



# Most People With Long-Duration Type 1 Diabetes in a Large Population-Based Study Are Insulin Microsecretors

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## OBJECTIVE

Small studies using ultrasensitive C-peptide assays suggest endogenous insulin secretion is frequently detectable in patients with long-standing type 1 diabetes (T1D), but these studies do not use representative samples. We aimed to use the stimulated urine C-peptide-to-creatinine ratio (UCPCR) to assess C-peptide levels in a large cross-sectional, population-based study of patients with T1D.

## RESEARCH DESIGN AND METHODS

We recruited 924 patients from primary and secondary care in two U.K. centers who had a clinical diagnosis of T1D, were under 30 years of age when they received a diagnosis, and had a diabetes duration of >5 years. The median age at diagnosis was 11 years (interquartile range 6–17 years), and the duration of diabetes was 19 years (11–27 years). All provided a home postmeal UCPCR, which was measured using a Roche electrochemiluminescence assay.

## RESULTS

Eighty percent of patients (740 of 924 patients) had detectable endogenous C-peptide levels (UCPCR >0.001 nmol/mmol). Most patients (52%, 483 of 924 patients) had historically very low undetectable levels (UCPCR 0.0013–0.03 nmol/mmol); 8% of patients (70 of 924 patients) had a UCPCR  $\geq$ 0.2 nmol/mmol, equivalent to serum levels associated with reduced complications and hypoglycemia. Absolute UCPCR levels fell with duration of disease. Age at diagnosis and duration of disease were independent predictors of C-peptide level in multivariate modeling.

## CONCLUSIONS

This population-based study shows that the majority of long-duration T1D patients have detectable urine C-peptide levels. While the majority of patients are insulin microsecretors, some maintain clinically relevant endogenous insulin secretion for many years after the diagnosis of diabetes. Understanding this may lead to a better understanding of pathogenesis in T1D and open new possibilities for treatment.

Recent studies have challenged the traditional view of type 1 diabetes (T1D) leading to absolute insulin deficiency. Sensitive C-peptide assays have shown that 43–74% people with long-standing (>5 years) T1D are microsecretors of endogenous insulin (1,2) with C-peptide levels in a range not detected by previous assays (1–30 pmol/L).

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A slide set summarizing this article is available online.

\*A complete list of the members of the UNITED Team can be found in the APPENDIX.

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Importantly, we showed that these low levels increased during a mixed meal, suggesting that there are a very small number of functional  $\beta$ -cells (1). The implications of these studies could be important, because if  $\beta$ -cells remain in most people with T1D, then they are either regenerating or evading immune attack. Either of these possibilities might open up new avenues of treatment in T1D.

The studies to date have not been able to give an accurate estimate of the prevalence of very low levels of C-peptide secretion in long-duration T1D. There have been only two small studies using sufficiently sensitive C-peptide assays from clinic-based populations (Oram et al. [1]  $n = 74$ ; and Wang et al. [2]  $n = 182$  [144 patients with disease duration of  $>5$  years]). The 382 Joslin Medalists studied by Keenan et al. (3) were defined on the basis of their long-term survival ( $>50$  years): the high prevalence of retained C-peptide (67%  $>30$  pmol/L) is likely to reflect survival bias because the risk of complications is reduced in patients with maintained endogenous C-peptide levels. There are no large community-based studies examining low-level insulin production in patients with T1D.

Measurement of postmeal urine C-peptide-to-creatinine ratio (UCPCR) is an alternative to serum C-peptide testing (4–7). The UCPCR involves a single, spot urine measurement and has the advantage of long-term stability (3 days) at room temperature, which facilitates large-scale community studies because samples can be posted. We have shown that a home UCPCR correlates with 90-min serum C-peptide measurement in the mixed-meal tolerance test (MMTT) (6,7). UCPCR and serum C-peptide levels identified similar long-duration patients with T1D as having detectable C-peptide levels in an MMTT (1).

We aimed to assess the prevalence of detectable endogenous C-peptide levels using UCPCR in a large, nonselected, population-based study of T1D patients and to assess the clinical associations.

## RESEARCH DESIGN AND METHODS

### Study Participants

We recruited 924 patients, with T1D for  $\geq 5$  years, from primary and secondary care in the catchment area of two U.K. hospitals in Tayside (Ninewells Hospital,

Dundee, U.K.,  $n = 474$ ) and Devon (Royal Devon and Exeter Hospital, Exeter, Devon, U.K.,  $n = 450$ ). These patients were recruited as part of the UNITED (Using Pharmacogenetics to Improve Treatment in Early-Onset Diabetes) study. All patients had received a diagnosis of diabetes before 30 years of age and were under 50 years of age at the time of study recruitment. T1D patients were included on the basis of having received a clinical diagnosis of T1D, being  $<30$  years of age at the time of diagnosis, and being treated with insulin since diagnosis. To exclude monogenic diabetes, patients with a UCPCR of  $\geq 0.2$  nmol/mmol (8) who did not have GAD or IA2 antibodies were tested for monogenic diabetes as previously described (9). To avoid inadvertent inclusion of patients with young-onset type 2 diabetes, patients with a UCPCR of  $\geq 0.2$  nmol/mmol who were GAD and IA2 autoantibody negative were excluded if their BMI was  $>30$  kg/m<sup>2</sup>. Ninety-seven percent of participants were white Europeans. More than 60% of eligible participants were recruited.

Informed consent was obtained from all participants, and the study was approved by the National Research Ethics Service Committee South West and the East of Scotland Research Ethics Committee (references 10/H0106/63 and NRS10/DI33, respectively). Clinical and demographic data were collected at the time of consent.

### C-Peptide Assessment

We assessed C-peptide levels using a home postmeal UCPCR. Participants voided their bladder before their largest (i.e., highest carbohydrate content) meal of the day, and collected a urine sample 2 h after the meal in a sample pot containing boric acid preservative. As in previous validation studies (6,7), the content of the meal was not specified, and the patients took their normal basal and prandial insulin (10). Patients returned the sample to the laboratory within 36 h usually by mail. Samples were analyzed within 36 h (on the same day or subsequent day). C-peptide analysis was performed using an electrochemiluminescence assay on the Roche E170 Analyzer (Roche, Mannheim, Germany) in the Royal Devon and Exeter Hospital Blood Sciences Laboratory, as previously described (4).

### C-Peptide Thresholds

We considered a UCPCR of  $>0.001$  nmol/mmol to have analytically detectable UCPCR, this reflected being able to detect a urine C-peptide concentration of  $>3.3$  pmol/L in the  $\times 10$  diluted urine (4). In addition, we analyzed two other thresholds: UCPCRs  $\geq 0.03$  and  $\geq 0.2$  nmol/mmol, which are equivalent to serum C-peptide levels of 30 pmol/L (a common historical limit of detection [3,11,12]) and 200 pmol/L (a clinically defined level associated with reduced microvascular complications and hypoglycemia [13]). The UCPCR equivalent cutoffs were derived using linear regression—calculated UCPCR values from previous studies comparing UCPCR and serum C-peptide measurements (6,7,14).

### Statistical Analysis

We tested the independence of the effects of age at diagnosis and diabetes duration on UCPCR with a logistic regression model using either an analytically detectable UCPCR or a UCPCR  $\geq 0.2$  nmol/mol as the outcome variable. The age at diagnosis and diabetes duration were treated as continuous predictor variables. The model fit was assessed using a Hosmer-Lemeshow goodness-of-fit test. We assessed the impact of age at diagnosis and diabetes duration on retained endogenous C-peptide production by comparing proportions of detectable and undetectable UCPCRs across diabetes duration and age at diagnosis quintiles. We used a Kruskal-Wallis test and a nonparametric trend test as age at diagnosis and diabetes duration data were non-normally distributed.

Differences in HbA<sub>1c</sub> level, BMI, and insulin dose between groups defined by UCPCR C-peptide values were assessed using a *t* test. A linear regression was used to test the independence of UCPCR to predict insulin dose allowing for BMI, age at diagnosis, diabetes duration, and HbA<sub>1c</sub> level. For all pediatric patients, we calculated a BMI z score relative to the 1990 U.K. reference population (15). We then calculated a BMI adjusted to age 22 years for all pediatric patients, and this was included in reported values of BMI and used for any analysis involving BMI.

All statistical analysis was performed using STATA version 12.1 (StataCorp, College Station, TX). All CIs reported are 95% CIs.

## RESULTS

The clinical characteristics of patients recruited are given in Table 1.

### Prevalence of Detectable C-Peptide

Eighty percent of participants (740 of 924 participants [95% CI 77–83%]) had detectable C-peptide levels (UCPCR >0.001 nmol/mmol). The majority of patients (52%, 483 of 924 patients [49–55%]) had a UCPCR between 0.001 and 0.03 nmol/mmol (Fig. 1). Twenty percent of participants (187 of 924 participants [18–23%]) had a UCPCR between 0.03 and 0.2 nmol/mmol, and 8% (70 of 924 participants [6–9%]) had a UCPCR  $\geq$ 0.2 nmol/mmol.

### Associations of Detectable C-Peptide

The presence of a detectable C-peptide level was inversely associated with a shorter duration of diabetes but was unrelated to age at diagnosis or BMI. Patients with a detectable UCPCR (>0.001 nmol/mmol) had a shorter diabetes duration than those without (17.8 vs. 20.9 years;  $P = 0.0003$ ; Table 2). The percentage of patients with a detectable UCPCR within each quintile of duration of T1D is given in Fig. 2. There was a trend for decreasing prevalence of detectable C-peptide level with the diabetes duration quintile ( $P < 0.0001$ ). The apparent increase between the fourth decade (72% [95% CI 66–78%]) and the fifth decade (79% [73–85%]) was not significant ( $P = 0.1$ ).

In logistic regression with duration of diabetes, age at diagnosis, and BMI as covariates, only diabetes duration was associated with the presence of a detectable C-peptide level (Supplementary Table 1).

### Associations of Higher Levels of C-Peptide

Patients with a UCPCR  $\geq$ 0.2 nmol/mmol had a shorter diabetes duration than those without (13.9 vs. 18.9 years;  $P < 0.0001$ ) and received a diagnosis at an older age (16 vs. 11 years;  $P < 0.0001$ ; Table 2). In a logistic regression with duration of diabetes, age at diagnosis, and BMI as covariates, diabetes duration and age at diagnosis were both associated with a UCPCR  $\geq$ 0.2 nmol/mmol (Supplementary Table 2, multivariate logistic regression). The odds of having a UCPCR  $\geq$ 0.2 nmol/mmol increased by 7% (odds ratio 1.07 [95% CI 1.04–1.11];  $P < 0.0001$ ) for each increase in year of age at diagnosis and decreased by 4% (0.96 [0.92–0.99];  $P = 0.01$ ) for each year of increase in duration.

### Association With Insulin Dose and Glycemia

Insulin dose and glycemia were similar in those with and without detectable C-peptide levels (insulin dose 0.77 vs. 0.78 units/kg/24 h, Table 1; HbA<sub>1c</sub> level 8.7% vs. 8.9% [72 vs. 74 mmol/mol]; Table 2). There was no association between UCPCR level and either HbA<sub>1c</sub> level or insulin dose in univariate or multivariate regression.

Patients receiving continuous subcutaneous insulin infusion (CSII) had better median glycemic control (HbA<sub>1c</sub> level 8.2% [interquartile range (IQR) 7.5–9.2%] vs. 8.8% [7.9–9.9%],  $P < 0.001$ ) and used lower doses of insulin (0.66 units/kg/day [0.53–0.86 units/kg/day] vs. 0.79 units/kg/day [0.60–0.98 units/kg/day];  $P < 0.001$ ), but did not have higher UCPCR values (0.01 nmol/mmol [0.004–0.04 nmol/mmol] vs. 0.01

nmol/mmol [0.003–0.02 nmol/mmol];  $P = 0.1$ ).

## CONCLUSIONS

This large study, using home postmeal UCPCRs, found that 80% of all people with T1D for  $\geq$ 5 years had measurable endogenous C-peptide levels. Across the range of durations in the study, the prevalence did not fall below 72%. These findings provide strong evidence that complete  $\beta$ -cell loss does not develop in most people with T1D, and that they will continue to secrete low levels of insulin for decades after receiving a diagnosis. These results support the histological data (3,16) that occasional insulin-producing  $\beta$ -cells are visible in most histological pancreas samples of people with long-duration T1D.

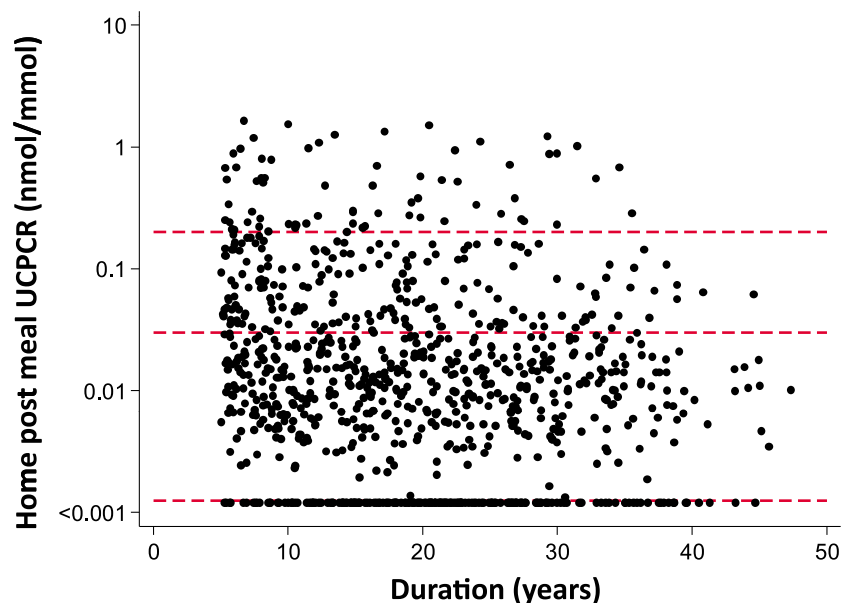
The two previous smaller studies using sensitive C-peptide assays in patients with T1D support the high prevalence in our study. Our initial study (1) on 74 people (median duration 30 years) showed that 73% had detectable post-mixed-meal serum C-peptide levels. The study by Wang et al. (2) found a lower proportion of patients (43% of 182 patients, median diabetes duration 15 years), which probably reflected the use of a fasting sample and that the ELISA used was less sensitive than the chemiluminescence assay used in our two studies (1). The absolute levels of C-peptide in our study are lower than those seen in the very long-duration participants (>50 years) in the Joslin Medalists studied by Keenan et al. (3), where 67% had serum C-peptide levels >30 pmol/L. Only 28% of our participants had a UCPCR equivalent to or above this level. This probably reflects the increased survival of those participants with retained C-peptide levels at the longer durations found in the Joslin Medalists (3). The failure to find an increase in C-peptide secretion in those of very long diabetes duration in our study probably reflects that we did not recruit patients with >50 years diabetes duration as recruitment was limited to patients under 50 years of age.

Even after 5 years of diabetes, duration of diabetes is a predictor of both C-peptide level and the presence of a detectable C-peptide level. There was a decline in absolute C-peptide levels and in the likelihood of a detectable C-peptide level with longer durations of diabetes.

**Table 1—Clinical characteristics of cohort**

Total number	924
Male sex, <i>n</i> (%)	492 (53)
Age at diagnosis (years)	11 (6–17)
Duration of diabetes (years)	18.6 (11.2–26.7)
BMI (kg/m <sup>2</sup> )*	24.8 (23.1–27.6)
HbA <sub>1c</sub> level	
%	8.7 (7.9–9.8)
mmol/mol	72 (63–84)
Insulin dose (units/kg/24 h)	0.78 (0.60–0.97)
CSII use (%)	13
Postmeal UCPCR (nmol/mmol)	0.012 (0.004–0.036)

Data are the median (IQR), unless otherwise specified. \*BMI results for pediatric patients adjusted to equivalent BMI for age 22 years.



**Figure 1**—Scatterplot of UCPCR against duration. Red dashed reference line at UCPCR = 0.2 nmol/mmol is equivalent to a stimulated serum C-peptide level of 200 pmol/L; UCPCR values of 0.03 are equivalent to serum values of 30 pmol/L, the lower limit of many historical assays; and UCPCR of 0.001 nmol/mmol is the effective lower limit of detection of this assay). UCPCR values are plotted on a log scale to allow separation of the range of low levels found.

Age at diagnosis was not associated with whether C-peptide was detectable but did associate with higher C-peptide levels. Age at diagnosis is associated with HLA risk, and may reflect the strength and intensity of the underlying autoimmune process (17). This may explain the relationship seen in our data and numerous other studies (3,18,19). If age at diagnosis is a marker of the rate of immune destruction of the  $\beta$ -cells, then the lack of association between age at diagnosis and the detection of low levels of C-peptide may suggest that other factors are more important in determining whether a few functional  $\beta$ -cells remain, or it may reflect that the impact of age at diagnosis is

small and not detectable by our sample size.

Some participants in our study continue to make relatively large amounts of C-peptide despite a long duration of T1D. Eight percent of participants in our study with a diabetes duration  $>5$  years had a UCPCR  $\geq 0.2$  nmol/mmol, and another 20% had a UCPCR  $\geq 0.03$  pmol/L. This is similar to the 8% of adults over 5 years post diagnosis who had a stimulated serum C-peptide level  $>200$  pmol/L when screened for the Diabetes Control and Complications Trial (DCCT) (20). However, it is important to recognize that these levels are considerably lower than seen in patients with T1D in the first year after diagnosis. The

median postmeal UCPCR was 1.04 nmol/mmol (IQR 0.44–2.3 nmol/mmol) in 100 individuals within the first year from the diagnosis of T1D (unpublished data from the UNITED study), and the median post-oral glucose tolerance test ( $n = 38$ ) UCPCR was 3.8 nmol/mmol (IQR 2.4–7.0 nmol/mmol) in 38 nondiabetic control subjects (21). The high level of C-peptide was unlikely to be an incorrect diagnosis of T1D in a patient with type 2 diabetes or monogenic diabetes, as these patients were aggressively excluded from this study. It is not known why some patients with T1D retain relatively high endogenous insulin levels for so long; potential explanations include that these individuals have a less aggressive autoimmune process leading to slower  $\beta$ -cell destruction, that the autoimmune process has subsided through “burnout,” or that  $\beta$ -cells in these individuals have a greater ability to regenerate.

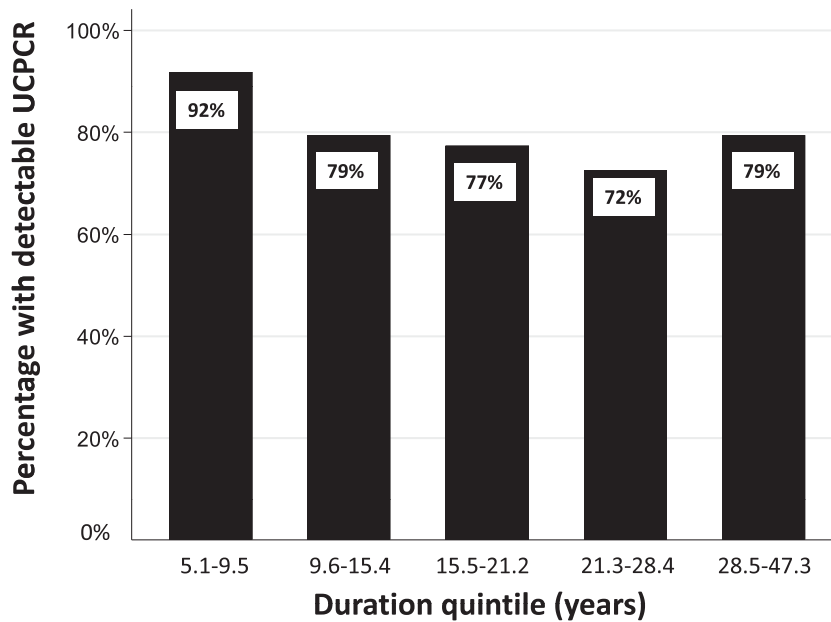
We did not find an association of persistent C-peptide secretion with either insulin dose or HbA<sub>1c</sub> level. This is in contrast with studies of a recent analysis of the DCCT by Lachin et al. (12) that demonstrates a continuous relationship between stimulated serum C-peptide and HbA<sub>1c</sub> levels and insulin dose, but only in the patients assigned to intensive therapy. The difference probably reflects 1) that our study was cross-sectional so that only a proportion of the patients will undergo intensive glycemic management and 2) that the low cutoff level we used means the majority of the patients in our study also had very low levels of C-peptide, which are unlikely to have a clinically significant effect.

There are some limitations in our study. The home postmeal UCPCR does not involve a fixed high-carbohydrate

**Table 2**—Table showing clinical characteristics across groups defined by UCPCR result

	UCPCR group				P value*
	$<0.001$ nmol/mmol ( $n = 184$ )	$\geq 0.001$ to $<0.03$ nmol/mmol ( $n = 483$ )	$\geq 0.03$ to $<0.2$ nmol/mmol ( $n = 187$ )	$\geq 0.2$ nmol/mmol ( $n = 70$ )	
Age at diagnosis (years)	10 (6–16)	11 (6–16)	12 (8–21)	16 (13–21)	0.0001
Diabetes duration (years)	20.9 (14.9–26.9)	19.1 (11.5–27.7)	15.0 (8.2–23.4)	13.9 (7.9–21.6)	0.0001
Insulin dose (units/kg/24 h)	0.77 (0.61–0.93)	0.78 (0.60–0.97)	0.77 (0.60–1.00)	0.74 (0.55–1.01)	0.9
CSII use (%)	16	15	9	10	0.2
HbA <sub>1c</sub> level					
%	8.9 (7.8–10.2)	8.6 (7.9–9.7)	8.7 (7.9–9.8)	9.1 (7.6–10.3)	0.3
mmol/mol	74	70	72	76	
BMI (kg/m <sup>2</sup> )†	24.0 (22.6–26.5)	24.8 (23.2–27.7)	25.3 (23.2–27.8)	24.8 (23.0–26.6)	0.7

Data are the median (IQR). \*Kruskal-Wallis test. †BMI results for pediatric patients adjusted to equivalent BMI for age 22 years.



**Figure 2**—Bar chart of the proportion of subjects with detectable UCPCR (>0.001 nmol/mmol) against the duration quintile.  $P < 0.0001$  for trend of decreasing proportion across duration groups.

meal, does involve taking prandial insulin, and is not supervised, and so is likely to be less sensitive than a formal MMTT assessment of serum C-peptide levels that has been performed in previous studies. However, a home UCPCR is highly correlated with MMTT serum C-peptide level in patients with T1D (6), and both urine and serum were equally sensitive in detecting very low levels of C-peptide in long-standing T1D (1). Any bias is small from the variable meal (6) or insulin administration (10) and would only result in C-peptide being less likely to be detected. We did not assess renal function in this study. Urine C-peptide levels are lower in those with chronic kidney disease, so this could lead to an underestimation of the prevalence of patients with retained endogenous insulin secretion. Participants in this study were mainly white Europeans, and our results may not be generalizable to other racial groups and other geographical regions.

The presence of a spectrum of endogenous insulin production at all durations of T1D is relevant to the study and treatment of the disease process in T1D patients. A pressing question is why some patients still have significant levels of endogenous insulin many years after diagnosis. The factors that cause the variation from undetectable or very low levels in most patients to very high

levels in a few patients may inform ongoing attempts to prevent, halt, or reverse the pathological process in T1D. Given that these individuals with higher levels of C-peptide are in a minority, large studies such as the UNITED study may be required to identify enough patients for future study. Identifying outliers with the highest or lowest levels of endogenous insulin will allow study of their immunology, genetics, and clinical phenotype in more detail. This may provide valuable insights into the biology of disease progression in T1D patients.

In conclusion, this population-based study confirms that the majority of people with long-duration T1D are insulin microsecretors and have detectable endogenous C-peptide levels. The presence of detectable C-peptide levels in most people with T1D may have important clinical and scientific implications, and warrants further investigation.

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**Author Contributions.** R.A.O. helped to design the study and perform the data analysis and interpretation; wrote the first draft of the manuscript; and read and modified the manuscript. T.J.M. helped to design the study, performed all biochemical analyses, helped to perform the data analysis and interpretation, and read and modified the manuscript. B.M.S., M.M.H., and E.R.P. helped to perform the data analysis and interpretation and read and modified the manuscript. M.H.S. and S.H. recruited patients, collected samples, and read and modified the manuscript. A.T.H. helped to design the study and perform the data analysis and interpretation and read and modified the manuscript. A.T.H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Prior Presentation.** Parts of this study were presented in abstract form at the 74th Scientific Sessions of the American Diabetes Association, San Francisco, CA, 13–17 June 2014.

## Appendix

The UNITED team includes all authors of the article, the nursing staff in Exeter (Tina Sanders and Sarah Tiley) and Dundee (Emma Gellatly, Lynsey Beall, and Bridget Shepherd), the Dundee Type 1 Diabetes Bioresource (Principal Investigator Professor Helen Colhune), the UNITED database manager Keith Milburn, and the genetic testing team (Kev Colclough and Professor Sian Ellard).

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