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TITLE: The relationship between islet autoantibody status and the clinical characteristics of children and adults with incident type 1 diabetes in a UK cohort

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ABSTRACT

Objectives: To describe the characteristics of children and adults with incident type 1 diabetes in contemporary, multi-ethnic UK, focusing on differences between the islet autoantibody negative and positive.

Design: Observational cohort study.

Setting: 146 mainly secondary care centres across England and Wales.

Participants: 3,312 people aged ≥5 years were recruited within 6 months of a clinical diagnosis of type 1 diabetes via the National Institute for Health Research Clinical Research Network. 3,021 were of white European ethnicity and 291 (9%) were non-white. There was a small male predominance (57%). Young people <17 years comprised 59%.

Main outcome measures: Autoantibody status and characteristics at presentation.

Results: The majority presented with classical osmotic symptoms, weight loss, and fatigue. Ketoacidosis was common (42%), especially in adults, and irrespective of ethnicity. Of the 1,778 participants who donated a blood sample, 85% were positive for one or more autoantibodies against glutamate decarboxylase, islet antigen-2, and zinc transporter 8. Presenting symptoms were similar in the autoantibody positive and negative participants, as was the frequency of ketoacidosis (43% vs 40%, p=0·3). Autoantibody positivity was less common with increasing age (p=0·0001), in males compared with females (82% vs 90%, p<0·0001) and in people of non-white compared with white ethnicity (73% vs 86%, p<0·0001). Body mass index was higher in autoantibody negative than positive adults (median, IQR 25·5, 23·1-29·2 vs 23·9, 21·4-26·7 kg/m^2; p=0·0001). Autoantibody negative participants were more likely to

have a parent with diabetes (28% vs 16%, p<0·0001) and less likely to have another autoimmune disease (4% vs 8%, p= 0.01).

Conclusions: Most people assigned a diagnosis of type 1 diabetes presented with classical clinical features and islet autoantibodies. Although indistinguishable at an individual level, autoantibody negative participants as a group demonstrated features more typically associated with other diabetes subtypes.

STRENGTHS AND LIMITATIONS OF THE STUDY

- We have studied a large multi-ethnic cohort of adults and children ≥5 years with clinically diagnosed incident type 1 diabetes in whom pancreatic islet autoantibodies were measured in a central laboratory.
- In routine practice, the initial assignment of a diagnosis of type 1 diabetes is a purely clinical one. The lack of further selection before inclusion in this study (e.g. based on autoantibody status and/or genetic testing) renders the results of particular relevance to standard clinical care.
- Individual autoantibody positive and negative patients were indistinguishable clinically but the size and diversity of the cohort permitted group differences to be detected at high levels of statistical significance, suggesting diagnostic heterogeneity.
- As this was a volunteer study recruiting from mainly secondary care centres, ascertainment bias could have been introduced.
- Provision of a blood sample was optional and autoantibody status was therefore available in just over half of the patients. Other than having a higher median age, this sub-group was representative of the whole cohort.

INTRODUCTION

Type 1 diabetes (T1D) is an autoimmune disease that develops at any age, but most frequently in children and young adults.[1] Autoantibodies against islet antigens are typically present before, and for a variable time following, diagnosis.[2-6] Once initiated, beta cell damage classically leads to progressive loss of insulin secretion and a need for lifelong insulin treatment.

The diagnosis of T1D is a clinical one, but may be supported by the presence of one or more of the autoantibodies to islet-cell antigens. In routine care, autoantibody status may not be available at diagnosis, and may never be checked (management guidelines differ, with some not recommending their routine measurement or restricting measurements to situations where there is clinical doubt).[7-9] Previous studies suggest that 80-90% have detectable autoantibodies at disease onset,[2 10] with a background autoantibody prevalence of around 2% in the young general population.[11] Autoantibody positivity may be lower in some non-white ethnic groups.[12-14] There is however uncertainty around the clinical and demographic correlates of autoantibody status in incident disease in an unselected multi-ethnic cohort including children and adults, using well characterised, validated assays. The After Diabetes Diagnosis REsearch Support System (ADDRESS), supported by the National Institute for Health Research (NIHR) Clinical Research Network (CRN), recruits people with incident T1D from centres across England and Wales. We aimed to characterise these people with reference to their autoantibody status.

METHODS

Ethics approval

Ethical approval was obtained from the South Central – Berkshire NHS Research Ethics Committee (reference 10/H0505/85). The project complies with the recommendations for research on human subjects by the 18th World Medical Assembly, Helsinki 1964 and later revisions and the International Conference on Harmonization Guideline for Good Clinical Practice (Topic E6 - 10 June 1996). Protocol details have been reported previously[15] and are therefore described in brief only.

Inclusion and exclusion criteria

People with a clinician-assigned diagnosis of T1D aged ≥5 years were recruited within 6 months of diagnosis. Written, informed consent was obtained for all participants.

Data collection

On recruitment: demographic information; medications including insulin(s); medical history, including that of autoimmune disease; family history of diabetes; blood pressure; weight and height; HbA1c; fasting or random blood glucose. A diagnosis of ketoacidosis was recorded if clinically assigned or if hyperglycaemia was accompanied by acidosis and either ketonaemia or ketonuria.[15] Ethnicity was self-reported as one of 16 categories.[15]

Project-specific blood sampling and measurement of islet autoantibodies

Sample donation was voluntary.[15] Where collected, autoantibodies to glutamate decarboxylase (GADA), islet antigen-2 (IA-2A), and zinc transporter 8 (ZnT8A) were measured in sera using established radiobinding assays[16 17] in a single central laboratory. Antibodies to both major ZnT8 isoforms, defined by the polymorphic amino

acid at position 325 (Arginine, ZnT8RA or Tryptophan, ZnT8WA), were measured separately. Thresholds for autoantibody positivity were set at the 97th percentile of 974 control samples for GADA, the 98th percentile of 500 control samples for IA-2A, and the 97·5th percentile of 523 healthy schoolchildren for both ZnT8RA and ZnT8WA. Positive autoantibody status was defined as positive for one or more of GADA, IA-2A or either form of ZnT8A. In the 2015 Islet Autoantibody Standardization Program Workshop, the assay sensitivities and specificities achieved were 74% and 96·7% for GADA; 72% and 100% for IA-2A; 60% and 100% for ZnT8RA, and 46% and 100% for ZnT8WA, respectively.

Data analysis

Children were defined as aged <17 years. Body mass index (BMI) was derived as a z-score for children using World Health Organisation (2007) reference data.[18] As a criterion for adiposity shared between children and adults, we applied a definition of 'normal' weight (z-score <1 for children, BMI <25kg/m^2 for adults, both including underweight) as distinguished from 'overweight' (z-score ≥1 for children,[19] BMI ≥25kg/m^2 for adults,[20] both including obese). Parental and sibling history of diabetes was recorded. No attempt was made to differentiate between diabetes types in the family history. Variables were categorized as 'Individual Characteristics' and 'Diabetes Presentation'. We analysed data from participants recruited between 1st September 2011 and 30th April 2016, with data querying and verification completed in November 2016.

Statistical analysis

Statistical analysis was carried out using StataCorp. 2013 (*Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP). Median and interquartile ranges

(IQRs) were used to summarize continuous variables. Categorical variables were summarized as percentages. The Mann-Whitney U test and Kruskal-Wallis test were used for between-group comparisons of continuous variables. Chi-square testing was used for comparisons of categorical variables. Individual characteristics were explored as predictors of diabetes presentation and antibody status in univariate logistic and linear regression analyses. Multiple logistic and linear regression were used to establish the independence of predictors. For regression analyses, non-normally distributed continuous variables were transformed to normalize their distributions. A significance level of $p<0{\cdot}05$ (two-sided) was taken as a guide to interpretation (actual p values down to $p<0{\cdot}0001$ are reported throughout).

Patient involvement

Patient and public involvement groups within the NIHR CRN representing people with diabetes, and representing children and young people, had input into the design of the patient information sheets, consent forms, and recruitment strategies. After the start of recruitment, a patient advocate group was established to have input into aspects of study design and conduct, such as the procedures for accessing the data and stored biological samples, and communication with and engagement of participants, people living with T1D, and healthcare professionals. The group is made up of adults with type 1 diabetes and the parents of children with type 1 diabetes. Results are disseminated to participants via newsletters and other information about the study is published on the study website and on social media.

RESULTS

Cohort characteristics

Data were analyzed for 3,312 participants recruited with incident T1D (1,879 (57%) males, 1,946 (59%) children, from 146 centres, Table 1). The slight male predominance was more prominent in adults than in children (61% versus 54%, p<0·0001, Table 2). Islet autoantibodies were measured in the 1,778 participants who donated an optional blood sample. For individual characteristics, data recording was >98% complete for all variables except BMI (and 'overweight' - 88%) and records of having a sibling with diabetes (91%). Data recording for diabetes presentation features was >98% complete for all variables except symptom duration (94%). Sample sizes for incomplete data are reported in Figure 1 and Tables 1-3. Of the total cohort, people of white European origin comprised 91% (n=3021), Asian (not Chinese) 3% (n=107), African-Caribbean 2% (n=63) and other or mixed ethnicity 3% (n=121). Median time from diagnosis to recruitment was 71 days (IQR 40-119) and to blood sampling, 75 days (IQR 42-126). Of those with body weight measured (n=2911), 35% were classified as overweight or obese, more commonly in adults than children (41% versus 31%, p<0·0001, Table 2).

Table 1. Clinical and demographic characteristics of the cohort (n=3312).

	median (IQR) / percentage (n)
INDIVIDUAL CHARACTERISTICS	
Age (years) (n=3312)	14·6 (10·4, 26·4)
Male (n=3312)	57 (1879)
Children (<17y) (n=3312)	59 (1946)
Body mass index (n=2911)	
Children (z score, n=1676)	0·44 (-0·28, 1·23)
Adult (kg/cm^2, n=1235)	24·1 (21·5, 27·1)
Overweight or obese (n=2911)	35 (1033)
White European ethnicity (n=3312)	91 (3021)
Other autoimmune disease present (n=3270)	6 (204)
Parent(s) with any diabetes (n=3261)	15 (499)
Sibling with any diabetes (n=3003)	8 (229)
DIABETES PRESENTATION	
Clinical presentation	
Ketoacidosis (n=3242)	42 (1348)
Osmotic symptoms (n=3286)	96 (3158)
Weight loss (n=3251)	85 (2753)
Fatigue (n=3252)	82 (2682)
Symptom duration (weeks, n=3105)	3 (2, 6)
Antibody positive (n=1778)	85 (1510)

Abbreviations: IQR: interquartile range; BMI, body mass index.
Sample sizes (n) are given for each variable if data collection was incomplete.
Data collection for ketoacidosis at diabetes presentation was based on a record of it being assigned clinically, or of hyperglycaemia accompanied by acidosis and either ketonaemia or ketonuria.

Table 2. Characteristics of children and adults; percentages (n) or medians (IQR) are shown.

	Children (n=1946)	Adults (n=1366)	p
INDIVIDUAL CHARACTERISTICS			
Age (years)	11·1 (8·5, 13·5)	29·6 (22·9, 39·8)	NA
Male	54 (1048)	61 (831)	<0·0001
Overweight (n=1676, 1235)	31 (527)	41 (506)	<0·0001
White European ethnicity	90 (1750)	93 (1271)	0·001
Other autoimmune disease (n=1915, 1355)	4 (82)	9 (122)	<0·0001
Parent(s) with any diabetes (n=1920, 1341)	12 (223)	21 (276)	<0·0001
Siblings with any diabetes (n=1769, 1234)	6 (100)	10 (129)	<0·0001
DIABETES PRESENTATION			
Clinical presentation			
Ketoacidosis (n=1912, 1330)	39 (744)	45 (604)	0·0002
Osmotic symptoms (n=1935, 1351)	97 (1877)	95 (1281)	0·001
Weight Loss (n=1907, 1344)	82 (1556)	89 (1197)	<0·0001
Fatigue (n=1904, 1348)	78 (1493)	88 (1189)	<0·0001
Symptom Duration (weeks, n=1844, 1261))	3 (2, 4)	4 (2·5, 8)	0·0001
Antibody positive (n=680, 1098)	90 (614)	82 (896)	<0·0001

Sample sizes (n) are given for each variable if data collection was incomplete.

Relationships between clinical presentation and individual characteristics

The main presenting features (Table 1) were: osmotic symptoms (polyuria and/or polydipsia) 96%; weight loss 85%; and fatigue 82%. Ketoacidosis was identified at clinical presentation in 42%. Another autoimmune disease was present in 6%; 15% had a parent with diabetes; 8% had a sibling with diabetes. One or more autoantibodies were present in 85% of those in whom they were measured.

Effect of age, and characteristics of children versus adults: Increasing age was independently associated with an increased prevalence of ketoacidosis, weight loss, and fatigue at presentation, and decreased prevalence of osmotic symptoms, longer symptom duration, and lower antibody positivity (Figure 1). Although significant statistically, the differences between children and adults in presenting symptoms were small, as were significant differences in symptom duration (Table 2). Ketoacidosis was less common in children than adults (Table 2, 39% versus 45%, p=0·0002). Children were more likely than adults to be positive for one or more islet autoantibodies (90% vs 82%, p<0·0001).

Sex: Female sex was independently associated with longer symptom duration and increased prevalence of autoantibodies (Figure 1). Median symptom duration in females and males were 4 and 3 weeks, respectively (p=0·0001); the prevalence of islet autoantibody positivity was 90% and 82%, respectively (p<0·0001).

Ethnicity: Ethnicity was a significant determinant of autoantibody status (on chi-squared analysis, p<0·0001). Amongst the 3 major non-white ethnic groups (Asian; African-Caribbean; other or mixed ethnicity) numbers with autoantibodies measured were small (n=46, 36 and 51, respectively) and the proportion with autoantibody positivity did not differ significantly (70%, 64% and 82%, respectively; p=0·1). People of non-white ethnic origin were therefore grouped and comparisons limited to white European versus non-white ethnic origin. White European ethnicity was independently

associated with a higher prevalence of autoantibody positivity compared with the combined non-white group (86% and 73%, respectively; p<0·0001, Table 3 and Figure 1). There were no other significant associations between ethnicity and initial clinical presentation and ketoacidosis was equally likely (white Europeans, 41%; non-white, 44%; p=0·3, Table 3).

Other autoimmune disease: Another autoimmune disease was present in 204 participants and 117 of those in whom autoantibodies were measured. A history of another autoimmune disease was positively associated with autoantibody positivity (p=0·01, Figure 1), being present in 8% of the autoantibody positive and 4% of the autoantibody negative participants (Table 4).

Family history of diabetes: Having a parent with any diabetes was associated with a lower probability of presenting with ketoacidosis; such people were also less likely to have autoantibodies (Figure 1). The proportion of those with ketoacidosis at presentation who had a parent with diabetes was 12% versus 18% of those without ketoacidosis (p<0·0001). The proportion of those who were autoantibody positive and who had a parent with any diabetes was 16% versus 28% of those who were autoantibody negative (p<0·0001, Table 4). Having a sibling with diabetes was also independently negatively associated with presentation with ketoacidosis (Figure 1). The proportion of those with ketoacidosis who had a sibling with any diabetes was 5% compared with 10% of those without ketoacidosis (p<0·0001).

Table 3. Characteristics of participants of white European ethnicity and non-white ethnicity; percentages (n) or medians (IQR) are shown.

	White European (n=3021)	Non-white ethnicity European (n=291)	p
INDIVIDUAL CHARACTERISTICS			
Age (years)	14.8 (10.5, 26.6)	12.7 (9.0, 23.7)	<0.001
Male	57 (1713)	57 (166)	0.9
Children	58 (1750)	67 (196)	0.002
Body mass index			
Children (z score, n=1507, 169)	0.42 (-0.28, 1.20)	0.48 (-0.32, 1.45)	0.5
Adult (kg/cm^2, 1151, 84)	24.0 (21.5, 27.1)	24.8 (22.6, 27.2)	0.1
Overweight (n=2658, 253)	35 (935)	39 (98)	0.2
Other autoimmune disease (n=1981, 289)	6 (184)	7 (20)	0.6
Parent(s) with any diabetes (n=2976, 285)	15 (444)	19 (55)	0.05
Siblings with any diabetes (n=2751, 252)	7 (204)	10 (25)	0.1
DIABETES PRESENTATION			
Clinical presentation			
Ketoacidosis (n=2960, 282)	41 (1224)	44 (124)	0.3
Osmotic symptoms (n=2997, 289)	96 (2879)	97 (279)	0.6
Weight loss (n=2964, 287)	84 (2503)	87 (250)	0.2
Fatigue (n=2970, 282)	82 (2449)	83 (233)	0.9
Symptom duration (weeks, n=2840, 265))	4 (2, 6)	3 (2, 6)	0.02
Antibody positive (n=1645, 133)	86 (1413)	73 (97)	<0.001

Sample sizes (n) are given for each variable if data collection was incomplete.

Table 4. Characteristics of pancreatic autoantibody (Ab) positive and negative participants (n=1778 with known antibody status); percentages (n) or medians (IQR) are shown.

	Ab positive (n=1510)	Ab negative (n=268)	p
INDIVIDUAL CHARACTERISTICS			
Age	20·1 (13·1, 31·1)	31·4 (17·7, 41·0)	0·0001
Male	56 (851)	72 (192)	<0·0001
Children	41 (614)	25 (66)	<0·0001
Body mass index			
Children (z score, n=545, 56))	0·41 (-0·35, 1·19)	0·47 (-0·48, 0·97)	0·4
Adult (kg/cm^2, n=825, 184)	23·9 (21·4, 26·7)	25·5 (23·1, 29·2)	0·0001
Overweight (n=1370, 240)	36 (490)	48 (114)	0·0005
White European ethnicity	86 (1413)	14 (232)	<0·0001
Other autoimmune disease (n=1495, 265)	8 (117)	4 (10)	0·01
Parent(s) with any diabetes (n=1493, 261)	16 (233)	28 (74)	<0·0001
Siblings with any diabetes (n=1374, 238)	9 (117)	8 (20)	0·9
DIABETES PRESENTATION			
Clinical presentation			
Ketoacidosis (n=1483, 260)	43 (639)	40 (104)	0·3
Osmotic symptoms (n=1495, 267)	97 (1444)	94 (250)	0·02
Weight loss (n=1480, 267)	87 (1285)	88 (235)	0·5
Fatigue (n=1490, 265)	86 (1282)	80 (213)	0·01
Symptom duration (weeks, n=1424, 246)†	6·8 (10·5)	10·4 (32·2)	0·004

† median and IQRs for symptom duration were identical: 4 (2, 8); mean and SD is shown to clarify the direction of difference
Sample sizes (n) are given for each variable if data collection was incomplete.

Relationships between autoantibodies, diabetes presentation and individual characteristics

Children comprised 38% of the sub-group of 1,778 participants who provided a blood sample and the sub-group with blood samples was, accordingly, significantly older than the full cohort (21·6 (13·4, 32·8) vs 14·6 (10·4, 26·4) years, p<0·0001). Other parameters were similar.

The relationship between positive autoantibody status and diabetes presentation was restricted to an increased prevalence of osmotic symptoms (97% versus 94%, p=0·02, Table 4). There was no significant difference in rates of ketoacidosis at presentation between autoantibody positive and negative participants (43% versus 40%, p=0·3, Table 4). The rate of autoantibody positivity was higher among children than adults, females versus males, and white Europeans versus people of non-white ethnicity (see above). Autoantibody positivity decreased progressively with age in adults (Figure 2). Of the individual autoantibodies, GADA were the most frequently observed in adults, with IA-2A and ZnT8A being relatively more common in children (Figure 2). The autoantibody positive adults were of lower BMI than autoantibody negative adults (BMI, median (IQR) 23·9 (21.4, 26·7) vs 25·5 (23·1, 29·2), p<0·0001), but no relationship to z-score was observed in children (Table 4). Autoantibody positivity was less prevalent amongst those who had a parent with diabetes.

DISCUSSION

For the first time, relationships between autoantibody status (measured centrally in a single reference laboratory) and phenotypic features in incident T1D are reported from a large, unselected, multi-ethnic population of both children and adults. The study was conducted with support from the NIHR CRN with most participants recruited from specialist centres. Based on estimates of T1D incidence and population demographics,[21 22] 20-25% of eligible incident cases in England and Wales were recruited.

Male predominance is unusual for an autoimmune condition but has been reported in adults with incident T1D.[23] In young children, the sex ratio has been reported to be close to unity.[24] In the present study the male excess was observed also in children, although the excess was less marked than in adults. Symptoms at presentation were as expected. Although weight loss was common, average body weight at the time of recruitment was normal, and many participants were overweight or obese, especially adults. An association between increased BMI and increased risk of progression from autoantibody positivity to development of diabetes in at-risk relatives has been reported previously.[25] Symptom duration was similar to that reported previously by others and was shorter in children than adults.[26 27] This may reflect parental vigilance of unwell children or a more insidious onset of clinical disease in older people.

The overall frequency of ketoacidosis at diagnosis (42%) was high, and slightly more so in adults than children. It occurred with similar frequency in white European and non-white ethnic groups. The figure of 42% is higher than in previous reports from the UK (23% in a recent national paediatric audit,[28] 26-27% in regional studies[29 30]) and a range of 13-80% in those aged <20 years has been reported internationally.[31] A very similar figure (40.3%) has been reported recently for children in Italy.[32] Ketoacidosis at

diagnosis is a quality issue as it reflects lack of awareness of diabetes features amongst professionals and the general population[28] and efforts to increase awareness lead to reductions in ketoacidosis at first presentation.[33] All methods of estimating the frequency of ketoacidosis at diagnosis have limitations, often leading to under-reporting.[28] Strengths of the current study include the large number of patients and the ability to confirm or refute the diagnosis where this was in doubt. A limitation is that ascertainment bias could be introduced because those who are the most ill at diagnosis may be the most likely to volunteer or to be referred. The higher ketoacidosis rate in adults versus children in our study appears at variance with the observation that ketoacidosis or severe ketoacidosis is more common in younger than in older children.[29 32 34-36] The current study did not include children <5 years of age, the group in childhood in whom ketoacidosis at diagnosis occurs most frequently[37], and this may have contributed to the apparent children to adult difference. Of course, if such younger children had been included, this could have been increased the overall rate of ketoacidosis even higher. The lower rate associated with having a parent or a sibling with diabetes could result from a heightened awareness of symptoms leading to earlier clinical referral.[31] The absence of any significant ethnic influence on ketoacidosis is at variance with some previous reports where higher rates were observed in non-white sub-groups.[38 39]

One or more islet autoantibodies were observed in 85% of participants, more commonly in female than male and in younger compared with older participants. This is compatible with previous literature from the UK and other countries,[40-42] although assay differences make such comparisons difficult. The positivity rate is higher than reported in people with T1D of non-white ethnic origin,[12 13] albeit with the same caveats and bearing in mind that the previous studies of ethnic influences on antibody

status have been limited in size or age range of population studied. The slight female autoantibody preponderance has been observed in other studies in young children, but not in older children and young adults.[43] The higher autoantibody frequency in those with a coexistent autoimmune disease reflects the clustering of autoimmune disorders observed in T1D and shared genetic susceptibility.[44] Overall, GADA were the antibodies most commonly present; while IA-2A and ZnT8A were seen most frequently in children, findings compatible with previous studies.[43] Insulin itself is considered a potential primary autoantigen as insulin autoantibodies are observed in incident T1D, especially in children[26 27 45]. In the current study, most participants had received insulin therapy for weeks before study entry and as they could have developed antibodies to the exogenous insulin, insulin autoantibodies were not measured. In prospective studies of infants at high genetic risk of T1D, insulin autoantibodies were often detected earlier than the other islet autoantibodies[46 47] and in consequence we may have underestimated the frequency of autoantibody positivity at diagnosis, especially in children.

Although autoantibodies can be present for years in people with diabetes who do not require insulin treatment immediately,[48 49] and are present in some diagnosed clinically with type 2 diabetes,[50] they are generally regarded as a biomarker for T1D. In prospective studies they precede and predict the onset of T1D.[51] They typically disappear, or titres drop to very low levels, in the years following diagnosis. In the autoantibody negative participants studied here, several explanations may be proposed. *First*, insulin autoantibodies were not measured. *Second*, autoantibodies to as yet unknown antigens may have been present.[52] The identification of tetraspanin-7 as an autoantigen could, for example, account for some apparently antibody negative participants,[53] although recent data suggest this is unlikely to account for large

numbers.[54] *Third*, autoantibodies may have disappeared or their levels diminished, by the time of sampling. As all participants were recruited within six months of diagnosis, this is unlikely to be a major factor. *Fourth*, autoantibodies might develop subsequently, as reported previously for islet cell antibodies in a small proportion of patients.[55] *Fifth*, some people may have autoimmune T1D without a humoral response. *Finally*, people may actually have another diabetes sub-type. The autoantibody negative participants as a group tended to be older and, if adult, more overweight. These features are compatible with type 2 diabetes. They were more likely to have a parent with diabetes, typical of type 2 or monogenic diabetes. They were more likely to be of non-white ethnicity, more associated with type 2 diabetes. Those with ketoacidosis could have ketosis-prone diabetes (so-called idiopathic diabetes)[56] as this is difficult to distinguish from T1D at first presentation. Further studies and follow-up of the cohort are planned to explore the extent to which T1D without detectable autoantibodies describes a sub-group of T1D that is distinct from other diabetes sub-types.

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DATA SHARING

The full anonymous dataset is available to access via a management committee, which includes people living with type 1 diabetes, scientists, clinicians and funder representatives as members.[15] Participants gave informed consent for data sharing subject to conditions described in the access procedure documents available from the study website: www.address2.org.

COMPETING INTERESTS

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: funding from Diabetes UK and the Juvenile Diabetes Research Foundation administered as grants to Imperial College London for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

CONTRIBUTORSHIP

Author contributions

Literature searches were carried out by authors VB, HCW, IFG, NSO and DGJ. Authors AK, HCW, IFG, AJKW, PJB, DBD and DGJ were involved in the study design. Data collection was coordinated by authors AK and HCW. Authors AK, HCW and IFG were responsible for data management. Authors AJKW and PJB were responsible for autoantibody measurements. The statistical analysis plan was developed by IFG. Analysis of the data was by authors VB, AK and IFG. Data were interpreted by all authors (VB, AK, HCW, IFG, SM, PJB, AJKW, DBD, CMD, MP, NSO and DGJ). The manuscript was prepared by authors VB, AK, HCW, IFG, AJKW and DGJ. Critical revisions were made by authors PJB, DBD, CMD, MP and NSO. Figures were prepared by authors AK, HCW and IFG.

The Management Committee oversaw access to the data and stored biological samples. The Patient Advocate Group had input into aspects of study conduct. Local investigators were responsible for the recruitment of participants and collection of data.

GUARANTORSHIP

Dr Walkey accepts full responsibility for the finished article and confirms that she had full access to all the data in the study and was responsible for the decision to submit for publication. Dr Walkey affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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Figure captions

Figure 1. Individual characteristics as predictors of diabetes presentation and autoantibody status.

Univariate logistic regression odds ratios or linear regression coefficients (circles), 95% CIs (horizontal lines)[#] and statistical significances are shown. Red circles signify that a significant univariate relationship was sustained on multivariable analysis with all individual characteristics included as predictor variables (participants with complete data: n=2911-3312) and in the sub-group with antibodies measured (participants with complete data including antibody status: n=1610-1778)

Footnotes: [#] for 'Age' 95% CIs did not extend beyond the odds ratio circles
* not significant in sub-group with antibodies measured on multivariable analysis
** significant in sub-group with antibodies measured on multivariable analysis
[†] not significant on multivariable analysis
[††] significant on multivariable analysis
[§] coefficient derived from square-root-transformed data

Figure 2. The percentage of participants exhibiting islet autoantibodies (any and individual) autoantibody in relation to age at diagnosis.

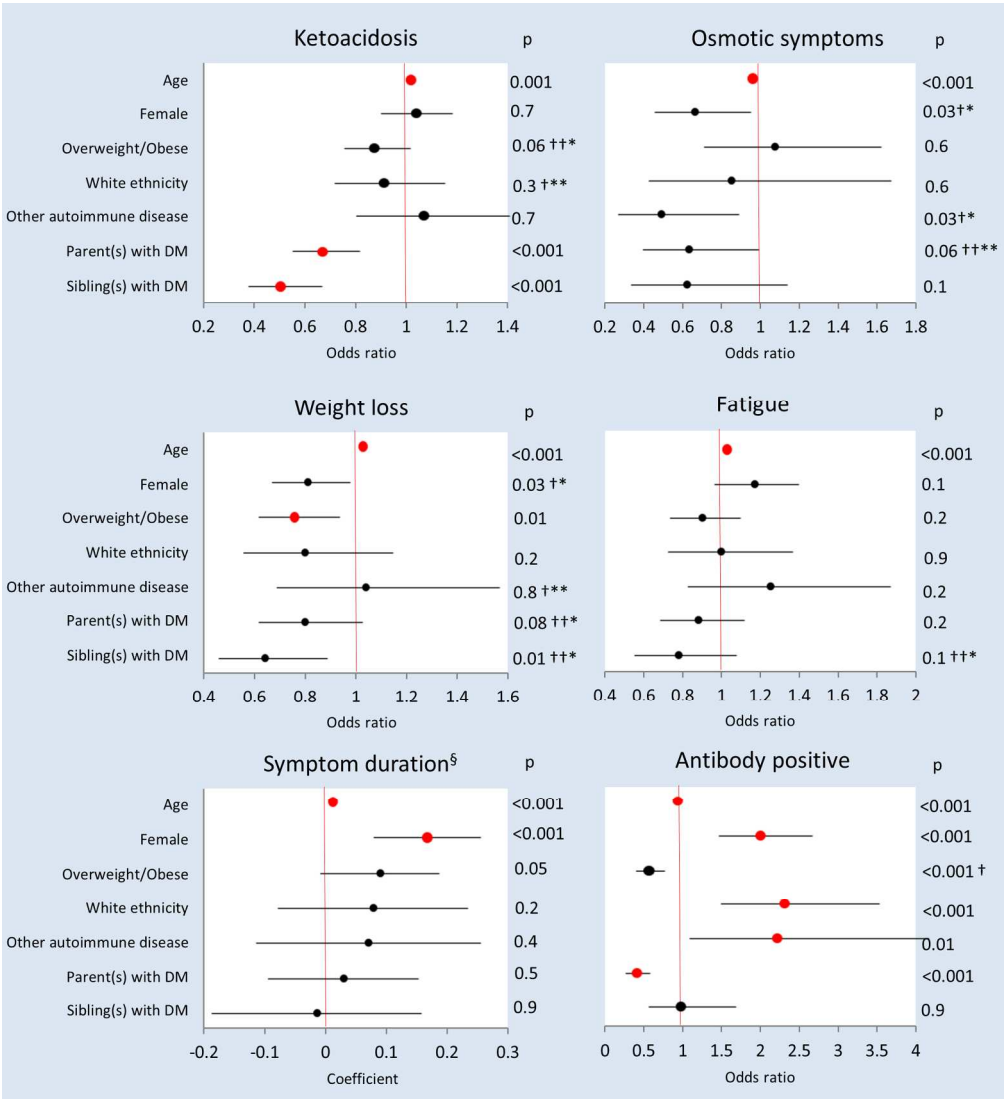

Figure 1. Individual characteristics as predictors of diabetes presentation and autoantibody status.

171x187mm (300 x 300 DPI)

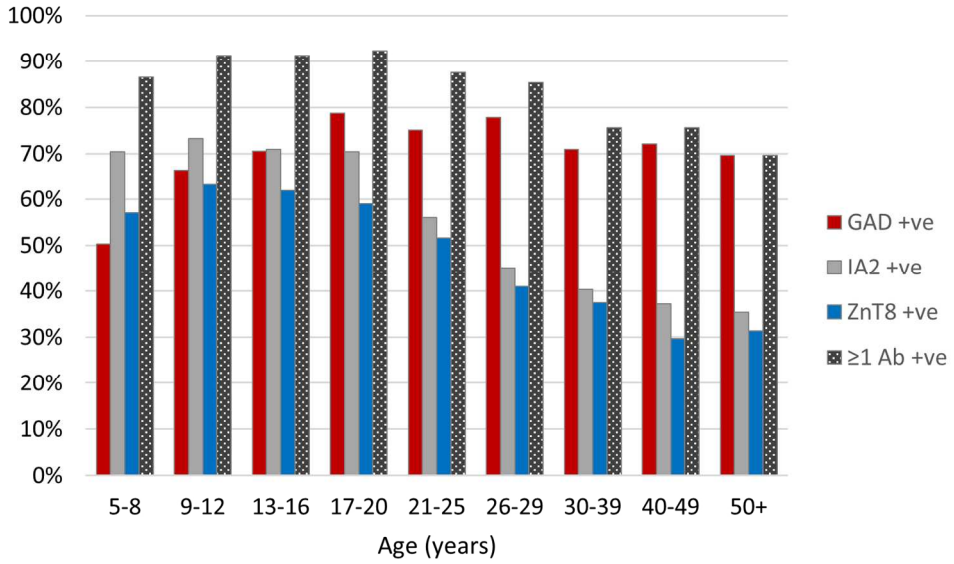

Figure 2. The percentage of participants exhibiting islet autoantibodies (any and individual) autoantibody in relation to age at diagnosis.

141x90mm (300 x 300 DPI)

Open Access Protocol

BMJ Open

Rationale and protocol for the After Diabetes Diagnosis REsearch Support System (ADDRESS): an incident and high risk type 1 diabetes UK cohort study

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ABSTRACT

Introduction Type 1 diabetes is heterogeneous in its presentation and progression. Variations in clinical presentation between children and adults, and with ethnic group warrant further study in the UK to improve understanding of this heterogeneity. Early interventions to limit beta cell damage in type 1 diabetes are undergoing evaluation, but recruitment is challenging. The protocol presented describes recruitment of people with clinician-assigned, new-onset type 1 diabetes to understand the variation in their manner of clinical presentation, to facilitate recruitment into intervention studies and to create an open-access resource of data and biological samples for future type 1 diabetes research.

Methods and analysis Using the National Institute for Health Research Clinical Research Network, patients >5 years of age diagnosed clinically with type 1 diabetes (and their siblings) are recruited within 6 months of diagnosis. Participants agree to have their clinical, laboratory and demographic data stored on a secure database, for their clinical progress to be monitored using information held by NHS Digital, and to be contacted about additional research, in particular immunotherapy and other interventions. An optional blood sample is taken for islet autoantibody measurement and storage of blood and DNA for future analyses. Data will be analysed statistically to describe the presentation of incident type 1 diabetes in a contemporary UK population.

Ethics and dissemination Ethical approval was obtained from the independent NHS Research Ethics Service. Results will be presented at national and international meetings and submitted for publication to peer-reviewed journals.

Strengths and limitations of this study

- Children and adults (including older adults) are characterised soon after disease onset.
- There is wide geographical spread of ascertainment.
- The study allows differences in presentation of type 1 diabetes to be assessed by ethnic group.
- There is no attempt to capture information on all incident cases.
- Assessment of C-peptide and genetic risk markers is desirable in the characterisation of new-onset type 1 diabetes, but beyond the scope of this protocol. However, the After Diabetes Diagnosis REsearch Support System resource will enable precisely such future studies.

INTRODUCTION

Type 1 diabetes (T1D) is the most common form of diabetes in childhood, but it is frequently diagnosed in adults.[1] In the UK it is most common in people of white European descent, but also affects other ethnic groups,[2] and it is heterogeneous in initial clinical presentation and in its progression.[3] Markers of autoimmunity, such as antibodies to islet antigens, most commonly glutamate decarboxylase (GADA), insulin itself, islet antigen 2 (IA-2A) and zinc transporter-T8 (ZnT-8A), are frequently, but not always detectable at onset in people with a clinical diagnosis of T1D.[4–6] Measurement of autoantibodies is advocated by many to aid diabetes classification,[3 7–9] but only recommended in the UK when knowledge of autoantibody status (positive/negative) would have implications for clinical management or access to treatment (eg, 'insulin pump' therapy).[10] Few T1D cohorts include both children and adults and, where studied, clinical characteristics and autoantibody frequencies have been found to vary between children and adults at onset.[11–15] Additionally, descriptions of adult-onset diabetes with autoantibodies have highlighted differences in T1D phenotype at onset between adults and children.[16 17] Reports of the variation of autoantibody status

and frequency with ethnicity are also scarce and have revealed a somewhat conflicting picture.[6 18–20] Differences in presenting features of T1D with autoantibody status warrant further investigation in children and adults in the multiethnic UK population to improve understanding of the heterogeneity of T1D. People with T1D have a lifelong dependency on treatment with exogenous insulin, resulting from an autoimmune destruction of pancreatic beta cells. Early intervention therapies are emerging that aim to limit this autoimmune destruction and preserve beta cell function.[21 22] Preservation of even modest levels of insulin secretion has been shown to reduce the risk of developing diabetic complications, improve glycaemic control and may also protect against severe hypoglycaemia.[23 24] Many investigational therapies target new-onset T1D — at a stage when there is still significant insulin secretion to preserve. Recruitment to trials in new-onset T1D is challenging, in part because individual centres see a relatively small number of incident cases per year. The aims of the After Diabetes Diagnosis REsearch Support System (ADDRESS) are therefore the following:

1. to characterise people with clinician-assigned, new-onset T1D, demographically, clinically and by islet autoantibody status, in a national, multiethnic cohort, and to perform hypothesis-generating analyses investigating the heterogeneity of clinical presentation
2. to use the cohort of children and adults with incident T1D and their siblings to support recruitment into T1D trials and other clinical research studies by providing them with information about studies for which they might be eligible (initially in new-onset T1D, and with time, studies in established T1D, or studies for first-degree relatives), thereby also increasing awareness of opportunities to participate in research among patients and their families
3. to establish an open-access resource of data and biological samples, including DNA, collected close to diagnosis for use in other T1D research, forming, in particular, a foundation for prospective studies from the time of diagnosis.

There are a number of ongoing initiatives in the USA and Europe to characterise people with incident T1D, some of which include the banking of biological samples and open access to samples or data, some that include the study of first-degree relatives, and some that also support the conduct of clinical trials. Exemplar initiatives are summarised in table 1, in comparison with ADDRESS. There are T1D registries in the UK primarily set up to drive improvements in clinical care, and the notable open-access Warren repository,[25] established to further understanding of T1D genetic susceptibility. There are, however, no national, multiethnic collections of data and biological samples from both children and adults with incident T1D in the UK. The Scottish Health Research register SHARE is an example of another national resource that supports recruitment to clinical research, although unlike ADDRESS it does not focus on new-onset T1D.[26] The features of these UK-specific T1D registries and resources are summarised in table 2.

In 2006 the National Institute for Health Research Clinical Research Network (NIHR CRN) was set up as the research delivery arm of the National Health Service (NHS) in England. The universal health coverage of the NHS, coupled with the NIHR CRN, makes the UK a particularly suitable environment for studies in new-onset T1D. This is further strengthened via linkage with the ADDRESS new-onset T1D ascertainment network, which is dedicated to identifying people with incident T1D shortly after diagnosis and offering them and their siblings entry into T1D clinical research studies. ADDRESS is a partner in the T1DUK Consortium, formed in 2015 to promote, support and develop immunotherapy research in the UK via a network of centres to conduct clinical trials, and a mechanistic core to conduct state-of-the-art assays to study the immunology of T1D and the mechanisms of action of immunotherapies.

METHODS AND ANALYSIS

Establishment of the cohort

The After Diabetes Diagnosis REsearch Support System (previously referred to as ADDRESS or ADDRESS-2, hereafter as ADDRESS) started in pilot form in 2008 to establish the 'proof of concept' that ascertainment and recruitment of people with incident T1D shortly after diagnosis were feasible in substantial numbers using the resources of the NIHR CRN. Patients with a clinician-assigned diagnosis of T1D were recruited if they agreed to have their data on a database and to be contacted about other research projects for which they might be eligible. Clinical and demographic information, including the participant's unique NHS identifier, was obtained for analysis, but no biological samples were collected. This pilot was established in 78 recruiting hospitals in England and Wales. NIHR CRN staff recruited 645 participants between 2008 and 2010. With cofunding from Diabetes UK and the Juvenile Diabetes Research Foundation, and with separate NHS Research Ethics Committee review, the project was expanded to its present and ongoing form in January 2011 in which 156 hospitals are participating in 124 NHS Trusts across England and Health Boards in Wales. Figure 1 illustrates the timeline of development of ADDRESS, and figure 2 shows the locations of centres participating currently. The NIHR CRN structure provides research support via 15 local branches in England and a related system exists to support diabetes research in Wales.

Inclusion and exclusion criteria

During the first 3 years of the current study (2011–2014), people with a clinician-assigned diagnosis of T1D aged 5–60 years were recruited within 6 months of diagnosis. From October 2014 the upper age limit was removed to allow newly diagnosed adults over the age of 60 to participate. People initially diagnosed with another diabetes

Table 1 Exemplar initiatives to characterise incident T1D, in comparison with ADDRESS

Title	Country	Geographical coverage	Diabetes subtypes	Relatives of proband	Recruitment age (years)	Multiethnic	Biological sample collection	Accessible data or biological samples	Consent to be approached about other research	Year initiated	Aims
SEARCH for Diabetes in Youth Study[32]	USA	Five regional centres	All	No	<20	Yes	Yes	Unknown	Unknown	2000	To determine temporal changes in incidence and prevalence of diabetes in young people by subtype, age, gender and ethnicity, and to aid classification of subtype
T1D Exchange registry[33]	USA	77 centres in 35 states	T1D	No	Children and adults of any age	Yes	Yes	Yes	Yes (75% of cohort)	2010	To identify issues of clinical relevance, generate hypotheses and characterise patients for future studies
Belgian Diabetes Registry[34]	Belgium	Belgium-wide with national network of centres to conduct clinical trials	T1D	First-degree relatives	<40	Unknown	Yes	Unknown	Yes	1989	To detect trends in incidence over time, to identify markers of T1D onset and progression, and to conduct trials
BOX[35]	UK	Six centres in the former Oxford Regional Health Authority area	T1D	First-degree relatives	≤21	Unknown (there is little ethnic diversity in the region)	Yes	No	Yes	1985	To study the natural history of T1D, including initiation and progression of islet autoimmunity to identify those at risk of developing T1D, and to determine temporal changes in incidence
ADDRESS	UK	156 centres across England and Wales	T1D	Siblings	≥5	Yes	Yes	Yes	Yes	2007	To study the heterogeneity of clinical presentation, to support recruitment into clinical trials and other studies, and to facilitate genetic and biomarker research

Table 2 UK-specific type 1 diabetes (T1D) registries and open-access resources

Title	Type of collection	Diabetes subtypes	Relatives of proband	Recruitment age (years)	Incident/prevalent	Multiethnic	Biological sample collection	Accessible data or biological samples	Consent to be approached about other research
Type 1 diabetes Warren repository[25]	DNA and cell lines (collected during 1990–1995)	T1D	Multiplex families (two affected children): at least one diagnosed <17 years of age and other(s) diagnosed <29 years, with two living parents	Children and adults	Prevalent	No: white European only	Yes (DNA)	Yes	Unknown
National Children and Young People's Diabetes Network, www.cypdiabetesnetwork.nhs.uk	National register in Wales[36] and regional registries in England[37]	All	No	<19	Incident and prevalent	Yes	No	No	No
The Scottish Care Information – Diabetes Collaboration, www.sci-diabetes.scot.nhs.uk	Register and shared electronic patient record	All	No	Children and adults	Incident and prevalent	Yes	No	No	Unknown
The Scottish Health Research register SHARE[26]	Register of people living in Scotland who have declared their interest in taking part in health research	All (supports recruitment to research across all clinical specialties, not diabetes-specific)	Unknown	>16	Unknown	Yes	Storage of remainder of routine clinical samples	Unknown	Yes

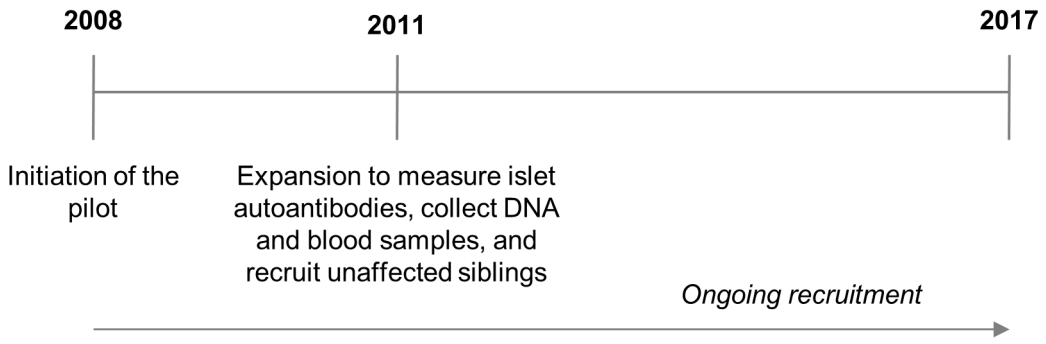

Figure 1 Study timeline.

subtype and later reassigned a diagnosis of T1D are eligible if still within 6 months of the initial diagnosis. Adults who are not competent to give consent are excluded. People who took part in the pilot form of ADDRESS are eligible to be recruited to the current study, and their individual data are transferred from the pilot into the current study.

Sample size

The annual incidence of T1D in the UK is 22–30 per 100 000 in the 0–14 years age group, with an estimated 76% of incident cases aged 5–14 years[27]. There is a paucity of incidence data for adults worldwide, but one UK study reported an incidence of 12 per 100 000 for those aged 15–34 years.[28] Therefore, we estimate that 3900–4500 children and adults will be diagnosed per year in the current recruitment age range in England and Wales.

Recruitment

Methods vary depending on local resources and preferences, but local researchers (NIHR CRN research nurses and/or other local research nurses and physicians) perform the recruitment mainly via secondary care and specialist diabetes centres, as patients (both children and adults) are mostly seen clinically in these settings. Recruiting staff in the NIHR CRN have been trained to approach patients and families with sensitivity soon after diagnosis and to take informed consent. Permission is sought from the patient or parent/guardian to approach siblings. The patients, their siblings and the parent/guardian for children aged 5–15 years are provided with age-specific information sheets about the study by the local researcher. Informed consent is sought at least 1 week after diagnosis and at least 48 hours after the information sheets have been given. Recruitment support initiatives have included providing NHS clinical care teams with information about T1D research, displaying posters about ADDRESS and providing patients with leaflets introducing clinical research and ADDRESS in clinic and in packs for newly diagnosed patients. A website provides additional information.

Consent

ADDRESS operates a two-tier consent model: for tier 1, participants provide written informed consent for their health and contact details to be held on a database and for their being contacted about studies. They also consent to follow-up using information held by NHS Digital and the Office for National Statistics (ONS). With additional second-tier consent, participants may choose to donate blood samples for islet autoantibody analysis and storage of blood and DNA for future use in diabetes research.

Data collection

At the time of recruitment participant information is collected via interview with the participant or parent/guardian, and from specialist care medical records, as shown in table 3. Ethnicity is self-reported as one of 16 categories, following UK ONS guidance on collecting ethnic group data. A diagnosis of parental or grandparental diabetes is recorded without subtype because historical classifications may be incorrect or incomplete.[29] Laboratory test results are recorded from those tests performed locally as part of routine clinical care. A diagnosis of ketoacidosis at presentation is recorded if assigned by the local clinical team, or if all of the following were documented in the clinical records: glucose >11 mmol/L; blood ketone bodies >3 mmol/L or urine ketones levels positive ++; pH <7.3; and/or bicarbonate <15 mmol/L. Except for the additional specialist care information available for patients, similar data are collected at baseline for siblings.

Project-specific blood sampling

Sample donation is a voluntary component of ADDRESS, currently for participants with T1D only (prior to October 2014, siblings were also given the option of donating samples). Random blood samples are collected and sent at ambient temperature via standard post to the Public Health England European Collection of Cell Cultures at Porton Down, UK, which operates in accordance with the UK Human Tissue Act 2004. Serum (from a serum separation tube) and peripheral blood lymphocytes (PBLs, from an acid citrate dextrose tube) are separated on arrival. An EDTA tube is frozen for later DNA extraction (median: 2 (IQR: 1–3) days after sampling). The samples are stored in coded, pseudoanonymised format and include serum from coagulated blood, extracted and

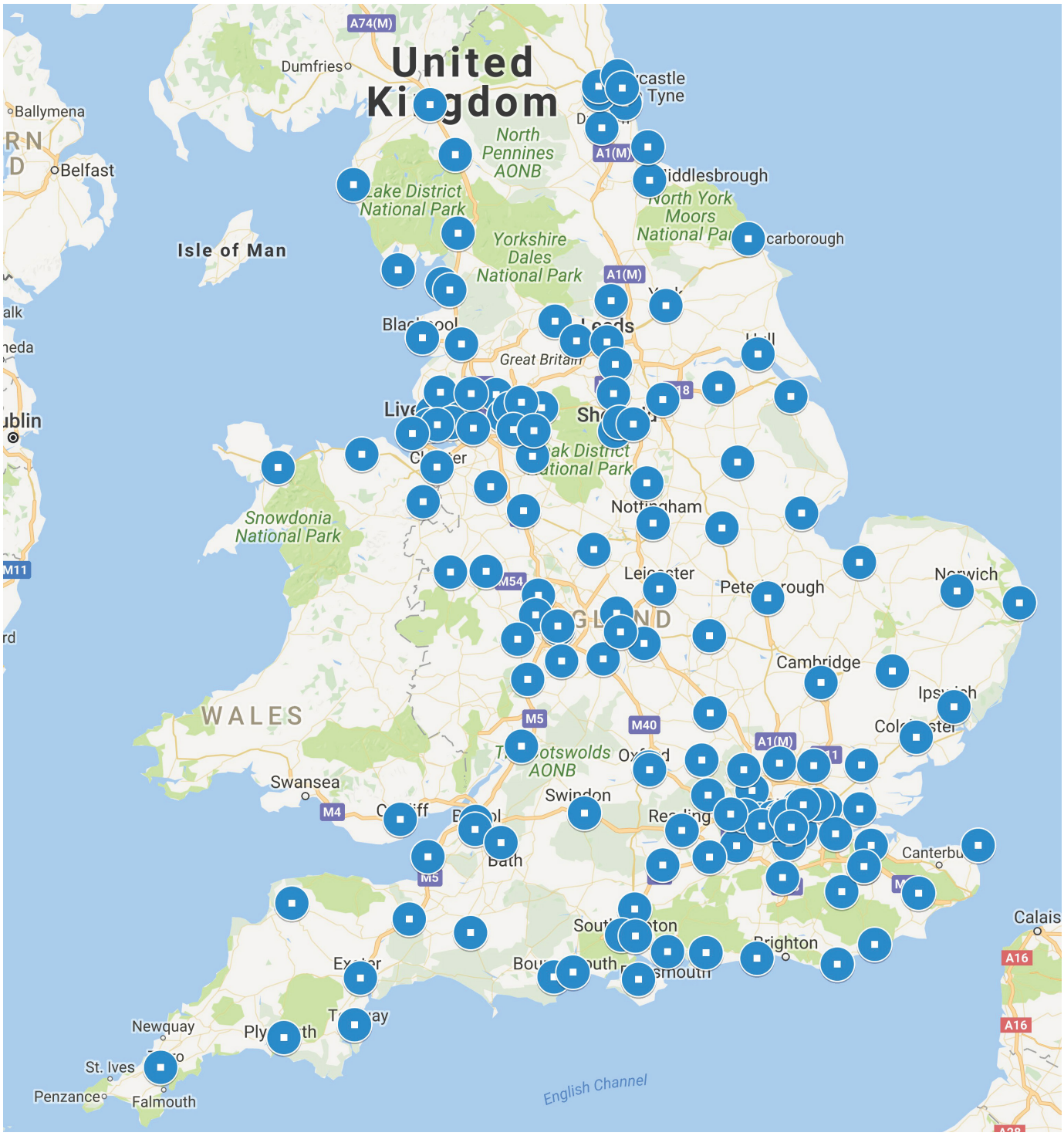

Figure 2 Map with circles showing the locations of the 156 recruiting centres in 124 English NHS Trusts and Welsh Health Boards (an estimated 84% of the total number that provide acute care services). Numbers of centres recruiting from both paediatric and adult clinics, the paediatric clinic only, and the adult clinic only are 80, 32 and 44, respectively.

resuspended DNA, PBLs, and lymphoblastoid cell lines (LCLs) derived by Epstein-Barr virus transformation of thawed PBLs. Samples are stored at −80°C and PBLs and LCLs are placed in liquid nitrogen for cryopreservation for the duration of the study. An aliquot (0.5 mL) of serum from each blood sample is sent to the University of Bristol for autoantibody measurements.

Islet autoantibody measurement

Autoantibodies to GADA, IA-2A and ZnT-8A are measured in participants with T1D using established radiobinding assays.[30 31] Insulin autoantibodies are not measured as these people are receiving exogenous insulin therapy, which could itself induce an immune response.

Table 3 Data and associated methods of collection for participants with incident T1D and for sibling participants at the time of recruitment

Data	Incident T1D		Sibling
	Via interview or at visit	Via or validated against medical records	Via interview or at visit
Contact details and unique NHS identifier	X		X
General practitioner (GP) details (GP is informed of the person's participation)	X		X
Diabetes care physician details (physician is informed of the person's participation if not the principal investigator at the research site)	X	X	
Demographic information including self-reported ethnic origin	X		X
Date of diagnosis, clinical presentation and duration of symptoms (presentation with diabetic ketoacidosis, polyuria/polydipsia, weight loss, fatigue, abdominal pain,* fasting or random plasma glucose)	X	X	
Current diabetes treatment regimen, including date insulin first administered	X	X	
Non-diabetic medication	X	X	X
Medical history including history of autoimmune diseases (Addison's disease, coeliac disease, hyperthyroidism, hypothyroidism and vitiligo) and of gestational diabetes	X	X	X
Family medical history including parental and grandparental history of diabetes, hypertension, myocardial infarction and stroke, along with sibling demographics and diabetes history	X		X
Clinical measures, including blood pressure, weight and height using standard protocols	X		X
Blood biochemistry including glycated haemoglobin (HbA1c) (mmol/mol), fasting or random plasma glucose (mmol/L), oral glucose tolerance test results (mmol/L) if performed locally and C-peptide levels (nmol/L) if performed locally		X	
Details of diabetes structured education offered/scheduled/completed*	X	X	

*Collected for participants recruited from February 2015 onwards.
T1D, type 1 diabetes; NHS, National Health Service.

Antigens radiolabelled with ^{35}S methionine are expressed using a TNT in vitro reticulocyte lysate quick coupled transcription/translation system kit (Promega, Madison, Wisconsin, USA) with plasmids encoding full-length GAD65, the intracytoplasmic region of IA-2 (aa 606–979), and the 325-arginine (ZnT8R) and 325-tryptophan (ZnT8W) isoforms of the C-terminal region of ZnT8 (aa268-379). Immunocomplexes formed following incubation of sera with radiolabelled antigens are precipitated with Protein A-Sepharose (GE Healthcare Life Sciences, Little Chalfont, Bucks, UK), washed and counted in a TopCount beta counter (PerkinElmer, Waltham, Massachusetts, USA).

Results are expressed in digestive and kidney (DK) units/mL (GADA and IA-2A) or arbitrary units (ZnT8A) after reference to standard curves consisting of dilutions of patient sera in antibody-negative sera from healthy donors. Thresholds are set at the 97th percentile of 974 control samples for GADA, the 98th percentile of 500 control samples for IA-2A and the 97.5th percentile of 523 healthy schoolchildren for ZnT8A. The GADA assay achieves a sensitivity of 74% at 96.7% specificity, while the IA-2A, ZnT8RA and ZnT8WA assays achieve sensitivities of 72%, 60% and 46%, respectively, at 100% specificity, in the 2015 Islet Autoantibody Standardization Program Workshop. Interassay coefficients of variation of high and moderate/low positive samples, respectively, were 18% and 18% for GADA, 19% and 17% for IA-2A, 22% and 16% for ZnT8RA, and 25% and 20% for ZnT8WA.

Follow-up data collection and verification

Participants with T1D are reviewed via their medical records at 4–8 months following diagnosis to verify that their original clinical diabetes classification has not been changed by their clinicians. Data reviewed at the follow-up are shown in table 4.

An application to NHS Digital and the UK ONS is planned for tracing change of postal address, mortality flagging and access to hospital admission data (Hospital Episode Statistics).

Database

Data are entered into a secure electronic data capture and management system designed specifically for clinical

Table 4 Data collection for participants with incident T1D at 4–8 months postdiagnosis and at least 2 months after the time of recruitment

Data	Incident T1D
	Via medical records
Confirmation of, or change in classification of, diabetes subtype	X
Occurrence of diabetic ketoacidosis since diagnosis*	X
Any change in diabetes treatment or dose, and concurrent medication	X
Clinical measures, including blood pressure, weight and height	X
Blood biochemistry including glycated haemoglobin (HbA1c) (mmol/mol), fasting or random plasma glucose (mmol/L), oral glucose tolerance test results (mmol/L) if performed locally and C-peptide levels (nmol/L) if performed locally	X
Details of diabetes structured education offered/scheduled/completed*	X

All data are collected from medical records.
*Collected for participants recruited from February 2015 onwards.
T1D, type 1 diabetes.

research. All information is stored in accordance with the UK Data Protection Act (1998).

Recruitment into other studies

Eligible participants are given information at intervals about specific diabetes trials/research studies by the central study team or local researchers. Information is retained on those previously contacted about research and whether or not they are currently participating in a study.

Data analysis plan

The collected data allow generation and testing of hypotheses about the presentation of clinician-assigned T1D and other characteristics early after diagnosis. Our aim is to compare characteristics between groups: for example, islet autoantibody positive and negative; ethnicity groupings of white European, South Asian (including Indian, Pakistani and Bangladeshi) and African-Caribbean (black African or black Caribbean); and children and adults. We aim also to investigate the relationships between insulin dose and glycaemic control. Recorded variables are categorised as 'Individual Characteristics' (eg, age, gender, child/adult, birth weight, ethnicity, history of other autoimmune disease, parent with diabetes, sibling with diabetes), 'Diabetes Presentation' (eg, ketoacidosis at T1D presentation, osmotic symptoms (polydipsia/polyuria), weight loss, fatigue, symptom duration, initial insulin dose and autoantibody status) and 'Diabetes Characteristics' (eg, insulin dose, glycated haemoglobin (HbA1c) at time points after presentation).

ETHICS AND DISSEMINATION

Ethical approval was obtained from the NHS Research Ethics Committee - South Central-Berkshire (reference 10/H0505/85), and each participating NHS Trust submitted a Site-Specific Assessment in order to participate. The project is conducted in accordance with the recommendations for physicians involved in research on human subjects by the 18th World Medical Assembly, Helsinki 1964 and later revisions. The International Conference on Harmonisation Guideline for Good Clinical Practice (Topic E6 — 10 June 1996) and participant confidentiality are maintained throughout.

ADDRESS is an accessible resource for clinical and academic researchers, both as an infrastructure to identify and contact candidates for recruitment into T1D studies and as an open-access database and biological sample repository. Access is through a management committee comprised of lay people, scientists and clinical investigators representing relevant T1D research interests. Researchers wishing to use the ADDRESS-2 resource can apply to the ADDRESS-2 Management Committee. The access process and associated documents are described on the study website (www.address2.org).

Ethical approval will be sought for continuing use of the data every 5 years (current approval is to the end of 2019). Every effort will be made to sustain this open-access resource beyond the present funding term. The database will be maintained by Imperial College London. The biological samples will continue to be stored for as long as there is funding to support the repository.

The results arising from this project will be presented at national and international meetings, and submitted for publication to peer-reviewed medical journals.

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Contributors The vision for an ascertainment network was that of DGJ in consultation with funders. Recruitment, data collection and analysis were designed and refined by DGJ, HCW, NO, VB, AK, SM and DBD. Islet autoantibody analysis was designed by PJB and AJKW. The statistical analysis plan was written by IFG. Procedures for open access were drafted by HCW and ratified by the ADDRESS-2 Management Committee.

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Competing interests None declared.

Ethics approval NHS Research Ethics Committee (South Central-Berkshire).

Provenance and peer review Not commissioned; externally peer reviewed.

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BMJ Open

Rationale and protocol for the After Diabetes Diagnosis REsearch Support System (ADDRESS): an incident and high risk type 1 diabetes UK cohort study

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STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	4-5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	7
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	8-9 & protocol paper (BMJ Open 2017;7:e013956)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8-6, 11 & protocol paper (BMJ Open 2017;7:e013956)
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	8 & protocol paper (BMJ Open 2017;7:e013956)
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8-9 & protocol paper (BMJ Open 2017;7:e013956)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8-9 & protocol paper (BMJ Open 2017;7:e013956)
Bias	9	Describe any efforts to address potential sources of bias	20
Study size	10	Explain how the study size was arrived at	8, 11, 19 & protocol paper (BMJ Open 2017;7:e013956) (ongoing cohort)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8-9
		(*b*) Describe any methods used to examine subgroups and interactions	8-9, 14 (ethnic groups) 18 (autoantibody subgroup)
		(*c*) Explain how missing data were addressed	11, Tables 1-4 (12-13, 16-18) & Figure 1
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	N/A

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8, 19 & protocol paper (BMJ Open 2017;7:e013956)
		(b) Give reasons for non-participation at each stage	8 & protocol paper (BMJ Open 2017;7:e013956)
		(c) Consider use of a flow diagram	Not complex: diagnosed and recruited or not, blood sample given or not.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11 & Table 1 (12)
		(b) Indicate number of participants with missing data for each variable of interest	11, Tables 1-4 (12-13, 16-18) & Figure 1
		(c) Summarise follow-up time (eg, average and total amount)	N/A data from recruitment only
Outcome data	15*	Report numbers of outcome events or summary measures over time	N/A
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11-18, Tables 1-4 (12-13, 16-18) & Figure 1
		(*b*) Report category boundaries when continuous variables were categorized	9 & Figure 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	18 (autoantibody subgroup)
Discussion			
Key results	18	Summarise key results with reference to study objectives	19-22
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	19-22
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	19-22
Generalisability	21	Discuss the generalisability (external validity) of the study results	6, 22
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	23

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

BMJ Open

The relationship between islet autoantibody status and the clinical characteristics of children and adults with incident type 1 diabetes in a UK cohort

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Secondary Subject Heading:	Epidemiology
Keywords:	General diabetes < DIABETES & ENDOCRINOLOGY, EPIDEMIOLOGY, IMMUNOLOGY, Paediatric endocrinology < PAEDIATRICS, DIABETES & ENDOCRINOLOGY

TITLE: The relationship between islet autoantibody status and the clinical characteristics of children and adults with incident type 1 diabetes in a UK cohort

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ABSTRACT

Objectives: To describe the characteristics of children and adults with incident type 1 diabetes in contemporary, multi-ethnic UK, focusing on differences between the islet autoantibody negative and positive.

Design: Observational cohort study.

Setting: 146 mainly secondary care centres across England and Wales.

Participants: 3,312 people aged ≥5 years were recruited within 6 months of a clinical diagnosis of type 1 diabetes via the National Institute for Health Research Clinical Research Network. 3,021 were of white European ethnicity and 291 (9%) were non-white. There was a small male predominance (57%). Young people <17 years comprised 59%.

Main outcome measures: Autoantibody status and characteristics at presentation.

Results: The majority presented with classical osmotic symptoms, weight loss, and fatigue. Ketoacidosis was common (42%), especially in adults, and irrespective of ethnicity. 35% were overweight or obese. Of the 1,778 participants who donated a blood sample, 85% were positive for one or more autoantibodies against glutamate decarboxylase, islet antigen-2, and zinc transporter 8. Presenting symptoms were similar in the autoantibody positive and negative participants, as was the frequency of ketoacidosis (43% vs 40%, p=0·3). Autoantibody positivity was less common with increasing age (p=0·0001), in males compared with females (82% vs 90%, p<0·0001) and in people of non-white compared with white ethnicity (73% vs 86%, p<0·0001). Body mass index was higher in autoantibody negative than positive adults (median, IQR 25·5, 23·1-29·2 vs 23·9, 21·4-26·7 kg/m^2; p=0·0001). Autoantibody negative

participants were more likely to have a parent with diabetes (28% vs 16%, p<0·0001) and less likely to have another autoimmune disease (4% vs 8%, p= 0.01).

Conclusions: Most people assigned a diagnosis of type 1 diabetes presented with classical clinical features and islet autoantibodies. Although indistinguishable at an individual level, autoantibody negative participants as a group demonstrated features more typically associated with other diabetes subtypes.

STRENGTHS AND LIMITATIONS OF THE STUDY

- We have studied a large multi-ethnic cohort of adults and children ≥5 years with clinically diagnosed incident type 1 diabetes in whom pancreatic islet autoantibodies were measured in a central laboratory.
- In routine practice, the initial assignment of a diagnosis of type 1 diabetes is a purely clinical one. The lack of further selection before inclusion in this study (e.g. based on autoantibody status and/or genetic testing) renders the results of particular relevance to standard clinical care.
- Individual autoantibody positive and negative patients were indistinguishable clinically but the size and diversity of the cohort permitted group differences to be detected at high levels of statistical significance, suggesting diagnostic heterogeneity.
- As this was a volunteer study recruiting from mainly secondary care centres, ascertainment bias could have been introduced.
- Provision of a blood sample was optional and autoantibody status was therefore available in just over half of the patients. Other than having a higher median age, this sub-group was representative of the whole cohort.

INTRODUCTION

Type 1 diabetes (T1D) is an autoimmune disease that develops at any age, but most frequently in children and young adults.[1] Autoantibodies against islet antigens are typically present before, and for a variable time following, diagnosis.[2-6] Once initiated, beta cell damage classically leads to progressive loss of insulin secretion and a need for lifelong insulin treatment.

The diagnosis of T1D is a clinical one, but may be supported by the presence of one or more of the autoantibodies to islet-cell antigens. In routine care, autoantibody status may not be available at diagnosis, and may never be checked (management guidelines differ, with some not recommending their routine measurement or restricting measurements to situations where there is clinical doubt).[7-9] Previous studies suggest that 80-90% have detectable autoantibodies at disease onset,[5 10] with a background autoantibody prevalence of around 2% in the young general population.[11]

Autoantibody positivity may be lower in some non-white ethnic groups.[12-16] There is however uncertainty around the clinical and demographic correlates of autoantibody status in incident disease in an unselected multi-ethnic cohort including children and adults, using well characterised, validated assays. The After Diabetes Diagnosis REsearch Support System (ADDRESS), supported by the National Institute for Health Research (NIHR) Clinical Research Network (CRN), recruits people with incident T1D from centres across England and Wales. We aimed to characterise these people with reference to their heterogeneity, focusing on the associations of autoantibody status with variation in presentation characteristics.

METHODS

Ethics approval

Ethical approval was obtained from the South Central – Berkshire NHS Research Ethics Committee (reference 10/H0505/85). The project complies with the recommendations for research on human subjects by the 18th World Medical Assembly, Helsinki 1964 and later revisions and the International Conference on Harmonization Guideline for Good Clinical Practice (Topic E6 - 10 June 1996). Protocol details have been reported previously[17] and are therefore described in brief only.

Inclusion and exclusion criteria

People with a clinician-assigned diagnosis of T1D aged ≥5 years were recruited within 6 months of diagnosis. Written, informed consent was obtained for all participants.

Data collection

On recruitment: demographic information; medications including insulin(s); medical history, including that of autoimmune disease; family history of diabetes; blood pressure; weight and height; HbA1c; fasting or random blood glucose. A diagnosis of ketoacidosis was recorded if clinically assigned or if hyperglycaemia was accompanied by acidosis and either ketonaemia or ketonuria.[17] Ethnicity was self-reported as one of 16 categories.[17]

Project-specific blood sampling and measurement of islet autoantibodies

Sample donation was voluntary.[17] Where collected, autoantibodies to glutamate decarboxylase (GADA), islet antigen-2 (IA-2A), and zinc transporter 8 (ZnT8A) were measured in sera using established radiobinding assays[18 19] in a single central laboratory. Antibodies to both major ZnT8 isoforms, defined by the polymorphic amino

acid at position 325 (Arginine, ZnT8RA or Tryptophan, ZnT8WA), were measured separately. Thresholds for autoantibody positivity were set at the 97th percentile of 974 control samples for GADA, the 98th percentile of 500 control samples for IA-2A, and the 97·5th percentile of 523 healthy schoolchildren for both ZnT8RA and ZnT8WA. Positive autoantibody status was defined as positive for one or more of GADA, IA-2A or either form of ZnT8A. In the 2015 Islet Autoantibody Standardization Program Workshop, the assay sensitivities and specificities achieved were 74% and 96·7% for GADA; 72% and 100% for IA-2A; 60% and 100% for ZnT8RA, and 46% and 100% for ZnT8WA, respectively.

Data analysis

Children were defined as aged <17 years. Body mass index (BMI) was derived as a z-score for children using World Health Organisation (WHO) (2007) reference data.[20] As a criterion for adiposity shared between children and adults, we applied WHO-recommended definitions of 'normal' weight (z-score <1, equivalent percentile <84.13% for children; BMI <25kg/m^2 for adults, both including underweight) as distinguished from 'overweight' (z-score ≥1, percentile ≥84.13% for children,[21] BMI ≥25kg/m^2 for adults,[22] both including obese). Parental and sibling history of diabetes was recorded. No attempt was made to differentiate between diabetes types in the family history. Variables were categorized as 'Individual Characteristics' and 'Diabetes Presentation'. We analysed data from participants recruited between 1st September 2011 and 30th April 2016, with data querying and verification completed in November 2016.

Statistical analysis

Statistical analysis was carried out using StataCorp. 2013 (*Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP). Median and interquartile ranges (IQRs) were used to summarize continuous variables. Categorical variables were summarized as percentages. The Mann-Whitney U test and Kruskal-Wallis test were used for between-group comparisons of continuous variables. Chi-square testing was used for comparisons of categorical variables. Individual characteristics were explored as predictors of diabetes presentation and antibody status in univariate logistic and linear regression analyses. Multiple logistic and linear regression were used to establish the independence of predictors. A significance level of $p<0{\cdot}05$ (two-sided) was taken as a guide to interpretation (actual p values down to $p<0{\cdot}0001$ are reported throughout).

Patient involvement

Patient and public involvement groups within the NIHR CRN representing people with diabetes, and representing children and young people, had input into the design of the patient information sheets, consent forms, and recruitment strategies. After the start of recruitment, a patient advocate group was established to have input into aspects of study design and conduct, such as the procedures for accessing the data and stored biological samples, and communication with and engagement of participants, people with T1D, and healthcare professionals. The group is made up of adults with type 1 diabetes and the parents of children with type 1 diabetes. Results are disseminated to participants via newsletters and other information about the study is published on the study website and on social media.

RESULTS

Overview of the cohort

Individual characteristics

Data were analyzed for 3,312 participants recruited with incident T1D (1,879 (57%) males, 1,946 (59%) children, from 146 centres, Table 1). The slight male predominance (57%) was more prominent in adults than in children (61% versus 54%, p<0·0001). Islet autoantibodies were measured in the 1,778 participants who donated an optional blood sample. For individual characteristics, data recording was >98% complete for all variables except BMI (and 'overweight' - 88%) and records of having a sibling with diabetes (91%). Data recording for diabetes presentation features was >98% complete for all variables except symptom duration (94%). Sample sizes for incomplete data are reported in the Tables. Of the total cohort, people of white European origin comprised 91% (n=3021), Asian (not Chinese) 3% (n=107), African-Caribbean 2% (n=63) and other or mixed ethnicity 3% (n=121). Median time from diagnosis to recruitment was 71 days (IQR 40-119) and to blood sampling, 75 days (IQR 42-126). Of those with body weight measured (n=2911), 35% were classified as overweight or obese, more commonly in adults than children (41% versus 31%, p<0·0001). Where records of body weight were available within 28 days of diagnosis (n=554), 35% were also overweight or obese (adults 40% versus children 29%, p<0.005).

Table 1. Clinical and demographic characteristics of the cohort (n=3312).

	median (IQR) / percentage (n)
INDIVIDUAL CHARACTERISTICS	
Age (years) (n=3312)	14·6 (10·4, 26·4)
Male (n=3312)	57 (1879)
Children (<17y) (n=3312)	59 (1946)
Body mass index (n=2911)	
Children (z score, n=1676)	0·44 (-0·28, 1·23)
Adult (kg/m^2, n=1235)	24·1 (21·5, 27·1)
Overweight or obese (n=2911)	35 (1033)
White European ethnicity (n=3312)	91 (3021)
Other autoimmune disease present (n=3270)	6 (204)
Parent(s) with any diabetes (n=3261)	15 (499)
Sibling with any diabetes (n=3003)	8 (229)
DIABETES PRESENTATION	
Clinical presentation	
Ketoacidosis (n=3242)	42 (1348)
Osmotic symptoms (n=3286)	96 (3158)
Weight loss (n=3251)	85 (2753)
Fatigue (n=3252)	82 (2682)
Symptom duration (weeks, n=3105)	3 (2, 6)
Antibody positive (n=1778)	85 (1510)

Abbreviations: IQR: interquartile range; BMI, body mass index.
Sample sizes (n) are given for each variable if data collection was incomplete.
Data collection for ketoacidosis at diabetes presentation was based on a record of it being assigned clinically, or of hyperglycaemia accompanied by acidosis and either ketonaemia or ketonuria. The children's body mass index z-score expressed as a percentile (median (IQR)) was 67% (39%, 89%) (n=1676).

The relationships between diabetes presentation and individual characteristics

The main presenting features (Table 1) were: osmotic symptoms (polyuria and/or polydipsia) 96%; weight loss 85%; and fatigue 82%. Ketoacidosis was identified at clinical presentation in 42%. Another autoimmune disease was present in 6%; 15% had a parent with diabetes; 8% had a sibling with diabetes.

The influence of age: Increasing age was independently associated with an increased prevalence of ketoacidosis, weight loss, and fatigue at presentation, decreased prevalence of osmotic symptoms and longer symptom duration (Figure 1). In accord with its relationship with age, ketoacidosis was less common in children than adults (39% versus 45%, p=0·0002). Although significant statistically, the differences between children and adults in other presenting symptoms were small (osmotic symptoms, 97% vs 95%, p=0.001; weight loss, 82% vs 89%, p=0.0001; fatigue, 78% v 88%, p=0.0001, respectively), as were differences in symptom duration (median, 3 vs 4 weeks, p=0.0001).

The influence of gender: Female sex was independently associated with longer symptom duration (Figure 1). Median symptom duration in females and males were 4 and 3 weeks, respectively (p=0·0001).

Associations with ethnicity: There were no significant associations between ethnicity and initial clinical presentation, including ketoacidosis, which was equally likely: in white Europeans and non-whites (41% and 44%, respectively; p=0·3).

Family history of diabetes: Having a parent with any diabetes was associated with a lower probability of presenting with ketoacidosis (Figure 1; 12% versus 18%, p<0·0001). The same applied to those who had a sibling with diabetes (Figure 1: 5% versus 10%, p<0·0001).

Other autoimmune disease: The presence or absence of another autoimmune disease had no significant influence on diabetes presentation.

Table 2. Characteristics of pancreatic autoantibody (Ab) positive and negative participants (n=1778 with known antibody status); percentages (n) or medians (IQR) are shown.

	Ab positive (n=1510)	Ab negative (n=268)	p
INDIVIDUAL CHARACTERISTICS			
Age	20·1 (13·1, 31·1)	31·4 (17·7, 41·0)	0·0001
Male	56 (851)	72 (192)	<0·0001
Children	41 (614)	25 (66)	<0·0001
Body mass index			
Children (z score, n=545, 56)	0·41 (-0·35, 1·19)	0·47 (-0·48, 0·97)	0·4
Adult (kg/m^2, n=825, 184)	23·9 (21·4, 26·7)	25·5 (23·1, 29·2)	0·0001
Overweight (n=1370, 240)	36 (490)	48 (114)	0·0005
White European ethnicity	86 (1413)	14 (232)	<0·0001
Other autoimmune disease (n=1495, 265)	8 (117)	4 (10)	0·01
Parent(s) with any diabetes (n=1493, 261)	16 (233)	28 (74)	<0·0001
Siblings with any diabetes (n=1374, 238)	9 (117)	8 (20)	0·9
DIABETES PRESENTATION			
Clinical presentation			
Ketoacidosis (n=1483, 260)	43 (639)	40 (104)	0·3
Osmotic symptoms (n=1495, 267)	97 (1444)	94 (250)	0·02
Weight loss (n=1480, 267)	87 (1285)	88 (235)	0·5
Fatigue (n=1490, 265)	86 (1282)	80 (213)	0·01
Symptom duration (weeks, n=1424, 246)†	6·8 (10·5)	10·4 (32·2)	0·004

† median and IQRs for symptom duration were identical: 4 (2, 8); mean and SD is shown to clarify the direction of difference
Sample sizes (n) are given for each variable if data collection was incomplete.

The children's body mass index z-scores expressed as percentiles (median (IQR)) were 66% (36%, 88%) in the Ab positive (n=545) and 68% (32%, 83%) in the Ab negative (n=56).

Description of the cohort in whom autoantibodies were measured

Children comprised 38% of the sub-group of 1,778 participants who provided a blood sample for autoantibody measurement. The sub-group with blood samples was, accordingly, significantly older than the full cohort (median (IQR) 21·6 (13·4, 32·8) vs 14·6 (10·4, 26·4) years, p<0·0001). Other parameters were similar. One or more autoantibodies were present in 85% of those who donated a blood sample.

The relationships between autoantibody status and individual characteristics

The influence of age: Autoantibody positivity decreased with increasing age; adults were less likely than children to be positive for one or more antibodies (82% vs 90%, p<0·0001). The decline in autoantibody positivity continued throughout adult life (Figure 2). Of the individual autoantibodies, GADA were the most frequently observed in adults, with IA-2A and ZnT8A being relatively more common in children (Figure 2). The autoantibody positive adults were of lower BMI than autoantibody negative adults (BMI, median (IQR) 23·9 (21.4, 26·7) vs 25·5 (23·1, 29·2), p<0·0001) and they were less likely to be overweight or obese (40% vs 55%, p=0.0001). No relationship to BMI z-score was observed in children and there was no independent relationship between overweight/obesity and antibody positivity across children and adults (Figure 1).

The influence of gender: Females were more likely than males to be antibody positive (90% versus 82%, respectively (p<0·0001).

Associations with ethnicity: There was a statistically significant relationship between ethnicity and autoantibody status (on chi-squared analysis, p<0·0001). Amongst the 3 major non-white ethnic groups (Asian; African-Caribbean; other or mixed ethnicity) numbers with autoantibodies measured were small (n=46, 36 and 51, respectively) and the proportion with autoantibody positivity did not differ significantly (70%, 64%

and 82%, respectively; p=0·1). People of non-white ethnic origin were therefore grouped and comparisons limited to white European versus non-white ethnic origin. White European ethnicity was independently associated with a higher prevalence of autoantibody positivity compared with the combined non-white group (86% and 73%, respectively; p<0·0001; Figure 1).

Family history of diabetes: People who had a parent with diabetes were less likely than those without to have autoantibodies (Figure 1; 16% versus 28%, p<0·0001, Table 2).

Other autoimmune disease: Another autoimmune disease was present in 204 participants and 117 of those in whom autoantibodies were measured. A history of another autoimmune disease was positively associated with pancreatic islet autoantibody positivity (p=0·01, Figure 1), being present in 8% of the autoantibody positive and 4% of the autoantibody negative participants (Table 2).

The relationship between autoantibody status and diabetes presentation

A statistically significant relationship between positive autoantibody status and diabetes presentation was restricted to a very small increase in prevalence of osmotic symptoms (97% versus 94%, p=0·02, Table 2). There was no significant difference in rates of ketoacidosis at presentation between autoantibody positive and negative participants (43% versus 40%, p=0·3, Table 2).

DISCUSSION

For the first time, relationships between autoantibody status (measured centrally in a single reference laboratory) and phenotypic features in incident T1D are reported from a large, unselected, multi-ethnic population of both children and adults. The study was conducted with support from the NIHR CRN with most participants recruited from specialist centres. Based on estimates of T1D incidence and population

demographics,[23 24] 20-25% of eligible incident cases in England and Wales were recruited.

Male predominance is unusual for an autoimmune condition but has been reported in adults with incident T1D.[25] In young children, the sex ratio has been reported to be close to unity.[26] In the present study the male excess was observed also in children, although the excess was less marked than in adults. Symptoms at presentation were as expected.[27 28] Although weight loss was common, average body weight at the time of recruitment was normal, and many participants were overweight or obese, especially adults. This was apparent even in people with body weight measurements obtained within 28 days of diagnosis, belying the belief that patients presenting with type 1 diabetes are underweight. An association between increased BMI and increased risk of progression from autoantibody positivity to development of diabetes in at-risk relatives has been reported previously.[29] Symptom duration was similar to that reported previously by others and was shorter in children than adults.[30 31] This may reflect parental vigilance of unwell children or a more insidious onset of clinical disease in older people.

The overall frequency of ketoacidosis at diagnosis (42%) was high, and slightly more so in adults than children. It occurred with similar frequency in white European and non-white ethnic groups. The figure of 42% is higher than in previous reports from the UK (23% in a recent national paediatric audit,[32] 26-27% in regional studies[33 34]) and a range of 13-80% in those aged <20 years has been reported internationally.[35] A very similar figure (40.3%) has been reported recently for children in Italy.[36] Ketoacidosis at diagnosis is a quality issue as it reflects lack of awareness of diabetes features amongst professionals and the general population[32] and efforts to increase awareness lead to reductions in ketoacidosis at first presentation.[37] All methods of estimating the

frequency of ketoacidosis at diagnosis have limitations, often leading to under-reporting.[32] Strengths of the current study include the large number of patients and the ability to confirm or refute the diagnosis where this was in doubt. A limitation is that ascertainment bias could be introduced because recruitment was mainly from secondary care and those who are the most ill at diagnosis may be the most likely to volunteer or to be referred. The higher ketoacidosis rate in adults versus children in our study appears at variance with the observation that ketoacidosis or severe ketoacidosis is more common in younger than in older children.[28 33 36 38-40] The current study did not include children <5 years of age, the group in childhood in whom ketoacidosis at diagnosis occurs most frequently[41], and this may have contributed to the apparent children to adult difference. Of course, if such younger children had been included, this could have increased the overall rate of ketoacidosis even higher. The lower rate associated with having a parent or a sibling with diabetes could result from a heightened awareness of symptoms leading to earlier clinical referral.[35] The absence of any significant ethnic influence on ketoacidosis is at variance with some previous reports where higher rates were observed in non-white sub-groups.[42 43]

One or more islet autoantibodies were observed in 85% of participants, more commonly in female than male and in younger compared with older participants. This is compatible with previous literature from the UK and other countries,[8 44-46] although assay differences make such comparisons difficult. The positivity rate is higher than reported in people with T1D of non-white ethnic origin,[12 13] albeit with the same caveats and bearing in mind that the previous studies of ethnic influences on antibody status have been limited in size or age range of population studied. The slight female autoantibody preponderance has been observed in other studies in young children, but not in older children and young adults.[47] The higher autoantibody frequency in those

with a coexistent autoimmune disease reflects the clustering of autoimmune disorders observed in T1D and shared genetic susceptibility.[48] Overall, GADA were the antibodies most commonly present; while IA-2A and ZnT8A were seen most frequently in children, findings compatible with previous studies.[47] Insulin itself is considered a potential primary autoantigen as insulin autoantibodies are observed in incident T1D, especially in children[30 31 49]. In the current study, most participants had received insulin therapy for weeks before study entry and as they could have developed antibodies to the exogenous insulin, insulin autoantibodies were not measured. In prospective studies of infants at high genetic risk of T1D, insulin autoantibodies were often detected earlier than the other islet autoantibodies[50 51] and in consequence we may have underestimated the frequency of autoantibody positivity at diagnosis, especially in children.

Although autoantibodies can be present for years in people with diabetes who do not require insulin treatment immediately,[52-54] and are present in some diagnosed clinically with type 2 diabetes,[55] they are generally regarded as a biomarker for T1D. In prospective studies they precede and predict the onset of T1D.[56] They typically disappear, or titres drop to very low levels, in the years following diagnosis .[57] In the autoantibody negative participants studied here, several explanations may be proposed. *First*, insulin autoantibodies were not measured. *Second*, autoantibodies to as yet unknown antigens may have been present.[58] The identification of tetraspanin-7 as an autoantigen could, for example, account for some apparently antibody negative participants,[59] although recent data suggest this is unlikely to account for large numbers.[60] *Third*, autoantibodies may have disappeared or their levels diminished, by the time of sampling. As all participants were recruited within six months of diagnosis, this is unlikely to be a major factor. *Fourth*, autoantibodies might develop

subsequently, as reported previously for islet cell antibodies in a small proportion of patients.[61] *Fifth*, some people may have autoimmune T1D without a humoral response. *Finally*, people may actually have another diabetes sub-type. The autoantibody negative participants as a group tended to be older and, if adult, more overweight. These features are compatible with type 2 diabetes. They were more likely to have a parent with diabetes, typical of type 2 or monogenic diabetes. They were more likely to be of non-white ethnicity, more associated with type 2 diabetes. Those with ketoacidosis could have ketosis-prone diabetes (so-called idiopathic diabetes)[62] as this is difficult to distinguish from T1D at first presentation. Further studies and follow-up of the cohort are planned to explore the extent to which T1D without detectable autoantibodies describes a sub-group of T1D that is distinct from other diabetes sub-types.

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The funders were consulted about the vision for a UK-based type 1 diabetes ascertainment network. They had no involvement in study design, data collection, data analysis, data interpretation, or writing of the article, and took no part in the decision to submit for publication. The funders are represented on the management committee that oversees access to the data and stored biological samples. The funding was administered as a grant to Imperial College London. Imperial College London is the study Sponsor.

DATA SHARING

The full anonymous dataset is available to access via a management committee, which includes people living with type 1 diabetes, scientists, clinicians and funder representatives as members.[17] Participants gave informed consent for data sharing subject to conditions described in the access procedure documents available from the study website: www.address2.org.

COMPETING INTERESTS

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: funding from Diabetes UK and the Juvenile Diabetes Research Foundation administered as grants to Imperial College London for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

CONTRIBUTORSHIP

Author contributions

Literature searches were carried out by authors VB, HCW, IFG, NSO and DGJ. Authors AK, HCW, IFG, AJKW, PJB, DBD and DGJ were involved in the study design. Data collection was coordinated by authors AK and HCW. Authors AK, HCW and IFG were responsible for data management. Authors AJKW and PJB were responsible for autoantibody measurements. The statistical analysis plan was developed by IFG. Analysis of the data was by authors VB, AK and IFG. Data were interpreted by all authors (VB, AK, HCW, IFG, SM, PJB, AJKW, DBD, CMD, MP, NSO and DGJ). The manuscript was prepared by authors VB, AK, HCW, IFG, AJKW and DGJ. Critical revisions were made by authors PJB, DBD, CMD, MP and NSO. Figures were prepared by authors AK, HCW and IFG.

The Management Committee oversaw access to the data and stored biological samples. The Patient Advocate Group had input into aspects of study conduct. Local investigators were responsible for the recruitment of participants and collection of data.

GUARANTORSHIP

Dr Walkey accepts full responsibility for the finished article and confirms that she had full access to all the data in the study and was responsible for the decision to submit for publication. Dr Walkey affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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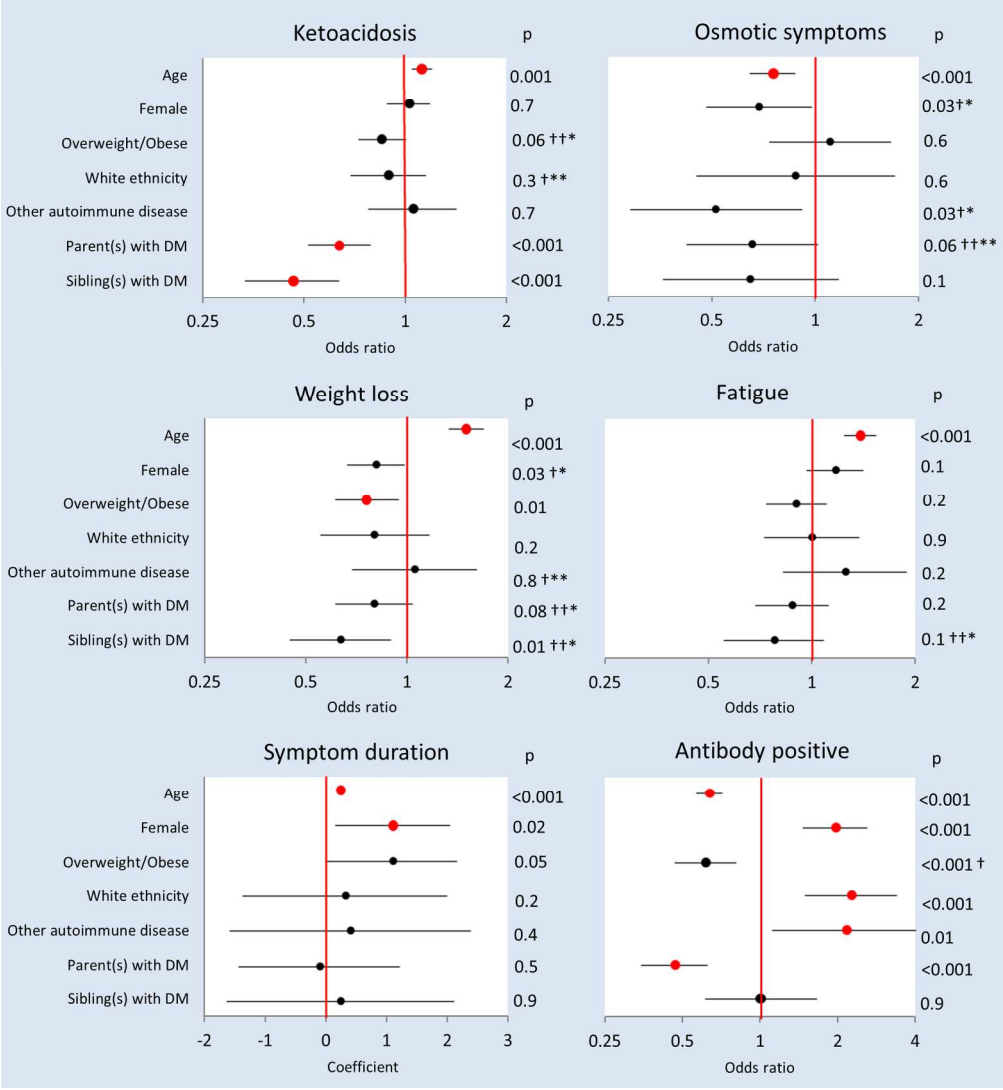
Figure captions

Figure 1. Individual characteristics as predictors of diabetes presentation and autoantibody status.

Univariate logistic regression odds ratios or linear regression coefficients (circles), 95% CIs (horizontal lines)[#] and statistical significances are shown. Red circles signify that a significant univariate relationship was sustained on multivariable analysis with all individual characteristics included as predictor variables (participants with complete data: n=2911-3312) and in the sub-group with antibodies measured (participants with complete data including antibody status: n=1610-1778)

Footnotes: [#] for 'Age' odds ratios or coefficients and 95% CIs were derived from standardised data
* not significant in sub-group with antibodies measured on multivariable analysis
** significant in sub-group with antibodies measured on multivariable analysis
[†] not significant on multivariable analysis
[††] significant on multivariable analysis

Figure 2. The percentage of participants exhibiting islet autoantibodies (any and individual) autoantibody in relation to age at diagnosis.



Univariate logistic regression odds ratios or linear regression coefficients (circles), 95% CIs (horizontal lines)# and statistical significances are shown. Red circles signify that a significant univariate relationship was sustained on multivariable analysis with all individual characteristics included as predictor variables (participants with complete data: n=2911-3312) and in the sub-group with antibodies measured (participants with complete data including antibody status: n=1610-1778)

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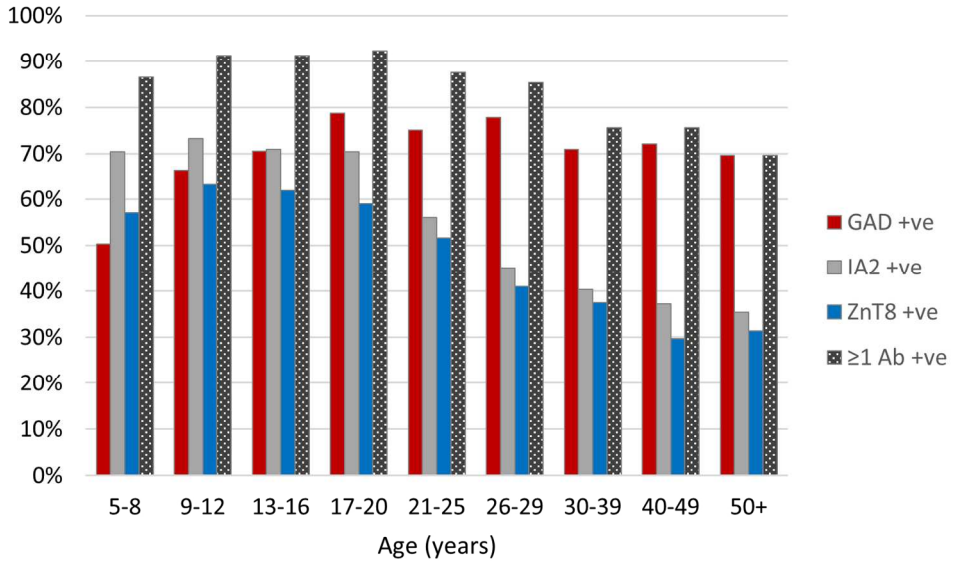

Figure 2. The percentage of participants exhibiting islet autoantibodies (any and individual) autoantibody in relation to age at diagnosis.

141x90mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	4-5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	7
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	8-9 & protocol paper (BMJ Open 2017;7:e013956)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8-~~6~~9, 11 & protocol paper (BMJ Open 2017;7:e013956)
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	8 & protocol paper (BMJ Open 2017;7:e013956)
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8-9 & protocol paper (BMJ Open 2017;7:e013956)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8-9 & protocol paper (BMJ Open 2017;7:e013956)
Bias	9	Describe any efforts to address potential sources of bias	19~~20~~
Study size	10	Explain how the study size was arrived at	8, 11, 18~~19~~ & protocol paper (BMJ Open 2017;7:e013956) (ongoing cohort)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8-9
		(*b*) Describe any methods used to examine subgroups and interactions	8-9, 13~~14~~ (ethnic groups) 16~~18~~ (autoantibody subgroup)
		(*c*) Explain how missing data were addressed	11, Tables 1-2~~4~~ (12-13, 15-16~~16-18~~) & Figure 1
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	N/A

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8, 18~~19~~ & protocol paper (BMJ Open 2017;7:e013956)
		(b) Give reasons for non-participation at each stage	8 & protocol paper (BMJ Open 2017;7:e013956)
		(c) Consider use of a flow diagram	Not complex: diagnosed and recruited or not, blood sample given or not.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11 & Table 1 (12-13)
		(b) Indicate number of participants with missing data for each variable of interest	11, Tables 1-24 (12-13, 15-16~~16-18~~) & Figure 1
		(c) Summarise follow-up time (eg, average and total amount)	N/A data from recruitment only
Outcome data	15*	Report numbers of outcome events or summary measures over time	N/A
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11-17~~18~~, Tables 1-~~4~~2 (12-13, 15-16~~16-18~~) & Figure 1
		(*b*) Report category boundaries when continuous variables were categorized	9 & Figure 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	16~~18~~ (autoantibody subgroup)
Discussion			
Key results	18	Summarise key results with reference to study objectives	17-21~~19-22~~
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17-21~~19-22~~
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17-21~~19-22~~
Generalisability	21	Discuss the generalisability (external validity) of the study results	6, 21~~22~~
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	22~~23~~

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.