Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 59 (2010) 1448 – 1455
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Presence or absence of a known diabetic ketoacidosis precipitant defines distinct syndromes of "A-β+" ketosis-prone diabetes based on long-term β-cell function, human leukocyte antigen class II alleles, and sex predilection

Ramaswami Nalini[a,b,1], Kerem Ozer[a,b,1], Mario Maldonado[a,c,1], Sanjeet G. Patel[a], Christiane S. Hampe[d], Anu Guthikonda[a,b], Jesus Villanueva[a,b], E. O'Brian Smith[e], Lakshmi K. Gaur[f], Ashok Balasubramanyam[a,b,*]

[a]*Translational Metabolism Unit, Baylor College of Medicine, Houston, TX 77030, USA*
[b]*Endocrine Service, Ben Taub General Hospital, Houston, TX, USA*
[c]*Novartis, Inc, Basel, Switzerland*
[d]*Department of Medicine, University of Washington, Seattle, WA 98195, USA*
[e]*Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030, USA*
[f]*Puget Sound Blood Center, University of Washington, Seattle, WA 98104, USA*

Received 1 October 2009; accepted 14 January 2010

Abstract

Ketosis-prone diabetes (KPD) is heterogeneous. Longitudinal follow-up revealed that patients with "A-β+" KPD (absent autoantibodies and preserved β-cell function) segregated into 2 subgroups with distinct evolution of β-cell function and glycemic control. Generalized linear analysis demonstrated that the variable that most significantly differentiated them was presence of a clinically evident precipitating event for the index diabetic ketoacidosis (DKA). Hence, we performed a comprehensive analysis of A-β+ KPD patients presenting with "provoked" compared with "unprovoked" DKA. Clinical, biochemical, and β-cell functional characteristics were compared between provoked and unprovoked A-β+ KPD patients followed prospectively for 1 to 8 years. Human leukocyte antigen class II allele frequencies were compared between these 2 groups and population controls. Unprovoked A-β+ KPD patients (n = 83) had greater body mass index, male preponderance, higher frequency of women with oligo-/anovulation, more frequent African American ethnicity, and less frequent family history of diabetes than provoked A-β+ KPD patients (n = 64). The provoked group had higher frequencies of the human leukocyte antigen class II type 1 diabetes mellitus susceptibility alleles DQB1*0302 (than the unprovoked group or population controls) and DRB1*04 (than the unprovoked group), whereas the unprovoked group had a higher frequency of the protective allele DQB1*0602. β-Cell secretory reserve and glycemic control improved progressively in the unprovoked group but declined in the provoked group. The differences persisted in comparisons restricted to patients with new-onset diabetes. "Unprovoked" A-β+ KPD is a distinct syndrome characterized by reversible β-cell dysfunction with male predominance and increased frequency of DQB1*0602, whereas "provoked" A-β+ KPD is characterized by progressive loss of β-cell reserve and increased frequency of DQB1*0302 and DRB1*04. Unprovoked DKA predicts long-term β-cell functional reserve, insulin independence, and glycemic control in KPD.

1. Introduction

Ketosis-prone diabetes (KPD) is defined by presentation with diabetic ketoacidosis (DKA) [1,2]. The recently validated "Aβ" classification scheme distinguishes 4 forms of KPD based on autoantibodies and β-cell functional reserve [3]. Two forms are characterized by complete, irreversible loss of β-cell function ("β−" KPD) and insulin dependence [3]. The other 2 forms comprise patients with substantially preserved β-cell functional reserve shortly after the index DKA ("β+" KPD): they include a group with autoantibodies (A+β+ KPD) and another without autoantibodies (A-β+ KPD). A-β+ KPD is a common and heterogeneous form; about half of these patients develop DKA without an evident precipitating factor, whereas the remainder have an

* Corresponding author. Translational Metabolism Unit, Division of Diabetes, Endocrinology and Metabolism, Baylor College of Medicine, Houston, TX 77030, USA. Tel.: +1 713 798 8654; fax: +1 713 798 8764.
E-mail address: ashokb@bcm.tmc.edu (A. Balasubramanyam).
[1] These authors contributed equally to this article.

0026-0495/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2010.01.009

associated precipitating event such as acute illness or treatment noncompliance [1,2].

We observed that about 35% to 40% of A-β+ KPD patients had persistent or sustained improvement in β-cell function over 12 to 24 months, with concomitant ability to attain good glycemic control or discontinue insulin therapy. Conversely, about 60% to 65% showed little improvement or progressive decline in β-cell function, with inability to

Table 1

A. Demographic, clinical, and biochemical characteristics of "unprovoked" and "provoked" A-β+ KPD patients at baseline and after 12 mo of treatment

	Unprovoked (n = 83)	Provoked (n = 64)	P
Age	41.6 ± 12.6	41.9 ± 12.8	.9
Age at diagnosis of diabetes	41.5 ± 12.5	37.5 ± 13.6	.06
Years with diabetes	0 ± 0	5.2 ± 6.8	<.0001
Index DKA was the first episode of DKA	83 (100%)	46 (67%)	<.0001
Male-to-female ratio	2.8:1	0.9:1	<.0001
Female with normal menstrual cycles at time of diagnosis of diabetes	3 (14.3%)	22 (57.9%)	.0007
Ethnicity (% by column)			.01
African American	42 (51%)	19 (28%)	
Hispanic	34 (41%)	42 (62%)	
White	7 (8.0%)	5 (7%)	
Asian	0	2 (3%)	
Family history of diabetes	58 (70%)	59 (92%)	.0007
BMI (kg/m^2)	33.2 ± 8.3	31.1 ± 8.9	.07
Weight category (% by column)			.04
Lean (BMI <25 kg/m^2)	17 (20.5%)	18 (28.1%)	
Overweight (BMI ≥25 and <30 kg/m^2)	11 (13.3%)	21 (32.8%)	
Obese (BMI ≥30 kg/m^2)	55 (66.3%)	25 (39.1%)	
Serum glucose at admission for index DKA	509.4 ± 187.3	418.6 ± 141.2	.001
HbA_{1c} at baseline (%)	13.9 ± 2.3	13.2 ± 2.4	.1
HbA_{1c} after 12 mo (%)	7.6 ± 2.7	8.8 ± 2.6	. 01
HbA_{1c} range at 12 mo after index DKA	(n = 81)	(n = 61)	<.001
HbA_{1c} ≤7.0%	52 (64.2%)	18 (29.5%)	
HbA_{1c} >7.0% and <9.0%	19 (23.5%)	20 (32.8%)	
HbA_{1c} ≥9.0%	10 (12.3%)	23 (37.8%)	
Fasting C-peptide at baseline (ng/dL)	1.9 ± 1.7	1.9 ± 1.5	.6
Fasting C-peptide at 12 mo (ng/dL)	3.3 ± 1.6	2.6 ± 1.6	.07
AUC-GST C-peptide at baseline (ng/[dL 10 min])	25.1 ± 17.3	27.9 ± 21.2	.3
AUC-GST C-peptide at 12 mo (ng/[dL 10 min])	43.6 ± 19.4	31.4 ± 15.4	.04
HOMA2-%β at baseline	35.2 ± 34.7	34.2 ± 39.7	.8
HOMA2-%β at 12 mo	128.9 ± 67.2	66.3 ± 47.1	<.0001
Change from baseline in HOMA2-%β	89.7 ± 74.8	30.1 ± 58.2	<.0001
HOMA2-IR at baseline	1.9 ± 1.8	1.9 ± 2.0	.8
HOMA2-IR at 12 mo	2.6 ± 1.7	2.7 ± 2.5	.8
Insulin discontinued	37 (44.6%)	15 (23.4%)	.008
Diabetes treatment at 12 mo			.001
Diet and exercise only	9 (10.8%)	0	
Oral antidiabetic medications only	25 (30.1%)	12 (18.8%)	
Insulin only	29 (34.9%)	33 (51.6%)	
Both insulin and oral antidiabetic medications	20 (24.1%)	19 (29.7%)	

B. HLA class II allele frequencies in unprovoked A-β+ KPD, provoked A-β+ KPD, and population controls

	HLA allele	Population controls	Unprovoked A-β+ KPD	Provoked A-β+ KPD
		n = 561	n = 53	n = 43
Susceptibility	DRB1*03	97 (17)	7 (13)	8 (19)
	DRB1*04	202 (36)	14 (26)	19 (44)†
	DRB1*07	104 (19)	8 (15)	6 (14)
	DQB1*02	154 (27)	15 (28)	9 (21)
	DQB1*0302	96 (17)	10 (19)	18 (42)*,†
Resistance	DRB1*15	137 (24)	11 (21)	6 (14)
	DQB1*0602	77 (14)	14 (26)*	7 (16)

Data are number (percentage).

* $P < .05$ (A-β+ KPD subset compared with population controls).

† $P < .05$ (unprovoked A-β+ KPD compared with provoked A-β+ KPD).

discontinue insulin. Generalized linear analysis of data at the 24-month time point, using age, sex, ethnicity, and body mass index (BMI) as covariates, demonstrated that the variable that most significantly differentiated the 2 subgroups with these distinct "natural histories" was whether or not the patient had had a clinically evident precipitating event for the index DKA; that is, categorization of patients with respect to "provoked" compared with "unprovoked" DKA yielded the most significant difference with respect to linear change across time ($P = .04$).

To understand the basis for this difference, we prospectively tracked a large number of patients belonging to these 2 subgroups over a prolonged period to delineate their clinical, biochemical, and genetic characteristics.

2. Methods

2.1. Patient identification

The protocol was approved by the Institutional Review Boards for Human Studies of Baylor College of Medicine and the Harris County Hospital District. Five hundred eighty-four consecutive adult patients admitted with DKA to the Ben Taub General Hospital from June 1, 1999, to October 31, 2006, were interviewed during the admission and offered follow-up in the KPD research clinic. Two hundred ninety-two patients gave informed consent. They were classified according to the Aβ scheme [3], based on fasting and stimulated C-peptide levels measured 3 to 4 weeks after correction of ketoacidosis as well as presence or absence of β-cell autoantibodies, into 4 categories described previously [1,3,4]. One hundred forty-seven patients were classified as having A-β+ KPD (ie, lacking autoantibodies and with β-cell functional reserve). These patients were followed prospectively for 1 to 8 years.

For the reasons described above, these longitudinally followed A-β+ KPD patients were classified as "provoked" if they had a clinically evident precipitant of the index DKA or "unprovoked" if they lacked a precipitant. The DKA-precipitating factors were either acute medical conditions in 55.7% of the provoked patients (infections, pancreatitis, steroid use, hypertriglyceridemia, seizures, upper gastrointestinal bleeding) or discontinuation of insulin or oral agents in persons previously known to have diabetes in 44.3%. The physicians who admitted and treated the patients during the index DKA episode performed the diagnosis and documentation of the acute medical conditions or of noncompliance with prior antidiabetic therapy. The investigators, who did not participate in the admission or inpatient management of patients at the time of the index DKA episode, reviewed postdischarge hospital charts (laboratory test results, microbiological, and radiological data) to confirm the primary inpatient physicians' diagnoses.

After the index episode of DKA, all patients were followed in a dedicated research clinic with a standard outpatient management protocol [3,4]. Patients were placed on twice-daily neutral protamine Hagedorn (NPH) insulin with or without premeal rapid-acting insulin, and self-monitoring of capillary blood glucose levels. If glucose values during the following 2-week period attained American Diabetes Association–defined goals [5], the insulin dose was reduced by 50%; and the patient was reassessed 1 week later. If the mean blood glucose values remained at American Diabetes Association goals at 2 consecutive clinic visits, insulin was discontinued; and the patient was monitored closely. Conversely, if the patient developed ketosis upon decreasing the insulin dose, the regimen was intensified; and no further attempts were made to discontinue insulin.

2.2. Measures

Age, age at diagnosis of diabetes, sex, ethnicity (by patient self-identification), duration of diabetes, family history of diabetes, height, weight, BMI, and treatment before and after DKA were recorded. A comprehensive reproductive history was obtained from all women, including age at menarche and menopause, menstrual frequency, and use of estrogen/progestin for contraception or replacement. Polycystic ovary syndrome was defined by 2 of the following: oligo- or anovulation, clinical or biochemical hyperandrogenism, and polycystic ovaries by ultrasound examination [6].

Laboratory values measured during the hospital stay for the index DKA were fasting serum glucose levels, hemoglobin A_{1c} (HbA_{1c}), total cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol. C-peptide concentration was measured in a fasting serum sample obtained 24 hours after complete correction of ketoacidosis and before the morning dose of subcutaneous insulin. Within 2 to 3 weeks after resolution of DKA, C-peptide response to glucagon stimulation was performed as described previously [1]. C-peptide concentrations were determined using a human C-peptide radioimmunoassay kit (Linco Research, St Louis, MO). Serum samples were analyzed for the presence of autoantibodies to the 65- and 67-kd isoforms of glutamic acid decarboxylase (GAD65 Ab, GAD67 Ab), tyrosine-phosphatase like protein IA-2 (IA-2 Ab), and Zn-T8 (Zn-T8 Ab to all 3 isotypes: ZnT8R, ZnT8W, and ZnT8G) by highly sensitive and specific quantitative radioligand binding assay methods [1,7]. β-Cell function and insulin resistance indices were calculated using the homeostasis model assessment (HOMA) 2 model [8].

Hemoglobin A_{1c} was measured every 3 months, using high-performance liquid chromatography. Fasting serum C-peptide and glucose levels (to calculate HOMA2-IR and HOMA2-%β) were measured 6 and 12 months after the index episode of DKA and yearly thereafter.

2.3. Analysis

We compared baseline and follow-up characteristics between the 2 subgroups using the JMP 6.0.2 statistical package (SAS, Cary, NC, 2006). Demographic, clinical, and biochemical data were analyzed using Student t test, analysis of variance, and χ^2 tests. P values were calculated using the likelihood ratio method, with significance at $P < .05$. A subanalysis of patients presenting with DKA as the first

manifestation of diabetes was also carried out, comparing the 16 provoked patients with new-onset diabetes to new-onset unprovoked patients in 2 ways: (*a*) to all 83 new-onset unprovoked patients and (*b*) to a subgroup of 18 representative new-onset unprovoked patients group selected based on their age, sex, waist circumference, total cholesterol, triglycerides, BMI, and initial HbA_{1c} being within 1.5 SD of the mean from the complete set of 83. Both the complete set of 83 new-onset unprovoked patients and the representative subgroup of 18 patients satisfied the Shapiro-Wilk test for normality.

The 4 forms of KPD differ in their frequencies of human leukocyte antigen (HLA) class II alleles known to confer susceptibility or resistance to autoimmune type 1 diabetes mellitus [9]. Hence, we compared the frequencies of key alleles between A-β+ KPD patients who presented with and without precipitating factors for DKA as well as those of a large control population of a similarly heterogeneous ethnic composition in Texas [10]. Genotyping was performed as described previously [9]. Fisher exact test was used to compare allele frequencies between A-β+ KPD subgroups and normal control subjects.

3. Results

Eighty-three patients (56.5%) had A-β+ KPD with unprovoked DKA, whereas 64 (43.5%) had A-β+ KPD with provoked DKA. The DKA precipitants that defined the latter group included acute illness in 35 patients and noncompliance with diabetes therapy in 29. All 83 unprovoked A-β+ KPD patients were "new onset," and none developed another episode of DKA during the follow-up period of this study. Of the 64 patients with provoked A-β+ KPD, 16 had new-onset diabetes at presentation with DKA (with a concomitant acute illness), whereas 48 had previously diagnosed diabetes (1 managed with diet/exercise, 21 on oral antidiabetic medications, 10 on insulin and oral medications, and 16 on insulin alone).

3.1. Clinical and metabolic differences

3.1.1. Baseline

Mean duration of diabetes was 0 year in the unprovoked A-β+ KPD subgroup compared with 5.2 ± 6.8 years in the provoked A-β+ KPD subgroup ($P < .0001$) (Table 1). Ethnic distribution in the unprovoked subgroup was 51% African American, 41% Hispanic, 8.0% white, and 0% Asian, compared with 28%, 62%, 7%, and 3%, respectively, among the provoked subgroup ($P = .01$). Fifty-eight (70%) of the unprovoked patients had a family history of diabetes compared with 59 (92%) of the provoked patients ($P = .0007$). Patients of the unprovoked subgroup had greater mean BMI (33.2 ± 8.3 compared with 31.1 ± 8.9 kg/m^2, $P = .07$) and a higher frequency of obesity (66.3% [95% confidence interval {CI}, 56.4%-75.2%] compared with 39.1% [95% CI, 28.5%-50.9%], $P = .04$).

Male predominance was apparent in the unprovoked A-β+ KPD subgroup, with a male-to-female ratio of 2.8:1 compared with 0.9:1 in the provoked subgroup ($P < .0001$). Among the 21 women in the unprovoked subgroup, only 3 (14.3%) had experienced regular menses during the previous year, whereas 22 (57.9%) of the 38 women in the provoked subgroup had experienced regular menses ($P = .0007$). Analysis of menstrual history at the time of the index DKA revealed that 61.9% of unprovoked A-β+ KPD women had attained menopause and 14.3% had polycystic ovary syndrome compared with 31.6% and 7.9%, respectively, of provoked patients ($P = .02$) (Fig. 1).

Patients of both A-β+ KPD subgroups had evidence of chronic hyperglycemia at the time of the index DKA, but unprovoked patients had higher serum glucose concentrations and HbA_{1c} levels. Baseline measures of β-cell functional reserve showed no group differences: mean fasting C-peptide levels measured 2 to 3 weeks after resolution of DKA were similar in unprovoked and provoked groups (1.9 ± 1.7 compared with 1.9 ± 1.5 ng/mL, $P = .6$), as were mean areas under the curve (AUCs) for C-peptide concentration during the initial glucagon stimulation test (25.1 ± 17.3 compared with 27.9 ± 21.2 ng/mL over 10 minutes, $P = .3$) and HOMA2-%β (35.2 ± 34.7 compared with 34.2 ± 39.7, $P = .8$) (Table 1). Baseline HOMA2-IR was also similar in the 2 subgroups.

3.1.2. Follow-up

At 12 months of follow-up, HbA_{1c} was obtained in 137 patients. Unprovoked patients (n = 76) had significantly lower mean HbA_{1c} than provoked patients (n = 61) (7.6% ± 2.7% compared with 8.8% ± 2.6%, $P = .0001$) (Table 1). The unprovoked subgroup also included a higher proportion of patients with HbA_{1c} less than 7% (64.2% [95% CI, 53.1%-73.2%] compared with 29.5% [95% CI, 20.6%-43.5%], $P < .001$). Insulin treatment was discontinued in a higher proportion of unprovoked patients (44.6% [95% CI,

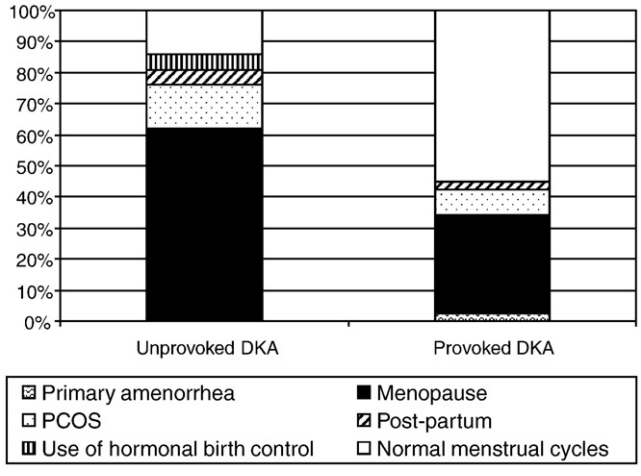

Fig. 1. Reproductive/menstrual status of women with "unprovoked" compared with "provoked A-β+ KPD at the time of index DKA. The different designs in each bar represent percentage of women with the indicated reproductive/menstrual status.

34.4%-55.7%] compared with 23.4% [95% CI, 15.9%-36.4%], $P = .01$). The distribution of diabetes treatment modalities at 12 months was 10.8% diet/exercise, 30.1% oral medications only, 24.1% insulin plus oral medications, and 34.9% insulin only in the unprovoked group compared with 0%, 18.8%, 29.7%, and 51.6%, respectively, in the provoked group ($P = .001$).

Despite similar levels of β-cell functional reserve at baseline, there were subgroup differences in these measures after 12 months. The unprovoked group showed greater improvement than the provoked group: mean serum fasting C-peptide, 3.3 ± 1.6 compared with 2.6 ± 1.6 ng/mL, $P = .07$; mean AUC for C-peptide during the glucagon stimulation test (GST), 43.6 ± 19.4 compared with 31.4 ± 15.4 ng/mL over 10 minutes, $P = .04$; HOMA2-%β, 128.9% ± 67.2% compared with 66.3% ± 47.1%, $P < .0001$.

3.2. Clinical and metabolic differences between new-onset patients of the 2 A-β+ KPD subgroups

Because duration of diabetes can affect β-cell functional reserve and glycemic control, we performed a subgroup comparison restricted to new-onset patients (Table 2). This comparison revealed the same differences between new-onset provoked and unprovoked patients as there were between all provoked and unprovoked patients. With regard to β-cell functional and glycemic parameters at 12 months, new-onset unprovoked patients had significantly higher fasting C-peptide values (3.3 ± 1.6 compared with 2.5 ± 1.2 ng/mL, $P = .04$), AUC-GST C-peptide levels (43.6 ± 19.4 compared with 28.4 ± 9.7 ng/mL over 10 minutes, $P = .04$), and HOMA2-%β scores (128.9% ± 67.2% compared with 79.8% ± 53.2%, $P = .01$), together with lower HbA_{1c} (7.6% ± 2.7% compared with 8.7% ± 3.5%, $P = .04$), than new-onset provoked patients. Because there was asymmetry in the number of new-onset patients in each subgroup (16 provoked and 83 unprovoked), we performed another comparison between the 16 new-onset provoked patients and 18 representative new-onset unprovoked patients selected as described in "Methods." This comparison yielded similar results and showed that after 12 months of follow-up these 18 representative new-onset unprovoked patients had significantly higher fasting C-peptide values (3.3 ± 1.1 compared with 2.5 ± 1.2 ng/mL, $P = .04$), AUC-GST C-

Table 2
Comparison of all 18 new-onset "provoked" to new-onset "unprovoked" A-β+ KPD patients at baseline and after 12 months of treatment

	New-onset provoked (n = 16)	All new-onset unprovoked (n = 83)	Representative new-onset unprovoked (n = 18)	P new-onset provoked (n = 16) vs all new-onset unprovoked (n = 83)	P new-onset provoked (n = 16) vs representative new-onset unprovoked (n = 18)
Age	37.1 ± 12.7	41.6 ± 12.6	41.9 ± 9.6	.2	.2
Age at diagnosis of diabetes	37.1 ± 12.3	41.5 ± 12.5	41.9 ± 9.6	.2	.2
Years with diabetes	0 ± 0	0 ± 0	0 ± 0	NA	NA
Male to female ratio	1.25:1	2.8:1	3.50:1	.1	.2
Ethnicity (% by column)				.01	.04
African American	2 (12.5%)	42 (51%)	11 (61.1%)		
Hispanic	10 (62.5%)	34 (41%)	5 (27.8%)		
White	2 (12.5%)	7 (8.0%)	2 (11.1%)		
Asian	2 (12.5%)	0	0		
Family history of diabetes	13 (81.2%)	58 (70%)	14 (77.8%)	.2	.4
BMI (kg/m^2)	31.4 ± 8.9	33.2 ± 8.3	32.6 ± 5.9	.5	.6
Weight category (% by column)					
Lean (BMI <25 kg/m^2)	3 (18.8%)	17 (20.5%)	2 (11.1%)	.03	.05
Overweight (BMI ≥25 and <30 kg/m^2)	8 (50%)	11 (13.3%)	2 (11.1%)		
Obese (BMI ≥30 kg/m^2)	5 (31.2%)	55 (66.3%)	14 (77.8%)		
Serum glucose at index DKA	438.3 ± 179.1	509.4 ± 187.3	559.2 ± 181.9	.1	.04
HbA_{1c} at baseline (%)	12.7 ± 2.4	13.9 ± 2.3	14.9 ± 1.9	.08	.02
HbA_{1c} at 12 mo (%)	8.7 ± 3.5	7.6 ± 2.7	7.2 ± 2.3	.04	.05
Fasting C-peptide at baseline (ng/dL)	1.8 ± 1.3	1.9 ± 1.7	1.8 ± 0.6	.7	.9
Fasting C-peptide at 12 mo (ng/dL)	2.5 ± 1.2	3.3 ± 1.6	3.3 ± 1.1	.04	.04
AUC-GST C-peptide at baseline (ng/[dL 10 min])	29.8 ± 16.1	25.1 ± 17.3	23.8 ± 11.8	.2	.3
AUC-GST C-peptide at 12 mo (ng/[dL 10 min])	28.4 ± 9.7	43.6 ± 19.4	44.3 ± 14.7	.04	.03
HOMA2-%β at baseline	39.2 ± 31.7	35.2 ± 34.7	32.9 ± 12.4	.2	.3
HOMA2-%β at 12 mo	79.8 ± 53.2	128.9 ± 67.2	109.7 ± 54.9	.01	.04
HOMA IR at baseline	2.0 ± 1.8	1.9 ± 1.8	2.0 ± 1.8	.5	.6
HOMA IR at 12 mo	2.6 ± 2.3	2.6 ± 1.7	2.7 ± 1.9	.7	.8

"Representative new-onset unprovoked" group refers to 18 patients of the new-onset unprovoked group selected based on their mean values for key phenotypic characteristics being within 1.5 SD of the means in the complete set of 88 new-onset unprovoked patients. See text for details.

peptide levels (44.3 ± 14.7 compared with 28.4 ± 9.7 ng/mL over 10 minutes, $P = .03$), and HOMA2-%β scores (109.7% ± 54.9% compared with 79.8% ± 53.2%, $P = .04$) and lower HbA_{1c} (7.2% ± 2.3% compared with 8.7% ± 3.5%, $P = .05$) than the 16 new-onset provoked patients.

3.3. Long-term evolution of β-cell function

Evolution of β-cell functional reserve in the 2 A-β+ KPD subgroups was assessed over follow-up periods from 1 to 8 years, and of glycemic control from 1 to 5 years (Fig. 2). Profiles of fasting C-peptide levels and HOMA2-%β scores were significantly different between the subgroups by repeated-measures analysis of variance ($P = .027$ and .035, respectively). The unprovoked subgroup demonstrated greater improvement after 12 months, followed by stability or further improvement over years 2 to 8, whereas the provoked subgroup demonstrated less improvement over years 1 and 2, followed by a decline in function.

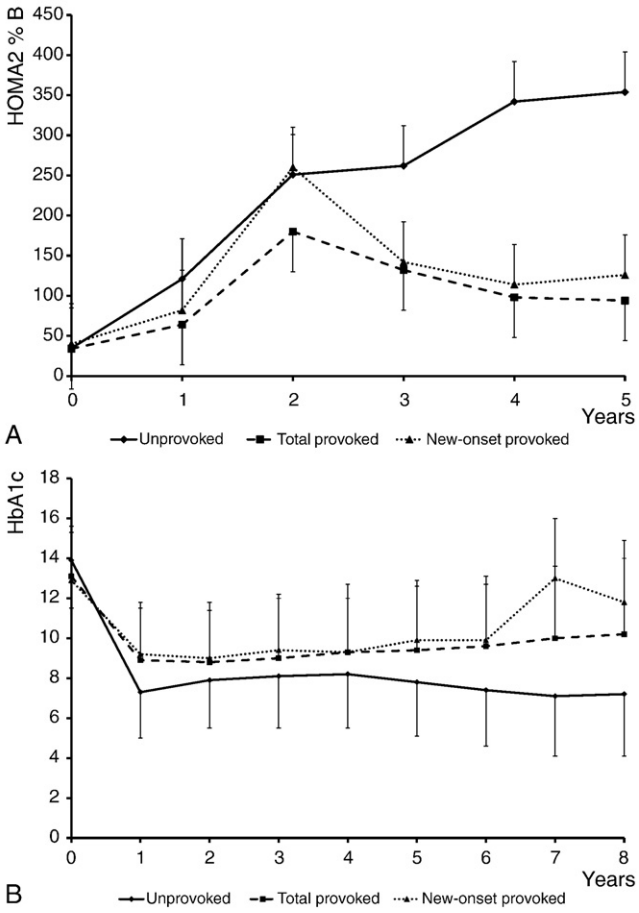

Fig. 2. Longitudinal measures of β-cell function (HOMA2%β) and glycemic control (HbA_{1c}) for the unprovoked A-β+ KPD (all new onset), total provoked A-β+ KPD, and subgroup of new-onset provoked A-β+ KPD patients. Data points are mean ± SD. Diamonds/solid line, unprovoked A-β+ KPD; squares/dashed line, total provoked A-β+ KPD; triangle/dotted line, new-onset provoked A-β+ KPD. By repeated-measures analysis of variance, $P = .035$ for differences in HOMA2%β and $P = .005$ for group differences in HbA_{1c}.

Concomitantly, unprovoked patients maintained better, sustained glycemic control over time ($P = .005$). The longitudinal patterns of β-cell function in the 16 provoked A-β+ KPD patients with new-onset diabetes were similar to those of all provoked patients taken together, except at the year 2 time point, when they were similar to the unprovoked A-β+ KPD patients.

3.4. HLA class II alleles

Frequency of the classic type 1 diabetes mellitus susceptibility allele DQB1*0302 was significantly higher in the provoked A-β+ KPD subgroup than either the unprovoked subgroup (42% compared with 19%, $P < .05$) or population controls (42% compared 17%, $P < .05$), with no difference between the latter 2 groups. Frequency of the classic type 1 diabetes mellitus susceptibility allele DRB1*04 was significantly higher in the provoked A-β+ KPD subgroup than the unprovoked subgroup (44% compared with 26%, $P < .05$). Conversely, frequency of the type 1 diabetes mellitus resistance allele DQB1*0602 was significantly higher in the unprovoked A-β+ KPD subgroup than the provoked subgroup (26% compared with 14%, $P < .05$), whereas the latter had a similar frequency of this allele as the population controls. Analysis of ethnic-specific allele frequencies, as performed previously [9], was not attempted here because of the small sample sizes in the A-β+ KPD subgroups when they were further divided by ethnicity.

4. Discussion

These data demonstrate that A-β+ KPD comprises 2 groups of patients distinguishable by whether the index DKA event was provoked by a clinically evident precipitating event. Patients with unprovoked A-β+ KPD have better recovery and sustained stability of β-cell function, with better long-term glycemic control and increased likelihood of becoming insulin independent, than those with provoked A-β+ KPD. There is male predominance in the unprovoked group but no sex predilection in the provoked group. Unprovoked A-β+ KPD patients frequently present with DKA as the initial manifestation of diabetes, whereas provoked patients often have a preexisting diagnosis of type 2 diabetes mellitus; however, duration of diabetes does not appear to affect subgroup differences in recovery and evolution of β-cell functional reserve. These distinctions persist over a prolonged period and point to different pathophysiologic bases for β-cell dysfunction in the 2 subgroups of A-β+ KPD.

We previously reported population-based and ethnic-specific differences in HLA class II allele frequencies between the 4 forms of KPD [9], including a higher-than-population frequency of the type 1 diabetes mellitus susceptibility allele DQB1*0302 in A-β+ KPD. The present data show that this high frequency of DQB1*0302 is largely accounted for by patients with provoked A-β+ KPD, whereas unprovoked A-β+ patients have a higher frequency of the protective allele

DQB1*0602. The provoked A-β+ KPD subgroup also has a higher frequency of the HLA class II susceptibility allele DRB1*04 compared with the unprovoked subgroup. The finding of these HLA class II allele differences between clinically distinct subgroups of an apparently "non-autoimmune" mediated form of KPD has pathophysiologic implications. The high prevalence of DQB1*0302 and DRB1*04 in the provoked patients suggests that specific immunologic factors may contribute to their inexorable decline in β-cell function. Conversely, reversibility of the β-cell functional defect in the unprovoked subgroup may be due in part to the protective effects of DQB1*0602. Li et al [11] have reported previously that persons with type 2 diabetes mellitus and a "mixed" family history of both type 1 and type 2 diabetes mellitus have a frequency of the DQB1*0302 allele that is higher than that among those with a family history of only type 2 diabetes mellitus, but lower than that among patients with type 1 diabetes mellitus; patients with type 2 diabetes mellitus who possess the DQB1*0302 allele have decreased insulin responses to oral glucose challenge compared with those who do not [11]. Although we lack details regarding "type" of diabetes in the family histories of our provoked A-β+ KPD patients, it is possible that they have an accelerated form of the β-cell dysfunction that develops progressively in patients with type 2 diabetes mellitus and "mixed" family history as described by Li et al. Immunologic studies are ongoing to identify mechanisms such as altered T-lymphocyte reactivity to islet antigens that may underlie the different autoantibody-negative forms of KPD.

Other investigators have reported male preponderance in cohorts of West African [12,13] and African American [14] patients with a phenotype of A-β+ KPD. The former study was confined to patients who presented with unprovoked DKA or ketosis, whereas the latter did not distinguish provoked from unprovoked presentation; and neither distinguished new-onset from previously known diabetes. The male-to-female ratios in these cohorts were 3.12 and 1.63, respectively. In our cohort of A-β+ KPD patients of mixed ethnicity (African American, Hispanic, and white), the separation of these 2 subgroups slightly increased the ratio in favor of men and restricted it to the unprovoked subgroup. Hence, "unprovoked, new-onset A-β+ KPD" further refines the phenotype of a unique, sex-associated syndrome, making it more amenable to accurate genetic and metabolic characterization. The potential sex effect in the etiology of unprovoked A-β+ KPD is heightened by the finding that 86% of women in this subgroup had abnormal menstrual cycles or amenorrhea, compared with only 40% in the provoked subgroup. Louet et al [13] have investigated the role of male sex/anovulation and variations in the neurogenin-3 gene in the pathogenesis of KPD and have demonstrated that the former was associated with diminished insulin secretion and the latter with lack of glycemic control. Collectively, the human data point to an intrinsic β-cell mechanism of dysfunction in the syndrome of male-predominant, unprovoked A-β+ KPD. These findings add significance to evidence from transgenic mice that altered sex hormone action due to altered tissue-specific signaling through their estrogen or androgen receptors may affect β-cell function or proneness to ketosis [15].

Unprovoked A-β+ KPD patients had a greater mean BMI and higher frequency of obesity, together with a lower frequency of family history of diabetes, than the provoked patients. Unprovoked A-β+ KPD may be influenced by specific metabolic factors related to increased adiposity or glycemic levels, or to dietary or environmental factors that may affect β-cell function or ketogenesis, whereas provoked A-β+ KPD may be influenced more by genetic factors related to the vulnerability of β-cells to damage. These differences could play a role in the reversibility of β-cell dysfunction in the former, and lack thereof in the latter.

In our multiethnic KPD cohort, African Americans were most highly represented in the unprovoked A-β+ subgroup; and Hispanics were most highly represented in the provoked subgroup. The increased association of African ancestry with unprovoked A-β+ KPD is consistent with reports of similar phenotypes of KPD among West Africans [12] and African Americans [16,17]; however, this syndrome is by no means restricted to such populations [16-19]. About 40% of our unprovoked A-β+ patients were of Hispanic decent, and 8% were white. Although not precisely specified, syndromes resembling unprovoked A-β+ KPD have been reported in persons of such diverse ethnicities as Asians [20], Apache Indians [21], and whites [22].

Mauvais-Jarvis et al [12] reported long-term clinical evolution in patients of sub-Saharan African origin with "KPD–non-insulin dependent" (KPD-NID), a condition defined very similarly to "unprovoked A-β+ KPD." The evolution of β-cell function in KPD-NID patients paralleled that of our cohort of unprovoked A-β+ KPD patients. Forty percent of the KPD-NID patients were insulin independent after long-term follow-up, consistent with the 45% rate of insulin discontinuation among the unprovoked A-β+ KPD patients in the present study.

These data indicate that A-β+ KPD comprises 2 subgroups of patients with distinct etiologies. The unprovoked subgroup represents a syndrome of severe but partially reversible β-cell dysfunction, with potential sex-related influences and effects of HLA-DQB1*0602 on its pathophysiology and clinical course. The provoked subgroup has a more irreversible and sex-independent trajectory of β-cell dysfunction, with potential contributions from the effects of HLA-DQB1*0302 and DRB1*04. Future studies should investigate metabolic and other genetic factors responsible for the distinct mechanisms of β-cell dysfunction and proneness to ketosis in these well-characterized KPD patients.

Acknowledgment

This study was supported by funds from the Alkek Foundation, RO1-HL73696, and R21-DK082827 (to AB). The authors thank the house officers and staff of the Ben

Taub General Hospital for excellent inpatient care of the patients. The long-term follow-up glycemic data of A-β+ KPD patients were presented in part as a poster at the American Diabetes Association's 68th Scientific Sessions, San Francisco, in June 2008.

References

[1] Maldonado M, Hampe CS, Gaur LK, D'Amico S, Iyer D, Hammerle LP, et al. Ketosis-prone diabetes: dissection of a heterogeneous syndrome using an immunogenetic and beta cell functional classification, prospective analysis, and clinical outcomes. J Clin Endocrinol Metab 2003;88:5090-8.

[2] Umpierrez GE, Smiley D, Kitabchi AE. Narrative review: ketosis-prone type 2 diabetes mellitus. Ann Intern Med 2006;144:350-7.

[3] Balasubramanyam A, Garza G, Rodriguez L, Hampe CS, Gaur L, Lernmark A, et al. Accuracy and predictive value of classification schemes for ketosis-prone diabetes. Diabetes Care 2006;29:2575-9.

[4] Balasubramanyam A, Nalini R, Hampe CS, Maldonado M. Syndromes of ketosis-prone diabetes mellitus. Endocr Rev 2008;29:292-302.

[5] Standards of medical care for patients with diabetes mellitus. Diabetes Care 2003;26(Suppl 1):S33-50.

[6] The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group: 2004 revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19:41-7.

[7] Sani FV, Oak S, Radtke J, Lernmark Å, Lynch K, Agardh CD, et al. ZnT8 autoantibody titers in type 1 diabetes patients decline rapidly after clinical onset. Autoimmunity 2009 [in press].

[8] Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care 1998;21:2191-2.

[9] Nalini R, Gaur LK, Maldonado M, Hampe CS, Rodriguez L, Garza G, et al. HLA class II alleles specify phenotypes of ketosis-prone diabetes. Diabetes Care 2008;31:1195-200.

[10] McCormack J., Osowski L.D.: US normal. In HLA 1998. Gjertson D. W., and Terasaki P.I., Eds. American Society of Histocompatibility and Immunogenetics, Lenexa, Kansas, 1998, p. 232, 258-59, 261.

[11] Li H, Lindholm E, Almgren P, Gustafsson A, Forsblom C, Groop L, et al. Possible human leukocyte antigen–mediated genetic interaction between type 1 and type 2 diabetes. J Clin Endocrinol Metab 2001;86:574-82.

[12] Mauvais-Jarvis F, Sobngwi E, Porcher R, Riveline JP, Kevorkian J, Vaisse C, et al. Ketosis-prone type 2 diabetes in patients of sub-Saharan African origin: clinical pathophysiology and natural history of beta cell dysfunction and insulin resistance. Diabetes 2004;53:645-53.

[13] Louet JF, Smith SB, Gautier JF, Molokhia M, Virally ML, Kevorkian JP, et al. Gender and neurogenin3 influence the pathogenesis of ketosis-prone diabetes. Diabetes Obes Metab 2008;10:912-20.

[14] Pinero-Pilona A, Raskin P. Idiopathic type 1 diabetes. J Diabetes Complications 2001;15:328-35.

[15] Le May C, Chu K, Hu M, Ortega CS, Simpson ER, Korach KS, et al. Estrogens protect pancreatic beta cells from apoptosis and prevent insulin-deficient diabetes mellitus in mice. Proc Natl Acad Sci U S A 2006;103:9232-7.

[16] Umpierrez GE, Woo W, Hagopian WA, Isaacs SD, Palmer JP, Gaur LK, et al. Immunogenetic analysis suggests different pathogenesis for obese and lean African-Americans with diabetic ketoacidosis. Diabetes Care 1999;22:1517-23.

[17] Ramos-Roman MA, Pinero-Pilona A, Adams-Huet B, Raskin P. Comparison of type 1, type 2, and atypical ketosis-prone diabetes at 4 years of diabetes duration. J Diabetes Complications 2006;20:137-44.

[18] Sobngwi E, Mauvais-Jarvis F, Vexiau P, Mbanya JC, Gautier JF. Diabetes in Africans. Part 2: ketosis-prone atypical diabetes mellitus. Diabetes Metab 2002;28:5-12.

[19] Banerji MA, Chaiken RL, Huey H, Tuomi T, Norin AJ, Mackay IR, et al. GAD antibody negative NIDDM in adult black subjects with diabetic ketoacidosis and increased frequency of human leukocyte antigen DR3 and DR4. Flatbush diabetes. Diabetes 1994;43:741-5.

[20] Aizawa T, Funase Y, Katakura M, Asanuma N, Yamauchi K, Yoshizawa K, et al. Ketosis-onset diabetes in young adults with subsequent non-insulin-dependency, a link between IDDM and NIDDM? Diabet Med 1997;14:989-91.

[21] Wilson C, Krakoff J, Gohdes D. Ketoacidosis in Apache Indians with non–insulin-dependent diabetes mellitus. Arch Intern Med 1997;157:2098-100.

[22] Pitteloud N, Philippe J. Characteristics of Caucasian type 2 diabetic patients during ketoacidosis and at follow-up. Schweiz Med Wochenschr 2000;130:576-82.