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Clinical features, biochemistry and HLA-DRB1 status in children and adolescents with diabetes in Dhaka, Bangladesh

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Abstract

Aims—Little information is published on diabetes in young people in Bangladesh. We aimed to investigate the demographic, clinical, and biochemical features, and HLA-*DRB1* alleles in new cases of diabetes affecting Bangladeshi children and adolescents <22years of age.

Methods—The study was conducted at Bangladesh Institute of Research and Rehabilitation of Diabetes, Endocrine and Metabolic Disorders (BIRDEM) in Dhaka. One hundred subjects aged <22years at diagnosis were enrolled. Demographic characteristics, clinical information,

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Author contributions

BZ, JN and KA implemented the study in Bangladesh and helped with writing the manuscript. DG conducted the data analysis and wrote the initial draft of the manuscript. MAA and CHW advised on the study protocol, implementation, and analysis and contributed to the manuscript. SM performed statistical analysis of HLA data and JL performed HLA genotyping. GDO designed and coordinated the study and co-wrote the manuscript. JN led the HLA analysis and co-wrote the manuscript.

Declaration of Competing Interest

No potential conflicts of interest relevant to this article exist.

Guarantor statement

As the guarantor of this work, Graham David Ogle assumes full responsibility for the ethical collection, analysis and reporting of data as well as the decision to publish.

biochemical parameters (blood glucose, HbA1c, C-peptide, and autoantibodies against glutamic acid decarboxylase 65 (GADA) and islet antigen-2 (IA-2A) were measured. High-resolution DNA genotyping was performed for *HLA-DRB1*.

Results—Eighty-four subjects were clinically diagnosed as type 1 diabetes (T1D), seven as type 2 diabetes (T2D), and nine as fibrocalculous pancreatic disease (FCPD). Of the 84 with T1D, 37 (44%) were males and 47 (56%) females, with median age at diagnosis 13years (y) (range 1.6–21.7) and peak age at onset 12–15years. 85% of subjects were assessed within one month of diagnosis and all within eleven months. For subjects diagnosed with T1D, mean C-peptide was 0.46±0.22nmol/L (1.40±0.59ng/mL), with 9 (10.7%) IA-2A positive, 22 (26%) GADA positive, and 5 (6%) positive for both autoantibodies. Analysis of HLA-*DRB1* genotypes revealed locus-level T1D association (p=6.0E−05); *DRB1*04:01* appeared predisposing (p<3.0E−06), and *DRB1*14:01* appeared protective (p=1.7E−02).

Conclusions—Atypical forms of T1D appear to be more common in young people in Bangladesh than in European populations. This will be helpful in guiding more specific assessment at onset and potentially, expanding treatment options.

Keywords

Childhood diabetes; Type 1 diabetes; Bangladesh; Autoantibody; C-peptide; Diabetes; Children; Bangladesh; Autoimmunity; HLA

1. Introduction

The vast majority of diabetes in children in European countries is classic type 1 diabetes (T1D) [1, 2]. However, there is evidence from various non-European populations that other types of diabetes, including atypical forms of the disease, occur more frequently than in European communities [3, 4, 5, 6].

There is limited published information on diabetes in young people in Bangladesh, the eighth most populous nation in the world [7]. Aside from classic T1D, type 2 diabetes (T2D) [8] and fibrocalculous pancreatic disease (FCPD) also occur [9, 10], and preliminary work indicates that autoantibody-negative forms of diabetes may be common [11]. Knowledge of the types of diabetes that occur and concurrent clinical, biochemical, and genetic factors will help in health professional training, organisation of care, and optimal treatment for each child or adolescent affected.

We conducted a prospective study of 100 consecutive new cases of diabetes diagnosed in Bangladeshi children and adolescents <22years of age, investigating demographic, clinical, and biochemical features as well as genotype at HLA-DRB1 alleles.

2. Methods

2.1 Study site

The study was conducted at the Bangladesh Institute of Research and Rehabilitation of Diabetes, Endocrine and Metabolic Disorders (BIRDEM) in Shahbagh, Dhaka, Bangladesh, which is part of the network of services provided by the Diabetes Association of Bangladesh

[12]. All procedures were approved by relevant Ethics Committees in Bangladesh, the United States and Australia, and were performed in accordance with the Declaration of Helsinki. Written informed consent was obtained for all subjects prior to enrolment in the study.

2.2 Subjects

A total of 100 subjects ≤22years (y) of age at diabetes diagnosis were enrolled. The subjects were diagnosed from September 2014 to May 2015. 10% were assessed on the same day as diagnosis, 36% within one week, 39% one week to one month, 15% one to six months. All subjects were of Bengali ethnicity.

One hundred and eight-one control subjects were also enrolled solely for HLA-DRB1 analysis. They were unrelated to the study subjects and to each other, and did not have diabetes or a family history of T1D. All subjects were of Bengali ethnicity.

2.3 Demographic data

Date of birth, sex, ethnicity, city and province of residence at diagnosis, date of diagnosis, distance, as well as distance and travel time to the BIRDEM centre were recorded.

2.4 Clinical parameters

Diabetes was diagnosed according to standard World Health Organisation (WHO) criteria [13]. Determination of the type of diabetes was made by the local investigators according to available clinical features and history: T1D was diagnosed upon abrupt onset of typical symptoms of diabetes with insulin required from diagnosis, and no acanthosis nigricans. They were usually non-obese. T2D was considered in pubertal or older subjects who were overweight or obese, who usually did not initially need insulin for blood glucose control, often had a slower onset of symptoms or were asymptomatic, and exhibited acanthosis nigricans, dyslipidaemia, and a family history of the disease. FCPD was diagnosed if there was pancreatic calcification on X-ray or ultrasonography, as reported by a radiologist, in the absence of alcohol intake, hypercalcemia, or biliary duct stones. A history of abdominal pain was also frequent in FCPD patients.

The presence of polyuria, polydipsia, weight loss, and ketoacidosis at the time of diagnosis were recorded. Ketoacidosis was defined as the presence of ketonuria, as well as acidosis (bicarbonate <15mmol/l). The following information pertaining to diabetes care was also recorded for each subject: date of insulin commencement, insulin dosage, number of insulin injections per day, type of insulin used, insulin storage method at homes, use of oral hypoglycaemic agents, and other medications or treatment. History of other medical conditions and family history were also recorded. Body weight and height were measured by electronic scales and stadiometer respectively with subjects wearing light-weight clothing and without shoes. Body Mass Index (BMI) was then calculated. BMI SD scores were calculated using the WHO standards for patients <5years of age [14] and for those 5.0–19.0years [15].

2.5 Sample collection

After an overnight fast, peripheral blood was collected by venepuncture into vacutainer tubes on the day of assessment. Serum samples were spun down immediately and stored at −20°C. For gentotyping, blood samples collected from patients and saliva samples from subjects selected as controls were preserved using DNAgard® reagents from BioMatrica, Inc. (San Diego, USA).

2.6 Biochemical parameters and serology

For diabetes patients, blood glucose was measured using an Evolution 3000 machine (Biochemical Systems International, Arezzo, Italy). HbA1c was measured using a Clover A1c analyzer (Infopia Co. Ltd. Kyunggi, Korea). C-peptide and autoantibodies against glutamic acid decarboxylase 65 (GADA) and islet antigen 2 (IA-2A) were measured by ELISA as previously described [16].

2.7 HLA-DRB1 genotyping

All 84 of the T1D patients and 109 of the 181 control samples were genotyped using previously described methods [16].

2.8 Statistical analysis

Demographic and biochemical data was analysed using Microsoft Excel (Redmond, USA).

For HLA-DRB1 locus analysis in T1D subjects, we used two approaches to perform locus-level tests of heterogeneity (comparing all alleles in cases to those in controls) and allele-level chi-squared ($\chi 2$) tests of association (comparing each individual allele against the set of all others in cases and controls) [17]. The preferred method for chi-squared analyses of case-control contingency tables for highly polymorphic genetic loci, such as HLA, is to combine low frequency alleles into a single “binned” category [18] to ensure that fewer than 20% of expected counts are less than five [19]. The BIGDAWG R package [20] combines alleles with expected counts less than five in either cases or controls into the binned category. However, because some alleles in this dataset that were present only in cases or controls were binned by BIGDAWG, we performed a set of ‘manual’ analyses in which only alleles with expected counts less than three in cases or controls were binned. Results of BIGDAWG analyses had no expected counts less than five, while only 14% (4/28) of expected counts were less than five using our manual analyses, a percentage appropriate for $\chi 2$ analysis. Because we applied both the BIGDAWG and manual approaches, significance for locus level (kx2) tests of association was evaluated at the 0.025 α-level. In cases where the threshold of significance is not met for kx2 tests, the p-values for 2×2 tests must be adjusted for the number of alleles (n) tested (0.05/n).

Hardy-Weinberg equilibrium (HWE) proportions of HLA-DRB1 genotypes in T1D subjects and control subjects were tested using PyPop (v0.8.0) [21]. The significance of locus-level HWE deviations was tested using Guo and Thompson’s exact method [22], and individual genotypes deviating significantly from HWE expectations were identified using Chen’s method [17, 23] with a threshold for significance of 0.05.

3. Results

3.1 Diagnosis

Eighty-four of the 100 enrolled diabetes patients were diagnosed as having T1D, seven with T2D, and nine with FCPD (Table 1).

3.2 Demographic characteristics

Of the 84 subjects with T1D, 37 (44.0%) were males and 47 (56.0%) females. The mean±SD and range of age of diagnosis are shown in Table 1. The median age at diagnosis was 13.0years and the peak age of onset was 12–15years (Fig. 1). Three T1D subjects (3.6%) were diagnosed at 0–4years, 11 (13.1%) from 5 to 9years, 42 (50.0%) from 10 to 14years, 23 (27.4%) from 15 to 19years, and 5 (6.0%) 20 to 22years. Table 1 shows the sex distribution and age of onset of the subjects diagnosed with T2D and FCPD. Of all 100 subjects with diabetes, 30 (30%) travelled <10km to access care, 11 (11%) 10–50km, 34 (34%) 50–200km, and 25 (25%) >200km.

3.3 Clinical parameters

The main symptoms preceding diagnosis of T1D were polyuria (n=77, 91.7%), polydipsia (n=76, 90.5%) and weight loss (n=77, 91.7%). Eight (9.5%) presented in DKA. Table 1 shows the BMI SD results for each diagnosis group. For the T1D subjects, two had a BMI SDS >2: (1) 2.63 in a girl aged 12y with height SD 0.99, no DKA, GADA and IA-2A negative, and C-Peptide 2.05ng/mL; and (2) 2.47 in a girl aged 13y with height SD −0.34, no DKA, GADA and IA-2A negative, and C-Peptide 1.2ng/mL.

Blood glucose and HbA1c values at diagnosis are also shown in Table 1. No subjects reported any other significant medical conditions.

All subjects with T1D and FCPD commenced insulin at diagnosis. Two subjects with T1D also received metformin. For the seven subjects with T2D, three were treated with metformin only, three with insulin only, and one with both insulin and metformin. Of the 96 who commenced insulin, 54 (56.3%) were on long-acting human insulin, 63 (65.6%) were on short-acting insulin, 20 (20.8%) on pre-mixed insulin, and 11 (11.5%) on analogue insulin. One (1.0%) was receiving four injections per day, six (6.3%) three injections, and 89 (92.7%) two injections. Of the 96 subjects receiving insulin, 49 (51.0%) were able to store insulin in a refrigerator at home, 7 (7.3%) in a refrigerator outside the home, and 40 (41.7%) using clay pot evaporative cooling [24].

3.4 C-peptide

Table 1 shows C-peptide levels for those with T1D, T2D and FCPD. Of the 84 subjects diagnosed with T1D, C-peptide levels were not measured in three of the subjects. For those with T1D, C-peptide was in the normal range (0.8–3.1ng/mL (0.26–1.03nmol/L)) in sixty-eight T1D subjects, with no subject having values higher than the normal range. All T2D subjects had C-peptide values in the normal range, with low and normal range values in FCPD subjects.

3.5 Autoantibody results

Table 1 shows the autoantibody values for each diagnostic group. Table 2 shows relationships between DKA and C-peptide status in the T1D subjects. One of the seven subjects diagnosed with T2D was positive for IA-2A (41.5IU/mL), and none were positive for GADA. Two of the nine FCPD subjects had a positive IA-2A (43.7 and 37.7IU/mL), with another subject being positive for GADA of 304.1IU/mL (Table 1, Table 2).

3.6 HLA results

A total of 33 unique DRB1 alleles were observed (Table 3 column A). As shown in Table 3 column B, overall locus-level association with T1D calculated using our manual approach was significant (p=0.00003). The most significantly associated allele was DRB1*04:01, which is well-established as a T1D risk allele in European populations [25, 26, 27]. No controls were observed to carry this allele, precluding calculation of an odds ratio (OR). When the statistical method of adding a count of 0.5 to the zero-count cell was applied, the estimated T1D OR for DRB1*04:01 was >45 (Supplementary Table). As expected from other studies [20, 25, 28, 29, 30], DRB1*14:01 showed a highly-protective effect for T1D (OR=0.000; p=0.017); no diabetes patients were observed with this allele. Of note, the DRB1*15:01 allele, usually observed to confer almost complete dominant protection in European cohorts [25, 28, 31], was not significantly protective in these data, while the closely-related, Asian DRB1*15:02 allele was significantly protective (OR=0.584; p=0.0477). Three other alleles, DRB1*03:01, DRB1*12:02 and DRB1*13:01, were suggestive of association, but the p-values were not significant.

These data were also analyzed using BIGDAWG (Table 3 column C) [20]. As described in the Methods, BIGDAWG bins all alleles that do not have expected counts of 5 in either the patient or control groups. Due to the small sample size of the T1D patient cohort, 24 of 33 observed alleles were binned by BIGDAWG, including DRB1*04:01 and DRB1*14:01, which were significantly T1D-associated in the manual analysis. The BIGDAWG approach resulted in nominal locus-level significance (p=0.049), which does not meet the 0.025 threshold of significance after correction for multiple kx2 tests. The only nominally significant allele identified by BIGDAWG analysis was DRB1*15:02 (p=0.049), which does not meet the corrected 0.005 threshold of significance for multiple 2×2 tests.

4. Discussion

The incidence of T1D in young people in Bangladesh is still being determined. The IDF Atlas [32] reports a rate of 3.0 per 100,000 children <15years per year, as derived from a study in India [33], but this is a different population. Preliminary data from our group indicates that the incidence of T1D in Dhaka is substantially lower, but rising quickly [34].

This study of 100 new- and recent-onset cases of diabetes in Bangladeshi children and youth found that in addition to T1D, T2D and FCPD are also common representing 7% and 9% of diabetes in youth, respectively. In those diagnosed with T1D, the age of onset is later (mean 13y, peak 12–15y) than in European populations, C-peptide values were often not clinically low (i.e., below 0.26nmol/L (0.8ng/ml)) at diagnosis, and autoantibody negativity was

common (70.2%). There is a suggestion of two peaks for T1D onset in Bangladesh– the first around 5–6years and the second at 10–15years. However, as this was not a consecutive series, this observation should be tested with a larger, consecutive series. A female preponderance for T1D was observed (male:female ratio=0.79). This is commonly seen in lower-incidence populations [35], and described in the preliminary data published by Balsa et al. [34]. Classic T1D is characterised by low C-peptide levels, autoantibody positivity, and the development of DKA unless diagnosis is made earlier. A number of the subjects clinically diagnosed with T1D in this study did not have these findings, and the rate of DKA was low at 10% [36]. This may be due to a higher incidence of atypical forms of diabetes, incomplete characterisation of these cases, or a combination of these reasons.

Aside from T1D, a number of subjects in this series were diagnosed with FCPD (n=9) or T2D (n=7). FCPD has been extensively reported in adults and adolescents in Bangladesh, India, and other countries [8, 10, 37], but the cause is not well understood. An association with the incompletely-understood condition of “malnutrition-related diabetes” was thought to be likely, but more recent evidence suggests that this is not so [37]. Pancreatic calcification and abdominal pain are characteristic. T1D autoantibodies have been raised in some subjects with FCPD in some but not all studies [37, 38], potentially reflective of pancreatic damage [37]. T2D is common in adults in Bangladesh [39], and cases in older children and adolescents have been reported [8].

Autoantibody-negative forms of T1D occur in many populations, but appear to be more common in India [4, 38, 40, 41, 42] and other non-European populations [6, 16, 43], and the insulin resistance of T2D can be seen in autoantibody-positive subjects [3, 40, 43]. Some studies have attempted to address this with subclassifications of “ketosis-resistant” diabetes [40], and type 1B (idiopathic) diabetes [4].

The fact that the overall DRB1 locus association was less strong in these data, compared to published results from other populations [31], supports the notion that the pathophysiology of T1D may differ in this population compared to more widely-studied populations, such as European and African cohorts [31, 44]. The differences in results observed between the two association analyses performed on these data illustrate the challenges involved with analysis of highly-polymorphic HLA genotyping data. The possibility exists that the T1D-associated alleles detected in the manual analysis could be spurious, resulting from type 1 statistical error. However, the striking differences in control versus patient counts for DRB1*04:01 (0 vs. 10) and DRB1*14:01 (12 vs. 0), combined with the facts that these statistically-significant associations are seen for alleles with well-established T1D associations and that the associations are in the expected direction (i.e., DRB1*04:01 is predisposing [25, 26, 27] and DRB1*14:01 is protective [20, 25, 28, 29, 30]), argue that the detected associations are likely real. An alternative explanation is that these alleles occur at relatively low frequencies in this population, which could also help explain the weak overall T1D association in this data set. BIGDAWG’s binning strategy may be overly conservative for a small population sample displaying a >2:1 difference in the number of controls and patients, and may result in type 2 statistical error (i.e., missing one or more true associations).

In the present study, the diagnosis of the type of diabetes was made by clinical features, and the boundary between T1D/atypical T1D and T2D was difficult to determine without assessment of insulin sensitivity and further testing of C-peptide at intervals beyond diagnosis. Also, due to funding considerations, only two autoantibodies were studied: GADA and IA-2A. The addition of zinc transporter-8 autoantibodies (ZnT8A) [45] and insulin autoantibodies (IAA) may reveal further evidence of autoimmunity. Analysis of DQ and other HLA alleles would also provide further information, however this has not been possible to date due to funding constraints.

In conclusion, distinguishing the type of diabetes in these non-classical “T1D” cases is not just of scientific interest. It is our experience that standard practice in many less-resourced countries is to start and continue such patients on insulin alone unless there are overt signs of T2D (in particular, obesity and acanthosis nigricans). In some cases though, oral agents, such as metformin or a sodium-glucose co-transporter 2 inhibitor may be of benefit. These subjects deserve to receive “personalised medicine”, with appropriate investigations taking into consideration the constraints of local resources.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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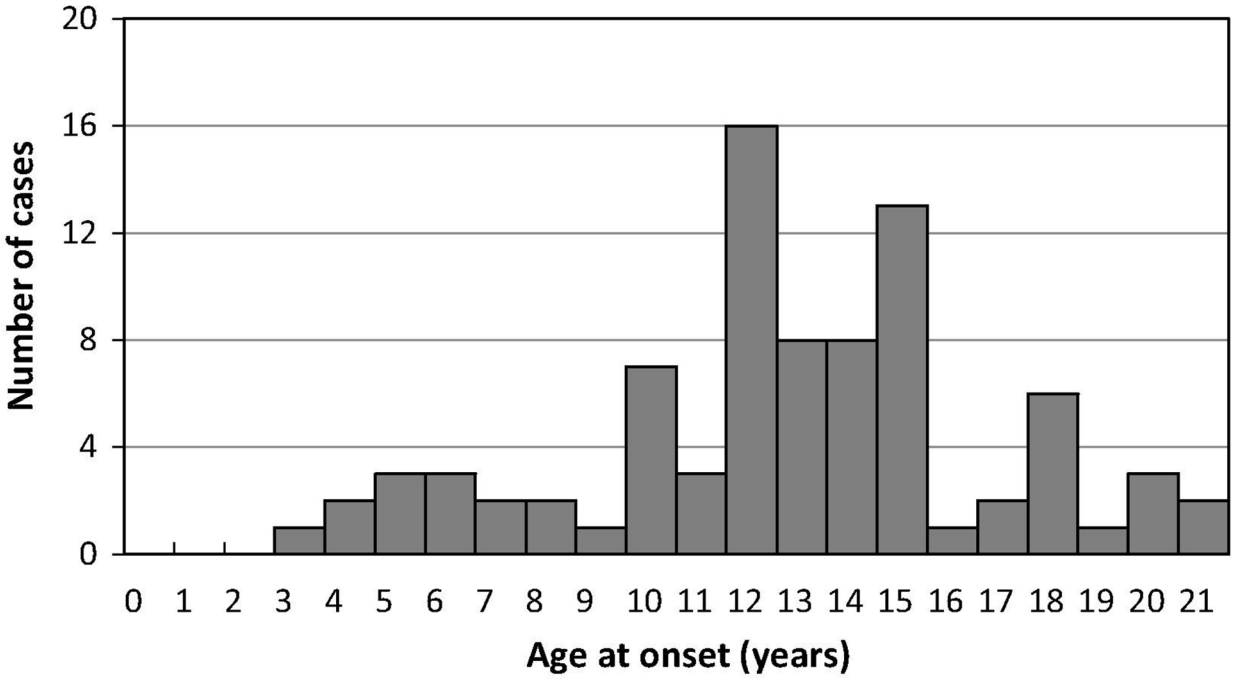

Figure 1.
The age of onset of type 1 diabetes in young people < 22 years of age in Bangladesh

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TABLE 1.

Demographic, clinical and biochemical characteristics.

		Type 1 Diabetes	Type 2 Diabetes	Fibrocalculous Pancreatic Disease
		n=84*	n=7	n=9
Demographic	Gender male:female	0.79	1.33	1.25
	Age		11.6±1.7 (9.0–14.0)	15.4±2.2 (12.0–19.0)
	BMI	12.7±4.1 (3.0–22.0)	25.6±2.5 (22.5–29.1)	18.9±4.0 (15.2–27.6)
	BMI SD	16.7±4.1 (10.3–29.1)	2.4±0.6 (1.7–3.3)	−0.9±1.4 (−2.9 to 1.8)
Biochemical	Blood glucose (mmol/L)	−1.4±2.1 (−8.9–2.6)	16.9±6.8 (8.8–28.5)	24.3±9.1 (15.7–33.8)
	HbA1c			
	(%)	12.7±2.5 (5.1–20.1)	9.4±2.5 (6.1–14.0)	12.6±2.1 (8.7–14.0)
	[mmol/mol])	115±27 (32–196)	79±27 (43–130)	15±23 (72–130)
	DKA (n, %)	8 (9.5%)	0 (0%)	0 (0%)
	C-peptide levels			
	<0.13nmol/L (<0.4ng/mL)	5 (6.2%)	0 (0%)	3 (33.3%)
	0.13–0.23nmol/L (0.4–0.7ng/mL) (n=8)	8 (9.9%)	0 (0%)	1 (11.1%)
	0.26–1.03nmol/L (0.8–3.1ng/mL)	68 (84.0%)	7 (100%)	5 (55.6%)
	>1.03nmol/L (>3.1ng/mL)	0 (0%)	0 (0%)	0 (0%)
	Autoantibodies	22 (26.2%)	0 (0%)	1 (11.1%)
	GAD65 positive (n, %)	9 (10.7%)	1 (14.3%)	2 (22.2%)
	IA-2 positive (n, %)	5 (6.0%)	0 (0%)	0 (0%)
	Both autoantibody positive (n, %)	25 (29.8%)	1 (14.3%)	3 (33.3%)
	One or more autoantibodies positive (n, %)	59 (70.2%)	6 (85.7%)	6 (66.7%)
	Neither autoantibody positive (n, %)			

Data is expressed as mean±SD followed by the range in brackets. Descriptive data analysis was analysed with MSOffice Excel.

*n=84 for type 1 diabetes for all parameters except for BMI SD (n=83) and C-peptide (n=81).

TABLE 2.

The relationships of diabetic ketoacidosis, C-peptide and autoantibodies for 81 T1D subjects.

	C-peptide status			**Autoantibody status**				
	<0.13nmol/L (<0.4ng/mL)	**0.13–0.23nmol/L (0.4–0.7ng/mL)**	**0.26–1.03nmol/L (0.8–3.1ng/mL)[1]**	**GAD-65 ≥30 IU/m**	**IA2 ≥30 IU/m**	**Both autoantibodies**	**Either/both autoantibodies**	**Neither autoantibody**
Diabetic Ketoacidosis (n=8) (9.5%)	0 (0%)	1 (12.5%)	7 (87.5%)	3 (37.5%)	2 (25.0%)	1 (12.5%)	4 (50.0%)	4 (50.0%)
C-peptide <0.13nmol/L (<0.4ng/mL) (n=5)				2 (40.0%)	1 (20.0)	1 (20.0%)	2 (40.0%)	3 (60.0%)
C-peptide 0.13–0.23nmol/L (0.4–0.7ng/mL) (n=8)				1 (12.5%)	1 (12.5%)	0 (0%)	2 (25.0%)	6 (75.0%)
C-peptide 0.26–1.03nmol/L (0.8–3.1ng/mL)[1] (n=68)				19 (27.9%)	7 (10.3%)	4 (5.9%)	21 (30.9%)	46 (67.6%)

1: Normal range. No T1D subjects had C-peptide values >1.03nmol/L (>3.1ng/mL).

Table 3.

DRB1 genotyping of Type 1 diabetic patients

A. Allele	B. patient	control	OR (95% CI)	p	C. OR (95% CI)	p
	frequency (n)	frequency (n)				
DRB1*01:01	0.018 (3)	0.022 (8)	0.805 (0.136 – 3.408)	n.s.		
DRB1*03:01	0.054 (9)	0.025 (9)	2.220 (0.764 – 6.435)	0.0895	2.22 (0.76 – 6.44)	0.0895
DRB1*04:01	0.060 (10)	0.000 (0)	Inf (5.040 - Inf)	2.7810E-06		
DRB1*04:02						
DRB1*04:03	0.042 (7)	0.069 (25)	0.586 (0.210– 1.433)	n.s.	0.59 (0.21 – 1.43)	n.s.
DRB1*04:04						
DRB1*04:05	0.030 (5)	0.019 (7)	1.556 (0.383 – 5.787)	n.s.		
DRB1*04:06						
DRB1*04:07						
DRB1*04:08						
DRB1*07:01	0.238 (40)	0.199 (72)	1.259 (0.788 – 1.991)	n.s.	1.26 (0.79 – 1.99)	n.s.
DRB1*08:01						
DRB1*08:03						
DRB1*08:04						
DRB1*09:01						
DRB1*10:01	0.071 (12)	0.050 (18)	1.470 (0.629– 3.314)	n.s.	1.47 (0.63 – 3.31)	n.s.
DRB1*11:01						
DRB1*11:04						
DRB1*11:08						
DRB1*12:01						
DRB1*12:02	0.071 (12)	0.119 (43)	0.571 (0.266 – 1.141)	0.0962	0.57 (0.27 – 1.14)	0.0962
DRB1*13:01	0.012 (2)	0.039 (14)	0.299 (0.033 – 1.330)	0.0938	0.3 (0.03 – 1.33)	0.0938
DRB1*13:02						
DRB1*14:01	0.000 (0)	0.033 (12)	0.000 (0.000 – 0.762)	0.0170		
DRB1*14:04	0.042 (7)	0.028 (10)	1.530 (0.485 – 4.543)	n.s.	1.53 (0.48 – 4.54)	n.s.
DRB1*14:05						
DRB1*14:07						
DRB1*15:01	0.125 (21)	0.094 (34)	1.378 (0.733 – 2.539)	n.s.	1.38 (0.73 – 2.54)	n.s.
DRB1*15:02	0.119 (20)	0.188 (68)	0.584 (0.323 – 1.019)	0.0477	0.58 (0.32 – 1.02)	0.0477
DRB1*15:04						
DRB1*15:06						
DRB1*15:20						
DRB1*16:02						
Binned1	0.119 (20)					
0.116 (42)						
1.030 (0.552– 1.866)	0.9197	1.24 (0.77 – 1.98)	0.3423			

A. Allele	B. patient	control	OR (95% CI)	p	C. OR (95% CI)	p
	frequency (n)	frequency (n)				
			p for DRB1 locus	3.04E-05	p for DRB1 locus	0.0492

A. all observed alleles B. Results of manual chi-squared association analysis C. Results of BIGDAWG analysis (n= 181 controls, n= 84 TD1 patients)

1: Frequency and count values for the binned alleles in the 'manual' analysis (Table 3B) are shown. For the BIGDAWG analysis (Table 3C), frequencies and counts for binned alleles in T1D patients and controls totaled 0.226 (38) and 0.191 (69), respectively.