch M, Payton M, Hawkes C, Ziegler A: Perinatal autoimmunity in offspring of diabetic parents: the German multicenter BABY-DIAB study: detection of humoral immune responses to islet antigens in early childhood. *Diabetes* 45:967–973, 1996
19. Christie M, Roll U, Payton M, Hatfield E, Ziegler A: Validity of screening for individuals at risk for type I diabetes by combined analysis of antibodies to re-

combinant proteins. *Diabetes Care* 20: 965–970, 1997

20. Bingley P, Bonifacio E, Williams A, Genovese S, Bottazzo G, Gale E: Prediction of IDDM in the general population: strategies based on combinations of autoantibody markers. *Diabetes* 46:1701–1710, 1997

21. Rowe R, Leech N, Nepom G, McCulloch D: High genetic risk for IDDM in the Pacific Northwest: first report from the Washington State Diabetes Prediction Study. *Diabetes* 43:87–94, 1994

22. Schatz D, Krischer J, Horne G, Riley W, Spillar R, Silverstein J, Winter W, Muir A, Derovanesian D, Shah S: Islet cell antibodies predict insulin-dependent diabetes in United States school age children as powerfully as in unaffected relatives. *J Clin Invest* 93:2403–2407, 1994

23. Samuelsson U, Sundkvist G, Borg H, Fernlund P, Ludvigsson J: Islet autoantibodies in the prediction of diabetes in school children. *Diabetes Res Clin Pract* 51:51–57, 2001

24. Kupila A, Muona P, Simell T, Arvilommi P, Savolainen H, Hamalainen A, Korhonen S, Kimpimaki T, Sjoroos M, Ilonen J, Knip M, Simell O: Feasibility of genetic and immunological prediction of type I diabetes in a population-based birth cohort. *Diabetologia* 44:290–297, 2001

25. Woo W, LaGasse J, Zhou Z, Patel R, Palmer JP, Campus H, Hagopian WA: A novel high-throughput method for accurate, rapid, and economical measurement of multiple type 1 diabetes autoantibodies. *J Immunol Methods* 244:91–103, 2000

26. Mire-Sluis A, Das R, Lernmark A: The development of a World Health Organization international standard for islet cell antibodies: the aims and design of an international collaborative study. *Diabete Metab Res Rev* 15:72–77, 1999

27. Immunology of Diabetes Society: Diabetes Autoantibody Standardization Program. *Diabetes Metab Res Rev* 17:S6, 2001

28. Lernmark Å, Molenaar JL, vanBeers WAM, Yamaguchi Y, Nagataki S, Ludvigsson J, Maclaren NK: The Fourth Interna-

tional Serum Exchange Workshop to standardize cytoplasmic islet cell antibodies. *Diabetologia* 34:534–535, 1991

29. Boehm B, Manfras B, Seissler J, Schoffling K, Gluck M, Holzberger G, Seidl S, Kuhnl P, Trucco M, Shcerbaum W: Epidemiology and immunogenetic background of ICA positive non-diabetic schoolchildren. *Diabetes* 40:1435–1439, 1991

30. Kimura A, Sasazuki T: HLA 1991. In *11th International Histocompatibility Workshop.* Tsuji K, Aizawa M, Sasazuki T, Eds. Oxford, U.K., Oxford University Press, 1992, p. 397–419

31. Gambelunghe G, Ghaderi M, Consentino A, Falorni A, Brunetti P, Falorni A, Sanjeevi CB: Association of MHC class I chain-related A (MIC-A) gene polymorphism with type 1 diabetes. *Diabetologia* 43:507–514, 2000

32. Kemp EH, Ajjan R, Waterman EA, Gawkrodger DJ, Cork MJ, Watson PF, Weetman AP: Analysis of a microsatellite polymorphism of the cytotoxic T-lymphocyte antigen-4 gene in patients with vitiligo. *Br J Dermatol* 140:73–78, 1999

33. McIndoe R, Stanford J, Gibbs M, Jarvik G, Brandzel S, Neal C, Li S, Gammack J, Gay A, Goode E, Hood L, Ostrander E: Linkage analysis of 49 high-risk families does not support a common familial prostate cancer susceptibility gene at 1q24–25. *Am J Hum Genet* 61:347–353, 1997

34. Kahn S, Pridgeon R, McCulloch D, Boyko E, Bergman R, Schwartz M, Neifing J, Ward W, Beard J, Palmer J, Porte DJ: Quantification of the relationship between insulin sensitivity and β-cell function in human subjects. *Diabetes* 42: 1663–1672, 1993

35. Casella G, Berger R: *Statistical Inference.* Belmont, CA, Duxbury Press, 1990

36. Begg CB, Greenes RA: Assessment of diagnostic tests when disease verification is subject to selection bias. *Biometrics* 39: 207–215, 1983

37. Dahlquist G, Gustavsson K, Holmgren G, Hagglof B, Larsson Y, Nilsson K, Samuelsson G, Sterky G, Thalme B, Wall S: The incidence of diabetes mellitus in Swedish

children 0–14 years of age: a prospective study 1977–1980. *Acta Paediatr Scand* 71: 7–14, 1982

38. Marshall M, Hxyer P, Petersen J, Hejnes K, Genovese S, Dyrberg T, Bottazzo G: Contribution of GAD antibodies to the reactivity of ICA. *J Autoimmun* 7:497–508, 1994

39. Bonifacio E, Lampasona V, Genovese S, Ferrari M, Bosi E: Identification of protein tyrosine phosphatase-like IA2 (islet cell antigen 512) as the IDDM-related 37/40K autoantigen and a target of islet-cell antibodies. *J Immunol* 155:5419–5426, 1995

40. Gardner S, Gale E, Williams A, Gillespie K, Lawrence K, Bottazzo G, Bingley P: Progression to diabetes in relatives with islet autoantibodies. Is it inevitable? *Diabetes Care* 22:2049–2054, 1999

41. Bennett S, Lucassen A, Gough S, Powell E, Undlien D, Pritchard L, Merriman M, Kawaguchi Y, Dronsfield M, Pociot F, et al.: Susceptibility to human type 1 diabetes at IDDM2 is determined by tandem repeat variation at the insulin gene minisatellite locus. *Nat Genet* 9:284–292, 1995

42. Bruining G, Grobbee D, Scheffer G, De-Bruyn A, Molenaar J, Hofman A, Bruining H, Valkenburg H: Ten-year follow-up study of ICA and childhood diabetes mellitus. *Lancet* i:1100–1103, 1989

43. Levy-Marchal C, Dubois F, Noel M, Tichet J, Czernichow P: Immunogenetic determinants and prediction of IDDM in French schoolchildren. *Diabetes* 44:1029–1032, 1995

44. Kulmala P, Rahko J, Savola K, Vahasalo P, Sjoroos M, Reunanen A, Ilonen J, Knip M: Beta-cell autoimmunity, genetic susceptibility, and progression to diabetes in unaffected schoolchildren. *Diabetes Care* 24: 171–173, 2001

45. Schwartz R. The new immunology: the end of immunosupressive drug therapy. *N Engl J Med* 340:1754–1756, 1999

46. Williams A, Bingley P, Bonifacio E, Palmer J, Gale E: A novel micro-assay for insulin autoantibodies. *J Autoimmun* 10: 473–478, 1997