Glutamic Acid Decarboxylase and ICA512/IA-2 Autoantibodies as Disease Markers and Relationship to Residual β-Cell Function and Glycemic Control in Young Type 1 Diabetic Patients

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Circulating autoantibodies (Ab) to islet autoantigens, glutamic acid decarboxylase (GAD_{65}), and tyrosine phosphatase ICA512/IA-2 have been proposed as predictive markers of type 1 diabetes mellitus. To ascertain residual β-cell function and the clinical relevance for monitoring autoimmunity after clinical manifestation of disease, we studied 63 children at diagnosis of type 1 diabetes (mean SD age 7.5 ± 4 years) and 91 adolescent patients with type 1 diabetes (age 14.7 ± 1.6 years) with a mean duration of disease of 7 ± 3.5) years. Forty-two normal adolescent subjects (age 14.6 ± 1.8 years) without a family history of diabetes were the control group. Anti-GAD_{65} and ICA512/IA-2 Ab were assessed by a quantitative radioimmunoprecipitation assay. The relationship between humoral autoimmunity and clinical parameters was explored. GAD_{65} and ICA512/IA-2 Ab were detected in 56% and 63% of newly diagnosed children and the prevalence was not different in relationship to clinical characteristics. Levels of GAD_{65} Ab positively correlated with diagnosis age ($P < .05$). Both Ab were associated with islet cell antibodies (ICA) ($P < .05$), but one fifth of patients had at least 1 of the 2 Ab and absent ICA. At onset, only age showed a significant relationship to residual C-peptide secretion. Among the cohort of patients with diabetes of short-mid duration, GAD_{65} and ICA512/IA-2 Ab were present in 44% and 45% of cases ($P > .05$ and $P < .05$ *v* newly diagnosed children, respectively) and more patients were identified by these Ab (68%) than by ICA alone (34%) ($P < .05$). In this cohort, levels of ICA512/IA-2 Ab negatively correlated with levels of glycosylated hemoglobin (HbA_{1c}) ($P < .005$) and with daily insulin requirement ($P < .05$). Moreover, the presence of some residual C-peptide secretion was significantly associated with the presence of ICA512/IA-2 Ab ($P < .05$). Our findings confirm that positivity for either GAD_{65} or ICA512/IA-2 Ab is a highly sensitive marker of type 1 diabetes in the pediatric age group, identifying a group of patients with absent ICA immunofluorescence. The persistence of Ab to islet tyrosine phosphatase possibly represents a marker of better glycemic control and less insulin requirement, indicating residual β-cell function, thus conferring clinical and prognostic relevance to these Ab, as well as potential usefulness in intervention strategies.

CONVENTIONAL ISLET CELL antibodies (ICA)[1] have been extensively evaluated as marker for type 1 diabetes mellitus and for prediction of development of the disease. Recent studies indicate that ICA represent a heterogeneous population of autoantibodies (Ab),[2,3] and to date, a growing number of biochemically defined islet autoantigens, target of the autoimmune process leading to type 1 diabetes, have been identified. These include insulin,[4] glutamic acid decarboxylase (GAD), [5] carboxypeptidase H,[6] ICA69,[7] Imogen 38,[8] the glycolipid GM2-1,[9] and tyrosine phosphatase-related enzyme proteins, that is ICA512/IA-2[10] and IA-2β/Phogrin.[11] Among these autoantigens, Ab directed against GAD_{65} and ICA512/IA-2 have been proposed as sensitive predictive markers of type 1 diabetes.[12] These Ab are detected using quantitative radiobinding assays, overcoming some of the limitations of the ICA test[13] and potentially representing an alternative strategy for primary screening in large-scale population studies.

However, little is known about the natural history of β -cell autoimmunity, and at present, there is little information on the relationship between clinical parameters and islet-related Ab to further assess their clinical and prognostic relevance, as well as the indication for monitoring them after the diagnosis of diabetes.[14-18] In particular, studies assessing the relationship between humoral autoimmunity, residual β-cell insulin secretion, and glycemic control give inconsistent and conflicting results.[16,17,19-24] Moreover, these reports mainly focused on the relationship with ICA or insulin Ab and, at present, there are few studies, some of which conducted in non-caucasoid cohorts, with respect to the recently identified humoral markers.

The aim of the present study was to explore the relationship between Ab to GAD_{65} and islet tyrosine phosphatase ICA512/IA-2 and residual β-cell function, glycemic control, or clinical characteristics in a cohort of diabetic children at disease onset and a cohort of adolescent patients with diabetes of short-mid duration. The sensitivity of these Ab to identify type 1 diabetes was also assessed.

PATIENTS AND METHODS

Patients

Sera were obtained from 63 (31 males, 32 females) consecutive newly diagnosed type 1 diabetic children (mean age 7.5 ± 4 years; range, 3 months to 15 years), attending the Paediatric Diabetic Clinic of the University of Turin. Blood was drawn within 3 days from diagnosis. Twenty-one children (33%) presented clinically at onset with diabetic ketoacidosis. Mean glycosylated hemoglobin (HbA_{1c}) for the cohort was 11.6% ± 2.7%. Levels of serum C peptide, fasting (mean value, 0.52 ± 0.4 ng/mL) and at 90 minutes after standardized breakfast (0.98 ± 0.8 ng/mL) were measured by radioimmunoassay using a commercially available kit (DPC, Llanberis, UK; detection limit 0.05

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ng/mL, normal fasting value 0.5 to 3.2 ng/mL) after euglycemia was achieved. Partial clinical remission was defined as HbA_{1c} levels $< 6\%$ and insulin requirement < 0.3 IU/Kg/24 h and occurred in 23 children (37%) within 1 month from diagnosis.

Four children (6%) had antithyroglobulin and/or antithyroid peroxidase Ab, and 1 was hypothyroid. Another child had celiac disease. Twelve children (19%) had one first- or second-degree relative with type 1 diabetes.

All 91 insulin-dependent diabetic adolescent patients (47 males, 44 females) attending the same Diabetic Clinic (mean age, 14.7 ± 1.6 years; range, 11 to 18 years; mean duration of disease, 7 ± 3.5 years) were also studied. Informed consent for participation was obtained. Antithyroglobulin and/or antithyroid peroxidase Ab were detected in 16 patients (18%), and 4 patients were hypothyroid on replacement therapy. Two other patients had celiac disease. Mean HbA_{1c} for the cohort, obtained from the mean value for each patient for the preceding year was 8.8% ± 1.5%. Twenty-three patients (25%) still had detectable fasting serum C peptide, with a mean value of 0.11 ± 0.09 ng/mL, while in the other 68 patients C peptide was undetectable. All patients were treated with multiple injection therapy, and for 50 patients, daily insulin requirement at the time of the study was available (mean dose 0.96 ± 0.3 IU/Kg/24 h).

The healthy control population consisted of 42 (20 males, 22 females) adolescents (mean age, 14.6 ± 1.8 years; range, 11 to 17) recruited from among adolescent students attending secondary school in the area of Turin and without a family history of diabetes mellitus.

GAD_{65} and ICA512/IA-2 Ab Radioimmunoassays

$GAD_{65\ Ab}$ Ab were measured in triplicate using in vitro transcribed/translated recombinant human glutamic acid decarboxylase (65 Kd isoform) as described.[25, 26] The results are expressed as an index (index = sample cpm - negative control cpm/ positive control cpm - negative control cpm). The cut-off point for this assay was established as the 99th percentile of Ab levels calculated using 280 control subjects (index of 0.069). The interassay coefficient of variation (CV) was 13.2% (n = 7), and the intra-assay CV was 12.2% (n = 11). The construct used for ICA512/IA-2 Ab, ICA512bdc,[27] is a splice variant lacking exon 13 of the published IA-2 sequence.[10] The ICA512/IA-2 Ab radioimmunoassay has a similar assay format to that for measuring GAD_{65} Ab, and the results are expressed as an index calculated in an identical fashion. The upper limit of the normal range for ICA512/IA-2 Ab was established as the 99th percentile of the levels in 280 healthy control subjects and corresponded to an index of 0.032. The interassay CV was 9.5% (n = 9), and the intra-assay CV was 12.4% (n = 11).

Results of the proficiency workshops organized by the University of Florida, Gainsville, FL, (1995, 1996, and 1997) and the Diabetes Autoantibody Standardisation Programme (DASP, 2000), organised by the World Health Organization (WHO), were: 76% to 100% sensitivity, 90% to 100% specificity (100% specificity 3 times), and 100% validity for GAD_{65} Ab; 48% to78.5% sensitivity, 98% to 100% specificity, 87.5% validity, and 91.6% consistency in 1996 and 2000 for IA-2 Ab.

ICA were detected in a 2-step indirect immunofluorescence assay on cryostat sections of blood group O human pancreas, as previously described.[28] This assay was tested in the stage III Immunology and Diabetes Workshop on ICA Proficiency, performing with 100% specificity, a detection limit of 5 JDF units, and a correlation of observed with consensus JDF units of 0.74. Results are reported here as positive (levels > 5 JDF units) or negative.

Antithyroglobulin Ab were detected by immunoradiometric assay (IRMA) technique (ERIA Diagnostics Pasteur, Marnes La Coquette, France) and antithyroid peroxidase Ab by radioimmunoassay (Brahms Diagnostica, Berlin, Germany).

Table 1. Prevalence of Each Ab and Their Combinations in Both Diabetic Study Groups

	Newly Diagnosed Diabetic Children (n = 63)	Diabetic Adolescent Patients (n = 91)
ICA positive	44 (70%)*	31 (34%)
GAD_{65} Ab positive	35 (56%)	40 (44%)
ICA512/IA-2 Ab positive	40 (63%)*	41 (45%)
GAD_{65} and ICA512/IA-2 Ab positive	24 (38%)*	19 (21%)
GAD_{65} and/or ICA512/IA-2 Ab positive	51 (81%)	62 (68%)†
ICA, GAD_{65} and ICA512/IA-2 Ab positive	23 (37%)*	8 (9%)
ICA negative and GAD_{65} and/or ICA512/IA-2 Ab positive	13 (21%)*	36 (40%)
ICA positive and GAD_{65} and ICA512/IA-2 Ab negative	6 (10%)	6 (6%)

NOTE. Data are shown as numbers and percentages.
*$P < .05$ *v* diabetic adolescent patients.
†$P < .05$ *v* ICA positive adolescent patients.

Statistical Analysis

Values of GAD_{65} and ICA512/IA-2 Ab index among the study groups did not satisfy the hypothesis of normality, as tested using the Kolmogorov-Smirnov goodness of fit test and were compared using the Mann-Whiney *U* test. Correlations between Ab index and clinical parameters were analyzed using the Spearman correlation test, and multiple linear regression analysis was performed to assess correlation between parameters with HbA_{1c} as the dependent variable. The distribution of Ab among the clinical groups was compared using Fisher's exact test or χ^2 test, when appropriate. *P* values less than .05 were considered significant.

RESULTS

Prevalences and Relationship Between Ab

The frequencies and combinations of various Ab are given in Table 1 for both diabetic groups. Among the newly diagnosed children, GAD_{65} and ICA512/IA-2 Ab were detected in 35 (56%) and in 40 (63%) patients, respectively, and both Ab were simultaneously present in 24 children (38%). One and/or the other Ab were detected in 51 (81%) newly diagnosed children.

Among the diabetic adolescent patients, GAD_{65} and ICA512/IA-2 Ab were present in 40 (44%) and 41 (45%) patients, respectively, and significantly more patients were identified by GAD_{65} and/or ICA512/IA-2 Ab (68%) than by ICA alone (34%) ($P < .05$). In this group, only the prevalence and levels of ICA512/IA-2 Ab were significantly lower (median, -0.003; range, -0.04 to 1.37) compared with that among the newly diagnosed patients (median, 0.78; range, -0.02 to 3.23) ($P < .05$ for prevalence, and $P < .0001$ for levels) (Fig 1).

Remarkably, positivity for GAD_{65} and/or ICA512/IA-2 Ab identified one fifth of the children (21%) and two fifth of the adolescent patients (40%) with absent ICA. All 3 antibodies were simultaneously present in 23 children (37%) and in 8 adolescent patients (9%).

One of the control subjects had GAD_{65} Ab, and another

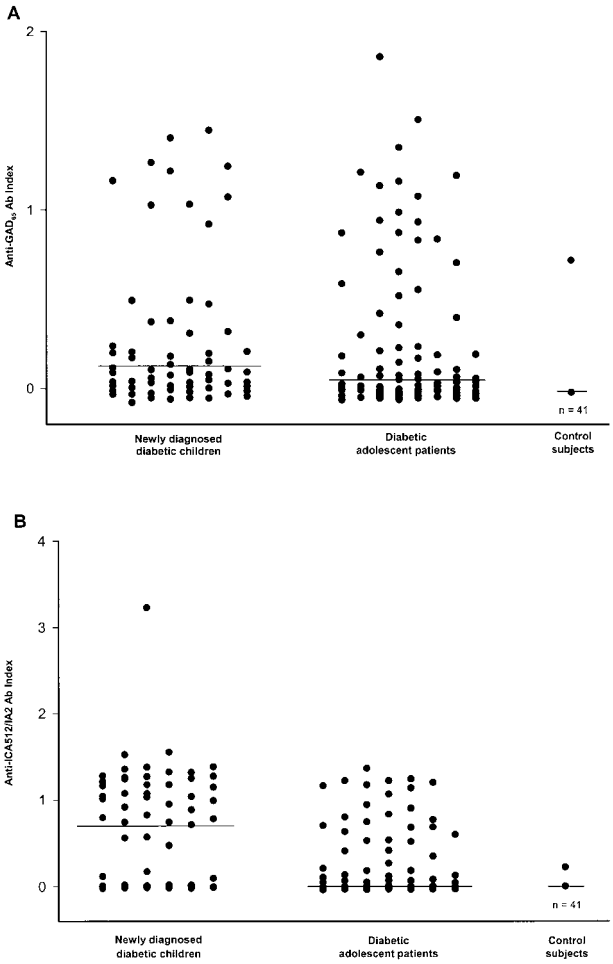

Fig 1. (A) Anti-GAD_{65} Ab levels and (B) anti-ICA512/IA-2 Ab levels in the subjects of the 3 study groups. The upper limit of normal was 0.069 for anti-GAD_{65} Ab, and 0.032 for anti-ICA512/IA-2 Ab. Horizontal bars represent the median levels of each study group. Levels of anti-ICA512/IA-2 Ab were significantly higher among the newly diagnosed diabetic children compared with the adolescent patients ($P < .0001$).

control had ICA512/IA-2 Ab. This prevalence was significantly lower compared with the 2 diabetic study groups ($P < .001$).

Analyzing the relationships between Ab and ICA, prevalence and levels of ICA512/IA-2 Ab were higher in ICA positive patients in both diabetic groups ($P < .05$ and $P < .02$, respectively). GAD_{65} Ab were associated with ICA only in the newly diagnosed children ($P < .05$).

Relationship Between Ab Positivity and Clinical Characteristics

There were no gender differences for Ab prevalence in both study groups. At diagnosis, levels of GAD_{65} Ab positively correlated with age ($r = .21$, $P < .05$). Clinical onset with diabetic ketoacidosis, a positive family history of type 1 diabetes, and clinical remission were not associated with a different prevalence of Ab. Onset age showed a significant relationship to residual C-peptide secretion ($r = .43$, $P < .005$).

Table 2. Metabolic Parameters in Both Diabetic Study Groups and Correlation With Ab Levels

	Newly Diagnosed Diabetic Children (n = 63)	Diabetic Adolescent Patients (n = 91)
HbA_{1c}	11.6% ± 2.7%	8.8% ± 1.5%
Fasting C peptide	0.52 ± 0.4 ng/mL	0.11 ± 0.09 ng/mL (n = 23)*
Postprandial C peptide	0.98 ± 0.8 ng/mL	—
GAD_{65} index		
v HbA_{1c}	*r* −0.043	−0.03
v U/Kg	—	−0.05 (n = 50)
ICA512/IA-2 index		
v HbA_{1c}	*r* −0.03	−0.33†
v U/Kg	—	−0.29‡ (n = 50)

NOTE. Data are shown as mean ± SD. U/Kg, daily insulin requirement.

*In the other 68 patients C peptide was undetectable

†$P < .005$.

‡$P < .05$.

Fasting and postprandial C peptide or HbA_{1c} levels did not correlate with Ab levels, nor were there significant differences according to combination of Ab. However, patients positive for ICA512/IA-2 Ab and simultaneously negative for GAD_{65} Ab had the highest levels of C peptide (0.62 ± 0.4) compared with all other patients (0.47 ± 0.5), although without reaching statistical significance ($P = .2$).

Among the adolescent patients, only levels of ICA512/IA-2 Ab declined with diabetes duration ($r = -.2$, $P < .05$), independently from age.

Levels of these Ab negatively correlated with HbA_{1c} ($r = -.33$, $P < .005$), independently from disease duration and age (Table 2, Fig 2). The correlation persisted among the 68 patients without detectable C peptide ($r = -.31$, $P < .05$). Moreover levels of ICA512/IA-2 Ab negatively correlated with daily insulin requirement among the 50 patients for whom the dose

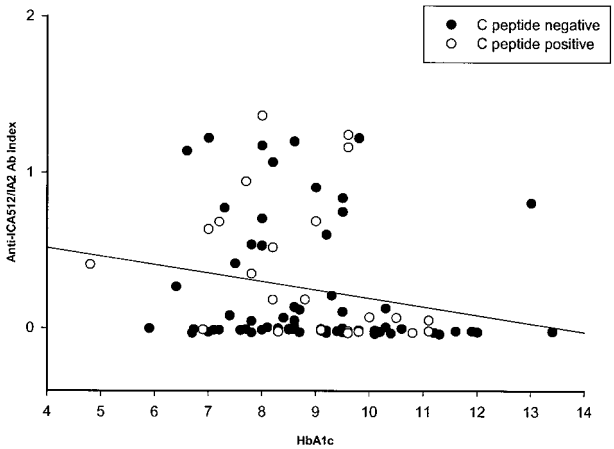

Fig 2. Scatter plot of anti-ICA512/IA-2 Ab and HbA_{1c} levels in the diabetic adolescent patients, r -.33, $P < .005$ (Spearman correlation test). Fifteen of the 23 patients with detectable C peptide (○) were ICA512/IA-2Ab positive (15/41, 37%) and the association was significant ($P < .05$).

was available ($r = -.29$, $P < .05$). Glycemic control and insulin requirement were not modified by combination of Ab. Furthermore, detection of residual C-peptide secretion in this cohort was significantly associated with the presence of ICA512/IA-2 Ab (15/41, 37% in ICA512/IA-2 Ab positive v 8/50, 18% ICA512/IA-2 Ab negative) ($P < .05$) and not with the presence of GAD_{65} Ab (12/40, 30%) or other Ab combinations. The 15 ICA512/IA-2 Ab positive patients with detectable C-peptide secretion did not differ in disease duration compared with the other patients.

In both study groups, there was no association between concomitant presence of antithyroid Ab and ICA, GAD_{65} and/or ICA512/IA-2 Ab, and the presence of ICA was not associated with differences in HbA_{1c} values, insulin requirement, or C-peptide levels.

DISCUSSION

During the 1990s, significant advances in the knowledge of pancreatic islet cell autoantigens in type 1 diabetes were achieved, although the questions of which autoantigens are central to the pathogenesis of the disease and of the clinical relevance of the Ab remain unanswered. In the present investigation, we evaluated the potential link between β-cell function and β-cell autoimmunity using a sensitive quantitative radioimmunoassay for Ab to recombinant GAD_{65} and islet tyrosine phosphatase-like protein ICA512/IA-2. Inconsistent and conflicting results on the ability of Ab to predict the duration of endogenous insulin secretion or to signify a more aggressive disease, are, in fact, reported.[16,20-23,29,30] In the present study, at diabetes onset, no differences in residual C-peptide secretion, clinical remission, or abrupt disease onset, could be detected according to the Ab status. In agreement with previous reports, we confirm that younger onset age is associated with poor residual β-cell function.[24,31]

The major finding of the present report is that persistence of ICA512/IA-2 Ab in diabetes of short-mid duration appears to indicate a better glycemic control, assessed by level of HbA_{1c}. Furthermore, these Ab may indicate a preserved β-cell function, directly assessed by fasting C-peptide secretion, and, indirectly, by HbA_{1c} and insulin requirement, as shown by studies negatively correlating levels of C peptide and levels of HbA_{1c}[32,33] and insulin requirement.[19] Our results are therefore in line with the observation that some type 1 diabetic patients retain residual β-cell secretory function.[34]

The associations identified in the present study support the only report assessing, so far, the relationship of residual β-cell function and Ab against tyrosine phosphatase, which indicates that children with these antibodies have the highest C-peptide levels during the first year of disease.[24] In the present investigation, newly diagnosed children positive for ICA512/IA-2 Ab and simultaneously negative for GAD_{65} Ab showed the highest levels of C peptide; however, a significant association between the 2 parameters was not present. This may be related to the smaller size of the cohort or the narrower age range. It will be of interest to evaluate future glycemic control and residual endogenous insulin secretion in this, possibly larger, cohort by a longitudinal study, which is currently being undertaken.

Our findings confer potentially clinical and prognostic relevance to humoral immunity against tyrosine phosphatase in overt disease, especially in relationship to future recruitment into therapeutic trials.

Considering the relationship between ICA or GAD_{65} Ab and preservation of β-cell function, previous studies have led to non-univocal findings. In particular, Ab to GAD have been described to be associated either with β-cell protection[22] or with a more aggressive β-cell destruction.[20,23,24] The discrepancy may be related to the fact that these studies were conducted in adult patients or in groups comprising both childhood and adult-onset type 1 diabetes[20,23,30] or in non-caucasoid patients.[21,22] The present investigation in young patients, however, does not corroborate any association with GAD_{65} Ab, possibly again indicating that antibodies may carry a different significance in different age, disease duration, and race groups.

As for the value of Ab to GAD_{65} and islet tyrosine phosphatase as disease markers, the presence of Ab to one and/or the other islet autoantigens, identified a proportion of newly diagnosed children and adolescent patients with diabetes of short-mid duration in whom the ICA immunofluorescence assay was negative. Moreover, a higher proportion of patients in both study groups tested positive for one and/or the other Ab than for ICA alone. These data further support the notion that a combined test is likely to be useful for screening purposes.[12] Lower prevalence of GAD_{65} Ab in newly diagnosed diabetic children were detected compared with previous studies in adult patients,[35-37] corroborating, however, the findings indicating that these antibodies appear to increase with age and, unusually, tend to persist in sera after diagnosis of the disease.[37,38] Their determination provides, therefore, a more objective marker for type 1 diabetes at older ages, possibly suggesting their preferential use as a marker for identifying and following adult diabetic patients with latent autoimmune disease.[39] We did not confirm the preliminary report of an association between GAD_{65} Ab and thyroid autoimmunity.[40]

In conclusion, the present study on humoral autoimmunity in childhood diabetes indicates that Ab to GAD_{65} and ICA512/IA-2 identifies a group of patients with absent ICA immunofluorescence in a complementary pattern. The 2 definite Ab appear to persist after clinical diagnosis, with percent positivity for ICA512/IA-2 Ab decreasing with disease duration. This might indicate differences in tissue expression of the antigens outside the endocrine pancreas, serving for antigenic stimulus. This report, above all, highlights an association between persistence of ICA512/IA-2 Ab, better glycemic control and, possibly, residual β-cell function by mechanisms to be elucidated. These findings need further confirmation by larger cohorts and, above all, follow-up studies and could be taken into account for disease prognosis and in intervention strategies aiming to preserve or ameliorate β-cell function, before or after the onset of type 1 diabetes.

REFERENCES

1. Bottazzo GF, Florin-Christensen A, Doniach D: Islet cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiency. Lancet 2:1279-1283, 1974

2. Genovese S, Bonifacio E, Mcnally JM, et al: Distinct cytoplasmic islet cell antibodies with different risks for type 1 (insulin-dependent) diabetes mellitus. Diabetologia 35:385-388, 1992

3. Gianani R, Pugliese A, Bonner-Wier S, et al: Prognostically significant heterogeneity of cytoplasmic islet cell antibodies in relatives of patients with type 1 diabetes. Diabetes 41:347-353, 1992

4. Palmer JP, Asplin CM, Clemons P, et al: Insulin antibodies in insulin dependent diabetes before treatment. Science 222:1337-1339, 1983

5. Baekkeskov S, Aanstoot H-J, Christgau S, et al: Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature 347:151-156, 1990

6. Castaño L, Russo E, Zhou L, et al: Identification and cloning of a granule autoantigen (carboxypeptidase H) associated with type 1 diabetes. J Clin Endocrinol Metab 3:1197-1201, 1991

7. Pietropaolo M, Castaño L, Babu S, et al: Islet cell autoantigen 69 kD (ICA69). Molecular cloning and characterization of a novel diabetes-associated autoantigen. J Clin Invest 92:359-371, 1993

8. Arden SD, Roep BO, Neophytou PI, et al: Imogen 38: A novel 38-kD islet mitochondrial autoantigen recognized by T cells from a newly diagnosed type 1 diabetic patient. J Clin Invest 97:551-561, 1996

9. Dotta F, Previti M, Lenti L, et al: GM2-1 pancreatic islet ganglioside: Identification and characterization of a novel islet-specific molecule. Diabetologia 38:1117-1121, 1995

10. Lan MS, Lu J, Goto Y, Notkins AL: Molecular cloning and identification of a receptor-type protein tyrosine phosphatase, IA-2, from human insulinoma. DNA Cell Biol 13:505-514, 1995

11. Lu J, Li Q, Xie H, et al: Identification of a second transmembrane protein tyrosine phosphatase, IA-2β, as an autoantigen in insulin-dependent diabetes mellitus: Precursor of the 37-kD tryptic fragment. Proc Natl Acad Sci USA 93:2307-2311, 1996

12. Christie MR, Genovese S, Cassidy D, et al: Antibodies to islet 37k antigen, but not to glutamate decarboxylase, discriminate rapid progression to IDDM in endocrine autoimmunity. Diabetes 43:1254-1259, 1994

13. Bonifacio E, Bingley P, Shattock M, et al: Quantification of islet-cell antibodies and prediction of insulin-dependent diabetes. Lancet 335:147-149, 1990

14. Schmidli RS, DeAizpurua HJ, Harrison LC, et al: Antibodies to glutamic acid decarboxylase in at-risk and clinical insulin-dependent diabetic subjects: Relationship to age, sex, and islet cell antibody status, and temporal profile. J Autoimmun 7:55-66, 1994

15. Zanone MM, Petersen JS, Peakman M, et al: High prevalence of autoantibodies to glutamic acid decarboxylase in long-standing IDDM is not a marker of symptomatic autonomic neuropathy. Diabetes 43: 1146-1151, 1994

16. Örtqvist E, Falorni A, Scheynius A, et al: Age governs gender-dependent islet cell autoreactivity and predicts the clinical course in childhood IDDM. Acta Paediatr 86:1166-1171, 1997

17. Martin S, Pawlowski B, Greulich B, et al: Natural course of remission in IDDM during the 1st yr after diagnosis. Diabetes Care 15:66-74, 1992

18. Zerbini G, Bazzigaluppi E, Mangili R, et al: Prevalence of GAD65 and IA-2 autoantibodies in IDDM patients affected by diabetic nephropathy. Diabetologia 40:485, 1997

19. Marner B, Agner T, Binder C, et al: Increased reduction of fasting C peptide is associated with islet cell antibodies in type 1 (insulin-dependent) diabetic patients. Diabetologia 28:875-880, 1985

20. Petersen JS, Dyrberg T, Karlsen AE, et al: Glutamic acid decarboxylase (GAD65) autoantibodies in prediction of β-cell function and remission in recent-onset IDDM after cyclosporin treatment. Diabetes 43:1291-1296, 1994

21. Urakami T, Miyamoto Y, Matsunaga H, et al: Serial changes in the prevalence of islet cell antibodies and islet cell antibody titer in children with IDDM of abrupt or slow onset. Diabetes Care 18:1095-1099, 1995

22. Yokota I, Shirakawa N, Shima K, et al: Relationship between GAD antibody and residual β-cell function in children after overt onset of IDDM. Diabetes Care 19:74-75, 1996

23. Hoeldtke RD, Bryner KD, Hoervath GG, et al: Antibodies to GAD and glycemic control in recent-onset IDDM. Diabetes Care 20:1900-1903, 1997

24. Bonfanti R, Bazzigaluppi E, Calori G, et al: Parameters associated with residual insulin secretion during the first year of disease in children and adolescents with type 1 diabetes. Diabet Med 15:844-850, 1998

25. Grubin CE, Daniels T, Toivola B, et al: A novel radiobinding assay to determine diagnostic accuracy of isoform-specific glutamic acid decarboxylase antibodies in childhood IDDM. Diabetologia 37: 344-350, 1994

26. Verge CF, Gianani R, Kawasaki E, et al: Prediction of type I diabetes mellitus in first degree relatives using a combination of insulin, glutamic acid decarboxylase and ICA512bdc/IA-2 autoantibodies. Diabetes 45:926-933, 1996

27. Pietropaolo M, Hutton JC, Eisenbarth GS: Protein-tyrosine phosphatase-like proteins: Link with insulin-dependent diabetes mellitus. Diabetes Care 20:208-214, 1997

28. Millward BA, Hussain MJ, Peakman, M, et al: Characterization of islet cell antibody in insulin dependent diabetes: Evidence for IgG1 subclass restriction and polyclonality. Clin Exp Immunol 71:353-356, 1988

29. Mauricio D, Corcoy R, Codina M, et al: Islet cell antibodies and beta-cell function in gestational diabetic woman: comparison to first-degree relatives of type 1 (insulin-dependent) diabetic subjects. Diabet Med 12:1009-1014, 1995

30. Mauricio D, Carreras A, Pérez A, et al: Association of islet-cell and glutamic acid decarboxylase antibodies to β-cell function after the onset of type 1 diabetes in adult subjects. Diabetes Nutr Metab 10:189-192, 1997

31. Sochett EB, Daneman D, Clarson C, et al: Factors affecting and patterns of residual insulin secretion during the first year of type 1 (insulin-dependent) diabetes mellitus in children. Diabetologia 30:453-459, 1987

32. Gonen B, Goldman J, Baldwin D, et al: Metabolic control in diabetic patients. Effect of insulin-secretory reserve (measured by plasma C-peptide levels) and circulating insulin antibodies. Diabetes 28:749-753, 1979

33. Knip M, Puukka R, Kaar ML, et al: Remission phase, endogenous insulin secretion and metabolic control in diabetic children. Acta Diabetol Latina 19:243-251, 1982

34. Faber OK, Binder C: C-peptide: an index of insulin secretion. Diabetes Metab Rev 2:331-345, 1986

35. Seissler J, Amann J, Mauch L, et al: Prevalence of autoantibodies to the 65- and 67-kD isoforms of glutamate decarboxylase in insulin-dependent diabetes mellitus. J Clin Invest 92:1394-1399, 1993

36. Petersen JS, Hejnæs KR, Moody A, et al: Detection of GAD_{65} antibodies in diabetes and other autoimmune diseases using a simple radioligand assay. Diabetes 43:459-467, 1994

37. Vandewalle CL, Falorni A, Svanholm S, et al: High diagnostic sensitivity of glutamate decarboxylase autoantibodies in insulin-dependent diabetes mellitus with clinical onset between age 20 and 40 years. J Clin Endocrinol Metab 80:846-851, 1995

38. Leslie RDG, Atkinson MA, Notkins AL: Autoantigens IA-2 and GAD in type 1 (insulin-dependent) diabetes. Diabetologia 42:3-14, 1999

39. Tuomi T, Groop LC, Zimmet PZ, et al: Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with non-insulin-dependent onset of disease. Diabetes 42:359-362, 1993

40. Vähäsalo P, Miettinen A, Kumala P, et al: GAD antibodies are associated with thyroid antibodies in children with IDDM and their siblings. Diabetologia 40:A77, 1997 (abstr, suppl 1)