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Authors

Fawwad, Asher
Govender, Denira
Ahmedani, Mohammad Yakoob
et al.

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Clinical features, biochemistry and HLA-*DRB1* status in youth-onset type 1 diabetes in Pakistan.

Asher Fawwad[1,2,*], **Denira Govender**[3,4,*], **Mohammad Yakoob Ahmedani**[2], **Abdul Basit**[2], **Julie Ann Lane**[5], **Steven John Mack**[5], **Mark Alvin Atkinson**[6,7], **Clive Henry Wasserfall**[6], **Graham David Ogle**[3,8], and **Janelle Annette Noble**[5]

[1]Biochemistry Department, Baqai Medical University, Gadap, Karachi, Pakistan

[2]Baqai Institute of Diabetology and Endocrinology, Nazimabad, Karachi, Pakistan

[3]Life for a Child, Glebe, NSW 2037, Australia

[4]Sydney Medical School, University of Sydney, NSW 2006, Australia

[5]Children's Hospital Oakland Research Institute, Oakland, CA, USA 94609.

[6]Department of Pathology, Immunology and Laboratory Medicine, College of Medicine, Diabetes Institute, University of Florida, Gainesville, FL, USA 32610.

[7]Department of Pediatrics, College of Medicine, Diabetes Institute, University of Florida, Gainesville FL, USA 32610.

[8]Diabetes NSW, Sydney, Australia

Abstract

Published information on diabetes in Pakistani youth is limited. We aimed to investigate the demographic, clinical, and biochemical features, and *HLA-DRB1* alleles in new cases of diabetes affecting children and adolescents <22 years of age.

The study was conducted at Baqai Institute of Diabetology and Endocrinology in Karachi from June 2013-December 2015. One hundred subjects aged <22 years at diagnosis were enrolled. Demographic characteristics, clinical information, biochemical parameters (blood glucose, HbA1c, C-peptide, glutamic acid decarboxylase 65 (GAD65) and islet antigen 2 (IA-2) autoantibodies) were measured. DNA from 100 subjects and 200 controls was extracted and genotyped for *HLA-DRB1* using high-resolution genotyping technology.

Correspondence to: Dr. Graham Ogle, 22 Howard PI, North Epping, NSW 2121, Australia, Telephone: +61 400 683 574, Fax: +61 2 9660 3633, grahamo@diabetesnsw.com.au.

AF, MYA and AB implemented the study in Pakistan and helped with writing the manuscript. DG did 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 designed and coordinated the genetic portion of the study, including sample collection, DRB1 genotyping, and HLA analysis, and co-wrote the manuscript.

[*]Indicates equal contribution to the paper and recognition as co-first author

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Ninety-nine subjects were clinically diagnosed as type 1 diabetes (T1D) and one as type 2 diabetes (T2D). Of the 99 with T1D, 57 (57.6%) were males and 42 (42.4%) females, with mean age at diagnosis 11.0±5.2 years (range 1.6–21.7 years) and peaks at six and fifteen years. Fifty-seven subjects were assessed within one month of diagnosis and all within eleven months. For the subjects diagnosed as T1D, mean C-peptide was 0.63±0.51 nmol/L (1.91±1.53 ng/mL), with 16 (16.2%) IA2 positive, 53 (53.5%) GAD-65 positive, and 10 (10.1%) positive for both autoantibodies. In T1D patients, the allele *DRB1*03:01* demonstrated highly significant T1D association ($p < 10^{-16}$), with no apparent risk conferred by *DRB1*04:xx* alleles.

Conclusions: Heterogeneous forms of T1D appear more common in children and youth in Pakistan than in European populations. Individual understanding of such cases could enablie improved management strategies and healthier outcomes.

Keywords

childhood diabetes; Pakistan; HLA; autoantibody; C-peptide

Introduction

Youth onset diabetes has multiple etiologies with varied geographic distribution (1,2). Considering this variation, it is important to understand the pathophysiology in each part of the world. From this, education and training of healthcare professionals can be tailored to target the local situation. Furthermore, improved understanding of disease heterogeneity will enable the stakeholders to ensure the optimum utilisation of available resources.

Little published information exists on diabetes in Pakistani young people, the sixth most populous nation in the world (3). Two incidence studies were performed in Karachi some years ago, in 1989–93 (4) and 1990–1999 (2), suggesting low incidence rates compared to Caucasian populations (2). Beyond this, Shera et al.(5) reported on clinical presentation and complications of a group of young people with type 1 diabetes (T1D) in Karachi, Pakistan's capital, in 2008. There was also a small study on diabetes autoimmunity markers (6) which discusses the difficulty of distinguishing diabetes types. Finally, Lone et al. (7) reported on rates of diabetic ketoacidosis.

We conducted a prospective study of 100 consecutive new cases of diabetes diagnosed in children and adolescents <22 years of age, investigating demographic, clinical, biochemical features, and *HLA-DRB1* alleles. This study was conducted at the Baqai Institute of Diabetology and Endocrinology (BIDE) in Karachi, which was the first specialty-oriented diabetes centre to be established in Pakistan (8).

Methods

1.1 Study site

The study was conducted at The Baqai Institute of Diabetology and Endocrinology (BIDE) in Karachi, Pakistan, which is a private tertiary care unit. All procedures were approved by relevant Ethics Committees in Pakistan, the United States, and Australia and were performed

in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects prior to enrolment in the study. *HLA-DRB1* genotyping was performed at Children's Hospital Oakland Research Institute with IRB approval.

1.2 Study subjects

A total of 100 subjects <22 years of age at diabetes diagnosis were enrolled. All subjects were diagnosed in a consecutive series from June 23, 2013 to December 15, 2015. Date of biochemical assessment for this study ranged from December 30, 2013 to December 23, 2015 such that 14% were assessed on the same day as diagnosis, 19% within one week, 24% one week to one month, 32% one to six months and 11% after six months (maximum eleven months) from diagnosis. One patient was classified as having type 2 diabetes (T2D) after enrolment. Data for this patient was excluded from the genetic analyses. A set of 200 control subjects were collected as controls for the HLA genotyping studies. Inclusion criteria for the controls included 1) no diabetes, 2) both parents born in Pakistan, 3) not related to a T1D patient, and 4) not related to another control subject. Age and gender were not included and this detail was not revealed to the genotyping laboratory.

1.3 Demographic data

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

1.4 Clinical parameters

Diabetes was diagnosed according to standard World Health Organisation (WHO) criteria (9). Determination of the type of diabetes was made by the local investigators according to available clinical features and history. The presence of polyuria, polydipsia, weight loss, malnutrition and ketoacidosis at the time of diagnosis were recorded. Ketoacidosis was defined as the presence of ketonuria as well as acidosis (anion gap calculation and/or measurement of arterial blood gases).

The following information pertaining to diabetes care was recorded for each subject: date commencement of insulin, use of oral hypoglycaemic agents, and other medications or treatment. History of other medical conditions, and family history of type 1 diabetes were also recorded. Body weight and height at diagnosis 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 those < 5 years of age (10), and for 5–19 years (11). For those 19–21 years of age, BMI SD was calculated using an age of 19.0 years.

1.5 Sample collection

Peripheral blood was collected by venepuncture into vacutainer tubes on the day of assessment, after an overnight fast. Serum samples were spun down immediately and stored in a −20°C freezer. For genotyping, approximately 200ul of peripheral blood was preserved by mixing with DNAgard® blood (BioMatrica, San Diego, CA), then drying for storage and

shipment. For controls, approximately 1 ml of saliva was mixed with DNAgard® saliva stabilizing reagent (BioMatrica, San Diego, CA) for storage and shipment.

1.6 Biochemical parameters and serology

For diabetes patients, blood glucose was measured at diagnosis with a Selectra Pro S ELITech Group (Dieren, Netherlands). HbA1c was collected on the day of study assessment, and assayed using a BioRad D-10 analyser (Biorad Laboratories Inc., Hercules, USA). Fasting C-peptide and autoantibodies against glutamic acid decarboxylase 65 (GAD65) and islet antigen 2 (IA-2) were measured from frozen samples by commercially available ELISA kits (IBL, Hamburg, Germany) in Pakistan. Fasting C-peptide was measured in ng/mL. GAD65 and IA-2 autoantibodies were considered positive if levels were ≥30 IU/mL based on the standard curve, according to manufacturer's recommendation. Similar ELISA formats have been challenged in Islet Autoantibody Standardization Programs with comparable sensitivity and specificity to radioimmunoassays (12).

1.7 HLA-DRB1 genotyping

Blood samples collected from patients and saliva samples from controls were preserved using DNAgard® reagents from BioMatrica, Inc. (San Diego, USA). DNA was extracted from preserved blood and saliva samples using QIAamp® blood kits (Qiagen). The samples were genotyped for *HLA-DRB1* alleles with high-resolution genotyping technology at the Children's Hospital Oakland Research Institute in California. HLA sequence data for *DRB1* exon 2 were generated using next-generation sequencing on the 454 GS Junior System (Roche, Basel, Switzerland) and on the MiSeq® platform (Illumina, San Diego, CA).

For the Roche 454 platform, PCR products (amplicons) were generated from genomic DNA using *DRB* generic, exon 2 454 fusion primers. The 454 fusion primers consist of a locus-specific primer on the 3' end, a 10-bp multiplex ID (MID) tag, and an "A" or "B" 454-specific sequence on the 5' end. Amplicons were purified with AMPure beads (Becton Dickinson, Franklin Lakes, USA), quantified using the Quant-iT PicoGreen dsDNA reagent (Life Technologies, Foster City, USA), and mixed with capture beads after dilution. Individual *DRB* exon 2 amplicon molecules were captured by these beads and amplified in an emulsion PCR. DNA-containing beads were pyrosequenced on the 454 GS Junior instrument to obtain sequence reads originating from a single molecule (13,14).

For the Illumina platform, DNA samples were amplified with primers containing the same locus-specific sequence used for the 454 platform but containing adapters to allow a second round of amplification. The second round of amplification served to attach individual "barcode" identifiers and sequences specific for sequencing on the Illumina platform. Second-round amplicons were purified and quantified as above, then mixed in equal proportions before sequencing on the MiSeq instrument.

DRB1 genotype calls for data generated on the Roche 454 platform were assigned with a combination of two customized software packages: Assign™ ATF (Conexio Genomics, Freemantle, Western Australia) and Sequence COmpilation and REarrangement (SCORE™) software (Graz, Austria) (15). Genotype calls for data generated on the Illumina MiSeq

platform were assigned using HLA Twin™ software, version 2.1 (Omixon, Budapest, Hungary).

1.8 Statistics

The data were assessed for bimodality using likelihood ratio tests for nested finite mixture models. Models were fitted using an Expectation-Maximisation algorithm implemented in R 3.3.1 (R Core Team, Vienna, Austria) and with the package 'mixtools' (16). Significance was assessed using Wilks' chi-square approximation and by parametric bootstrap with 1,000 samples. To assess sensitivity to distributional assumptions, the analysis was performed in triplicate, assuming mixtures of Normal, LogNormal and Gamma populations in turn. Locus-level tests of heterogeneity and allele-level chi-squared (χ^2) tests of association between T1D subjects and control subjects were performed for the *HLA-DRB1* locus using the BIGDAWG R package (17). Alleles were analysed at the two-field (peptide) level. For each allele-level comparison, alleles with expected counts less than 5 in cases or controls were combined into a common "binned" category for analysis (18).

Hardy-Weinberg equilibrium (HWE) proportions of *HLA-DRB1* genotypes in T1D subjects and control subjects were tested using PyPop (v0.8.0) (19). The significance of locus-level HWE deviations was tested using Guo and Thompson's exact method (20), and individual genotypes deviating significantly from HWE expectations were identified using Chen's method (21,22), using a threshold of significance of 0.05. Graphs were created using Microsoft Excel software.

Results

2.1 Diagnosis

Ninety-nine of the 100 enrolled diabetes patients were diagnosed as having T1D. One male who presented at 11.9 years of age was diagnosed with T2D.

2.2 Demographic characteristics

The 99 subjects with T1D included 57 (57.6%) males and 42 (42.4%) females. There was no discernible seasonal pattern in disease incidence (data not shown). 61.6% were of Muhajir ethnicity, 13.1% Sindhi, 10.1% Pashtun, 8.1% Punjabi, 2.0% Balochi and 5.1% other ethnic groups.

The mean±SD age of diagnosis of T1D was 11.0±5.2 years (range 1.6 – 21.7 years). The median age at diagnosis was 11.0 years (Figure 1) with a suggestion of two peaks, the first at 6–10 years and the second at 12–16 years. On bimodality analysis, the binary mixture model was significantly preferred to the single population model ($p<0.05$) using both the Wilks' chi-square approximation and parametric bootstrap tests. We report the model with the most favourable Bayesian Information Criterion. Under this model, 48% of the data come from a Normal distribution with mean 6.4 and SD 2.7, and 52% from a Normal distribution with mean 15.2 and SD 2.8.

Fifteen subjects (15.2%) were diagnosed at 0–4 years, 31 (31.3%) from 5–9 years, 29 (29.3%) from 10–14 years, 20 (20.2%) from 15–19 years and 4 (4.0%) 20–21 years. Of all

100 subjects, 41 (41%) travelled <10km to access care, 42 (42%) 10–50 km, 4 (4%) 50–200 km and 13 (13%) >200km.

2.3 Clinical parameters

The main symptoms preceding T1D diagnosis were polyuria (n=98, 99.0%), polydipsia (n=97, 98.0%) and weight loss (n=99, 100%). Twenty-one (21.2%) presented in diabetic ketoacidosis (DKA).

For the 91 subjects with T1D who had both weight and height measured at diagnosis, the mean±SD BMI was 16.5±3.3 (range 10.2–27.3). Mean BMI standard deviation score (SDS) ranged from −7.41 to +2.61 (mean= −1.04). Two subjects had a BMI SDS > 2: 1) 2.61 in a boy aged 5 years with height SD −0.25, no DKA, both autoantibodies negative, and C-peptide 0.40 nmol/L (1.2 ng/mL) and 2) 2.27 in a boy aged 5 years with height SD −3.72, no DKA, both autoantibodies negative, and C-peptide 0.53 nmol/L (1.6 ng/mL). Two subjects had a BMI SDS < −5.0: 1) −6.18 in a boy aged 14 years with height SD −2.33, with malnutrition, DKA present, autoantibodies negative, C-peptide 0.40 nmol/L (1.2 ng/mL); and 2) −7.41 in a girl aged 16 years with height SD −1.80, malnutrition, no DKA, autoantibodies negative, C-peptide 0.93 nmol/L (2.8 ng/mL).

For T1D subjects, the mean±SD blood glucose at diagnosis was 28.7±6.3mmol/L (range 13.6–45.9 mmol/L). The mean±SD HbA1c was 12.7±2.7 % (115.0±29.4 mmol/mol), with a range 7.1–21.0 (range 54.1–206.0 mmol/mol).

Four T1D subjects had other medical conditions: two with cerebral palsy, one with thalassaemia (diagnosed after the diagnosis of diabetes), and one with both cerebral palsy and thalassaemia, diagnosed after diabetes. One T1D subject had a sister with T1D.

All T1D subjects were treated with insulin. Eighty-seven (87.9)% started on insulin on the same day as diagnosis, 10 (10.1%) within 1 week, and two (2.0%) within 2 weeks.

The single case diagnosed with T2D was a boy diagnosed at 12.0 years with a BMI SD of 2.78 and a height SD of 2.07, no DKA, both autoantibodies negative, and C-Peptide 1.62 nmol/L (4.9 ng/mL). He was initially commenced on insulin but then moved to metformin.

3.3 C-peptide

The mean±SD for C-peptide was 0.63±0.51 nmol/L (1.91±1.53 ng/mL). Of T1D subjects, 3 (3.0%) had a C-peptide value <0.13 nmol/L (<0.4 ng/mL), 9 (9.1%) between 0.13–0.26 nmol/L (0.4-<0.8 ng/mL), 64 (64.6%) between 0.26–0.66 nmol/L (0.8-<2.0 ng/mL), 13 (13.1%) between 0.66–1.03 nmol/L (2.0-<3.1 ng/mL), and 10 (10.1%) >1.03 nmol/L (>3.1 ng/mL). There was no relationship between C-peptide and duration of diabetes before assessment (r=0.0.7, p=0.5 on linear regression). Table 1 shows relationships with DKA and autoantibody status.

3.4 Autoantibody results

For T1D subjects, 16 (16.2)% were IA-2 autoantibody positive, 53 (53.5%) were GAD65 autoantibody positive, and 10 (10.1%) were positive for both autoantibodies (see Table 1 for

further relationships). Amongst seropositive subjects, the mean±SD titre was 105.8±137.0 IU/mL for GAD65 autoantibodies and 74.7±198.8 IU/mL for IA-2 autoantibody levels. Table 1 shows relationships with DKA and C-peptide status.

3.5 HLA-DRB1 results

HLA-DRB1 genotypes were generated successfully for 100 patients and 188 control subjects. Association analysis for 99 T1D patients and 188 control subjects is shown in Table 2. As expected, the association of the DRB1 locus with T1D was highly significant ($p<2.22 \times 10^{-16}$). The risk for T1D was primarily driven by the allele *DRB1*03:01*, which is part of the conserved *DRB1*03:01-DQA1*05:01-DQB1*02:01* haplotype (sometimes abbreviated as DR3-DQ2). *DRB1*03:01* was seen at 51% frequency in T1D subjects but only 12% in controls (OR= 7.86; 95% CI = 5.06–12.25; $p<2.22 \times 10^{-16}$). The less frequent *DRB1*14:04* allele (observed in 6.6% of T1D subjects but only 1.6% of controls) also predisposed to T1D (OR = 4.33; 95% CI = 1.5–14.1; p=1.56E-03). Coincidentally, both alleles are roughly four times more frequent in T1D patients than controls (*DRB1*03:01*: 4.4×; *DRB1*14:04*: 4.1×). In Asians, *DRB1*14:04* is commonly found in a haplotype with *DQB1*05:01*. This haplotype was not found to be significantly associated with T1D in the Type 1 Diabetes Consortium data (23), but is significantly positively associated with juvenile autoimmune thyroiditis in North India (24). Notably, the similar European haplotype *DRB1*14:01~DQB1*05:01* exhibits a very strong protective association with T1D (23). As anticipated, the Asian allele *DRB1*04:03* was significantly negatively associated with T1D. Other DRB1*04 alleles, particularly *DRB1*04:01/02/04/05*, which are usually positively associated with T1D, were rare in these data and not significantly predisposing for T1D. Of the T1D patients, 66.7% contained at least one copy of *DRB1*03:01*, and 35.4% were homozygous for the *DRB1*03:01* allele. This is in stark contrast to the typical HLA association result seen in European-derived populations, where up to 40% of patients are heterozygous for *DRB1*03:01* and *DRB1*04:01/04:02/04:04/04:05/04:08* (23).

While *DRB1* genotype proportions of the control subjects comported to HWE expectations (p = 0.35), *DRB1* genotypes in the T1D subjects displayed an overall HWE deviation ($p = 4.6 \times 10^{-05}$) characterized by an excess of homozygotes (39 observed, 27.57 expected, p = 0.03). In particular, a significant excess of *DRB1*03:01*+*DRB1*03:01* homozygous genotypes (35 observed, 25.8 expected, $p = 2.5 \times 10^{-04}$) and significant decrease in *DRB1*03:01* heterozygotes (31 observed, 49.5 expected, $p = 8.6 \times 10^{-03}$) was observed for T1D subjects. Deviations were not observed for these genotypes in control subjects (*DRB1*03:01* homozygotes: 4 observed, 2.57 expected, p = 0.47; *DRB1*03:01* heterozygotes: 36 observed, 38.85 expected, p = 0.65).

Discussion

This study investigated the clinical, biochemical, and genetic characteristics of new and recent onset diabetes in children and youth in Pakistan.

The pattern of age of onset of subjects diagnosed with T1D suggested a bimodal distribution with peak diagnosis at approximately six to nine years in age with a second peak occurring in the early teenage years, a finding similar to the pattern observed in some other studies

(25,26). However, previous studies in Pakistan have reported the peak age of T1D onset as 10–14 years (2) and 10–12 years (4). The male excess seen in this study was also observed in Karachi by Shera et al. (5), and in some studies in northern India (27–29). However, Staines et al. (4) in Karachi found a slight female excess as did and some studies in northern India. Globally, low incidence countries tend to have a female excess (30).

Understanding of T1D in Pakistan is hampered by the lack of recent incidence studies. The two past studies suggested low incidence rates: 1.0 cases per 100,000 children <17 years of age per year in Karachi between 1989–1993 (4), and 0.5 cases per 100,00 children <15 years of age per year in Karachi from 1990–1999 (2) compared to rates of 4.2–40.9 in the European populations reported in the same period (2).

In our study, most T1D subjects (61.6%) were from the Muhajir ethnic group, who came from northern India at the time of the partition with Pakistan in 1947. This group represents only 7.6% of Pakistan's population (3) but in Karachi, it is estimated to be 50% of the population (31).

The incidence of T1D can change in migrant populations. The reported incidences in Karachi (with 50% of Indian descent) are lower than those reported in India: 3.0 per 100,000 <15 years in Karnal (northern India) in 2008 (27,32) and 10.5 in 1991–1994 in Madras (southern India) (33). In children of South Asian migrants to the United Kingdom and Sweden, rates are substantially higher than those observed in Pakistan or northern India but less than those in children born to parents from the country of immigration. For example, in Yorkshire (United Kingdom), the rate was 14.7 per 100,000 children <15 years of age of South Asian parents compared to 21.5 for non-south Asians (34) and in Sweden, the rate in children of South Asians was 34% of that in children of parents born in Finland (with an overall rate of 29 per 100,000 <15 years of age) (35). Such data show the potent impacts of both genetic and environmental effects in incidence of T1D (1,36).

In this cohort, all but one of the 100 patients were clinically diagnosed with T1D. However, the data suggest that those classified as T1D are a heterogeneous group, consisting of typical/classic T1D, atypical T1D, and possibly other forms of diabetes such as malnutrition-related diabetes and T2D. The rate of DKA at onset was 21.2%, which is relatively low by international standards (37). The two previous Pakistan studies reported markedly different rates: 57.2% (7) and 5.8% (5). The GAD65 autoantibody positivity of 54% and IA-2 autoantibody positivity of 16% (with 10.1% of all 99 cases having both autoantibodies) are also relatively low (38). Only 12.1% had a fasting C-peptide at diagnosis of 0.25 nmol/L (<0.8 ng/mL), which is typically the upper threshold for insulin requiring T1D (39) and in 10.1% of cases, the C-peptide was >1.03 nmol/L (>3.1 ng/mL).

Such heterogeneity of those diagnosed with T1D could be partly due to incomplete characterisation. Further autoantibody studies, of insulin autoantibodies and particularly Zinc transporter 8 (ZnT8), could reveal unrecognised autoimmunity. Shivaprasad et al. (40) documented rates of 64.7%, 31.8% and 19.3% for GAD-65, ZnT8 and IA-2 autoantibodies, respectively, with one quarter of those positive for ZnT8 being negative for the other two autoantibodies. However, autoantibody rates lower than those seen in children of European

descent are not uncommon in studies from India (41–45). Even in Western countries, some T1D patients are autoantibody negative; in the SEARCH study for instance, 15.6% of those who had diabetes and were insulin sensitive were autoantibody negative (46). C-peptide studies after the honeymoon period has passed could show whether some of these subjects just have a slowly-progressive T1D, T2D, or possibly a different type of diabetes that is neither T1D nor T2D. T2D occurs at lower BMIs in Asian as compared to European populations (47). According to the Second National Diabetes Survey of Pakistan in 2016–17, rates of T2D in adults ≥ 20 years of age in Pakistan are 26.3% (males 26.2% and females 26.3%) (48). The incompletely understood and possibly overlapping entities of malnutrition-related diabetes and fibrocalculous pancreatic disease (FCPD) (49) are seen in India and Bangladesh (41,42,49), but have not been reported from Pakistan. None of the subjects in this study had the history of chronic abdominal pain that is characteristic of FCPD, although two subjects had a BMI SDS <5.0 and so it is possible that malnutrition was occurring. Complicating diagnosis further, these two conditions may also have autoantibodies (42).

In a small study from northern Pakistan, Fatima et al. (6) found that distinguishing between T1D and T2D was difficult even when autoantibodies and insulin resistance were studied. For individual subjects, it is important to be able to recognise if diabetes is not a classic T1D pattern with very low insulin secretion. Inexpensive oral medicines such as metformin or gliclazide may be of added benefit in a subset of individuals. If good control can be achieved from such medicines, then insulin may not be needed.

The T1D patients in this study seem to have an unusually high representation of *DRB1*03:01*, with more homozygotes than expected. Kelly et al. (50) studied the T1D immunological features in Indo-Aryan children, with both parents originally from Pakistan or Indian Punjab, residing in the UK and found that the DR3-DQ2 haplotype (*DRB1*03:01-DQA1*05:01-DQB1*02:01*) was significantly positively associated with T1D in this population, as it is in most populations (24). Rasmussen et al. (51) examined HLA in newborns of Pakistani migrants in Norway to look for DR-DQ haplotypes considered to confer high-risk for T1D. They found the DR3-DQ2 haplotype in high frequency (18.5%); no instances of DR3/4 heterozygotes with a predisposing DR4 (expectation would be 2 or 3), and an excess of DR3/DR3 homozygous individuals (n =13, with expectation of 6). These data are somewhat consistent with the results presented here; however, because the study was of newborns, diabetes status was unable to be determined.

Data from a population of Somali immigrants to the United States were quite similar to our Pakistani data in that nearly all of the T1D susceptibility was attributable to *DRB1*03:01*. Nearly every patient genotype (93%) included at least one *DRB1*03:01* allele, and 50% of patients were homozygotes for *DRB1*03:01* (52). One interpretation of this observation could be that the Pakistani and Somalian populations may partly share a common ancestry and genetic predisposition for T1D. Further genotyping of the HLA region as well as other T1D susceptibility loci in both populations will address this issue.

Funding constraints limited this study to two autoantibodies (GAD65 and IA-2), a single HLA gene (*DRB1*), and a one-time measurement of C-peptide, with no external quality control. When funds are identified, samples will be genotyped for all classical HLA loci.

Expansion to include other autoantibodies (particularly ZnT8) (40) and other HLA and genetic loci (53) could reveal further information. Longitudinal studies of C-peptide and insulin requirements would help further discrimination of the types of diabetes occurring in this population. Another limitation is that C-peptide and autoantibodies were not always measured at diagnosis. Particualrly for C-peptide, this may have resulted in a few subjects being in the “honeymoon phase” with some recovery of insulin secretion.

In conclusion, this study shows that children and adolescents diagnosed with type 1 T1D in Pakistan may be a heterogenous group, as GAD-65 and IA2 autoantibody positivity and low C-peptide values are less common than in populations of European ancestry. HLADRB1 shows highly significant T1D association, but the overall allele distribution and individuals allele associations differ from Europeans, with *DRB1*03:01* showing exceptionally strong risk. If there is greater heterogeneity, individualised assessment of atypical “T1D” cases could lead to improved management strategies and healthier outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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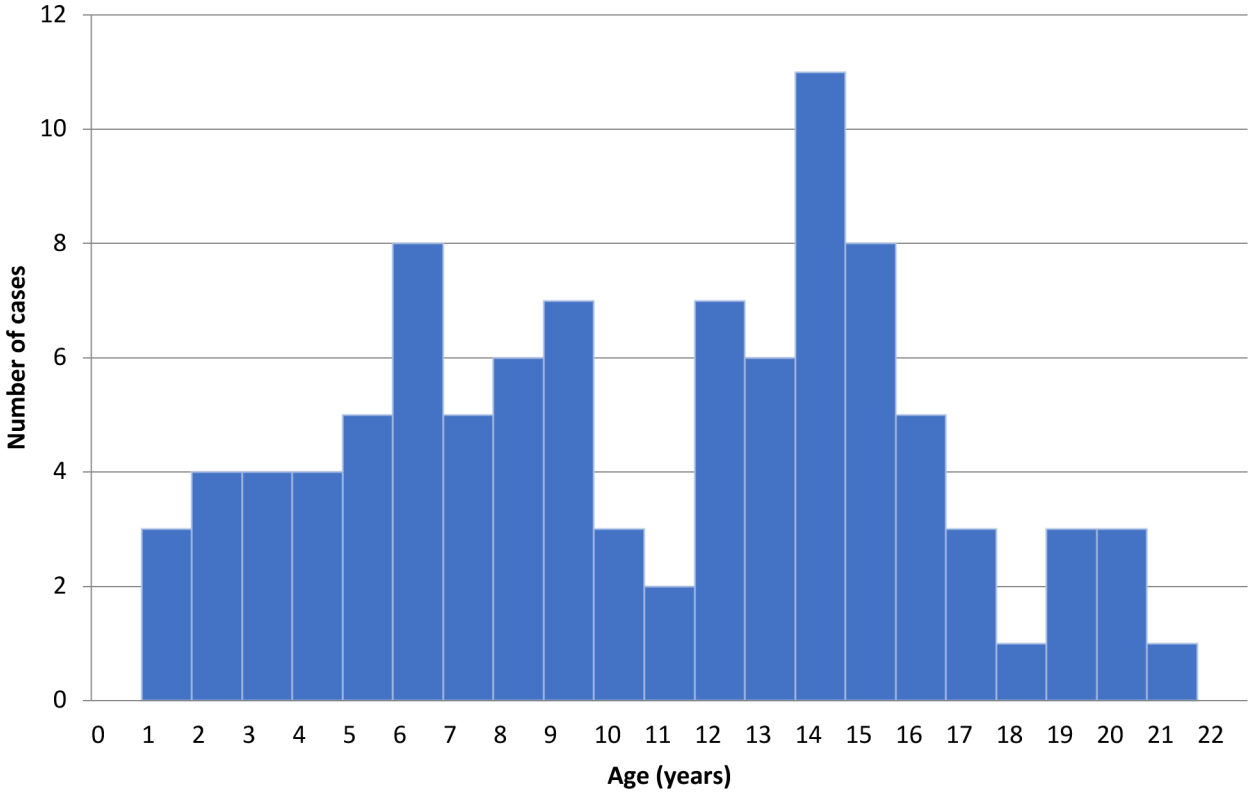

Figure 1.
The age of onset of T1D in young people <22 years of age in Pakistan.

Table 1.

The relationships of diabetic ketoacidosis, C-peptide and autoantibodies for 99 T1D subjects.

		C-peptide				Autoantibody status			
	C-peptide <0.13 nmol/L (<0.4 ng/mL)	**C-peptide 0.13–0.26 nmol/L (0.4–0.8 ng/mL)**	**C-peptide 0.26–1.03 nmol/L (0.8–3.1 ng/mL)[1]**	**C-peptide >1.03 nmol/L) (> 3.1 ng/mL)**	**GAD ≥ 30**	**IA2 ≥ 30**	**Both autoantibodies**	**Either/both autoantibodies**	**Neither autoantibodies**
Diabetic Ketoacidosis (n=21)	1 (5%)	1 (5%)	15 (71%)	4 (19%)	11 (52%)	3 (10%)	2 (5%)	12 (57%)	9 (43%)
C-peptide <0.13 nmol/L (<0.4 ng/mL) (n=3)					1 (33%)	0 (0%)	0 (0%)	1 (33%)	2 (67%)
C-peptide 0.13–0.26 nmol/L (0.40.8 ng/mL) (n=9)					4 (44%)	1 (11%)	0 (0%)	5 (56%)	4 (44%)
C-peptide 0.26–1.03 nmol/L (0.8–3.1 ng/mL)[2] (n=77)					42 (55%)	13 (17%)	8 (10%)	47 (61%)	30 (39%)
C-peptide >1.03 nmol/L (>3.1 ng/mL) (n=10)					6 (60%)	2 (20%)	2 (20%)	6 (60%)	4 (40%)

[1] For those who presented in DKA, C-Peptide was measured within one month in 16 subjects, 1–4 months in 4 subjects, and eight months in one subject.

[2] Normal range

Table 2.

Association of DRB1 Alleles with Type 1 Diabetes in Pakistani Patients and Controls

Allele	Controls		Patients		OR	95% C.I.	p-value	Significance[1]
	Count	Frequency	Count	Frequency				
DRB1*01:01	15	0.03989	8	0.04040	1.01	0.37 – 2.6	9.76E-01	NS
DRB1*03:01	44	0.11702	101	0.51010	7.86	5.06 – 12.25	< 2.22E-16	*
DRB1*04:03	16	0.04255	1	0.00505	0.11	0 – 0.75	1.18E-02	*
DRB1*07:01	61	0.16223	12	0.06061	0.33	0.16 – 0.65	5.13E-04	*
DRB1*09:01	9	0.02394	9	0.04545	1.94	0.67 – 5.62	1.60E-01	NS
DRB1*10:01	21	0.05585	5	0.02525	0.44	0.13 – 1.22	9.38E-02	NS
DRB1*11:01	27	0.07181	5	0.02525	0.33	0.1 – 0.9	2.08E-02	*
DRB1*11:04	17	0.04521	2	0.01010	0.22	0.02 – 0.92	2.54E-02	*
DRB1*13:01	31	0.08245	5	0.02525	0.29	0.09 – 0.77	7.22E-03	*
DRB1*14:04	6	0.01596	13	0.06566	4.33	1.5 – 14.1	1.56E-03	*
DRB1*15:01	26	0.06915	3	0.01515	0.21	0.04 – 0.69	4.99E-03	*
DRB1*15:02	32	0.08511	2	0.01010	0.11	0.01 – 0.44	2.96E-04	*
Binned	71	0.18883	32	0.16163	0.83	0.51 – 1.33	4.19E-01	NS
Total	376		198					

OR: Odds Ratio

CI: Confidence Interval

Binned: Alleles with expected counts less than five in cases or controls were combined into a common “Binned” category for analysis, as described in Methods section 1.9. Counts and frequencies for all detected alleles are included in Supplementary Table S1.

[1]: The p-values for DRB1 alleles were not corrected for the number of comparisons, as the DRB1 locus displayed significant overall heterogeneity (p-value < 2.22E-16). Significant p-values are indicated with asterisks. NS. Not significant

Total: The total number of alleles identified in controls and patients is shown in the last row.