Follow-up of Children Diagnosed with Diabetes

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I. Hypotheses and Specific Aims

Abstract

Type 1 diabetes is associated with a pre-clinical period marked by the production of autoantibodies against insulin, GAD65, IA-2 and ZnT8. Prospective studies such as The Environmental Determinants of Diabetes in the Young (TEDDY) and TrialNet have used the production of these autoantibodies to identify children who are at a very high risk for the development of type 1 diabetes. We and others have shown that children diagnosed through studies that employ longitudinal monitoring of at-risk subjects have a lower hemoglobin A1c at onset, lower insulin dose through the first year of diabetes, and less hospitalization for diabetic ketoacidosis at onset than a similar group of children diagnosed in the community.

Preservation of C-peptide production has been shown to be associated with decreased complications such as retinopathy and microalbuminuria and decreased episodes of severe hypoglycemia. Hyperglycemia is toxic to the β-cell and prolonged hyperglycemia may lead to decreased C-peptide production. Through our previous analysis, we hypothesize that children diagnosed with diabetes through the TEDDY study are diagnosed at an earlier stage of type 1 diabetes (T1D) and that as such they will maintain the ability to produce C-peptide longer. We propose to analyze preservation of C-peptide over time in the children diagnosed with T1D through the TEDDY study and compare to a group of T1D controls from the community matched by age at diagnosis and by clinical center[1]. In addition we propose to collect samples to investigate immunological changes that occur after diagnosis and whether these changes are related to the earlier diagnosis of T1D. We also plan to examine the extent to which earlier diagnosis leads to easier control as measured by continuous glucose monitoring. We also propose to analyze children diagnosed with T1D through TEDDY to compare the decline of C-peptide over time between the earlier and later stage of T1D diagnosis. Furthermore, we propose to analyze children diagnosed with T1D through TEDDY to compare the decline of C-peptide over time between the earlier and later stage of T1D diagnosis.

The children in the TEDDY cohort represent a unique resource in that they have been carefully followed and monitored for immunological (antibodies), metabolic (insulin and glucose) and gene expression changes prior to diabetes onset and through careful systematic follow-up after diagnosis. We propose to add to our understanding whether early diagnosis will improve their disease course after diagnosis. Our preliminary data suggests that prospective studies such as TEDDY, the DPT-1 and TrialNet do lead to a reduction in the incidence of diabetic ketoacidosis, but we do not know whether the benefit of close monitoring will lead to better outcomes beyond diagnosis. Should we establish this benefit, then it will lead to recommended changes in surveillance and monitoring of high risk pre-diabetes populations and perhaps a re-definition of the time point in the development of diabetes that exogenous insulin therapy is needed. The potential is to make a dramatic improvement in the lives of children diagnosed with type 1 diabetes.

Hypotheses for TEDDY Cases and Community Controls:

We hypothesize that children diagnosed with type 1 diabetes through the TEDDY study are diagnosed earlier in the time course of diabetes. We hypothesize that they will have a higher level of C-peptide at diagnosis of type 1 diabetes compared with a control population of children diagnosed through the community. We hypothesize that this prolonged production of C-peptide will continue through the early years following diabetes diagnosis.

[1] Washington controls may not be matched by clinical center.

We also hypothesize that the prolonged production of C-peptide will result in better glycemic control, reduced levels of insulin dosages, fewer hypoglycemic and hyperglycemic episodes, but will be accompanied by a longer period of islet cell antibody production. We propose that earlier diagnosis of diabetes in children participating in the TEDDY study compared to community controls will predict better glycemic control and lower glycemic variability as assessed by continuous glucose monitoring (CGM) over the follow-up period.

Furthermore, we hypothesize that parents of children diagnosed through the TEDDY study will have higher levels of health-related quality of life and psychological functioning than community controls.

Secondary Hypotheses for TEDDY Cases:
We hypothesize that TEDDY children diagnosed earlier in the course of the disease (prior to diabetic ketoacidosis and symptoms) will maintain a higher level of insulin secretion than their TEDDY peers who were diagnosed later in the course of the disease. Among these subjects, we hypothesize that the C-peptide level at diagnosis will predict the rate of C-peptide loss and duration of C-peptide decline. We further predict that the level of C-peptide at diagnosis will affect the subject's ability to obtain optimal glucose control as measured by incidence and frequency of episodes of hyperglycemia, hypoglycemia, and HbA1c at desired levels. This will generate support for the conclusion that early intervention (diagnosis and treatment) lends to better short term metabolic outcomes.

We hypothesize that TEDDY children who are diagnosed earlier in the course of the disease will have better health-related quality of life and psychological functioning than those TEDDY cases diagnosed later in the course of the disease. We also predict that parents of TEDDY children diagnosed earlier will also have better health-related quality of life, psychological functioning, and less parenting stress.

Specific Aim
Aim 1: To evaluate subjects diagnosed with diabetes through the prospective TEDDY study and control children with type 1 diabetes of similar age for factors including C-peptide production at diagnosis, diabetic ketoacidosis, symptoms at diagnosis and HbA1c at diagnosis and to correlate these factors with decline and duration of decline of C-peptide loss.
Aim 2: To assess the impact of 'early' diagnosis on glucose control, quality of life and psychological functioning.
Aim 3: To collect and store samples for correlative studies to assess changes that occur before and after diagnosis of type 1 diabetes in TEDDY children with respect to T-cell and B-cell activity and gene expression as indicators of active autoimmunity.

II. Background and Significance

Type 1 diabetes has been hypothesized to be a chronic autoimmune disease characterized by a pre-clinical period identifiable by the presence of diabetes associated autoantibodies to insulin, GAD65, IA-2 (ICA-512) and ZnT8(1). Prospective studies such as The Environmental Determinants of Diabetes in the Young (TEDDY) and the TrialNet have used the production of these autoantibodies to identify children who are at a very high risk for the development of type 1 diabetes (2-4). To date, TEDDY has identified and enrolled over 8,000 infants with increased genetic risk who will be followed prospectively for 15 years. Those who develop autoantibodies will be followed more closely according to protocol for the development of T1D. To date, 91 children in TEDDY have developed diabetes during follow-up. An expected 400 children will develop T1D over the course of the TEDDY study.

Table 1. Current and expected numbers of cases from TEDDY by site

T1D Cases	Currently Diagnosed (as of May 31, 2011)	Projected over The next 5 years	Projected over The next 10 years
Denver	13	32	51
Finland	31	43	59
Germany	12	21	34
Seattle	7	29	45
Georgia	4	22	36
Sweden	24	56	92
Total	91	203	317

C-peptide production is a marker of the native pancreas' ability to produce insulin. C-peptide can be measured in the basal and stimulated states and the production of C-peptide in response to stimulus has been extensively studied in the Diabetes Complications and Control Trial (DCCT) (6;7). Through the DCCT, it has been shown that patients who have sustained production of C-peptide have lower rates of severe hypoglycemia, microalbuminuria and retinopathy regardless of being in the intensively treated or conventionally treated cohorts(8). In addition, intensive treatment prolonged the production of C-peptide in the cohort with C-peptide production at baseline. This observation has led to the statement that good control begets good control, i.e. that the production of C-peptide is associated with easier care of diabetes, which in turn is associated with a sustained production of C-peptide.

In the Diabetes Prevention Trial-Type 1 (DPT-1), oral glucose tolerance tests (OGTTs) were performed every 6 months for diagnostic surveillance. Post-challenge C-peptide levels begin to decrease appreciably in the 6 months before diagnosis (9). C-peptide levels were higher in subjects diagnosed by surveillance (in the DPT-1) compared to those diagnosed in the community. C-peptide levels continued to decrease within 3 months after diagnosis; however, no data is available after 3 months post diagnosis. The decline in stimulated C-peptide during the first year after the diagnosis of type 1 diabetes as reported in the literature is highly variable (10). Most of the available C-peptide data is from the control arm of these intervention studies. Several studies have reported modest reductions at 1 year (11;12), whereas other studies report a 50% decline in stimulated C-peptide over the first year post-diagnosis (13-15). Several factors have been proposed (but not confirmed) to affect the rate of loss of beta-cell function in patients with type 1 diabetes, including age of onset, degree of metabolic control, immune status and antibody levels, genetic factors and individual variation (16;17). Our study will assess the influence of these factors on C-peptide loss.

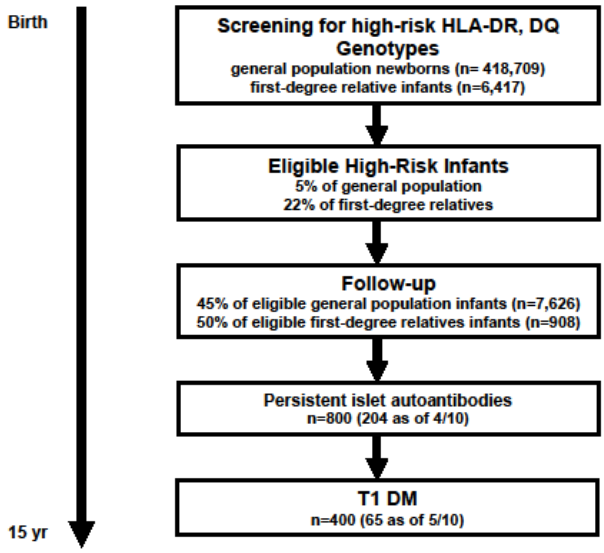
Most children and parents experience some level of psychological distress following the diagnosis of type 1 diabetes (18;19). In fact, it has been well documented that families of children diagnosed in the general population experience psychological symptoms such as shock, anxiety, and negative mood (20). In the general population, the diagnosis of type 1 diabetes in children has typically involved hospitalization and an acutely ill child, due to diabetic ketoacidosis. However, TEDDY participants who are diagnosed earlier in the disease process may avoid hospitalization and diabetic ketoacidosis. As a result, these families may experience less distress and may have improved psychosocial outcomes. Our study will measure psychological distress in families of children diagnosed with diabetes through a prospective design.

III. Preliminary Studies

The motivation for the proposed follow-up to the TEDDY study is based on the TrialNet and in part on results from the DAISY study being conducted (A.S. and M.R.) at the Barbara Davis center.

TEDDY and TrialNet. TEDDY is an international study recruiting young children (before the age of 4.5 months) with HLA-DR,DQ genotypes associated with type 1 diabetes through 6 clinical centers located in Denver (Colorado), Augusta (Georgia)/Atlanta (Georgia)/Gainesville (Florida), Seattle (Washington) in the United States and in Finland (Turku), Sweden (Malmo) and Germany (Munich). TEDDY has enrolled and followed, since 2004, 908 young first degree relatives of patients with type 1 diabetes (FDR) and 7,626 children identified through newborn screening for HLA-DR,DQ genotypes associated with type 1 diabetes (newborn cohort).

Figure 1. TEDDY Study Design



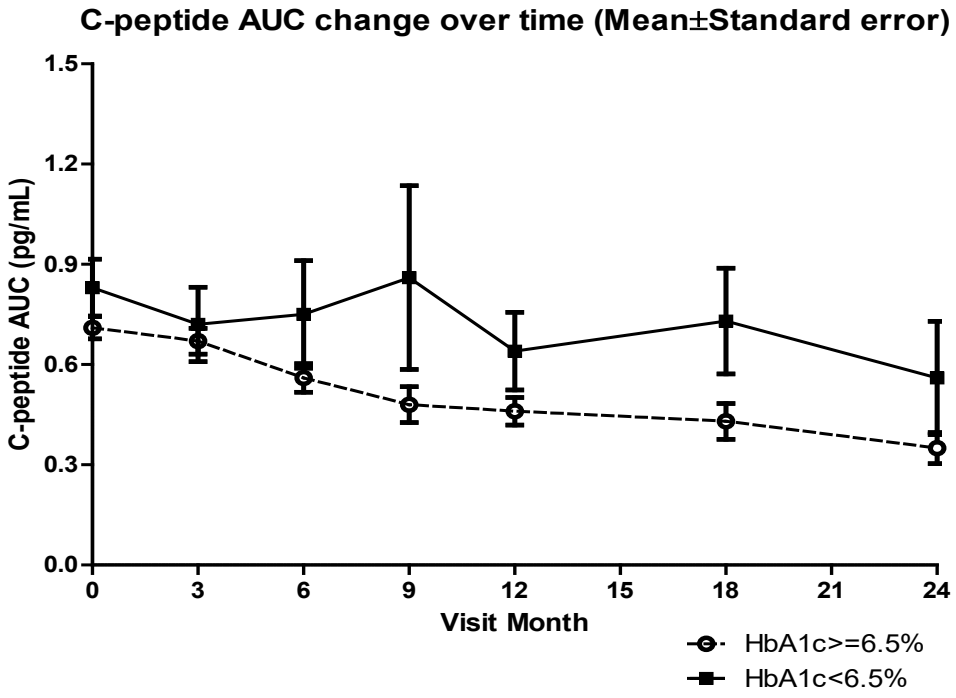
All newborns with high risk HLA genotype are invited to participate in the TEDDY follow-up. Participants in TEDDY are seen every 3 months up to 4 years of age, with subsequent visits every 6 months until the subject is 15 years of age. Individuals who are persistent positive for autoantibodies on two consecutive visits remain on the quarterly visit schedule after age 4 (22). They are screened for autoantibodies to pancreatic islet antigens at each visit. Children who are autoantibody positive also have HbA1c and random blood glucose measurements done at their quarterly visits. Participants who are 3 years old or older and are positive for two or more autoantibodies will have a two hour OGTT (Oral Glucose Tolerance Test) performed every 6 months. Serum, plasma, PBMC and mRNA samples are obtained at every visit to assess environmental exposures and changes that reflect active autoimmunity during follow-up.

Data supporting this study's hypotheses has also come from a comparison of TrialNet, (Natural History and the new onset trials) and early TEDDY results. The following data suggests that the children enrolled onto the TrialNet intervention studies from the community tend to be more symptomatic, have higher HbA1c values and lower C-peptides at diagnosis than do children diagnosed from the TrialNet or TEDDY studies where there is careful monitoring of at risk subjects.

Table 2. Comparison of TEDDY and TrialNet Subjects Diagnosed with Type 1 Diabetes.

	TEDDY (N=91)	TRIALNET	
		Natural history study (n=220)	3 Intervention studies (n=97)
Age at dx (Months)	29±15 25 (Q1=17, Q3=37)	201±146 150(Q1=102, Q3=243)	199±100 172 (Q1=145, Q3=221)
Female	44 (48%)	124 (56%)	35 (36%)
Asymptomatic	31 (34%)	133 (60%)	14 (14%)
HbA1c within 90days of diagnosis <6.5%	27 (30%)	165 (75%)	15 (16%)
Mean C-Peptide* AUC within 90 days of diagnosis		1.20±0.0.65 1.13(Q1=0.70, Q3=1.53)	0.73±0.31 0.70 (Q1=0.53, Q3=0.92)

Furthermore, from TrialNet anti-cd-20, GAD and ctla-4 IG new onset studies (control arms), we compared the rate of C-peptide AUC decline between earlier and later stage of T1D. The decline was estimated using the linear mixed effects model including random intercept and random slope. The decline in cases whose HbA1c were less than 6.5% was significantly slower than HbA1c greater than or equal to 6.5% (steeper decline, p-value=0.03). This lends support to our hypothesis that earlier diagnosis may help retain C-peptide production longer.



DAISY. Since 1993, DAISY has enrolled and followed 1120 young first degree relatives of patients with type 1 diabetes (sibling/offspring cohort – SOC) and 1422 children identified through newborn screening for HLA-DR,DQ genotypes associated with type 1 diabetes (newborn cohort – NEC). NEC participants were identified by screening of umbilical cord blood obtained from over 30,000 newborns at St. Joseph's Hospital in Denver, Colorado. EDTA sample of cord blood was sent for HLA typing, as previously described (21).

In DAISY follow-up, all children are tested at 9, 15, and 24 months of age and annually thereafter for autoantibodies to pancreatic islet antigens. Children who are autoantibody positive are placed on an

accelerated clinic visit schedule on which they return every 3-6 months for autoantibodies, HbA1c and random blood glucose testing. Individuals who are negative for the autoantibodies on two consecutive visits return to the annual clinic visit schedule. All newborns with high risk and a subset with moderate or average risk HLA genotypes are invited to participate in the follow-up.

Through a comparative analysis of these children with children diagnosed with type 1 diabetes in the community, we have demonstrated that children diagnosed through DAISY have a lower hemoglobin A1c at onset and through the first month of therapy, a lower insulin dose through the first year of therapy and decreased hospitalization for diabetic ketoacidosis compared with the community group (5).

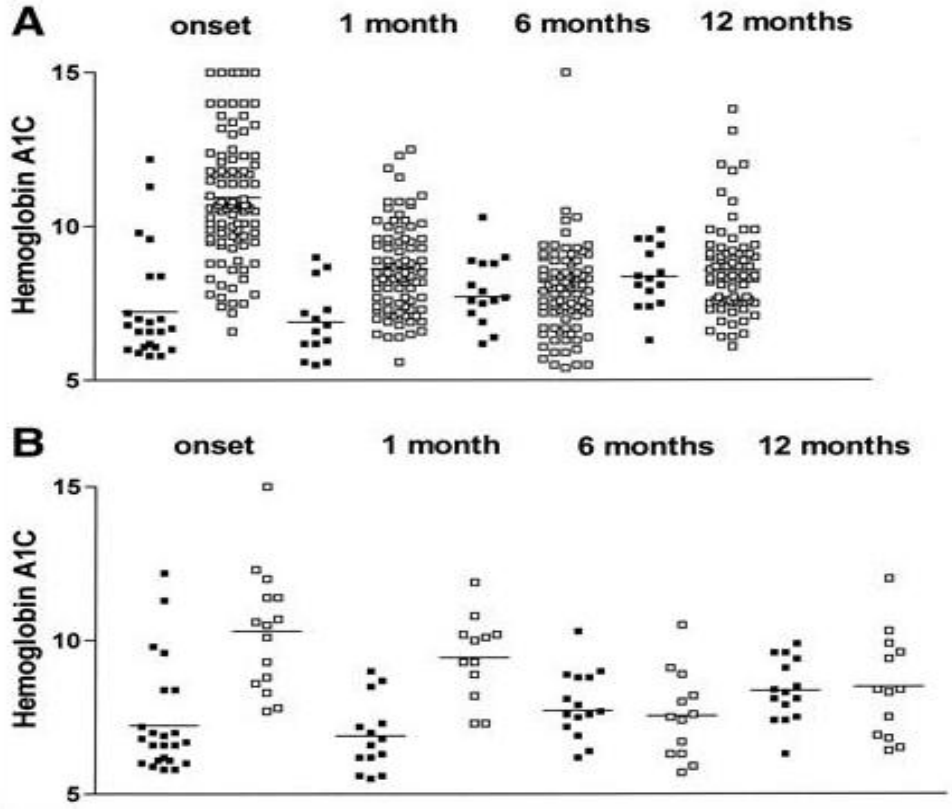


Figure 2. HbA1c in children diagnosed with diabetes in the DAISY (▪) and community (▫) groups at onset, 1, 6, and 12 months after diagnosis. A: HbA1c in the entire group, significant differences are noted at onset and 1 month after diagnosis. B: HbA1c in the group of individuals with a diabetic relative. Significant differences exist at onset and 1 month after diagnosis.

IV. Research Methods

A. Outcome Measure(s)

Primary outcome measure
The primary outcome measure will be the level of C-peptide over time from diagnosis of diabetes.

Secondary outcome measures
Secondary outcome measures include presence of diabetic ketoacidosis at onset. C-peptide, islet cell antibodies, hemoglobin A1c and insulin dose throughout the study will be collected and analyzed. Glycemic control and glycemic variability will also be assessed through continuous glucose monitoring at predetermined study visits. Health-related quality of life and psychological functioning

questionnaires will be administered to the children and their parents and compared between case and control groups. Specific constructs to be measured include diabetes-specific quality of life (child and parent), anxiety related to diabetes (child and parent), psychological well-being (parent), and pediatric parenting stress (parent). Among the TEDDY cases, we will also compare participants diagnosed earlier in the course of the disease (prior to diabetic ketoacidosis and symptoms) with TEDDY participants diagnosed later in the disease course.

Among the TEDDY children for whom samples have been obtained prior to and after diagnosis of type 1 diabetes, comparisons will be made of T-cell activity and gene expression prior to antibody development, post antibody development, post diagnosis of type 1 diabetes during which insulin secretory capability continues and after loss of C-peptide. As possible, stool samples will be collected to explore changes in the human biome for future studies focusing on changes in the biome that may predict C-peptide loss over time.

B. Description of Population to be Enrolled: Study Design and Research Methods

The study has a case-control design. Cases are children diagnosed with type 1 diabetes through the prospective TEDDY study and controls are patients diagnosed with type 1 diabetes from the community and followed at selected TEDDY centers. The TEDDY centers that will be participating in this study are Sweden, Finland and Denver, CO. The Pacific Northwest Diabetes Research Institute center in Seattle, WA will participate in this study, but currently only enrolls cases. If the Washington site is unable to enroll controls, then the Colorado site will be asked to enroll community controls to match the cases enrolled at the Seattle center. Participants will undergo visits with lab draws at regular intervals, i.e. at baseline, 3 months, 6 months, 12 months, 18 months, 24 months and every 6 months thereafter up until 60 months following their diagnosis of diabetes. For TEDDY participants, their post-diagnosis TEDDY visit will be used in lieu of the baseline visit for this study. Controls will be matched by age of diagnosis and by their clinical center location[2].

Study population description

Cases: Cases are children diagnosed with type 1 diabetes through the TEDDY study. They will be identified at onset of diabetes and referred by the TEDDY study coordinator to the study coordinator for the follow-up study.

Controls: Controls are children diagnosed with type 1 diabetes and treated at a TEDDY clinical center. Controls will be approached by their TEDDY treating physician for participating in this study and if interested will be invited to participate. Controls will be matched by age and their clinical center location*.

Subject recruitment

Children diagnosed with type 1 diabetes through TEDDY will be followed by the primary care practitioner, depending upon the local type of health care system, for care of their diabetes. In addition, they will be informed of a follow-up study that is in place and referred to the study coordinator for the TEDDY follow-up study. We will make every attempt to enroll all patients diagnosed with type 1 diabetes through TEDDY in the follow-up study.

Children diagnosed with type 1 diabetes external to TEDDY and followed at a TEDDY clinical center will be informed of this study shortly after diagnosis of diabetes. They will be identified as eligible based on matching with the cases by age of diagnosis and clinical center location[2]. Participants who meet the inclusion criteria will be eligible for enrollment.

[2] Washington controls may not be matched by clinical center.

Inclusion Criteria

Inclusion criteria for TEDDY case subjects:

1. Participated in regular follow-up through the TEDDY study, i.e. seen within a year prior to diagnosis, and enrolled within 3 months of diagnosis.
2. Diabetes diagnosed:
    a. with symptoms of diabetes (e.g. polyuria, polydipsia) and confirmatory blood sugar greater than or equal to 200 mg/dL (11.1 mmol/L).
    b. fasting glucose greater than or equal to 126 mg/dL (7 mmol/L) and/or random blood sugar greater than or equal to 200 mg/dL (11.1 mmol/L) at least twice.
    c. abnormalities of oral glucose tolerance testing (OGTT) with fasting glucose greater than or equal to 126 mg/dL (7 mmol/L) and/or 2 hour post blood sugar greater than or equal to 200 mg/dL (11.1 mmol/L) at least twice.
    d. unequivocal hyperglycemia with acute metabolic decompensation (diabetic ketoacidosis).
3. Informed consent and assent of subjects where appropriate
4. Children greater than or equal to age 3 will be eligible.

Inclusion criteria for Control subjects:

1. Diabetes diagnosed:
    a. with symptoms of diabetes (e.g. polyuria, polydipsia) and confirmatory blood sugar greater than or equal to 200 mg/dL (11.1 mmol/L).
    b. fasting glucose greater than or equal to 126 mg/dL (7 mmol/L) and/or random blood sugar greater than or equal to 200 mg/dL (11.1 mmol/L) at least twice.
    c. abnormalities of oral glucose tolerance testing (OGTT) with fasting glucose greater than or equal to 126 mg/dL (7 mmol/L) and/or 2 hour post blood sugar greater than or equal to 200 mg/dL (11.1 mmol/L) at least twice.
    d. unequivocal hyperglycemia with acute metabolic decompensation (diabetic ketoacidosis).
2. Autoimmunity documented with positive GAD65, IA-2, ZnT8 and/or insulin autoantibodies within the first 3 months of diabetes onset[3].
3. Matched to case subjects by age of diagnosis within one year and clinical center location[4].
4. Followed and recruited in the clinic with informed consent and assent of subjects where appropriate.
5. Did not participate in any other prospective studies such as TrialNet, DAISY, TRIGR, etc.
6. Children greater than or equal to age 3 will be eligible.
7. Enrolled within 3 months of diagnosis.

Exclusion criteria:

1. Not diagnosed with diabetes.
2. Do not provide informed consent.
3. Children less than 3 years of age.
4. The parent or primary caretaker refuses to have the child's samples stored at the Central Repository.

Duration of the study

The planned duration of the study is 5 years with recruitment during the first 3 years. The minimum duration of participation for each subject will be 2+ years or until loss of c-peptide production. The

[3] Autoantibody results from the samples collected at the first visit of this study will be used to determine if the control subject meets this inclusion criterion or not; the control subject will be allowed to enroll in the study and will be disenrolled should the first visit's autoantibody results be deemed negative.

[4] Washington controls will not be matched by clinical center, since they will be recruited at the Colorado site.

goal is to follow all subjects until loss of C-peptide production. HbA1c data will be collected at one year and two years after loss of c-peptide production.

It is anticipated that at the end of the 5 year project, application will be made to continue the study. As noted in Table 1, some TEDDY children have already developed diabetes. We propose to enroll all such children who have developed diabetes within 3 months of the time this follow-up study begins (expected number 15). The TEDDY protocol provides for comprehensive data collection at diagnosis and up to 6 weeks post diagnosis. For these subjects the TEDDY post diagnosis visit will be used in lieu of the Follow-up Study baseline visit. Prospectively, if we allow recruitment to extend over three years, we would expect another 57 cases to be identified by TEDDY sites, bringing the total anticipated enrollment of cases to 72. We anticipate that 60 of these cases will be at centers participating in recruiting community controls, with the Denver site possibly enrolling 12 controls to match the Seattle center's cases. Thus, we aim to recruit 72 community controls matched by age to the TEDDY cases, with at least 60 of those controls matched by clinical center location. Allowing for a 15% dropout rate, we aim to retain 60 subjects in the TEDDY case group and will enroll 60 community controls.

C. Description, Risks, and Justification of Procedures and Data Collection Tools

Study procedures

Visits

Baseline visits for control participants will begin within 3 months of diagnosis of type 1 diabetes when metabolic control is generally attained. For cases the TEDDY post-diagnosis visit data will be utilized in lieu of the baseline visit. All subjects will then be followed at 3 months after diagnosis, 6 months, 12 months, 18 months, 24 months, 30 months and 36 months. If there remains C-peptide at the 36 month visit the subject will be followed every 6 months until the disappearance of C-peptide response or 60 months post-diagnosis.

At baseline and each subsequent visit, blood draws for fasting and stimulated C-peptide (if possible to obtain stimulated c-peptide at baseline visit), antibody determination and PBMCs will be performed (see below). For control participants HLA typing will be conducted at the baseline visit to assess participants for the HLA DR, DQ genotypes. At the visit closest to onset of diabetes, an onset questionnaire will also be performed describing the clinical course at onset of diabetes; additional questionnaires regarding insulin dose, interval changes in history, including diabetes treatment, height, weight and HbA1c will be obtained at all visits. Quality of life and psychological questionnaires will be obtained at enrollment and at 3-month, 6-month, 12-month, 24-month, and 36-month visits. For subjects with C-peptide remaining, the Quality of Life and psychosocial questionnaires will also be collected at the 48 month visits and 60 month visits. If participants are unable to fully complete psychological questionnaires at the baseline visit due to time constraints, they may take these questionnaires home and mail back to the clinical center within 2 weeks.

A one-time stool sample may be collected within 3 months of the diagnosis of type 1 diabetes, if possible. Participants may opt not to participate in the stool sample collections. This will not affect the participant's ability to participate in the study.

Following the 3 month, 6 month, 12 month,18 month, 24 month, 30 month, 36 month, 42 month, 48 month, 54 month and 60 month visits, participants will be asked to complete a 5-7 day period of continuous glucose monitoring (CGM) immediately following the visit with MMTT assessment of C-peptide. Participants may opt not to participate in the continuous glucose monitoring. This will not affect the participant's ability to participate in the study.

In case the optimal proposed blood volume is not available, particularly at the earliest time points, the priorities for blood samples are as follows:

1. MMTT (c-peptide, glucose)
2. Autoantibodies
3. HbA1c
4. PBMC
5. RNA
6. HLA
7. Storage

Table 3. Blood Draw Requirements by Study Visit

		Months After Type 1 Diabetes Diagnosis											
Sample Type	**Blood Collection Tube**	**Baseline Visit***		**3 months**		**6 months**		**12 months**		**18 months**		**24 – 60 months - Every 6 month Tests**	
Auto-antibodies /Serum storage (GAD65, IA-2, ZnT8)	SST (Red)	**4 ml**		**4 ml**		**4 ml**		**4 ml**		**4 ml**		**4 ml**	
		0.4ml tests	1.5ml store	0.4ml tests	1.5ml store	0.4ml tests	1.5ml store	0.4ml tests	1.5ml store	0.4ml tests	1.5ml store	0.4ml tests	1.5ml store
PBMC /Plasma Storage	CPT(Blue/ Black)	**6 ml**		**6 ml**		**6 ml**		**6 ml**		**6 ml**		**6 ml**	
		1-3 vials PBMC	3-4ml store	1-3 vials PBMC	3-4ml store	1-3 vials PBMC	3-4ml store	1-3 vials PBMC	3-4ml store	1-3 vials PBMC	3-4ml store	1-3 vials PBMC	3-4ml store
RNA	ABI (Blue)	**2.5 ml**		**2.5 ml**		**2.5 ml**		**2.5 ml**		**2.5 ml**		**2.5 ml**	
HbA1c	EDTA (Purple or Pink)	**0.25 ml**		**0.25 ml**		**0.25 ml**		**0.25 ml**		**0.25 ml**		**0.25 ml**	
MMTT c-peptide	EDTA (Purple or Pink)	**7.0 ml total**		**7.0 ml total**		**7.0 ml total**		**7.0 ml total**		**7.0 ml total**		**7.0 ml total**	
		1.0ml x 7 timept		1.0ml x 7 timept		1.0ml x 7 timept		1.0ml x 7 timept		1.0ml x 7 timept		1.0ml x 7 timept	
MMTT Glucose	Fluoride (Grey)	**7.0 ml total**		**7.0 ml total**		**7.0 ml total**		**7.0 ml total**		**7.0 ml total**		**7.0 ml total**	
		1.0ml x 7 timept		1.0ml x 7 timept		1.0ml x 7 timept		1.0ml x 7 timept		1.0ml x 7 timept		1.0ml x 7 timept	
HLA	EDTA (Purple or Pink)	**1.0 ml**											
Total Blood Volume		**27.75 ml**		**26.75 ml**		**26.75 ml**		**26.75 ml**		**26.75 ml**		**26.75 ml**	

***TEDDY Cases will use the post-diagnosis visit data from the TEDDY study in lieu of the baseline visit; all items listed are for controls only.**

Table 4. Visit Schedule and Summary of Contents

	Months after Type 1 Diabetes Diagnosis						
Sampling Frequency	**Baseline Visit***	**3 months**	**6 months**	**12 months**	**18 months**	**24 – 60 months – Every 6 month Tests**	**24 – 60 months – Yearly Tests**
Blood Samples Collected	X	X	X	X	X	X	
Registration Form	X+						
Enrollment Form	X+						
Diagnosis of Diabetes Form	X						
Medical History Form	X	X	X	X	X	X	
Demographic Form	X			X			X
Family History Questionnaire	X						
Diabetes Management	X	X	X	X	X	X	
Physical Exam	X	X	X	X	X	X	
Continuous Glucose Monitoring		X	X	X	X	X	
Stool		X†					
PedsQL for parents (complete age appropriate form)	X	X	X	X			X
PedsQL for children (complete age appropriate form)	X^	X^	X^	X^			X^
STAI for parents and Well being question for parents	X	X	X	X			X
STAI for children	X^	X^	X^	X^			X^
PIP for parents	X	X	X	X			X

*** For cases TEDDY Study post-diagnosis visit data will be used in lieu of the follow-up study baseline visit; all items listed are for controls only.**
+ Registration and Enrollment forms for TEDDY cases are not completed as part of the TEDDY post-diagnosis visit; these forms must be completed for the TEDDY cases within 3 months of diagnosis.
^ Should only be completed by children 8 years of age and older.
† For cases TEDDY Study diagnosis and post-diagnosis visit data will be used in lieu of the follow-up study stool sample collection; a one-time stool sample will be collected from controls within 3 months of T1D diagnosis.

Assays
All samples will be assayed for glucose, C-peptide, autoantibodies (GAD65, IA-2 and ZnT8) and hemoglobin A1c. Extra serum will be stored. PBMC and RNA collection will also occur at each visit and frozen for future analysis. Whenever possible, assays will be done in the same laboratories currently performing like assays for TEDDY. Glucose and C-peptide determinations will be made at the TrialNet metabolic laboratory. Samples will be stored at the NIDDK repository as they are for all TEDDY participants.

A major effort (6 mL at each visit) will be to prepare, freeze and store PBMC for future analysis. It is imperative that each participating center will determine the white blood cell count (WBC) of the fresh blood before freezing. The WBC differential count determines the number of each type of white blood cell present in the blood and should be carried out with a local cell hematology analyzer. The data will be expressed both as a percentage (relative numbers of each type of WBC in relationship to the total WBC) or as an absolute value (percentage x total WBC). The absolute value is more important than the relative value.

PBMC will be prepared according to the current TEDDY protocol and the cells frozen. Future analysis of the frozen cells will include a thawing step followed by recovery of cells and a total cell count. A cell hematology analyzer may be used to determine recovery of cells as well as the cellular composition of the thawed cells. Thawed cells will be stained with fluorescent antibodies against cell specific surface markers and subjected to both phenotyping and functional assay (such cytokine secretion assays or similar analysis) and gene expression studies using single-cell gene expression technologies (such as http://www.fluidigm.com/single-cell-expression.html or similar microwell-based methods to detect copies of mRNA transcripts directly from individual cells by one-step, single-cell, reverse transcription polymerase chain reaction (RT-PCR).Novel gene expression approaches to sorted subsets of lymphocytes should yield specific information and answer questions related to function rather than gene expression arrays of total PMBC. This may be particularly relevant to PBMC which have been frozen and then thawed.

Stool Sample Collection
For cases, stool sample data collected during the TEDDY Study diagnosis and post-diagnosis visits will be used.

For controls, the child's parent(s) will collect a one-time stool sample of at least 5g of the child's stool within 3 months of the diagnosis of type 1 diabetes. Samples will be collected in the three plastic stool containers provided by the clinical center.

In the United States, parents will send the containers at either ambient or +4°C temperature with guaranteed delivery within 24 hours in the appropriate shipping box to the NIDDK repository. In Europe, parents will send the containers at ambient or +4°C temperature with guaranteed delivery within 24 hours in the appropriate shipping box to the local center they are affiliated with. The European clinical center will store the stool samples and will send monthly bulk shipments of frozen stool to the NIDDK Repository.

Continuous Glucose Monitoring (CGM)
Within this study, participants will be asked to complete a 5-7 day (minimum 72 hours) period of continuous glucose monitoring immediately after the visit with MMTT assessment of C-peptide, using continous glucose monitors (CGM), other CGMs or flash glucose monitors (FGM). This will occur following the 3 month, 6 month, 12 month, 18 month, 24 month, 30 month, 36 month, 42 month, 48 month, 54 month and 60 month visits. Participants may opt not to participate in the continuous glucose monitoring. This will not affect the participant's ability to participate in the study.

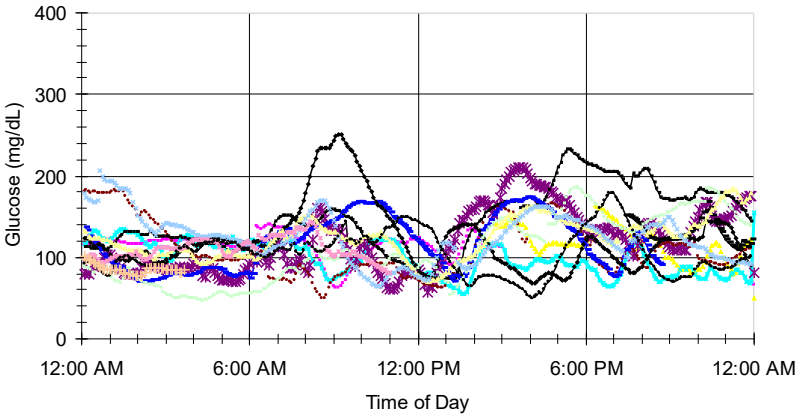
We anticipate that less than 5% of the participants will be using their own CGM at that time and they will not be switched to the study CGM sensor for the period. An increase in use of the FGM devices may also be noted in some areas. A CGM transmitter and receiver, sensors and in-person training by the study research nurse will be provided to the study participants on the day of the visit by a certified CGM trainer. In addition, the research nurse will be available by pager to answer questions or assist with problems during the 5-7 days of CGM. Our study teams have extensive experience with all currently available sensors: the Guardian® REAL-Time (MiniMed Medtronic), the SEVEN Plus ® System (DexCom) and the FreeStyle Navigator® (Abbott). The Barbara Davis center has played a key role in clinical trials testing the CGM technology.

For this study, we will select the best system available at the time, taking into account accuracy, long sensor life (7-10 days), and ease of download. Following the observation period, a study physician will review the CGM results with the participant and monitor the results for safety. Patients and their usual diabetes providers will be notified if significant hypoglycemia is observed, following standards used in our clinic. Participants will be given a glucose meter similar to the one they use and be asked to use the study's meter for all glucose measurements for one week while wearing the CGM.

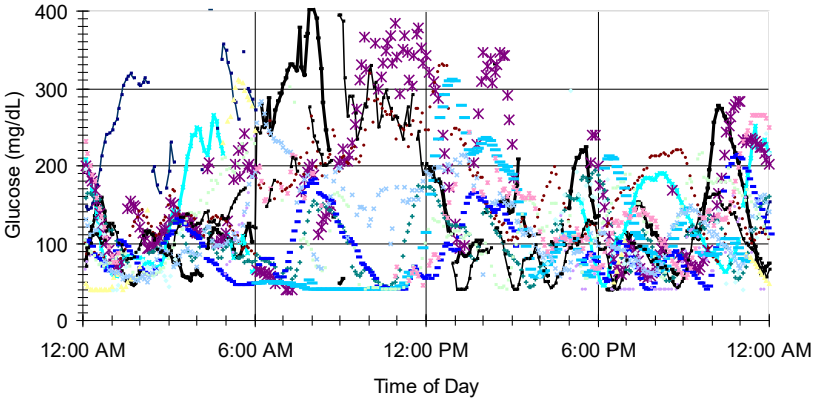
Measures of glycemic control will include HbA1c, the overall mean of glucose values, % of values within target range (60-180 mg/dl), % of values <60 mg/dl (hypoglycemic range), and % of values >180 mg/dl (hyperglycemic). Primary variables to characterize glycemic variability will include the overall standard deviation (SD), the mean of daily differences (MODD) and the mean amplitude of glycemic excursions (MAGE). While multiple additional computed measures have been proposed in this dynamically evolving analytical field, they do not appear to offer a particular advantage and we will limit the number of comparisons to reduce the chance of type I error.

Figure 3 provides contrasting patterns of glycemic control and variability in patients with T1D who used CGM in their daily living. Each line represents one day of monitoring. Pattern A illustrates the optimal diabetes control with good glycemic control (HbA1c 6.4%) and low glycemic variability; while Pattern B suggests good control (HbA1c 5.8%), but high variability, demonstrating the limitations of HbA1c as a sole measure of glycemic control.

Figure 3. Patterns of glycemic control and variability - examples from CACTI participants



A. Good control & low variability ID 2013; 3443 observations in 15 days	
Mean glucose	**117 mg/dl**
Overall standard deviation	**33 mg/dl**
% within 70 – 180 mg/dl	**96%**
HbA1c	**6.4%**



B. Good control & high variability ID 1049; 2805 observations in 15 days	
Mean glucose	**131 mg/dl**
Overall standard deviation	**72 mg/dl**
% within 70 – 180 mg/dl	**78%**
HbA1c	**5.8%**

In order to simplify the analyses, we selected only a few metrics to illustrate the proposed approach. Primary metrics of glycemic control will include: the overall mean of glucose values, the % of values within target range (60-180 mg/dl), and HbA1c. Primary metrics of glycemic variability will include: the overall standard deviation (SD), the mean of daily differences (MODD) and the mean amplitude of glycemic excursions (MAGE, not shown).

D. Human Subjects

Consent procedure

Upon identification, participants will be given a written consent form by qualified study personnel (the study coordinator and/or investigator or other designee). The personnel will understand the research study and will complete any necessary courses required by their Institutional Review Board prior to implementing the consent process. The consent process will occur in a private setting, and the participant will be given time to review the written consent form and ask questions prior to the initiation of study procedures. The Informed Consent Form for this clinical study will be reviewed with patients (and their guardian in the case of children patients) prior to performing any study-related assessments. Asking the participant to explain the study in his/her own words will assess the patient's understanding and autonomy. Qualified personnel as listed above will then obtain written consent prior to the initiation of study procedures. The consent form requests consent for each time the individual has blood drawn for these studies. The participant will be given their signed copy of the written consent form (and assent forms where applicable).

Assent procedure

An assent form has also been developed for participants as has been done for TEDDY. The assent process for each participant will be completed at an appropriate age as determined by the local IRB/Ethics board for each participating center. Those within that age range will be given the consent and assent forms requested and will have the opportunity to discuss the study apart from their parent(s) or guardian(s). This will allow these individuals to ask questions they might not have felt comfortable asking previously. In addition, the parent(s) or guardian(s) will be given the opportunity to discuss the study apart from the child or adolescent.

Authorization will be obtained during the Consent process by qualified personnel in the calm environment described above. The person obtaining authorization will explain the type of PHI that will be collected, how it will be stored and to whom it may be disclosed. If the patient agrees to authorize the use of their PHI for research, a signed and dated copy of the form will be provided to the subject.

Potential Risks

Patients may feel brief pain at the time of the needle stick for the blood draw. In about 10% of cases, a small amount of bleeding under the skin will produce a bruise. The risk of temporary clotting of the

vein is about 1% and the risk of infection of the bruise or significant external blood loss is less than 1 in 1,000.

The MMTT may result in transient hyperglycemia at end of study (BG>300 mg/dL). Subjects will be monitored for ketones and given insulin as prescribed by the investigator.

Continuous glucose monitoring (CGM) requires the insertion of a small plastic tube with a needle that is then removed. The small plastic tube stays in place for 5-7 days. There is a low risk of developing a local skin infection at the site of the sensor needle replacement. Itchiness, redness, bleeding and bruising at the insertion site may occur as well as local tape allergies.

Potential Benefits

While there may be direct benefit to subjects participating in this study due to more careful surveillance through study visits, the study is not designed to provide direct benefit to subjects participating in the study. There is the potential for indirect benefit if the studies reveal reasons for development or aggravation of islet autoimmunity. Early identification of these abnormalities may lead to prevention of disease or enhanced blood glucose control. Participation in DPT-1, DAISY, TEDDY and TrialNet has been shown to greatly reduce the severity of presentation and the risk of life-threatening diabetic ketoacidosis at the diagnosis of diabetes (10,11). Participants will be given their test results at the end of the study. This may be beneficial in assisting them and their health provider in diabetes management.

Participant enrollment may only begin with IRB approved consent forms. This is an observational study that meets the federal definition of minimal risk.

Study Oversight

The Study Chair has primary oversight responsibility of this protocol. Each site's Primary Investigator and their research team (co-Investigators, research nurses, clinical trial coordinators, and data managers) must be certified to conduct research in human subjects annually and are responsible for identifying adverse events. Aggregate report- detailed by severity, attribution (expected or unexpected), and relationship to the study protocol will be available from the Data Coordinating Center (DCC) at USF for site review. Adverse events will be reviewed once a month by the research team. A separate report detailing protocol compliance will also be available from the USF Data Coordinating Center for site review on a monthly basis. The research team will then evaluate whether the protocol or informed consent document requires revision based on these reports.

TEDDY investigators must comply with local institutional requirements regarding conflicts of interest and the need to inform subjects about any perceived conflicts should they have any.

Definitions And Standards

The study team defines an adverse event as: "…an unfavorable and unintended sign, symptom or disease associated with a participant's participation in the study."

Serious adverse events include those events that: "result in death; are life-threatening; require inpatient hospitalization or prolongation of existing hospitalization; create persistent or significant disability/incapacity, or a congenital anomaly/birth defects".

An unexpected adverse event is defined as any adverse experience…the specificity or severity of which is not consistent with the risks of information described in the protocol. Expected adverse

events are those that are identified in the research protocol as having been previously associated with or having the potential to arise as a consequence of participation in the study.

All reported adverse events will be classified using the Common Terminology Criteria for Adverse Events (CTCAE), version 4, developed and maintained by CTEP at the National Cancer Institute.

Reporting Timeline

• Within *24 hours* (of learning of the event), investigators must report any reportable Serious Adverse Event (SAE) that: Is considered life-threatening/disabling or results in death of subject -OR- Is Unexpected/Unanticipated.
• Investigators must report all other reportable SAEs within *5 working days* (of learning of the event).
• All other (suspected) reportable AEs must be reported to the Data Coordinating Center within *20 working days* of the notification of the event or of the site becoming aware of the event.

Local institutional reporting requirements to IRBs, any GCRC oversight committee remain the responsibility of the treating physician and the Study Chair.

Adverse Event Data Management System (AEDAMS)

The DCC has developed and monitors an Adverse Event System that enables a specified reviewer(s) to access and review information on reported Adverse Events. The system is designed to electronically capture data on reported Adverse Events, forward it to the AE reviewer, and organize the communication and subsequent actions (if applicable) related to each reported Adverse Event

Additional individuals (specified by the Steering Committee or NIH) can be automatically notified of all Adverse Events, although they have no network responsibilities in the Adverse Event review. An Administrator at the DCC monitors the use of the system and can manually assign Adverse Events to Reviewers or re-assign Adverse Events to other Reviewers if the Adverse Event is not reviewed in a reasonable time frame. The Administrator also assigns the roles (e.g. Reviewer, Reader) to individuals using the system.

Non-serious expected adverse events: Except those listed above as immediately reportable, non-serious expected adverse events that are reported to or observed by the investigator or a member of his research team will be submitted in a timely fashion (within 20 working days). The events will be presented in tabular form and given to the local sites for IRB submission on an annual basis. Local site investigators are required to fulfill all reporting requirements of their local institutions.

Study Discontinuation

This study will not have study discontinuation rules as it is an observational study. Subjects may discontinue their participation at any time.
Reasons for discontinuation:
• Withdrawal of consent
• Withdrawal by the participant
• Withdrawal by the investigator
• The parent or primary caretaker withdraw consent to future sample collection and the storage
• Intercurrent illness or event that precludes further visits to the study site or ability to evaluate disease.

All data acquired prior to termination for the reasons outlined above will be included in the primary analysis unless patient withdraws consent. In the case of adverse events associated with early

termination, every effort will be made to follow the participant clinically until, all adverse events resolve and to conduct a final study visit with the participant.

Data Quality and Monitoring Measures
Participants will be entered into the USF Data Coordinating Center's data management system without identifying information to protect confidentiality. The Data Coordinating Center uses a system of coded study identification numbers which protect confidentiality. This system is used extensively in TEDDY, TrialNet and other studies in which the Data Coordinating Center participates.

As much as possible data quality is assessed at the data entry point using intelligent on-line data entry via visual basic designed screen forms. Data element constraints, whether independent range and/or format limitations or 'relative' referential integrity limitations, can be enforced by all methods employed for data input. QA reports assess data quality post-data entry. As we note, data quality begins with the design of the data collection forms and procedures and incorporates reasonable checks to minimize transcription and omission errors. Of the more important quality assurance measures are the internal validity checks for reasonableness and consistency. In addition to those described above, we propose to build these checks into the initial tables and cross tabulations that should reveal any remaining data quality issues.
• Data Monitoring: The Data Coordinating Center identifies missing or unclear data and generates a data query to the enrolling center.
• Data Delinquency Tracking: The Data Coordinating Center will monitor data delinquency on an ongoing basis.

Costs, incentives and remuneration for study subjects
All study related costs are borne by the study protocol without charge to the subjects. We have requested $100 per study subject per visit as an incentive to complete study visits. The actual type and amount of incentive is subject to approval by the IRB at each participating site and varies domestically and in Europe.

E. Potential Scientific Problems

Potential problems we have considered include 1) Recruitment problems, 2) Difficulty scheduling/completing study visits, and 3) Inadequate blood samples. We anticipate enrolling up to 144 eligible children into the study (72 cases and 72 controls). Since the study protocol involves up to 4 visits in the first year, we may have difficulty enrolling all eligible children due to the time involved. However, based on our experience with the ongoing cohort studies from which children will be recruited, we anticipate a favorable response and participation from parents of eligible children. We have also considered that some study participants may be as young as 3 years of age; the volume of blood that can be drawn will be limited and there is the possibility that inadequate blood samples/volume will be collected in some cases, particularly if children are dehydrated because of fasting. We will address this potential problem by recruiting a large enough sample to allow for some missing data, instructing parents to keep children hydrated prior to visits, and by prioritization of blood samples to be drawn in order to capture the most important samples.

F. Data Analysis Plan for TEDDY Cases and Community Controls

This is a case-control study. To improve study efficiency (primarily, for efficient control of confounders), the stratified sampling method will be used for participant selection. As age at diagnosis is an established confounder, the age distribution of subjects in community

group will be matched to the TEDDY group based on current data available by the TEDDY site to prevent confounding issue at start of follow-up.

Preservation of stimulated C-peptide over time will be the primary outcome of the study. Preservation of C-peptide will be examined both as a continuous outcome (mean C-peptide over time) and as a binary outcome (C-peptide value > 0.6 ng/ml: yes/no). Potential confounders such as HLA types and BMI will be adjusted in the model as secondary analyses.

For continuous outcome measures, we will use a linear mixed effects model to take into account within subject correlation of an outcome. Considering both random slope and random intercept, the model will test whether the slope of the outcome over time in the TEDDY group is the same as that in the community group. To fit the model, PROC MIXED procedure with Kenward-Roger correction will be implemented since the correction is known to perform better with missing data in terms of keeping the type 1 error close to the nominal significance level. Maximum likelihood estimates (MLE) will be obtained for regression coefficients for fixed effects and restricted MLE for the variance component. A linear predictor will be obtained for the random intercept and random slope to identify individuals with steeper loss in C-peptide. The sample sizes of 50 in each group is expected to achieve 88% power to detect at least 0.02 slope difference between two groups in this design with 7 repeated measurements assuming unit variance and a compound symmetry covariance structure at two-sided test allowing 5% of type 1 error rate (23).

For binary outcome measures, the method of generalized linear mixed effects model will be used to compare stimulated c peptide over-time between two groups, accounting for correlations among observations from the same subjects. C-peptide values above 0.6 ng/ml have been shown to correlate with better clinical outcome, i.e. lower rates of severe hypoglycemia, microalbuminuria and retinopathy.

Secondary outcome measures including quality of life, presence of diabetic ketoacidosis, hemoglobin A1c, presence of islet cell antibodies and insulin dose throughout the study will be collected and analyzed to study their interrelationship. Glycemic control and variability will also be assessed using continuous glucose monitoring following the 3, 6, 12, 18, 24, 30, 36, 42, 48, 54 and 60 month visits. Quality of life questionnaires will be compared between two cohorts. The change over the entire follow-up period for each outcome will be compared using a two-sample t-test or Wilcoxon Rank-SumTest as appropriate. We will also evaluate change from baseline to 1 year, as well as at each time point. Changes will also be compared between the groups using a general linear model to adjust for baseline levels and other confounder in the model. To study the pattern of change over time, the mixed effects modeling described above will be carried out. Time-dependent covariates will be also considered as additional analyses.

If data (visits) are missing, the analyses will be performed in several ways: We will first analyze the data assuming missing completely at random. However, when the missingness depends on the outcome, the parameter estimation will be most likely biased. As a secondary analysis, we will investigate the missing data mechanism given observed outcomes. When the missingness depends on the set of observed outcomes, a correctly specified covariance structure can accommodate the situation. But if the missingness is due to a specific outcome value that should have been obtained at the time, we will do a sensitivity analysis under various plausible assumptions concerning the missingness process. Dropout is also considered in a monotone missing data pattern. When the dropout is completely at random or unrelated to all future outcome values, an imputation will be incorporated to fill out the missing data. However, when the dropout depends on current and future unobserved outcomes, there will be no standard approach to accommodate this situation.

Data Analysis Plan for TEDDY Cases

Earlier diagnosis will be considered as HbA1c <6.5% measured within 90 days of the diagnosis or asymptomatic at the diagnosis. Applying that, about 50% of current TEDDY T1D cases were earlier stage of diagnosis. Preservation of stimulated C-peptide over time will be the primary outcome of the study. Preservation of C-peptide will be examined both as a continuous outcome (mean C-peptide over time) and as a binary outcome (C-peptide value > 0.6 ng/ml: yes/no). Potential confounders such as HLA types and BMI will be adjusted in the model as secondary analyses.

For continuous outcome measures, we will use a linear mixed effects model to take into account within subject correlation of an outcome. Considering both random slope and random intercept, we will examine whether the C-peptide decline over time was different between earlier and later diagnosis of T1D. To fit the model, PROC MIXED procedure with Kenward-Roger correction will be implemented since the correction is known to perform better with missing data in terms of keeping the type 1 error close to the nominal significance level. Maximum likelihood estimates (MLE) will be obtained for regression coefficients for fixed effects and restricted MLE for the variance component. A linear predictor will be obtained for the random intercept and random slope to identify individuals with steeper loss in C-peptide.

As current TEDDY data suggests, we assumed an equal number of earlier and later diagnosis of T1D. The total sample size of 60 will achieve greater than 80% power to detect at least 0.005 slope difference between two groups, at two-sided test allowing 5% of type 1 error rate (23). In this design with 7 repeated measurements, we assumed within subject correlation of 0.5 and the variance of 0.09.

For binary outcome measures, we will employ the generalized estimating equation with the logit link function to take into account within subject correlation. C-peptide values above 0.6 ng/ml have been shown to correlate with better clinical outcome, i.e. lower rates of severe hypoglycemia, microalbuminuria and retinopathy.

Secondary outcome measures including quality of life, presence of diabetic ketoacidosis, hemoglobin A1c, presence of islet cell antibodies and insulin dose throughout the study will be collected and analyzed to study their interrelationship. Glycemic control and variability will also be assessed using continuous glucose monitoring following the 3, 6, 12, 18, 24, 30, 36, 42, 48, 54 and 60 month visits. For secondary outcomes measured repeatedly, we will apply those methods for primary outcome analysis to consider within subject correlation as described above. Some of these measures will be interesting to compare before and after diagnosis of T1D (e.g. quality of life questionnaires). For the comparison of changes between earlier and later diagnosis, a two-sample t-test or Wilcoxon rank-sum test as appropriate. For comparison of proportions, chi-square test or Fisher's exact test as appropriate. In the participants, comparisons will be made of T-cell activity, gene expression, and decline of c-peptide prior to antibody development, post antibody development, post diagnosis of type 1 diabetes during which insulin secretory capability continues and throughout decline of c-peptide.

If data (visits) are missing, the analyses will be performed in several ways: We will first analyze the data assuming missing completely at random. However, when the missingness depends on the outcome, the parameter estimation will be most likely biased. As a secondary analysis, we will investigate the missing data mechanism given observed outcomes. When the missingness depends on the set of observed outcomes, a correctly specified covariance structure can accommodate the situation. But if the missingness is due to a specific outcome value that should have been obtained at the time, we will do a sensitivity analysis under various plausible assumptions concerning the missingness process. Dropout is also considered in a monotone missing data pattern. When the

dropout is completely at random or unrelated to all future outcome values, an imputation will be incorporated to fill out the missing data. However, when the dropout depends on current and future unobserved outcomes, there will be no standard approach to accommodate this situation.

G. Summarize Knowledge to be Gained:

Knowledge to be gained
The information gained through this study will better define the benefit experienced by participating in long term studies of the natural history of type 1 diabetes. It will define if patients who participate in these studies have a prolonged C-peptide production which may translate into fewer diabetes related complications.

Expected Results
We expect to determine the duration of C-peptide production in the group followed for type 1 diabetes in the TEDDY study compared with those diagnosed with type 1 diabetes in the community. We expect that the level of C-peptide and duration of response will be greater in the population diagnosed through TEDDY compared with community controls. Furthermore, we expect to determine the slope of the decline of c-peptide in TEDDY cases prior to antibody development, post antibody development, post diagnosis of type 1 diabetes during which insulin secretory capability continues and throughout decline in c-peptide.

H. Milestones and Deliverables

This project is designed to accrue subjects for three years and follow them until the loss of C-peptide. The budget and study visit schedule is built upon an assumption that many will lose C-peptide production by 24 months following diagnosis and the last study visit is planned at 36 months after enrollment (if there remains C-peptide at the 36 month visit the subject will be followed every 6 months until the disappearance of C-peptide response or 60 months post-diagnosis.). Children will be enrolled at diagnosis, but a window of time is provided in that the TEDDY cohort includes one post diagnosis visit. Community controls will be enrolled at diagnosis. Three TEDDY centers will participate in the case-control portion of this study because they have strong links to primary care providers who will treat T1D in a consistent fashion, thereby reducing treatment-related variability. If the Seattle center can only enroll cases for the secondary analysis of comparison between cases diagnosed through TEDDY, then the Denver site will be asked to enroll community controls to match Seattle cases (subject to review and coordination with the Data Coordinating Center). The enrollment by site is projected to be:

T1D Cases	Cases Projected over the next 3 years	Controls Projected over the next three years	Total Projected Study Subjects over the next three years	Total Projected Study Subjects over the next three years (assuming approximately 75% enroll)
Denver	19	19 (possibly 16 others)	38 (possibly 54)	28 (possibly 40)
Finland	26	26	52	40
Sweden	34	34	68	52
Seattle	16	0 (possibly 16 controls)	16 (possibly 32)	12 (possibly 24)
Total	95	95	190	144

Our projections are to enroll 72 cases and 72 control subjects. Allowing for a 15% dropout rate, we aim to have 60 subjects in the TEDDY case group and 60 controls followed through the course of the study.

The deliverables follow from the planned study visits and we will monitor feasibility and compliance with study visits and samples during the course of the study. The analysis deliverable will occur at the end of the study when sufficient data have been accumulated.

As noted elsewhere, the TEDDY population is unique in being able to assess the question of the effect of close monitoring and early diagnosis.

Should our results demonstrate the benefit of close monitoring and early diagnosis, we believe that we will be able to recommend changes in the standard of care for children identified as having pre-diabetes and improve the course of their disease.

Dr. Krischer, as PI, agrees to accept responsibility for the scientific and technical conduct of the research project and agrees to all terms and conditions of the award.

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