Latent Autoimmune Diabetes in Adults (LADA) in the South Asian population of the United Kingdom

Abigail C. Britten, BSc1, Karen Jones, BSc1, Carina Törn, PhD2, Magnus Hillman, PhD2, Birgitte Ekholm2, Sudhesh Kumar, MD, FRCP3, Anthony H. Barnett, MD, FRCP1,4, M. Ann Kelly, PhD1

1Medicine, University of Birmingham, Birmingham, UK
2Clinical Sciences, Lund University, Lund, Sweden
3Diabetes and Metabolism, University of Warwick, Coventry, UK
4Heart of England NHS Foundation Trust, Birmingham, UK

Running title: LADA in the South Asians

Corresponding author:
Abigail Britten
Diabetes Research Group
ELG54, The Medical School
Vincent Drive
Edgbaston
Birmingham
B15 2TT
Email: a.c.britten@bham.ac.uk

Received for publication 8 May 2007 and accepted in revised form 11 September 2007.
Type 2 diabetes is four- to six-fold more common in the South Asian population of the United Kingdom (UK) than the indigenous white Caucasian population. A subset of all patients initially diagnosed with type 2 diabetes show evidence of slowly-evolving islet autoimmunity, termed Latent Autoimmune Diabetes in Adults (LADA). LADA is characterised by the presence of circulating autoantibodies specific for islet proteins and insulin-independence for at least six months post-diagnosis (1).

A recent pilot study in Birmingham, UK, suggested that 27% of South Asians initially presenting with type 2 diabetes were positive for autoantibodies to GAD65 and/or 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 latent autoimmune diabetes in adults in a larger UK-resident South Asian population and to characterise the phenotypic features and genetic basis of the disease in this ethnic group.

**Research Design and Methods**

**Subjects**

Five hundred South Asian subjects with type 2 diabetes (mean age 55 years [range 31-89], mean disease duration 7 years [range 0-29]) (table 1) were consecutively recruited in Birmingham and Coventry as part of the United Kingdom Asian Diabetes Study (UKADS). Two hundred and six normoglycaemic control subjects (mean age 49 years [range 30-83]) were recruited in Birmingham. All subjects were of Punjabi ancestry. Type 2 diabetes was defined according to the WHO 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, UK). LADA was defined as above (1).

**Antibody analysis**

Plasma samples were incubated with an excess of calcium ions overnight, followed by centrifugation. The supernatants were analysed for autoantibodies to GAD65 and IA-2 using commercially-available ELISA kits, according to the manufacturer’s instructions (RSR Ltd, Cardiff, UK). The reference value for GAD65 antibodies was 10 U/ml and for IA-2 autoantibodies, 15 U/ml (based on the WHO standard).

**Genetic analysis**

DNA samples were typed for alleles of HLA-DRB1, -DQA1 and –DQB1 using the phototyping method (6,7). The INS-VNTR type was determined using restriction fragment length polymorphism analysis with HphI (8). Alleles of the GCT microsatellite in the MIC-A gene were typed using the method of Gambelunghe et al. (9).

**Statistical analysis**

Associations between genotype and autoantibody status were analysed using the χ² 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 Inc., Chicago, USA).
Results
Autoantibodies were detected in 13/500 (2.6%) individuals with type 2 diabetes (eight GAD65-positive [1.6%] and six IA-2-positive [1.2%], including one subject positive for both autoantibodies) and 8/206 (3.9%) control subjects (three GAD65-positive [1.5%] and five IA-2-positive [2.4%]). There was no significant difference in antibody titres between the 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 DQBI*0302 alleles were increased in frequency among the IA-2 autoantibody-positive diabetic subjects (p=0.020 and p=0.015 respectively) and control subjects (p=NS) compared with those 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 subjects than autoantibody-negative diabetic subjects (p=0.044).

Clinical, biochemical and anthropometric measurements were compared between the autoantibody-positive and autoantibody-negative diabetic subjects (table 1). Mean weight and body mass index (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 the individuals that were positive for GAD65 autoantibodies compared with autoantibody-negative subjects and IA-2 autoantibody-positive subjects (p=0.019 and p=0.009 respectively). A higher percentage of the autoantibody-positive diabetic subjects than autoantibody-negative diabetic subjects were 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 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%) (2). The reasons for these differences are unclear, as both methods were approved by the Diabetes Antibody Standardisation 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 with type 2 diabetes 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 to 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. Those 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 UK Punjabi population would offer little clinical benefit and is not routinely indicated.

Acknowledgements
This study was sponsored by Diabetes UK (grant BDA:RD03/0002693).

The recruitment of samples through the UKAD study was supported by Pfizer, Sanofi-Aventis, Servier, MSD/SP, Takeda UK, Merck and Co., Roche, Boehringer Ingelheim, Eli Lilly, Novo Nordisk, BMS and Daichi Sankyo.
Thank you to Dr. Anthony Dixon, Dr. Srikanth Bellary, Shanaz Mughal and Kam Johal for their role in the collection of samples.
Reference List


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

<table>
<thead>
<tr>
<th>Clinical parameter</th>
<th>Autoantibody positive n=13</th>
<th>Autoantibody negative n=479</th>
<th>GAD65 positive n=8</th>
<th>GAD65 negative n=484</th>
<th>IA-2 positive n=6</th>
<th>IA-2 negative n=486</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58 (40-78)</td>
<td>55 (31-89)</td>
<td>55 (46-73)</td>
<td>55 (31-89)</td>
<td>65 (40-78)</td>
<td>55 (31-89)</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>9 (2-25)</td>
<td>7 (0-29)</td>
<td>13 (6-25)</td>
<td>7 (0-29)</td>
<td>7 (2-25)</td>
<td>8 (0-29)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165 (146-177)</td>
<td>162 (152-173)</td>
<td>166 (157-177)</td>
<td>162 (146-173)</td>
<td>164 (146-174)</td>
<td>162 (152-177)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70 (61-78)</td>
<td>77 (43-139)</td>
<td>71 (64-78)</td>
<td>77 (43-139)</td>
<td>69 (61-76)</td>
<td>77 (43-139)</td>
</tr>
<tr>
<td>BMI (kgm$^{-2}$)</td>
<td>26 (23-32)</td>
<td>29 (16-49)</td>
<td>26 (23-30)</td>
<td>29 (16-49)</td>
<td>26 (23-32)</td>
<td>29 (16-49)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>98 (93-109)</td>
<td>102 (60-139)</td>
<td>99 (93-108)</td>
<td>102 (60-139)</td>
<td>95 (93-109)</td>
<td>102 (60-139)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80 (54-96)</td>
<td>83 (53-124)</td>
<td>81 (56-96)</td>
<td>83 (53-124)</td>
<td>75 (54-87)</td>
<td>83 (53-124)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>130 (105-170)</td>
<td>137 (80-203)</td>
<td>126 (107-146)</td>
<td>137 (80-203)</td>
<td>134 (105-170)</td>
<td>137 (80-203)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.3 (4.4-9.5)</td>
<td>7.0 (2.0-15.7)</td>
<td>6.8 (5.4-9.5)</td>
<td>7.0 (2.0-15.7)</td>
<td>5.6 (4.4-6.5)†</td>
<td>7.0 (2.0-15.7)</td>
</tr>
<tr>
<td>Cholesterol (mM)</td>
<td>4.9 (3.2-6.1)</td>
<td>4.8 (2.2-11.8)</td>
<td>4.7 (3.2-6.1)</td>
<td>4.8 (2.2-11.8)</td>
<td>4.7 (3.2-5.4)</td>
<td>4.8 (2.2-11.8)</td>
</tr>
<tr>
<td>HDL (mM)</td>
<td>1.3 (0.8-2.1)</td>
<td>1.2 (0.6-3.1)</td>
<td>1.4 (1.1-2.1)</td>
<td>1.2 (0.6-3.1)</td>
<td>1.1 (0.8-1.2)</td>
<td>1.2 (0.6-3.1)</td>
</tr>
<tr>
<td>LDL (mM)</td>
<td>2.3 (1.6-3.4)</td>
<td>2.4 (0.49-6.6)</td>
<td>2.1 (1.6-3.0)</td>
<td>2.5 (0.49-6.6)</td>
<td>2.5 (1.6-3.4)</td>
<td>2.4 (0.49-6.6)</td>
</tr>
<tr>
<td>Triglycerides (mM)</td>
<td>2.6 (0.9-3.9)</td>
<td>2.9 (0.3-11.6)</td>
<td>2.4 (0.9-3.7)</td>
<td>2.9 (0.3-11.6)</td>
<td>2.5 (0.9-3.9)</td>
<td>2.9 (0.3-11.6)</td>
</tr>
<tr>
<td>Treated with insulin (%)</td>
<td>53.8</td>
<td>18.8</td>
<td>75.0</td>
<td>19.0</td>
<td>33.0</td>
<td>19.9</td>
</tr>
<tr>
<td>Treated with oral hypoglycaemic agents (%)</td>
<td>38.5</td>
<td>78.2</td>
<td>25.0</td>
<td>78.9</td>
<td>50.0</td>
<td>78.7</td>
</tr>
<tr>
<td>Treated with diet (%)</td>
<td>7.7</td>
<td>18.6</td>
<td>6</td>
<td>0.0</td>
<td>18.8</td>
<td>16.7</td>
</tr>
</tbody>
</table>
All data presented as mean values with the range in brackets, except percentage of subjects receiving treatment.

* $p=0.019$ 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