Islet Cell Autoimmunity in a Triethnic Adult Population of the Third National Health and Nutrition Examination Survey

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Markers of humoral islet cell autoimmunity, such as autoantibodies (AAs) against the 65-kDa isoform of GAD (GAD65), serve as determinants of risk for autoimmune diabetes. Despite the high prevalence of diabetes in U.S. racial and ethnic minority adult populations, little is known concerning the prevalence of GAD65 AA in these groups. We estimated the prevalence of GAD65 AA in 1,064 diabetic and 1,036 nondiabetic participants who were 40–90 years of age from the Third National Health and Nutrition Examination Survey (NHANES III), which provides a representative ethnic sample of the U.S. diabetic population. The prevalence of GAD65 AA was higher in diabetic participants compared with nondiabetic participants in non-Hispanic whites (n = 920; 6.3% vs. 2.0%; P = 0.001) and non-Hispanic blacks (n = 534; 3.7% vs. 1.3%; P = 0.08) but not in Mexican Americans (n = 646; 1.2% vs. 2.6%; P = 0.18). Among diabetic non-Hispanic whites and non-Hispanic blacks, being GAD65 AA positive was associated with lower BMI and C-peptide (P < 0.05). These results may reflect the outcome of an autoimmune process leading to β-cell destruction/dysfunction in non-Hispanic white and non-Hispanic black adult diabetic patients as it occurs in a similar manner in type 1 diabetes. Among diabetic Mexican Americans, the lower prevalence of GAD65 AA suggests a lower frequency of autoimmune-related diabetes. *Diabetes* 53:1293–1302, 2004

The prevalence of diabetes (diagnosed and undiagnosed) in adults ≥20 years of age in the U.S. was estimated to be 7.8% in the period between 1998 and 1994 based on data collected for the Third National Health and Nutrition Examination Survey (NHANES III) (1). It is projected that the prevalence of diabetes will continue to grow and that the largest increase will be among those aged ≥75 years (2,3). It has increasingly become more evident that in adults and the elderly, clinical criteria alone are no longer sufficient to permit an accurate distinction of type of diabetes (4,5). Markers of humoral islet cell autoimmunity seem to hold great promise as determinants of risk for autoimmune diabetes and for enhancing diabetes classification, even in older populations (6).

Antibodies to islet cell autoantigens, such as the 65-kDa isoform of GAD (GAD65), serve as predictors of disease onset and are found in 70–80% of children and adolescents before and at clinical diagnosis of type 1 diabetes (7,8). Autoantibodies (AAs) to GAD65, which serve as determinants of risk for autoimmune diabetes, seem to be present in up to 12% of adults who have a clinical diagnosis of type 2 diabetes (6,9–11). This form of diabetes with initial type 2 diabetes presentation but with evidence of islet cell autoimmunity has been termed latent autoimmune diabetes in adults, or type 1.5 diabetes, and has been associated with progressive decline in β-cell function and future insulin requirement in some populations (10,12–15).

Diabetes is more prevalent among non-Hispanic blacks and Mexican Americans than non-Hispanic whites (1). It is projected that the increase in diabetes will disproportionately affect non-Hispanic blacks and Hispanics (2,3). Despite the fact that in the U.S. the number of patients being diagnosed with type 2 diabetes is approaching an epidemic level, little is known concerning the prevalence of islet cell autoimmunity in these groups. The aim of the present study was to estimate the prevalence of GAD65 AAs in non-Hispanic white, non-Hispanic black, and Mexican-American adults who were previously evaluated as part of the NHANES III.

RESEARCH DESIGN AND METHODS

NHANES III. NHANES III was conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention from 1988 to 1994 (16). The survey included a nationally representative sample of the U.S. civilian noninstitutionalized population. A complex, stratified, multistage probability cluster sampling design was used with oversampling of blacks and Mexican Americans. The NHANES III survey consisted of a home interview followed by a physical examination in a mobile examination center (MEC). Details of this survey and methods of operation have been published (16).

During the home interview, data collected included medical history, health-related behavior, and sociodemographics. During this interview, individuals were asked whether they had a history of diabetes, their age at diagnosis, their use of diabetes medication, and their family history of diabetes. During the MEC examination, anthropometric assessments were performed. The MEC examinations could take place in the morning, after-

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AA, autoantibody; ADA, American Diabetes Association; GAD65, 65-kDa isoform of GAD; MEC, mobile examination center; NCHS, National Center for Health Statistics; NHANES, National Health and Nutrition Examination Survey; OHGA, oral hypoglycemic agent; WHO, World Health Organization.

TABLE 1
Characteristics of study population by diabetic status and race/ethnicity, NHANES III participants (N = 2,100)*

	Non-Hispanic whites		Non-Hispanic blacks		Mexican Americans	
	No diabetes	Diabetes	No diabetes	Diabetes	No diabetes	Diabetes
n	491	429	239	295	306	340
Sex (% men)	260 (53)	220 (51)	118 (49)	118 (40)†	150 (49)	151 (44)
Age (years)	64 ± 12	69 ± 12‡	50 ± 11	62 ± 11†	57 ± 11	61 ± 11‡
BMI (kg/m^2)	27.7 ± 5.0	29.2 ± 5.7‡	28.6 ± 6.1	30.9 ± 6.5§	29.0 ± 4.8	29.7 ± 5.7
Fasting plasma glucose (mmol/l)	5.9 (5.4–6.3)	8.1 (6.4–11.4)‡	5.7 (5.2–6.3)	8.6 (6.4–13.0)‡	5.8 (5.4–6.3)	8.4 (6.2–13.4)‡
2-h glucose (mmol/l)	8.4 (6.5–9.7)	15.9 (11.6–20.2)‡	7.5 (5.8–9.3)	15.3 (10.6–20.5)‡	7.9 (6.1–9.5)	18.2 (13.3–22.8)‡
Fasting serum insulin (pmol/l)	64 (46–85)	106 (68–184)‡	62 (44–94)	118 (68–205)‡	70 (53–101)	95 (64–155)‡
HbA_{1c} (%)	5.4 (5.2–5.8)	6.9 (6.1–8.4)‡	5.7 (5.4–6.1)	7.5 (6.2–9.4)‡	5.6 (5.2–5.8)	7.7 (6.4–9.5)‡
C-peptide (pmol/ml)	0.86 (0.59–1.18)	1.19 (0.82–1.70)‡	0.76 (0.47–1.04)	0.92 (0.55–1.28)‡	0.92 (0.65–1.22)	1.11 (0.71–1.51)‡
Family history of diabetes (%)	204 (42.1)	253 (60.1)‡	91 (38.7)	193 (65.6)‡	129 (42.9)	219 (65.8)‡

*Data are n (%), mean ± SD, or median (interquartile range) for nonnormal variables. Diabetes status based on self-reported history of diabetes, diabetes medication use, or fasting ADA criteria. †$P < 0.05$, diabetic vs. nondiabetic participants. ‡$P < 0.0001$, diabetic vs. nondiabetic participants. §$P < 0.001$, diabetic vs. nondiabetic participants.

noon, or evening. For morning examinations, individuals 20 years of age or older were instructed to fast for 12 h. For the afternoon and evening examinations, individuals were instructed to fast for 6 h. Individuals who reported use of insulin were instructed not to fast. Fasting blood specimens obtained by venipuncture during the MEC examination were tested for lipid levels, glucose, insulin, C-peptide, and glycated hemoglobin concentration (HbA_{1c}). Plasma glucose and insulin levels 2 h after a 75-g (Dextol-75) oral glucose load were measured only in adult participants who were 40–74 years of age. A detailed description of laboratory techniques and quality assurance methods used in the NHANES III has been reported (16). NCHS placed a portion of the sera in a bank for unanticipated future research projects. With the permission of the NCHS, banked sera were used for this study.

Study population. All subjects included in the present study were part of the NHANES III. Available coded serum specimens (n = 2,182) from all eligible NHANES III participants with diabetes (n = 1,099) and a sample of nondiabetic participants (n = 1,083) ≥40 years of age were obtained from the NCHS and assayed for GAD65 AAs. The study population, for whom serum specimens were requested from the NCHS, was limited to NHANES III participants ≥40 years of age because the oral glucose tolerance test was performed only in adults who were 40–75 years of age and comparisons in the prevalence of GAD65 AA by both World Health Organization (WHO) and American Diabetes Association (ADA) diabetes diagnosis criteria were of interest (17,18). In this study population of NHANES III, individuals were classified with physician diagnosed diabetes if they reported a medical history of diabetes, including the utilization of insulin and oral hypoglycemic agents (OHGAs). Among participants not reporting a positive medical history of diabetes, individuals were diagnosed with diabetes using the ADA fasting glucose criteria (18). Based on these criteria, individuals with fasting glucose ≥126 mg/dl were classified as having undiagnosed diabetes. The prevalence of GAD65 AAs among new (undiagnosed) diabetic cases was similar irrespective of whether the ADA or WHO diabetes diagnosis criteria was used (data not shown). Therefore, all analyses in this article are presented in terms of the ADA diabetes diagnosis criteria.

Only serum samples from individuals with fasting ≥9 h were requested from NCHS and included in these analyses, therefore, participants attending the afternoon and evening sessions were not included in these analyses. A previous report by Harris et al. (1) did not find any statistically significant differences in sociodemographic and clinical variables between individuals assigned to the morning session as compared with those assigned to the afternoon/evening session. Moreover, for these analyses, 82 individuals of "other" race/ethnicity were excluded (47 nondiabetic and 35 diabetic participants).

The final NHANES III study population for this report consisted of 2,100 individuals, of whom 920 were non-Hispanic whites, 534 non-Hispanic blacks, and 646 Mexican Americans. The mean age was 63 years of age (SD = 12, range = 40–89) and the mean BMI was 29.1 (SD = 5.7). There were 1,017 men (48.4%) and 1,083 (51.6%) women in this NHANES III sample. Overall, non-Hispanic whites were leaner ($P < 0.0001$), older ($P < 0.001$), and had a greater percentage of males ($P < 0.05$) than non-Hispanic blacks and Mexican Americans.

Laboratory methods. AAs to GAD65 were detected in triplicate by immunobinding of serum with the in vitro transcribed/translated recombinant ^{35}S-[Met]-labeled recombinant human GAD65, as originally described by Grubin et al. (19). The GAD65 construct was kindly donated by Dr. Åke Lernmark. The results are expressed as an index (index = sample cpm − negative control cpm/positive control cpm – negative control cpm) as previously reported (6). The cutoff point for the assay was established as the 99th percentile of AA levels calculated using 280 control subjects for the radioimmunoassays and corresponded to 0.069. The cutoff point for being GAD65 AA positive used by our group is distinct from the cutoff point based on WHO standards and, as such, may not be directly comparable to other studies that use the WHO standard. The coefficient of variation of the GAD65 AA assay was previously reported (6). Results for our laboratory from proficiency workshops, organized by the University of Florida in Gainesville (1995–1997), the Diabetes Autoantibody Standardization Program (2000 and 2002), and WHO are summarized as follows: 76–100% sensitivity, 90–100% specificity (100% specificity three times), and 100% validity for GAD AAs (20).

Data analysis and statistical methods. Once the GAD65 AA assays were completed and results were returned to the NCHS, the sequence number associated with each serum sample, which then could be linked to the NHANES III database, was made available. All data presented in this article, with the exception of the GAD65 AA data, were originally obtained by NHANES III. The data were analyzed using Statistical Analysis System software (Release 8.00; SAS Institute, Cary, NC). Because of the complex race/ethnicity stratified sampling scheme used in NHANES III, most of the analyses are presented by race/ethnicity. Comparisons between groups were performed using the unpaired t test and ANOVA for normally distributed variables, the Mann-Whitney test for nonnormal variables, and the χ^2 test and Fisher's exact test, when appropriate, for categorical variables. Stepwise logistic regression analysis was used to assess independent correlates of GAD65 AA positivity within each racial/ethnic group. Variables included in the model were sex, age, BMI, diabetes status, fasting glucose and C-peptide levels, and HbA_{1c}. Models limited to diabetic groups also included diabetes duration and use of diabetes medication. All statistical tests were two tailed, and $P < 0.05$ was considered statistically significant.

RESULTS

Of the 1,064 diabetic individuals identified (n = 429 non-Hispanic whites, n = 295 non-Hispanic blacks, and n = 340 Mexican Americans), in 887 (83.4%) diabetes was physician diagnosed (reporting a positive history of diabetes). Of the 1,036 nondiabetic participants identified, 491 were non-Hispanic white, 239 were non-Hispanic black, and 306 were Mexican American. Characteristics of the study population by race/ethnicity and diabetes status are presented in Table 1. Diabetic participants across all racial/ethnic groups were older and had higher BMI and

TABLE 2
Characteristics of diabetic study population by race/ethnicity, NHANES III (n = 1,064)*

	Non-Hispanic whites	Non-Hispanic blacks	Mexican American	Overall P value
n	429	295	340	—
Sex (% men)	220 (51)†	118 (40)	151 (44)	0.009
Age (years)	69 ± 12†	62 ± 11	61 ± 11‡	<0.0001
BMI (kg/m^2)	29.2 ± 5.7†	30.9 ± 6.5§	29.7 ± 5.7	0.001
Fasting glucose (mmol/l)	8.1 (6.4–11.4)	8.6 (6.4–13.0)	8.4 (6.2–13.4)	NS
2-h glucose (mmol/l)‖	15.9 (11.6–20.2)	15.3 (10.6–20.5)§	18.2 (13.3–22.8)‡	0.002
Fasting insulin (pmol/l)¶	89 (62–133)	82 (55–133)	85 (56–126)	NS
HbA_{1c} (%)	6.9 (6.1–8.4)†	7.5 (6.2–9.4)	7.7 (6.4–9.5)‡	<0.0001
C-peptide (pmol/ml)	1.19 (0.82–1.70)†	0.92 (0.55–1.28)§	1.11 (0.71–1.51)‡	<0.0001
Diabetes by reported history (%)	344 (80.2)	245 (83.1)	298 (87.7)‡	0.02
Family history of diabetes (%)	253 (60.1)	193 (65.6)	219 (65.8)	NS
Physician-diagnosed diabetes#				
Age at diabetes diagnosis (years)	58 ± 13†	51 ± 13	51 ± 12‡	<0.0001
Diabetes duration (years)	11.1 ± 9.9	11.7 ± 10.7	10.6 ± 9.4	NS
Any diabetes medication (%)	258 (75.0)	191 (78.0)	223 (74.8)	NS
Insulin use (%)	103 (30)†	100 (41)§	70 (23)	0.0004
Use of OHGAs (%)	164 (48)	105 (43)§	167 (56)‡	0.02

*Data are n (%), mean ± SD, or median (interquartile range) for nonnormal variables. Diabetes status based on self-reported history of diabetes, diabetes medication use, or fasting ADA criteria. †$P < 0.05$, non-Hispanic white vs. non-Hispanic black. ‡$P < 0.05$, non-Hispanic white vs. Mexican American. §$P < 0.05$, non-Hispanic black vs. Mexican American. ‖2-h glucose data are based on 176 non-Hispanic whites, 141 non-Hispanic blacks, 201 Mexican Americans. ¶Excludes insulin users, resulting in insulin data on 323 non-Hispanic whites, 195 non-Hispanic blacks, 268 Mexican Americans. #Following data are based on a group of individuals with positive history of diabetes only (344 non-Hispanic whites, 245 non-Hispanic blacks, 298 Mexican Americans).

higher levels of fasting glucose and insulin (consistent results were obtained when excluding insulin users), 2-h glucose, HbA_{1c}, and C-peptide, and a greater percentage reported a positive family history of diabetes as compared with nondiabetic participants.

Table 2 presents demographic and clinical characteristics among participants with and without physician-diagnosed diabetes by race/ethnicity. Among diabetic participants, a greater percentage of non-Hispanic whites were male as compared with non-Hispanic blacks. Compared with non-Hispanic white diabetic participants, Mexican-American and non-Hispanic black diabetic participants were younger at the time of the survey and at diagnosis of diabetes and had higher levels of HbA_{1c} ($P < 0.05$). Moreover, non-Hispanic blacks were heavier than non-Hispanic whites and Mexican Americans. A greater percentage of Mexican Americans reported a history of diabetes (88%) as compared with non-Hispanic blacks (83%; NS) and non-Hispanic whites (80%; $P < 0.05$). Among participants with diagnosed diabetes, there was no statistically significant difference by race/ethnicity in duration of diabetes or the percentage of participants who reported use of any diabetes medication. However, Mexican-American patients (56%) were more frequently treated with OHGAs compared with non-Hispanic whites (48%; $P < 0.05$) and non-Hispanic blacks (43%; $P < 0.05$) and less frequently with insulin compared with non-Hispanic blacks (41%; $P < 0.05$).

Sixty-three individuals were classified as GAD65 AA positive in our study population (37 non-Hispanic whites, 14 non-Hispanic blacks, and 12 Mexican Americans). The overall prevalence of GAD65 AA positivity among diabetic (physician-diagnosed and undiagnosed diabetes) individuals (4.0%; $n = 42$) was statistically significantly ($P = 0.01$) higher than that in nondiabetic individuals (2.0%, $n = 21$). The prevalence of GAD65 AAs was higher in the non-Hispanic white (6.3% vs. 2.0%; $P = 0.001$; diabetes versus no diabetes) and non-Hispanic black (3.7% vs. 1.3%; $P = 0.08$) diabetic population compared with the nondiabetic population, a difference not evident in Mexican Americans (1.2% vs. 2.6%; $P = 0.18$; Fig. 1). This racial/ethnic pattern in the prevalence of GAD65 AAs was also seen when the analysis was limited to participants with physician-diagnosed diabetes (Fig. 1). The difference in prevalence of GAD65 AAs by diabetes status was found in both men and women, but the difference in GAD65 AA positivity by diabetes status was more pronounced in non-Hispanic white women and only statistically significant in this group (Fig. 2).

We further compared the prevalence of GAD65 AA positivity by diabetes status in different age-groups. In non-Hispanic whites, the prevalence of GAD65 AAs was consistently higher in diabetic versus nondiabetic individuals, with the exception of the oldest group (≥75 years of age; Fig. 3 and Table 3). It is interesting that in non-Hispanic blacks, the difference in GAD65 AA positivity by diabetic status was evident only in the older age-groups (>60 years of age; Fig. 3).

We then divided participants with physician-diagnosed diabetes ($n = 870$) into four groups by duration of diabetes as follows: 0–3 years ($n = 234$, 26.9%), 4–9 years ($n = 216$, 24.8%), 10–16 years ($n = 203$, 23.3%), and ≥17 years ($n = 217$, 24.9%). Among non-Hispanic whites, GAD65 AA prevalence rates were 8.6, 5.0, 5.0, and 8.0% in the group with 0–3, 4–9, 10–16, and ≥17 years' duration of diabetes, respectively. Among non-Hispanic blacks, GAD65 AA prevalence rates were 4.5, 3.9, 1.8, and 7.9% in the group with 0–3, 4–9, 10–16, and ≥17 years' duration of diabetes, respectively. In Mexican Americans, GAD65 AA positivity was limited to the first two groups of duration of diabetes, 0–3 years (2.7%) and 4–9 years (1.2%), with none of the diabetic participants in the groups of 10–16 and ≥17 years'

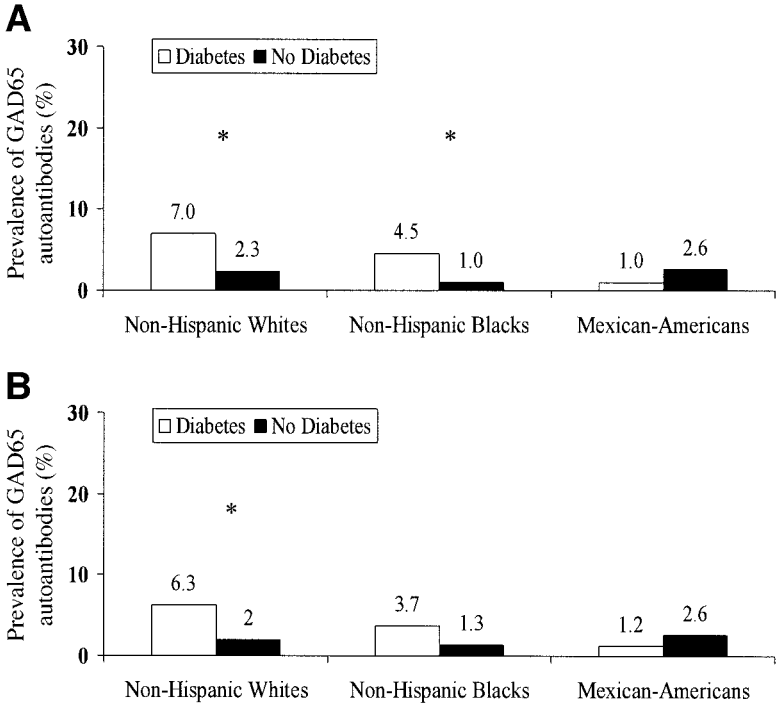


FIG. 1. Prevalence (%) of GAD65 AAs by diabetes status and race/ethnicity among adults ≥40 years of age, NHANES III population (N = 2,100), *P < 0.05. *A*: Physician-diagnosed diabetes. *B*: Physician-diagnosed and undiagnosed diabetes.

duration of diabetes positive for GAD65 AAs. None of these race-specific comparisons of GAD65 AA positivity by duration of diabetes were statistically significant. Similar results were obtained when including both participants with and without physician-diagnosed diabetes (data not shown).

Although the prevalence of GAD65 AAs was higher in non-Hispanic white and non-Hispanic black diabetic participants as compared with their nondiabetic counterparts, differences were attenuated for certain subgroups (as shown in Table 3). Non-Hispanic white diabetic participants were more likely to be GAD65 AA positive compared with nondiabetic participants when analyses were limited to participants <75 years of age and even when the diabetic group was limited to the following subgroups: *1*) those who diabetes was diagnosed after age 40, *2*) diabetic participants who were not on insulin, and *3*) those who had C-peptide levels ≥0.2 pmol/ml (as a cutoff point for insulin deficiency) and were not on insulin (21). Differences in GAD65 AA positivity by diabetes status persisted in non-Hispanic whites and non-Hispanic blacks when analyses were limited to *1*) participants with C-peptide

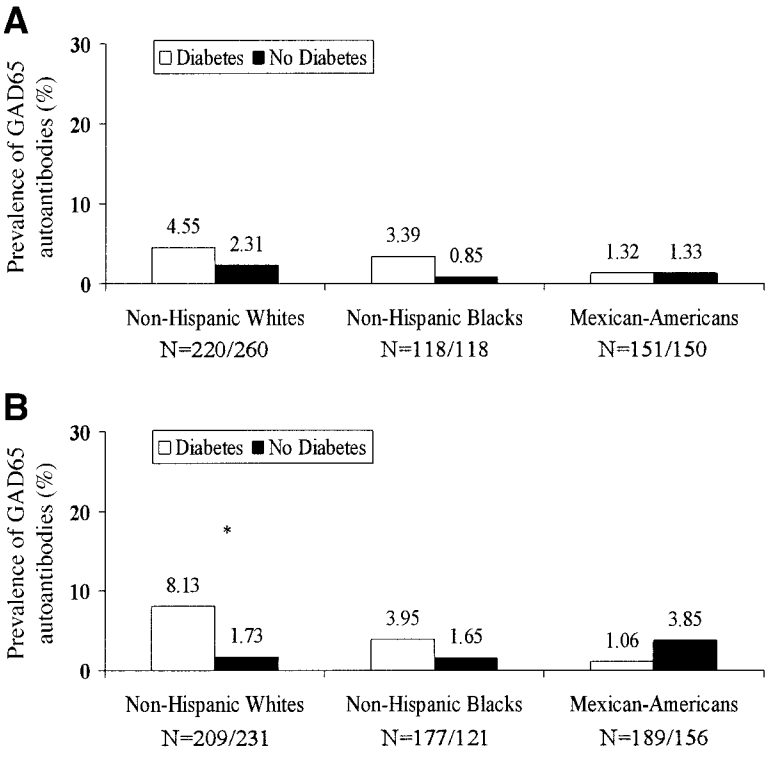


FIG. 2. Prevalence (%) of GAD65 AAs by self-reported diabetes status, fasting ADA criteria, and race/ethnicity among male and female adults ≥40 years of age, NHANES III population (N = 2,100), *P < 0.05. *A*: Men (n = 1,017). *B*: Women (n = 1,083).

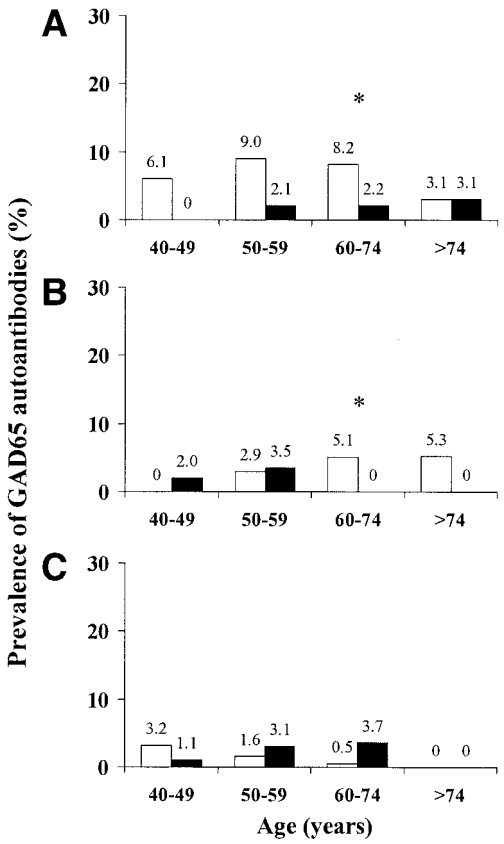


FIG. 3. Prevalence of GAD65 AAs by self-reported diabetes status and fasting ADA criteria, age and race/ethnicity among adults ≥40 years of age, NHANES III population (N = 2,100), *$P < 0.05$. *A*: Non-Hispanic whites (n = 920). *B*: Non-Hispanic blacks (n = 534). *C*: Mexican Americans (n = 646). □, diabetes; ■, no diabetes.

levels ≥0.2 pmol/ml (not statistically significant in non-Hispanic whites) and *2*) diabetic participants who were on insulin. There is consistently no difference in the prevalence of GAD65 AAs by diabetic status for Mexican Americans.

We also looked at the prevalence of GAD65 AAs by time from diagnosis of diabetes to initiation of insulin therapy. For these analyses, data on current duration of insulin use were used to estimate time from diagnosis to initiation of insulin therapy among current insulin users. Data on current duration of insulin use was available for 265 (97.8%) of the 271 current insulin users. Among current insulin users, the prevalence of GAD65 AAs was 7.5, 5.4, 8.0, and 6.8% in individuals who initiated insulin therapy 0, 1–2.9, 3–9.9, and ≥10 years after diagnosis of diabetes (P = 0.97). GAD65 AA prevalence data by race/ethnicity were limited by the small number of insulin users in each racial/ethnic category.

We then compared GAD65 AA–positive diabetic individuals with GAD65 AA–negative diabetic individuals and found that GAD65 AA positivity was associated with lower BMI, higher HbA_{1c}, and lower C-peptide among non-Hispanic whites and non-Hispanic blacks (Table 4). Non-Hispanic black diabetic participants who were GAD65 AA positive were more likely to have higher fasting glucose levels. Among non-Hispanic whites, the percentage of diabetic patients who were treated with insulin was higher in GAD65 AA–positive as compared with GAD65 AA–negative patients ($P < 0.05$). A similar pattern was also present in non-Hispanic blacks, although the difference was not statistically significant (Table 4). There were no statistically significant differences with regard to clinical characteristics between GAD65 AA–positive and –negative diabetic Mexican Americans, although GAD65 AA–positive diabetic Mexican Americans seem to have lower insulin and C-peptide levels. Moreover, although there were no statistically significant differences in duration of diabetes by GAD65 AA status in any of the racial/ethnic groups, GAD65 AA–positive diabetic non-Hispanic blacks tended to have longer duration of diabetes compared with their GAD65 AA–negative counterparts. Among diabetic participants, the prevalence of GAD65 AA did not differ statistically by duration of insulin treatment. The prevalence of GAD65 AAs was 4.9, 6.7, 4.3, and 6.8% among participants with prevalent diabetes with 0–2, 2–5, 5–10, and >10 years of insulin use, respectively.

We then evaluated the relationship between GAD65 AAs and some markers of insulin secretion. The mean (±SD)

TABLE 3
Prevalence (%) of GAD65 AA positivity by diabetes status and race/ethnicity among subgroups, NHANES III participants

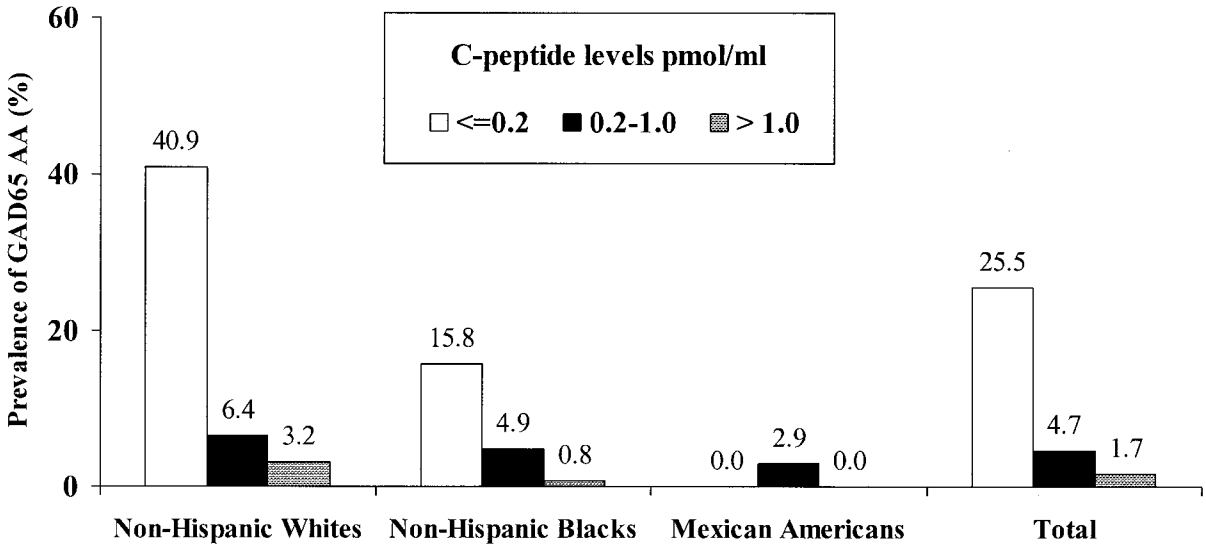
	Non-Hispanic whites		Non-Hispanic blacks		Mexican Americans	
	No diabetes	Diabetes	No diabetes	Diabetes	No diabetes	Diabetes
All diabetes*						
n	491	429	239	295	306	340
Participants age <75 years	1.8	8.2†	1.4	3.5	2.7	1.3
Participants age ≥75 years	3.1	3.1	0	5.3	0	0
C-peptide ≥ 0.2 pmol/ml	2.3	4.4	0.7	3.5†	2.6	1.0
Physician-diagnosed diabetes at ≥40 years of age‡						
n	491	402	239	248	306	296
All participants	2.0	5.7†	1.3	3.2	2.6	1.4
Diabetic participants not on insulin	2.0	4.8†	1.3	2.3	2.6	1.3
Diabetic participants on insulin	2.3	8.7†	1.0	5.4†	2.6	1.7
Participants with C-peptide ≥0.2 pmol/ml	2.1	4.4†	0.9	3.0	2.6	1.4
Diabetic participants not on insulin and C-peptide ≥0.2 pmol/ml	2.1	4.6†	0.9	2.4	2.6	1.3

*Diabetes status based on self-reported history of diabetes, diabetes medication use, or fasting ADA criteria. †$P < 0.05$, diabetic group vs. nondiabetic group. ‡Diabetes status based on positive history of diabetes only (self-reported history of diabetes and current diabetes treatment), and participants in diabetes group limited to those diagnosed after the age of 40.

TABLE 4
Characteristics of diabetic participants by race/ethnicity and GAD AA positivity, NHANES III participants (n = 1,064)*

	Non-Hispanic whites		Non-Hispanic blacks		Mexican Americans	
	GAD65 AA$^+$	GAD65 AA$^-$	GAD65 AA$^+$	GAD65 AA$^-$	GAD65 AA$^+$	GAD65 AA$^-$
n	27	402	11	284	4	336
Sex (% men)	37	52.2	36.4	40.1	50	44.4
Age (years)	65 ± 11	69 ± 12	68 ± 8	62 ± 12	53 ± 8	61 ± 11
BMI (kg/m^2)	27.2 ± 5.9	29.4 ± 5.7†	25.1 ± 4.5	31.1 ± 6.5†	28.1 ± 1.9	29.7 ± 5.7
Fasting glucose (mmol/l)	8.2 (6.6–14.5)	8.0 (6.4–11.4)	15.6 (8.5–19.4)	8.4 (6.4–12.9)†	8.2 (6.5–12.3)	8.4 (6.2–13.4)
2-h glucose (mmol/l)	16.4 (15.4–21.7)	15.8 (11.4–20.2)	18.9 (6.2–31.5)	15.2 (10.5–20.5)	18.4 (9.8–22.8)	18.2 (13.3–22.9)
Fasting insulin (pmol/l)‡	21.9 (9.8–47.6)	17.6 (11.4–30.5)	24.6 (8.0–62.0)	19.7 (11.3–33.2)	10.8 (7.7–19.4)	16.0 (10.8–25.9)
HbA_{1c} (%)	8.3 (6.2–9.1)	6.9 (6.0–8.3)†	9.4 (7.6–10.7)	7.5 (6.2–9.4)†	7.7 (5.5–10.5)	7.7 (6.4–9.5)
C-peptide (pmol/ml)	0.74 (0.11–1.15)	1.21 (0.84–1.71)†	0.47 (0.02–0.78)	0.92 (0.60–1.29)†	0.73 (0.63–0.80)	1.12 (0.72–1.52)
Systolic blood pressure (mmHg)	134 ± 20	141 ± 19	141 ± 19	141 ± 19	134 ± 23	141 ± 23
Diastolic blood pressure (mmHg)	73 ± 12	73 ± 10	76 ± 8	77 ± 11	81 ± 7	75 ± 11
LDL cholesterol (mmol/l)	2.77 (2.33–3.31)	3.59 (2.97–4.22)†	3.41 (3.13–3.70)	3.59 (3.03–4.37)	3.15 (2.82–3.47)	3.41 (2.84–4.01)
HDL cholesterol (mmol/l)	1.24 (0.83–1.66)	1.06 (0.88–1.32)	1.11 (0.85–1.81)	1.24 (1.03–1.55)	1.09 (1.01–1.19)	1.16 (0.98–1.37)
Triglycerides (mmol/l)	3.85 (2.53–7.71)	4.86 (3.44–7.37)	3.00 (1.86–4.58)	3.75 (2.48–5.25)	4.71 (2.66–5.35)	4.60 (3.44–7.06)
Physician-diagnosed diabetes (n = 887)§						
n	24	320	11	234	3	295
Age at diabetes diagnosis (years)	55 ± 13	58 ± 13	52 ± 16	51 ± 13	51 ± 8	51 ± 12
Diabetes duration (years)	11.1 ± 10.5	11.1 ± 9.8	16.2 ± 11.5	11.5 ± 10.5	3.7 ± 2.1	10.7 ± 9.4
Use of any diabetes medication (%)	79.2	74.7	90.9	77.4	66.7	74.9
Insulin use (%)	50	28.4†	63.6	39.7	33.3	23.4
OHGA use (%)	29.2	49.1†	54.6	42.3	33.3	56.3

*Data are percentage, mean ± SD, or median (interquartile range) for nonnormal variables. Diabetes status based on self-reported history of diabetes, diabetes medication use, or fasting ADA criteria. †$P < 0.05$, GAD65 AA$^+$ vs. GAD65 AA$^-$. ‡Excludes insulin users, resulting in insulin data on 15 GAD65 AA$^+$ and 310 GAD65 AA$^-$ non-Hispanic whites, 4 GAD65 AA$^+$ and 191 GAD65 AA$^-$ non-Hispanic blacks, 3 GAD65 AA$^+$ and 267 GAD65 AA$^-$ Mexican Americans. §Diabetes status based on positive history of diabetes only (self-reported history of diabetes and current diabetes treatment).



GAD65AA +	9	8	9	3	7	1	0	4	0	12	19	10
N	22	125	279	19	144	132	6	139	193	47	408	604
P-value *	< 0.001			0.006			0.07			<0.0001		

FIG. 4. Prevalence of GAD65 AAs by C-peptide levels (pmol/ml) and race/ethnicity among all participants with diabetes, NHANES III (n = 1,059). *Overall race/ethnicity-specific comparisons

C-peptide levels was 1.05 ± 0.61 pmol/ml for the total population (n = 2,092; C-peptide levels were missing for 9 individuals). Seventy-three participants had C-peptide level ≤0.2 pmol/ml, 47 of whom had a history of diabetes and 26 of whom had no history of diabetes and were considered nondiabetic on the basis of ADA fasting criteria. Of the 47 with diabetes, 36 were insulin users, 5 used both insulin and OHGAs, and 2 used OHGAs only. Fourteen individuals had C-peptide levels equal to 0.021 pmol/ml (minimum detection limit), 11 of whom reported a history of diabetes (all insulin users) and 3 of whom had no history of diabetes and had fasting glucose <126 mg/dl. Among non-Hispanic white and non-Hispanic black diabetic participants, the prevalence of GAD65 AAs was significantly higher with decreasing levels of C-peptide (Fig. 4). Moreover, among the 21 nondiabetic individuals who were GAD65 AA positive, GAD65 AA titers were correlated with 2-h glucose (r_s = 0.49, P = 0.04, n = 18) and fasting insulin (r_s = 0.41, P = 0.07, n = 21). Among the 21 GAD65 AA–positive nondiabetic participants, 2 (9.5%) had 2-h glucose levels >200 mg/dl and 6 (28.9%) had fasting glucose levels >110 mg/dl. In addition, 10 (48%) had a family history of diabetes.

Stepwise logistic regression modeling of the total population resulted in the following variables being significantly independently associated with GAD65 AA positivity: lower C-peptide levels, white race, and lower BMI. This was a consistent finding regardless of diabetes status variable used and regardless of whether the analysis was limited to the diabetic or total population. In non-Hispanic whites, diabetes status and low C-peptide levels were significantly independently associated with GAD65 AA positivity. In non-Hispanic blacks, low C-peptide levels and low BMI were independently associated with GAD65 AA positivity. Duration of diabetes and use of diabetes medication were not independently associated with GAD65 AA positivity among the diabetic population. No variables were statistically significantly associated with GAD65 AA positivity in Mexican Americans.

DISCUSSION

Type 2 diabetes is the most common form of diabetes, accounting for ~90% of cases and in many industrialized countries affecting 10–20% of individuals aged >45 years (22). The number of patients being diagnosed with type 2 diabetes is increasing each year and is approaching an epidemic level; in adults, the prevalence of diabetes worldwide was estimated to be 4.0% in 1995 and it is projected to rise to 5.4% by 2025 (23). Type 2 diabetes is a heterogeneous disorder of glucose and metabolic homeostasis that is characterized by an intricate interaction between insulin resistance and pancreatic β-cell dysfunction (24).

In a subgroup of type 2 diabetic patients, there are signs of humoral islet cell autoimmunity (25–27). To date, GAD65 AAs represent the most commonly detected marker in this subgroup of type 2 diabetic patients (28–30) and seem to be present in up to 12% of adults who received an initial diagnosis of type 2 diabetes (6,9,10,31). On the basis of these and older studies, it has been proposed that this subgroup of type 2 diabetes with islet cell autoimmunity may represent a different disease with type 1–like autoimmune pathogenesis. This form of diabetes with initial type 2 diabetes presentation is termed latent auto-

immune diabetes in adults, type 1.5 diabetes, or slowly progressive type 1 diabetes and has been associated with progressive decline in β-cell function and future insulin requirement (10,12–15). This is significant when one considers the extraordinarily high prevalence of diabetes in U.S. adults and that up to 12% of these adults may actually have a diabetes of autoimmune nature.

Despite the high prevalence of type 2 diabetes in U.S. racial/ethnic minority adult populations, little is known concerning the prevalence of GAD65 AAs in these groups. We measured one of the most widely used markers for the diagnosis and prediction of type 1 diabetes, GAD65 AA (7,32,33), in a well-characterized diabetic and nondiabetic population aged ≥40 years from the NHANES III. We observed that the prevalence of GAD65 AAs was higher in diabetic participants compared with nondiabetic participants in non-Hispanic whites and non-Hispanic blacks but not in Mexican Americans. The prevalence of GAD65 AAs in the triethnic adult population of NHANES III ranged from 1.2% in diabetic Mexican Americans to 6.3% in diabetic non-Hispanic whites. On the basis of our GAD65 AA prevalence estimates, data on the prevalence of diabetes in the U.S. reported by NHANES III, and the projected U.S. population for 2002 (34), we estimated that 720,000 non-Hispanic white, 67,000 non-Hispanic black, and 13,000 Mexican-American adults 40–74 years of age in the U.S. have evidence of islet cell autoimmunity, a prevalence as great as that of type 1 diabetes.

The prevalence of GAD65 AAs in different adult populations seems to be variable, being higher in Northern Europeans (10,25,31) and lower in Alaskan natives (35), Pima Indians (36), and populations from Northern Italy (37) and Southern Spain (38). It is not entirely clear why there is such a variation. Both genetic background and sample size of the populations examined in each study may account for these differences. In addition, the age of type 2 diabetic patients evaluated for GAD65 AAs or the inconsistency between studies about the criteria applied to exclude patients with clinical features of type 1 diabetes may explain differences in the prevalence reported in these studies. In our study, the prevalence of GAD65 AAs among diabetic whites was somewhat lower than that reported by other studies of white populations, including our study of older diabetic participants of the Cardiovascular Health Study (6,10,31). Similarly, the prevalence of GAD65 AAs in diabetic non-Hispanic blacks from the NHANES III was lower than that reported for blacks from the Cardiovascular Health Study (6).

We believe that one reason why Mexican Americans who are ≥40 years of age have lower prevalence of autoimmune phenomena compared with other American populations of white or black descent is because the incidence of type 1 diabetes in Mexican Americans is very low (39). In support of these findings, Hathout et al. (40) evaluated the prevalence of islet cell antibodies and GAD and insulin AAs at clinical diagnosis of type 2 diabetes in a group of white and Hispanic children and adolescents. They found that none of the Hispanic children with type 2 diabetes exhibited signs of humoral islet cell autoimmunity. A distinct genetic background in Mexican Americans compared with whites and blacks and differences in environmental factors such as diet and visceral adiposity, which are major determinants of the development of type 2 diabetes in Mexican Americans, are possible reasons for the difference in the prevalence of GAD65 AAs (41,42).

The possibility that other antibodies to islet autoantigens (which were not measured in this study) may be present in Mexican Americans cannot be excluded. We are currently measuring AAs to another islet cell antigen, insulinoma-associated protein 2 (43), in the NHANES III population. However, as we and others have shown, insulinoma-associated protein 2 AAs alone are not a strong marker for autoimmune diabetes in older-onset diabetes cases and tends to decrease with age to a much greater extent than GAD65 AAs, which tend to be much more stable in adults with diabetes (6,9). Given the cross-sectional nature of this study and that both new and prevalent diabetic individuals were identified, we believed that GAD65 AAs would be the first antibody marker of choice to detect in this population. Although data on sequential determination of GAD65 AAs are not available for this study, there is strong evidence to suggest that GAD AAs are stable for a number of years or even decades in patients who present with both type 1 (44,45) and type 2 diabetes (31,46).

Several key findings from our evaluation of the NHANES III population support the role of the outcome of an autoimmune process leading to β-cell damage and subsequent reduced insulin secretion in non-Hispanic whites and non-Hispanic blacks as it occurs in a similar manner in type 1 diabetes. Among diabetic non-Hispanic whites and non-Hispanic blacks, there was evidence of islet cell autoimmunity as measured through the presence of GAD65 AAs. We also found that among non-Hispanic whites and non-Hispanic blacks, the prevalence of GAD65 AAs was dramatically higher in diabetic individuals with low fasting levels of C-peptide, indicative of insulin secretion dysfunction. We also found that in non-Hispanic whites and non-Hispanic blacks, GAD65 AA positivity was independently associated with lower BMI and C-peptide levels, findings that are consistent with other studies (9,11).

In conclusion, in patients who are diagnosed with adult-onset diabetes, clinical and metabolic parameters alone may no longer be considered sufficient to allow an adequate classification of diabetes (27). The classification of diabetes continues to undergo changes, and markers of autoimmunity may be another tool for improving diabetes classification, which is important given the dependence of diabetes treatment on classification and initial diagnosis of diabetes. Furthermore, a more appropriate classification of diabetes will shed light on the heterogeneous pathoetiologic factors associated with autoimmune diabetes.

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