TITLE: Circulating C-peptide levels in living children and young people and pancreatic beta cell loss in pancreas donors across type 1 diabetes disease duration

SHORT TITLE: C-peptide decline and beta cell loss in T1D

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Tweet: The profile of decline in C-peptide in living children mirrors the profile of beta cell loss in pancreas donors across T1D duration, with the younger diagnosed having more severe loss @alicelouisejane @jinshaw16 @BesserBesser @SarahIbex @Exeter_Diabetes @HumanGeneticsOx

ABSTRACT

C-peptide declines in type 1 diabetes although many long-duration patients retain low, but detectable levels. Histological analyses confirm that beta cells can remain following type 1 diabetes onset. We explored the trends observed in C-peptide decline in UK Genetic Resource Investigating Diabetes (UK GRID) cohort (N=4,079), with beta cell loss in pancreas donors from the network for Pancreatic Organ donors with Diabetes (nPOD) biobank and the Exeter Archival Diabetes Biobank (EADB) (combined N=235), stratified by recently reported age at diagnosis endotypes (< 7, 7-12, ≥ 13 years) across increasing diabetes durations. The proportion of individuals with detectable C-peptide declined beyond the first year after diagnosis, but this was most marked in the youngest age group (< 1 year duration: age < 7 years: 18/20 (90%), 7-12 years: 107/110 (97%), ≥ 13 years: 58/61 (95%) versus. 1-5 years post diagnosis: < 7 years: 172/522 (33%), 7-12 years: 604/995 (61%), ≥ 13 years: 225/289 (78%)). A similar profile was observed in beta cell loss, with those diagnosed at younger ages experiencing more rapid loss of islets containing insulin-positive (insulin+) beta cells
< 1 year post diagnosis: age < 7 years: 23/26 (88%), 7-12 years: 32/33 (97%), ≥ 13 years: 22/25 (88%) versus. 1-5 years post diagnosis: < 7 years: 1/12 (8.3%) ,7-12 years: 7/13 (54%), ≥ 13 years: 7/8 (88%)). These data should be considered in the planning and interpretation of intervention trials designed to promote beta cell retention and function.

Circulating C-peptide, a marker of endogenous insulin secretion from pancreatic beta cells, is known to decline following a diagnosis of type 1 diabetes, but can persist for many years (1–8). It is frequently observed, however, that those diagnosed at the youngest ages have lower levels of C-peptide at diagnosis (2,3,5,6,9). Histological analyses of donor pancreata provide evidence for persistent immunoreactive insulin-positive (insulin+) beta cells; sometimes for many years after diagnosis (7,8,10,11). These findings challenge the dogma that all beta cells are destroyed at, or soon after, onset of type 1 diabetes. The centrepiece of many disease modifying intervention trials is to augment the survival of these residual beta cells, assessed via measures of preserved C-peptide secretion. However, currently there is little understanding of how C-peptide levels relate to absolute beta cell mass, as residual C-peptide alone cannot distinguish between loss of beta cell mass and reduced functionality. It is known that there are clear differences in disease progression between children and adults (3,5), but few studies have explored how this progression varies within children, particularly young children diagnosed under 7 years compared to those diagnosed around puberty (at or over 13 years) (6,11). In this study, we questioned whether trends of C-peptide decline observed in children and young people with type 1 diabetes from the UK Genetic Resource Investigating Diabetes (UK GRID) cohort were similar to trends of beta cell loss in pancreatic donors from the Network of Pancreatic Organ Donors (nPOD) and the Exeter Archival Diabetes Biobank (EADB), across wide ranges of age at diagnosis and durations.

RESEARCH DESIGN AND METHODS

Three independent resources were used to assess C-peptide levels in the plasma and beta cell loss within the pancreas, respectively: 1) plasma samples from the Genetic Resource Investigating Diabetes (GRID) collection (12), and 2) type 1 diabetes pancreas samples from the Exeter Archival Diabetes Biobank (EADB) (11,13) and Network for Pancreatic Organ Donors (nPOD) biobank (14). We stratified subjects by age at diagnosis (< 7, 7-12, ≥ 13 years) (6), and grouped them by diabetes duration (< 1, 1-5, 5-10, ≥ 10 years).

We report the proportion of individuals from the GRID collection with detectable C-peptide (> 9 pmol/l) and distribution of these levels. We report the proportion of donors from the combined biobanks: EADB and nPOD, retaining islets containing insulin+ beta cells and distribution of beta cell area, expressed as insulin+ area with respect to the sum of insulin+ and glucagon+ area.

Study cohorts

We analysed 4,079 random non-fasting plasma C-peptide measurements from people with clinically-defined type 1 diabetes (on insulin from diagnosis) from the GRID collection, diagnosed ≤ 16 years (12), and 235 native pancreas samples recovered from people with type 1 diabetes, diagnosed < 18 years, from the nPOD biobank (n=111) and EADB (n=124) (Table 1, ESM Table 1). Histopathology notes and slide digitization were available through nPOD as previously described (14).

Histological analyses

We studied 235 type 1 diabetes (non-transplant) donors diagnosed <18 years from the combined nPOD and EADB biobanks with native pancreas available or complete nPOD online pathology and age-at-disease-diagnosis information. We examined pancreas sections using either digitised slides via nPOD online pathology database or pancreas material, which was stained for the presence of insulin and/or glucagon using standard immunohistochemical approaches (14). Sections were double-stained for insulin/glucagon, or serial sections were stained for insulin and glucagon respectively, where alignment of the two stains allowed for identification of the insulin-negative (insulin-) islets. We defined type 1 diabetes histologically by the lobular loss of islets containing insulin+ beta cells with the presence of multiple (>10) insulin- islets in the section(s) studied. Insulin+ and insulin- islet counts were completed either by light microscopy or using high resolution digitised slides (via the Vectra® Polaris™ Automated Quantitative Pathology Imagining system (Akoya)) when appropriately stained sections were available. In some (n=12) older samples from the EADB collection islet count information was collated from historical studies (6,11,13,15). For light microscopy the total number of islets was quantified using the glucagon-stained section with the number of islets with residual beta cells assessed using the serially stained insulin section. In such slides islets were defined as comprising of >10 insulin and/or glucagon positive cells. When digitised slides were available, islets were identified using the random forest tissue classifier module of HALO V3.0 image analysis software (Indica Labs) and assessed for insulin positivity. In slides assess by the HALO V3.0 image analysis software (Indica Labs), islets were defined as groups of endocrine cells covering an area of $\geq$ 1000 μm^2. We identified 120 donors with islets containing insulin+ beta cells from collated recent and historical analyses and expressed the proportions of donors with islets containing insulin+ beta cells across diabetes duration, stratified by age at diagnosis.

100 out of the 120 donors with islets containing insulin+ beta cells, had slides of appropriate quality available for digitization. The Random Forest Classifier Module (Version 3.2.1851.354) was applied to tissue double-stained for insulin/glucagon or DenseNet AI V2 modules on serial single-stained tissue, within the Indica Labs HALO Image analysis platform (Version 3.2.1851.354), to identify insulin+ area and glucagon+ area for the sections per donor analysed across a total 38322 identified islets. We define insulin+ area relative to the sum of the insulin+ and glucagon+ area in the total section as: beta cell area with respect to total islet area. We make the assumption that insulin+ and glucagon+ area represents islet area. We report the distribution of beta cell area for these 100 donors across diabetes duration stratified by age at diagnosis.

In an additional sub-analysis, we selected 87 donors from nPOD that had been processed using the HALO Image analysis platform to identify beta cell area and who had random C-peptide measurements taken at the time of organ donation, without documented renal disease/failure or on dialysis, to assess if those with detectable C-peptide also had islets containing insulin+ beta cells.

C-peptide measurement

Plasma was obtained from 5,565 non-fasted blood samples from UK GRID patients, collected using the anticoagulant Acid Citrate Dextrose (ACD). Samples were excluded with C-peptide > 500 pmol/l (n=75), if time from blood draw to freeze > 72 hrs (n=1378) or data was incomplete (n=33). Samples were stored at -80°C. C-peptide was measured using the Diasorin Liaison C-peptide kit insert (product 316171, issued 24-02-2012) where the lower limit of the assay is 9 pmol/l, with a coefficient of variation of < 20%. C-peptide levels in nPOD donors were measured as described (14). Due to the variable limits of detection of C-peptide in nPOD,

we chose the minimum limit of detection (16.4 pmol/l) as the limit of detection for nPOD C-peptide in our sub-analysis, where detectable C-peptide is defined as ≥16.4 pmol/l. C-peptide levels are reported in pmol/l (1000 pmol/l = 3 ng/ml).

RESULTS

Patterns of beta cell loss mirror patterns of C-peptide decline in children and young people

C-peptide levels were detectable in some individuals across all age at diagnosis groups and diabetes durations. This was least common in those diagnosed < 7 years (Figure 1a, ESM Table 2), (detectable C-peptide: age < 7 years: 254/1666 (15%), 7-12 years: 838/1887 (44%), ≥ 13 years: 325/526 (62%)). In all age at diagnosis groups, the number of individuals with detectable C-peptide declined beyond the first year after diagnosis, but this trend was most marked in those diagnosed at younger ages (detectable C-peptide < 1 year duration: age < 7 years: 18/20 (90%), 7-12 years: 107/110 (97%), ≥ 13 years: 58/61 (95%) versus. detectable C-peptide 1-5 years post diagnosis: < 7 years: 172/522 (33%),7-12 years: 604/995 (61%), ≥ 13 years: 225/289 (78%)).

Across all diabetes durations, similar trends were observed in the proportions of individuals retaining islets containing insulin+ beta cells in the sections of pancreas studied. Although present in all groups irrespective of age at diagnosis or disease duration, fewer individuals diagnosed < 7 years retained islets containing insulin+ beta cells (Figure 1b, ESM Table 2), (retaining islets containing insulin+ beta cells: < 7 years: 30/86 (35%), 7-12 years: 50/89 (56%), ≥ 13 years: 41/61 (67%)). There was a more precipitous drop off in the number of individuals retaining islets containing insulin+ beta cells post 1 year diagnosis in those diagnosed at younger ages compared to those diagnosed older (retaining islets containing insulin+ beta cells < 1 year post diagnosis: age < 7 years: 23/26 (88%), 7-12 years: 32/33 (97%), ≥ 13 years: 22/25 (88%) versus. retaining islets containing insulin+ beta cells 1-5 years post diagnosis: < 7 years: 1/12 (8.3%) ,7-12 years: 7/13 (54%), ≥ 13 years: 7/8 (88%)).

The absolute levels of detectable C-peptide declined in all age groups across all diabetes durations (Figure 1c), and this mirrored the decline in beta cell area (as fraction of insulin+ and glucagon+ area), across the groups (Figure 1d, ESM Table 3).

Children diagnosed < 7 years had lower absolute levels of C-peptide and less insulin+ beta cells close to diagnosis

C-peptide decreased in all age groups over time (ESM Table 4). In those with detectable levels, C-peptide was markedly lower soon after diagnosis in children diagnosed < 7 years compared to those diagnosed ≥ 13 years (< 1 year post diagnosis: median (IQR) < 7 years: 61.5 (45.4-110.8) pmol/l vs. ≥ 13 years: 199.5 (114.3-282.3) pmol/l; $p=1x10^{-4}$) (Figure 1c). Similarly, among children diagnosed < 7 years who retained islets containing insulin+ beta cells close to diagnosis, as judged by beta cell area, was lower (< 1 year post diagnosis: median (IQR) < 7 years: 15% [6.7%,27%] vs. ≥ 13 years: 31% [12%,42%] p=0.025 (Figure 1d, ESM Table 3). This compares with a median beta cell area of 70.4% [64.0%, 79.1%] in 44 <18y donors without diabetes (median age of donors 9 years [4.6, 12.9]).

Approximately 5% of children diagnosed < 7 years retained detectable C-peptide 10 years post diagnosis

Across all age groups, a proportion of children retained C-peptide > 10 years post diagnosis and a similar proportion retained islets containing insulin+ beta cells over this time (Figure 1a, ESM Table 2). In long duration disease (≥10 years), children originally diagnosed < 7 years were more likely to be insulin deficient at the time of organ donation than those who were older at diagnosis (detectable C-peptide ≥ 10 years post diagnosis: < 7 years: 21/489 (4.3%), 7-12 years: 25/249 (10%), ≥ 13 years: 12/107 (11%)), and they were also less likely to retain islets

with insulin+ beta cells (retaining islets containing insulin+ beta cells ≥ 10 years post diagnosis: < 7 years: 3/34 (8.8 %), 7-12 years: 4/26 (15%), ≥ 13 years: 2/13 (15%)) (Figure 1b, ESM Table 2).

In nPOD pancreas donors with detectable C-peptide, the majority also had presence of insulin+ islets

Among a subset of nPOD donors (n=87), 17 had detectable C-peptide with 13 of these donors (76%) having presence of insulin+ beta cells, as determined by a > 0% beta cell area (ESM Table 5). There was a significant difference in presence or absence of insulin+ islets between the detectable/undetectable C-peptide groups (81.6% agreement, $p=1.5x10^{-6}$). The characteristics of 4 donors with detectable C-peptide but with no insulin+ beta cells in sections analysed are outlined in ESM Table 6. In these 4 donors, the C-peptide level was low (<100 pmol/l) and in 2 of the donors the histopathology notes state that, in some curated sections, islets containing insulin+ beta cells were seen but were rare (ESM Table 6).

DISCUSSION

We report that trends in C-peptide decline in living children and young people with type 1 diabetes are similar to the trends of loss of islets containing insulin+ beta cells within sections of donor pancreata, across all ages and disease durations. Our results support the proposition that C-peptide levels are a reliable, inexpensive and practical marker of retention of islets containing insulin+ beta cells in children and young adults with type 1 diabetes. Our results are consistent with those of other studies showing higher C-peptide levels in people diagnosed at older ages, but decline over time (2,3,5,9). Our study also supports the findings of Aida et al who demonstrated a significant correlation between beta cell volume and fasting serum C-peptide levels in Japanese patients with adult-onset type 1 diabetes (16). Our study is the first to provide a comparison of pancreatic histology with an independent clinical cohort, examining patterns of C-peptide loss according to age at diagnosis and duration in children with type 1 diabetes. Our study is also the largest to assess such disease progression trends in very young children (< 7 years). We demonstrate that, when compared with those who are older at diagnosis, children diagnosed < 7 years progress more rapidly towards total C-peptide loss and have minimal beta cell retention.

These data confirm that trialling a safe immunotherapy close to diagnosis to inhibit or halt the autoimmune destruction, as in recent clinical trials (17), is worthwhile to preserve pancreatic mass. The rapid depletion of C-peptide and beta cells in children diagnosed < 7 years, when comparing < 1 years and 1-5 years duration, emphasizes that early intervention close to (or before) diagnosis may be most time critical in those progressing to disease in very early life. Our results highlight that among children there are differences in progression which should be

considered in the planning and interpretation of intervention trials designed to promote beta cell retention and function.

We find that a small proportion of children retain some residual beta cells over > 10 years duration and a similar proportion retain C-peptide over this period. These proportions do not change markedly between disease durations of 5 or 10 years, in keeping with the concept that there are two phases of C-peptide decline: a rapid fall in the first 7 years after diagnosis, followed by a more stable phase (2). Our results are likely to be an underestimate given a higher limit of detection of C-peptide (9 pmol/l), compared to contemporary assays (2). It must be noted that the UK GRID cohort, included only those individuals with type 1 diabetes, and as such in this study we do not have access to a non-type 1 diabetes population for comparison of C-peptide levels. However, it is well established that levels of residual C-peptide in long duration type 1 diabetes are low and detectable using ultrasensitive assays (18,19).

We acknowledge that in histological analyses we have not been able to assess beta cell area for all 120 donors with islets containing insulin+ beta cells, calculating this for 100 such samples. Of the 20 samples we were unable to calculate beta cell area in, 12 were derived from the EADB biobank; a 50-year-old archival biobank mainly comprising of non-systematically collected autopsy samples from younger children very close to diagnosis. We were unable to include these archival sections due to deterioration of glass slides/ staining intensity which impacted on scan quality, and the rarity of material available from these donors precluded re-staining. In addition, we must emphasise that the standard error around the proportion estimates in the histological analyses are large, as influenced by the sample numbers. We also acknowledge that there is little information on the anatomical location of the sampled pancreas in the histological analyses of the EADB donors. However, as sampling was

random across the 235 donors, we think it is very unlikely that systematic sampling bias might explain our observations.

A further limitation of this study is its cross-sectional design and the dissociated biobanks used. Extensive, within donor, analysis is difficult in this setting, since there are no large systematic studies of C-peptide in clinical type 1 diabetes cohorts in whom post-death pancreas samples are available. Despite this, we are able to demonstrate that 81% (17/21) of donors from the nPOD biobank with detectable C-peptide also had islets containing insulin+ beta cells in the sections studied. 4 donors had detectable C-peptide and no islets containing insulin+ beta cells in the sections we were able to assess. It is reasonable to assume that, due to the nature of sampling, such islets could be present elsewhere in the pancreas. This is illustrated in 2 of the 4 donors studied, since the histopathology reports held by nPOD describe rare islets containing insulin+ beta cells in the sections they curate. In addition, it should be noted that C-peptide levels in nPOD organ donors may be influenced by end-of-life circumstances and must therefore be interpreted with caution. In donors with undetectable C-peptide but who retain insulin+ beta cells, acute glucotoxicity (20) and sample degradation may have contributed to false negative C-peptide results. Additionally, we accept that limited clinical data were available and that, in particular, no information was accessible on rates of diabetic ketoacidosis in the UK GRID cohort, which is known to be an independent predictor of C-peptide decline (20).

Despite these caveats, our data suggest that progressive loss of beta cells is the main contributory factor to the decline in endogenous insulin secretion observed in children and young people diagnosed with type 1 diabetes. Our results add weight to the proposal that intervention trials should be powered separately for each age at diagnosis group and highlight

that consideration of age at diagnosis is very important in the interpretation of outcomes. Interventions that delay diagnosis in "at-risk" individuals are likely to improve clinical outcomes by promoting the retention of beta cells and maintaining a higher C-peptide secretion rate.

ARTICLE INFORMATION

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Author Contributions

REJB, SJR, ALJC and JRJI designed the study. REJB and JRJI performed analysis of C-peptide data. CSF, BCW, PL, ALJC, BH, TW, DP, MP, LAR and SJR performed histological assessments and analysis. ALJC performed analysis of histological data and wrote the first draft. RAO and NGM helped with data interpretation and revision of manuscript. JAT, LSW and DBD provided access to the data from the GRID study and contributed to scientific discussion. All authors reviewed analysis and reviewed and contributed to final draft. REJB and SJR are responsible for the integrity of the work as a whole.

Disclosure Statement

The authors declare that there are no relationships or activities that might bias, or be perceived to bias, their work.

Ethics Statement

All procedures in nPOD were in accordance with federal guidelines for organ donation and the University of Florida Institutional Review Board. All EADB samples were used with ethical permission from the West of Scotland Research Ethics Committee ((ref: 20/WS/0074; IRAS project ID: 283620). Plasma samples were obtained from the Genetics Resource Investigating

Diabetes or GRID Study (Rec Reference 00/5/44) which encompassed the UK Nephropathy Family Study (Rec Reference 00/5/65).

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The content and views expressed are those of the authors.

Prior Presentation

Parts of this work were presented at the European Association for the Study of Diabetes conference (online) 21st-25th September 2020 and the Network for Pancreatic Organ Donors Conference (online) 22nd-24th February 2021.

Data Availability

Further information about the data is available from the corresponding author upon request.

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Table 1 Characteristics of UK GRID cohort and cohort from combined EADB and nPOD pancreas biobanks.

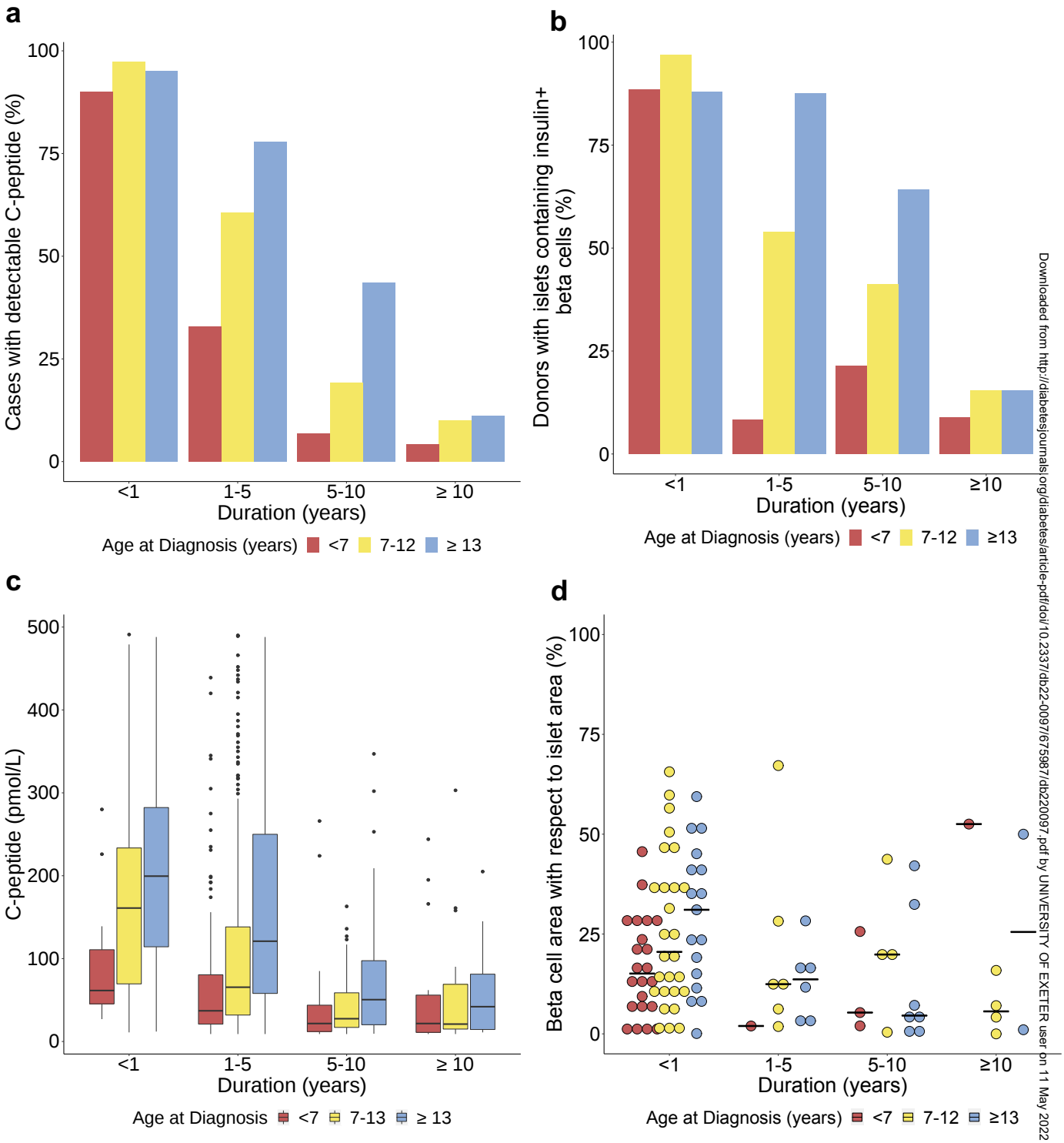
	UK GRID N=4079	**EADB** (n=124) **and nPOD** (n=111) N=235
Age (years), Median [25th;75th]	13 [10;16]	15 [10;22]
Diabetes Duration (years), Median [25th;75th]	5 [2;8]	5 [0.08;12]
Age at diagnosis (years), Median [25th;75th]	8 [4;11]	8 [4.9;13]
Sex, Male, N (%):	2149* (52.7%)	102 (43.4%)
C-peptide (pmol/L), Median [25th;75th]	<9‡ [<9‡, 31]	16.4†‡ [16.4†‡;16.4†‡]
Donors with islets containing insulin+ beta cells, N (%):		
None	-	115 (48.1%)
Present	-	120 (51.9%)

*missing data, n=2

† nPOD only, n=109

‡Limit of detection for UK GRID: 9 pmol/l , for nPOD 16.4 pmol/l

Figure 1 Comparison of proportions of individuals with detectable C-peptide (n=1417/4079) (a), proportions of donors retaining islets containing insulin+ beta cells (n=120/235) (b), absolute levels of detectable C-peptide (n=1417) (c) and within donor beta cell area, expressed as insulin+ area relative to the sum of the insulin+ and glucagon+ area (n=100) (d) stratified by age at diagnosis (< 7, 7-12, ≥ 13 years) and grouped by diabetes duration (<1, 1-5, 5-10, ≥ 10 years). Lines represent median and bars represent interquartile range. Proportions of donors with detectable C-peptide from UK GRID cohort (a) and donors with insulin+ beta cells from nPOD and EADB (b) are outlined in more detail in ESM Table 2. A summary of donors with available beta cell area (d) is outlined in ESM Table 3.



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ESM Table 1 Breakdown of characteristics for the EADB and nPOD biobanks (N=235) from people with type 1 diabetes diagnosed < 18 years.

	EADB n=124 (53%)	**nPOD** n=111 (47%)
Age (years), Median [25th;75th]	11.0 [6.9;15.0]	22.0 [16.3;32.5]
Diabetes Duration (years), Median [25th;75th]	0.2 [0.03;3.0]	12.0 [6.0;23.0]
Age-at-diagnosis (years), Median [25th;75th]	8.0 [4.0;13.0]	8.0 [5.0;12.8]
Sex, Male, N (%):	46 (37%)	56 (50%)
Donors with islets containing insulin+ beta cells, N (%):		
None	40 (32%)	75 (68%)
Present	84 (68%)	36 (32%)

ESM Table 2 Summary break down of proportions of donors from the UK GRID cohort (N=4079) with detectable C-peptide and pancreas donors from EADB and nPOD cohorts (N=235) with islets containing insulin+ beta cells within age-at-diagnosis (< 7, 7-12, ≥ 13 years) and diabetes duration (< 1,1-5,5-10, ≥ 10 years) groups.

		UK GRID (N=4079)		**EADB and nPOD (N=235)**	
Duration (years)	**Age-at-diagnosis (years)**	Total number of donors (N)	Number of donors with detectable C-peptide (n (%))	Total number of donors (N)	Number of donors with islets containing insulin+ beta cells (n (%))
<1	**<7**	20	18 (90%)	26	23 (88%)
<1	**7-12**	110	107 (97%)	33	32 (97%)
<1	**≥13**	61	58 (95%)	25	22 (88%)
1-5	**<7**	522	172 (33%)	12	1 (8.3%)
1-5	**7-12**	995	604 (61%)	13	7 (54%)
1-5	**≥13**	289	225 (78%)	8	7 (88%)
5-10	**<7**	635	43 (6.8%)	14	3 (21%)
5-10	**7-12**	533	102 (19%)	17	7 (41%)
5-10	**≥13**	69	30 (43%)	14	9 (64%)
≥10	**<7**	489	21 (4.3%)	34	3(8.8%)
≥10	**7-12**	249	25 (10%)	26	4 (15%)
≥10	**≥13**	107	12 (11%)	13	2 (15%)

ESM Table 3 Summary of pancreas donors from EADB and nPOD cohorts with islets containing insulin+ beta cells (n=120) and the donors of which beta cell area data was available (n=100) within age-at-diagnosis (< 7, 7-12, ≥ 13 years) and diabetes duration (< 1,1-5,5-10, ≥ 10 years) groups.

Duration (years)	**Age-at-diagnosis (years)**	Total number of donors, N=235 (N)	Number of donors with islets containing insulin+ beta cells, n=120 (N(%))	Number of donors with islets containing insulin+ beta cells and beta cell area data, n=100 (N)	Beta cell area, n=100 (Median % [IQR])
<1	**<7**	26	23 (88%)	22	15 [6.7,27]
<1	**7-12**	33	32 (97%)	27	21 [11,38]
<1	**≥13**	25	22 (88%)	17	31 [15,42]
1-5	**<7**	12	1 (8.3%)	1	2.0 [2.0,2.0]
1-5	**7-12**	13	7 (54%)	6	12 [7.5,25]
1-5	**≥13**	8	7 (88%)	6	14 [5.9,17]
5-10	**<7**	14	3 (21%)	3	5.3 [3.7,15]
5-10	**7-12**	17	7 (41%)	4	20 [14.5,26]
5-10	**≥13**	14	9 (64%)	7	4.6 [2.4,20]
≥10	**<7**	34	3(8.8%)	1	53 [53,53]
≥10	**7-12**	26	4 (15%)	4	5.6 [3.1,9.3]
≥10	**≥13**	13	2 (15%)	2	26 [13,38]

ESM Table 4 C-peptide in entire GRID cohort (N=4079) by age-at-diagnosis (< 7, 7-12, ≥ 13 years) and diabetes duration (< 1,1-5,5-10, ≥ 10 years).

Age-at-diagnosis, years (N)	**<7 (1666)**				**7-12 (1887)**				**≥13 (526)**			
Duration, years (N)	**<1 (20)**	**1-5 (522)**	**5-10 (635)**	**≥10 (489)**	**<1 (110)**	**1-5 (995)**	**5-10 (533)**	**≥10 (249)**	**<1 (61)**	**1-5 (289)**	**5-10 (69)**	**≥10 (107)**
C-peptide (pmol/l), Median [IQR]	54 [29-111]	<9* [<9*-21]	<9* [<9*-<9*]	<9* [<9*-<9*]	156 [65-233]	24 [<9*-88]	<9* [<9*-<9*]	<9* [<9*-<9*]	189 [102-282]	79 [14-209]	<9* [<9*-41]	<9* [<9*-<9*]

*Limit of detection

ESM Table 5 Two by two table of C-peptide detectability and presence of insulin+ beta cells, as determined by beta cell area positivity, in a subset of nPOD donors diagnosed <18 years without renal disease/failure, analysed by the HALO image analysis platform (n=87), (81.6% agreement, $p=1.5x10^{-6}$).

	Beta cell area >0%	Beta cell area of 0%
Detectable C-peptide (≥16.4pmol/L)	13	4
Un-detectable C-peptide (<16.4pmol/L)	12	58

ESM Table 6 Characteristics, including admission course, of nPOD donors identified as having detectable C-peptide and 0% islets containing insulin+ beta cells (n=4). Insulin (Ins) and Glucagon (Gluc) have been abbreviated.

Study Number	Donor Type	Sex	BMI (Kg/m^2)	Age (years)	Duration of diabetes (years)	Age-at-diagnosis (years)	Islets containing insulin+ beta cells (%)	C-peptide (pmol/l)	Transport Duration (Minutes)	nPOD Histopathology Notes
6074	T1D	F	19.5	73	66	7	0	70	NA	Ins-/Gluc+ islets, numerous. Occ. insulin+ cell in acinar regions or within 1 islet. Few CD3+ cells in acinar and parenchyma regions. Moderate arteriosclerosis.
6145	T1D	M	23.1	18	11	7	0	20	849	Ins-/Gluc+ islets, atrophic. No infiltrates.
6244	T1D	M	23.8	34	28	6	0	16.7	981	Ins-/Gluc+ islets. Exocrine atrophy moderate. Low Ki67. IHC- some may be repeated due to background.
6268	T1D	F	26.6	13	3	9	0	16.7	1050	Ins+ (very rare)/Gluc+ islets, possibly reduced islet numbers but increased glucagon+ single cells. Insulitis present at insulin+

			and insulin- islets. Ki67+ cells moderate numbers in acinar region, also in occasional islet and duct. Moderate acinar atrophy with prominent nerve fibres.