Latent Autoimmune Diabetes in Adults in a South Asian Population of the U.K.

ABIGAIL C. BRITTEN, BSC[1]
KAREN JONES, BSC[1]
CARINA TÖRN, PHD[2]
MAGNUS HILLMAN, PHD[2]
BIRGITTE EKHOLM[2]
SUDHESH KUMAR, MD, FRCP[3]
ANTHONY H. BARNETT, MD, FRCP[1,4]
MARILYN ANN KELLY, PHD[1]

Type 2 diabetes is four- to sixfold more common in the South Asian population of the U.K. than in the indigenous white Caucasian population. A subset of all patients initially diagnosed with type 2 diabetes shows evidence of slowly evolving islet autoimmunity, termed latent autoimmune diabetes in adults (LADA). LADA is characterized by the presence of circulating autoantibodies specific for islet proteins and by insulin independence for at least 6 months post-diagnosis (1).

A recent pilot study in Birmingham, U.K., suggested that 27% of South Asians initially presenting with type 2 diabetes were positive for autoantibodies to GAD65 and/or insulinoma-associated protein (IA)-2 (2). This is significantly higher than the islet autoimmunity frequency of 10% observed in white Caucasians diagnosed with type 2 diabetes (3,4). The study in South Asians was carried out in a very small cohort, however, and the findings require confirmation in a much larger study group. The aim of this study was to determine the prevalence of LADA in a larger U.K.-resident South Asian population and to characterize the phenotypic features and genetic basis of the disease in this ethnic group.

RESEARCH DESIGN AND METHODS

— A total of 500 South Asian subjects with type 2 diabetes (mean [range] age 55 years [31-89] and disease duration 7 years [0-29]) (Table 1) were consecutively recruited in Birmingham, U.K., and Coventry, U.K., as part of the U.K. Asian Diabetes Study. A total of 206 normoglycemic control subjects (age 49 years [30-83]) were recruited in Birmingham. All subjects were of Punjabi ancestry. Type 2 diabetes was defined according to World Health Organization criteria (5). The study was approved by the local ethics committee, and written informed consent was obtained from all participants. Venous blood samples were collected from each subject, plasma was removed for autoantibody analysis, and DNA was extracted from the remaining blood using an adaptation of the Nucleon protocol (Nucleon Biosciences, Coatbridge, U.K.). LADA was defined as described above (1).

Antibody analysis

Plasma samples were incubated with an excess of calcium ions overnight, followed by centrifugation. The supernatants were analyzed for autoantibodies to GAD65 and IA-2 using commercially available enzyme-linked immunosorbent assay kits according to the manufacturer's instructions (RSR, Cardiff, U.K.). The reference value was 10 units/ml for GAD65 antibodies and 15 units/ml for IA-2 autoantibodies (based on the World Health Organization standard).

Genetic analysis

DNA samples were typed for alleles of HLA-DRB1, -DQA1, and -DQB1 using the phototyping method (6,7). The insulin gene variable number tandem repeat (INS-VNTR) type was determined using restriction fragment–length polymorphism analysis with HphI (8). Alleles of the GCT microsatellite in the major histocompatibility complex class I chain-related gene-A (MIC-A) gene were typed using the method described by Gambelunghe et al. (9).

Statistical analysis

Associations between genotype and autoantibody status were analyzed using the χ^2 test or Fisher's exact test. Differences in continuous variables were investigated using the Mann-Whitney *U* test. All statistical analyses were performed using SPSS (version 13.0; SPSS, Chicago, IL).

RESULTS

— Autoantibodies were detected in 13 of 500 (2.6%) individuals with type 2 diabetes (of whom 8 were GAD65 positive [1.6%] and 6 were IA-2 positive [1.2%], including 1 subject who was positive for both) and 8 of 206 (3.9%) control subjects (of whom 3 were GAD65 positive [1.5%] and 5 were IA-2 positive [2.4%]). There was no significant difference in antibody titers between diabetic and control subjects.

The small number of autoantibody-positive subjects found in this cohort limited investigation of associations between genotype and antibody status in the South Asian population, but some trends were observed. The DRB1*04 and DQB1*0302 alleles were increased in frequency among the IA-2 autoantibody–positive diabetic ($P = 0.020$ and $P = 0.015$, respectively) and control ($P =$ not significant) subjects compared with those in subjects lacking these markers. The distribution of the INS-VNTR genotypes did not differ significantly between the autoantibody-positive and autoantibody-negative subjects in either the diabetic or control groups. The MIC-A6 allele was significantly less frequent among IA-2 autoantibody–positive diabetic than autoantibody-negative diabetic subjects ($P = 0.044$).

Clinical, biochemical, and anthropometric measurements were compared between the autoantibody-positive and autoantibody-negative diabetic subjects

From the [1]Department of Medicine, University of Birmingham, Birmingham, U.K.; [2]Clinical Sciences, Lund University, Lund, Sweden; [3]Diabetes and Metabolism, University of Warwick, Coventry, U.K.; and the [4]Heart of England NHS Foundation Trust, Birmingham, U.K.

Address correspondence and reprint requests to Abigail Britten, Diabetes Research Group, ELG54, The Medical School, Vincent Drive, Edgbaston, Birmingham, B15 2TT, U.K. E-mail: a.c.britten@bham.ac.uk.

Received for publication 8 May 2007 and accepted in revised form 11 September 2007.

Published ahead of print at http://care.diabetesjournals.org on 18 September 2007. DOI: 10.2337/dc07-0896.

Abbreviations: IA, insulinoma-associated protein; LADA, latent autoimmune diabetes in adults.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Clinical parameters recorded for autoantibody-positive and autoantibody-negative diabetic subjects*

Clinical parameter	Autoantibody positive	Autoantibody negative	GAD65 autoantibody		IA-2 autoantibody	
			Positive	Negative	Positive	Negative
n	13	479	8	484	6	486
Age (years)	58 (40–78)	55 (31–89)	55 (46–73)	55 (31–89)	65 (40–78)	55 (31–89)
Disease duration (years)	9 (2–25)	7 (0–29)	13 (6–25)*†	7 (0–29)	7 (2–25)	8 (0–29)
Height (cm)	165 (146–177)	162 (152–173)	166 (157–177)	162 (146–173)	164 (146–174)	162 (152–177)
Weight (kg)	70 (61–78)‡	77 (43–139)	71 (64–78)	77 (43–139)	69 (61–76)	77 (43–139)
BMI (kg/m^2)	26 (23–32)§	29 (16–49)	26 (23–30)	29 (16–49)	26 (23–32)	29 (16–49)
Waist circumference (cm)	98 (93–109)	102 (60–139)	99 (93–108)	102 (60–139)	95 (93–109)	102 (60–139)
Diastolic blood pressure (mmHg)	80 (54–96)	83 (53–124)	81 (66–96)	83 (53–124)	75 (54–87)	83 (53–124)
Systolic blood pressure (mmHg)	130 (105–170)	137 (80–203)	126 (107–146)	137 (80–203)	134 (105–170)	137 (80–203)
A1C (%)	6.3 (4.4–9.5)	7.0 (2.0–15.7)	6.8 (5.4–9.5)	7.0 (2.0–15.7)	5.6 (4.4–6.5)‖	7.0 (2.0–15.7)
Cholesterol (mmol/l)						
Total	4.9 (3.2–6.1)	4.8 (2.2–11.8)	4.7 (3.2–6.1)	4.8 (2.2–11.8)	4.7 (3.2–5.4)	4.8 (2.2–11.8)
HDL	1.3 (0.8–2.1)	1.2 (0.6–3.1)	1.4 (1.1–2.1)	1.2 (0.6–3.1)	1.1 (0.8–1.2)	1.2 (0.6–3.1)
LDL	2.3 (1.6–3.4)	2.4 (0.49–6.6)	2.1 (1.6–3.0)	2.5 (0.49–6.6)	2.5 (1.6–3.4)	2.4 (0.49–6.6)
Triglycerides (mmol/l)	2.6 (0.9–3.9)	2.9 (0.3–11.6)	2.4 (0.9–3.7)	2.9 (0.3–11.6)	2.5 (0.9–3.9)	2.9 (0.3–11.6)
Treatment						
Insulin	53.8	18.8	75.0	19.0	33.0	19.9
Oral hypoglycemic agents	38.5	78.2	25.0	78.9	50.0	78.7
Diet	7.7	18.6	0.0	18.8	16.7	18.6

Data are means (range) or percentages unless otherwise indicated. *$P = 0.019$, GAD65 autoantibody positive vs. GAD65 autoantibody negative; †$P = 0.009$, GAD65 autoantibody positive vs. IA-2 autoantibody positive; ‡$P = 0.029$, autoantibody positive vs. autoantibody negative; §$P = 0.032$, autoantibody positive vs. autoantibody negative; ‖$P = 0.046$, IA-2 autoantibody positive vs. IA-2 autoantibody negative.

(Table 1). Mean weight and BMI were significantly lower in the autoantibody-positive subjects ($P = 0.029$ and $P = 0.032$, respectively). A longer mean duration of diabetes was observed among individuals positive for GAD65 autoantibodies compared with that in autoantibody-negative and IA-2 autoantibody–positive subjects ($P = 0.019$ and $P = 0.009$, respectively). A higher percentage of the autoantibody-positive than autoantibody-negative diabetic subjects was treated with insulin (53.8 and 18.8%, respectively) (Table 1).

CONCLUSIONS — Our study shows that islet autoimmunity is considerably less common among type 2 diabetic individuals of Punjabi ancestry in Birmingham, U.K., than in those of white Caucasian origin. The differences observed between the two ethnic groups may reflect both the higher prevalence of classical type 2 diabetes in South Asians and their lower susceptibility to autoimmune disease. The overall prevalence of islet autoantibodies among the individuals diagnosed with type 2 diabetes in our study cohort (2.6%) was significantly lower than that observed in the pilot study (27%), as was the frequency in the control group (3.9 compared with 9%, respectively) (2). The reasons for these differences are unclear, as both methods were approved by the Diabetes Antibody Standardization Programme (10). The most likely explanation is that the high prevalence of autoimmunity observed in the pilot study is a spurious result due to the small number of individuals investigated (33 type 2 diabetic and 98 control subjects). A higher percentage of patients positive for diabetes autoantibodies has previously been reported in South Indian (GAD65 and ICA512) (11) and Eastern Indian (GAD65 and IA-2) (12) populations resident in India compared with that in the present study. It remains to be determined whether these differences are due to genetic, environmental, or population-selection influences.

The low frequency of islet autoantibodies in the current study made it difficult to detect statistically significant associations with the genetic loci studied and the clinical, biochemical, and anthropometric measurements. The trends that were observed, however, are generally consistent with previous associations with islet autoimmunity seen in other ethnic groups. Based on the findings of our study, screening for LADA in the U.K. Punjabi population would offer little clinical benefit and is not routinely indicated.

Acknowledgments— This study was sponsored by Diabetes UK Grant BDA:RD03/0002693). The recruitment of samples through the U.K. Asian Diabetes Study was supported by Pfizer, Sanofi-Aventis, Servier, MSD/SP, Takeda UK, Merck, Roche, Boehringer Ingelheim, Eli Lilly, Novo Nordisk, BMS, and Daichi Sankyo.

We thank Dr. Anthony Dixon, Dr. Srikanth Bellary, Shanaz Mughal, and Kam Johal for their roles in the collection of samples.

References

1. Pozzilli P, DiMario U: Autoimmune diabetes not requiring insulin at diagnosis (latent autoimmune diabetes of the adult): definition, characterization, and potential prevention. *Diabetes Care* 24:1460–1467,2001
2. Tica V, Hanif MW, Andersson A, Valsamakis G, Barnett AH, Kumar S, Sanjeevi CB: Frequency of latent autoimmune diabetes in adults in Asian patients diagnosed as type 2 diabetes in Birmingham, United Kingdom. *Ann N Y Acad Sci* 1005:356–358, 2003
3. Romkens TE, Kusters GC, Netea MG, Netten PM: Prevalence and clinical characteristics of insulin-treated, anti-GAD-positive, type 2 diabetic subjects in an outpatient clinical department of a Dutch teaching hospital. *Neth J Med* 64:114–118, 2006
4. Tuomi T, Carlsson A, Li H, Isomaa B, Miettinen A, Nilsson A, Nissen M, Ehrn-

strom BO, Forsen B, Snickars B, Lahti K, Forsblom C, Saloranta C, Taskinen MR, Groop LC: Clinical and genetic characteristics of type 2 diabetes with and without GAD antibodies. *Diabetes* 48:150–157, 1999
5. World Health Organization: *Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications, Part 1: Diagnosis and Classification of Diabetes Mellitus: Report of the WHO Consultation*. Geneva, World Health Org., 1999 (Tech. Rep. Ser., no. WHO/NCD/NCS/99.2)
6. Bunce M, O'Neill CM, Barnardo MC, Krausa P, Browning MJ, Morris PJ, Welsh KI: Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). *Tissue Antigens* 46: 355–367, 1995
7. Heward JM, Mijovic CH, Kelly MA, Morrison E, Barnett AH: HLA-DQ and DRB1 polymorphism and susceptibility to type 1 diabetes in Jamaica. *Eur J Immunogenet* 29:47–52, 2002
8. Julier C, Hyer RN, Davies J, Merlin F, Soularue P, Briant L, Cathelineau G, Deschamps I, Rotter JI, Froguel P, Boitard C, Bell J, Lathrop G: Insulin-IGF2 region on chromosome 11p encodes a gene implicated in HLA-DR4-dependent diabetes susceptibility. *Nature* 354:155–159, 1991
9. Gambelunghe G, Ghaderi M, Cosentino A, Falorni A, Brunetti P, Falorni A, Sanjeevi CB: Association of MHC class I chain-related A (MIC-A) gene polymorphism with type I diabetes. *Diabetologia* 43:507–514, 2000
10. Bingley PJ, Bonifacio E, Mueller PW: Diabetes Antibody Standardization Program: first assay proficiency evaluation. *Diabetes* 52:1128–1136, 2003
11. Das AK, Shtauvere-Brameus A, Sanjeevi CB: GAD65 and ICA512 antibodies in undernourished and normally nourished south Indian patients with diabetes. *Ann N Y Acad Sci* 958:247–250, 2002
12. Sanjeevi CB, Kanungo A, Berzina L, Shtauvere-Brameus A, Ghaderi M, Samal KC: MHC class I chain-related gene a alleles distinguish malnutrition-modulated diabetes, insulin-dependent diabetes, and non-insulin- dependent diabetes mellitus patients from eastern India. *Ann N Y Acad Sci* 958:341–344, 2002