# Autoantibodies to the IA-2 Extracellular Domain Refine the Definition of "A+" Subtypes of Ketosis-Prone Diabetes

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*Diabetes Care 2018;41:2637–2640 | https://doi.org/10.2337/dc18-0613*


## OBJECTIVE

**Autoantibodies directed against tyrosine phosphatase IA-2 antibody (IA-2 Ab) are diagnostic for autoimmune type 1 diabetes. Conventional assays target the intracellular domain of IA-2. Among patients with ketosis-prone diabetes (KPD), characterized by presentation with diabetic ketoacidosis (DKA), >60% of adults lack three classic islet autoantibodies—IA-2, GAD65, and ZnT8 Abs—associated with type 1 diabetes. We aimed to determine whether apparently autoantibody-negative ("A−") KPD patients possess occult IA-2 Ab directed against full-length IA-2 (IA-2FL) or its extracellular domain (IA-2EC).**

## RESEARCH DESIGN AND METHODS

**We developed an assay that targets IA-2FL and IA-2EC and used it to analyze 288 subjects with A− KPD.**

## RESULTS

**Ten A− KPD patients were positive for IA-2EC Ab (3.5%), and three were also positive for IA-2FL Ab (1.0%), similar to frequencies in type 1 and type 2 diabetes.**

## CONCLUSIONS

**Measurement of IA-2FL Ab and IA-2EC Ab improves the accuracy of the Aβ classification of KPD patients.**


Ketosis-prone diabetes (KPD) is a heterogenous syndrome characterized by presentation with diabetic ketoacidosis (DKA) and classified by the presence or absence of islet autoantibodies ("A+" or "A−") and presence or absence of β-cell functional reserve ("β+" or "β−") (1,2). Distinct from patients with type 1 diabetes, patients with KPD often present when older, have fewer recurrences of DKA, and can often discontinue insulin treatment while maintaining glycemic control (3). More than 60% of KPD adult patients lack evidence of islet autoimmunity (i.e., are A−) by testing for the presence of autoantibodies against the 65-kDa isoform of glutamate decarboxylase (GAD65), zinc transporter T8 (ZnT8), and the neuroendocrine autoantigen IA-2 (or ICA512) (1,2,4). Constructs used in conventional IA-2 autoantibody assays include intracellular fragments, but not the extracellular domain (IA-2EC), which has recently been investigated as a target for IA-2–specific autoantibodies (5). We reported that 1% of patients with autoimmune type 1 diabetes are positive only for the IA-2EC antibody (Ab), as were 4.7% of 258 patients with type 2 diabetes (5,6). Furthermore, we reported that full-length IA-2 (IA-2FL) Ab responses are

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*Received 20 March 2018 and accepted 10 September 2018.*

*This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc18-0613/-/DC1.*

**Table 1—Clinical characteristics of 10 IA-2EC autoantibody–positive KPD patients**

| Patient ID | IA-2FL Ab index ≥0.218 | IA-2EC Ab index ≥0.317 | Duration of DM at time of new IA-2 assay (years) | Ethnicity | Onset age (years) | Original KPD subtype | BMI (kg/m$^2$) | Duration of DM at first GST (years) | Fasting C-peptide (ng/mL) | Peak C-peptide (ng/mL) | Duration of DM at second GST (years) | Fasting C-peptide (ng/mL) | Peak C-peptide (ng/mL) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | 0.1139 | **0.3626** | 8.3 | Caucasian | 11.7 | A−β− | 18.0 | 8.7 | 0.09 | 0.09 | 9.6 | 0.09 | 0.09 |
| 118 | 0.1494 | **0.3700** | 15.0 | African American | 18.6 | A−β− | 25.0 | 15.0 | 0.22 | 0.23 | 15.5 | 0.09 | 0.09 |
| 346 | **0.2823** | **0.5331** | 24.3 | African American | 20.0 | A−β− | 23.5 | 33.2 | 0.1 | 0.1 | — | — | — |
| 409 | **0.2371** | **0.6161** | 1.7 | African American | 44.9 | A−β− | 21.1 | 7.9 | 0.5 | 0.5 | 11.3 | 0.1 | 0.1 |
| 60 | 0.1734 | **0.4683** | 4.3 | Hispanic | 51.7 | A−β+ | 28.8 | 4.3 | 0.71 | 1.27 | 5.2 | 2.59 | 4.55 |
| 148 | 0.1247 | **0.3172** | 13.6 | African American | 39.8 | A−β+ | 25.5 | 9.5 | 1.1 | — | 9.7 | 0.9 | — |
| 356 | **0.2191** | **0.4085** | 0.04 | Hispanic | 23.9 | A−β+ | 28.4 | 0.1 | 1.9 | 2.9 | 5.8 | 0.1 | 0.1 |
| 474 | 0.0599 | **0.6017** | 0.09 | Hispanic | 37.5 | A−β+ | 29.1 | 0.3 | 3.41 | 7.19 | — | — | — |
| 506 | −0.0030 | **0.4317** | 0.1 | Hispanic | 49.7 | A−β+ | 27.6 | 0.4 | 1.37 | 3.35 | 1.8 | 1.75 | 3.41 |
| 514 | 0.0250 | **0.3967** | 0.1 | Hispanic | 35.9 | A−β+ | 26.3 | 0.8 | 1.6 | 1.88 | 1.7 | 1.32 | 3.47 |

Boldface type indicates values that are above the threshold for positivity. DM, diabetes mellitus; GST, glucagon stimulation test; ID, identification.

associated with a high risk of progression to insulin-requiring diabetes among first-degree relatives of patients with type 1 diabetes (5). Because A− KPD patients include those with clinical phenotypes of both type 1 and type 2 diabetes (7), we sought to determine the rates of IA-2EC and IA-2FL autoantibody positivity (hence "occult" islet autoimmunity) among A− KPD patients.

## RESEARCH DESIGN AND METHODS

The study was approved by the Institutional Review Boards for Human Studies of Baylor College of Medicine and the Harris Health System, Houston, TX. Subjects with KPD were selected based on our published criteria (1,2) after presentation with DKA at Ben Taub General Hospital in Houston, TX, between January 1999 and May 2017. Subjects provided informed consent to be monitored prospectively in the KPD research clinic (2,8), and blood samples were obtained in the outpatient setting within 4 weeks of discharge from the hospital. As previously described, glucagon stimulation tests were performed on patients within 6 months of establishing outpatient care after their index episode of DKA (not necessarily congruent with the time of initial diagnosis of diabetes) (2).

All patients were classified according to the Aβ classification scheme for KPD as previously described (1,2), with A− status defined by absence of autoantibodies directed against GAD65, ZnT8, or IA-2 using the World Health Organization islet cell autoantibody standard (1,2,7,8). Radiobinding assays for IA-2FL and IA-2EC autoantibodies have been previously described (5,6). The cutoff points, established as the 99th percentile of 178 healthy individuals, are 0.218 for IA-2FL and 0.317 for IA-2EC autoantibodies. Interassay coefficients of variation are 8.3 and 13.4%, and intraassay coefficients of variation are 2.4 and 5.5%, for IA-2FL and IA-2EC autoantibodies, respectively. In the 2016 Islet Autoantibody Standardization Program workshop, these assays achieved ratings of 62 and 6% sensitivity and 99 and 100% specificity for the IA-2FL and IA-2EC autoantibodies, respectively.

Data are reported as mean ± SEM. The $\chi^2$ or Fisher exact tests were applied to compare proportions and evaluate statistically significant associations between

two categorical variables. $P < 0.05$ was considered significant.

## RESULTS

We identified 288 KPD patients (54% Hispanic, 35% African American, 9% Caucasian) as A− by conventional assays. The cohort was 62% male, with an average age at diagnosis of 36.6 ± 0.8 years, average $HbA_{1c}$ at diagnosis of 13.4 ± 0.2% (123 mmol/mol), and average duration of diabetes 4.4 ± 0.4 years. Ten patients were positive for IA-2EC Ab (3.5%). Of these, three (1.0%) were also positive for IA-2FL Ab. No patients were positive for IA-2FL Ab alone. The 10 patients positive for IA-2EC Ab were an average age at diagnosis of 33.4 ± 4.4 years, with a BMI 25.3 ± 1.1 kg/m$^2$, and were 50% Hispanic, 40% African American, and 10% Caucasian (Table 1). Of these 10, 2 were weaned off insulin treatment safely without loss of glycemic control (following a standard protocol [2]), and 8 (including the 3 IA-2FL Ab–positive patients) required long-term, uninterrupted insulin treatment. The remaining 278 patients in the original A− KPD cohort were negative for both IA2-EC Ab and IA2-FL Ab. In terms of Aβ phenotype, 6 of the 201 A-β+ KPD patients (3%) were IA2-EC positive, and 4 of the 85 A-β− patients (4.7%) were IA2-EC positive.

## CONCLUSIONS

Autoimmune diabetes is defined by the presence of circulating islet autoantibodies. Autoantibodies to IA-2EC identify autoimmune diabetes in patients with different clinical phenotypes whose sera are nonreactive in the conventional IA-2 Ab assay. In a large cohort of KPD patients who were previously considered A−, we were able to identify 10 patients (3.5%) to be IA2-EC Ab positive. This frequency is comparable to that of patients with type 1 diabetes (1%) as well as patients with "typical" type 2 diabetes (4.7%).

These 10 patients did not fit any of the American Diabetes Association–defined diabetes categories (9). Their average age at diagnosis was 33 years compared with patients with type 1 diabetes in the general population who have a mean age at diagnosis of 14 years (10). Also, their mean BMI was 25.3 kg/m$^2$ compared with patients with type 2 diabetes in the general population aged 20–44 years who have a mean BMI of 34.5 kg/m$^2$ (11). In addition, these patients with KPD all presented with DKA at some point in their clinical course (four presented within 1 month of diagnosis of diabetes), differentiating them from patients with latent autoimmune diabetes of adulthood (12,13). Patients with autoimmune diabetes of adulthood, as conventionally defined, do not require insulin so soon after diagnosis because their clinical course is characterized by slow, progressive β-cell failure without acute metabolic decompensation such as DKA. The IA-2EC Ab may identify a unique phenotype of autoimmune diabetes in a cohort of patients with heterogeneous phenotypes who are nonreactive in the conventional (intracellular domain epitope) IA-2 Ab assay. A previous report identified masked epitopes within the intracellular domain of IA-2 (14); hence, one explanation for the presence of IA-2EC autoantibodies in this new subset of A+ KPD patients is unmasking of these epitopes. Neoepitope recognition by T cells and autoantibodies is relevant for manifestation of autoimmunity in a variety of conditions, including rheumatoid arthritis and type 1 diabetes (15). We previously identified several T-cell epitopes within IA-2EC (6).

The three IA-2FL Ab–positive patients remained dependent on insulin therapy after the index DKA episode, consistent with our previous report of the high risk of progression to insulin requirement among first-degree relatives of patients with type 1 diabetes with IA-2FL Ab positivity (5). These IA-2FL Ab–positive KPD patients were first diagnosed with diabetes at the ages of 20, 24, and 44 years. IA-2FL Ab could be a marker of long-term insulin requirement in A+ KPD patients. This distinction is important for clinical management, because more than one-third of KPD patients who are A− become insulin independent 6–8 weeks after the index DKA and maintain excellent glycemic control for prolonged periods of time. Assessing the likelihood of remaining insulin dependent after the index episode of DKA among newly diagnosed KPD patients with islet autoimmunity is important for safe and cost-effective medical management (7).

Our study is limited by the small number of antibody-positive patients, which can compromise the establishment of clinical associations. These assays were run on stored samples that were collected at variable times after the index episode of DKA. Though DKA was the first manifestation of diabetes for some patients, many had had diabetes for varying lengths of time before their index episode of DKA. Also, we do not know the prevalence of IA-2EC or IA-2FL in the general adult population to compare them to the present cohort.

In conclusion, measurement of IA2-EC Ab and IA2-FL Ab provides useful clinical data in classifying and managing patients with KPD. Testing for IA2-EC Ab refines the definitions of the subtypes of KPD, and the presence of IA2-FL Ab predicts long-term insulin requirement.

**Funding.** This work was supported by the Robert and Janice McNair Foundation, JDRF grant 17-2012-688 (to M.P.), and National Institute of Diabetes and Digestive and Kidney Diseases grants R01-DK-53456 (to M.P.) and R01-DK-1041411 (to A.B.).

**Duality of Interest.** No potential conflicts of interest relevant to this article were reported.

**Author Contributions.** S.N.M. performed the calculations, collected the clinical data, and drafted the manuscript. M.A.-C. performed the IA2-EC and IA2-FL autoantibody analyses and calculations. C.S.H. performed the initial autoantibody analyses to phenotype the patients with KPD. M.P. and A.B. designed the research, interpreted the data, and had primary responsibility for the final content. All authors edited and approved the final manuscript. M.P. and A.B. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

**Prior Presentation.** Parts of this study were presented in abstract form at ENDO 2017: The 99th Annual Meeting of The Endocrine Society, Orlando, FL, 1–4 April 2017.

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