TEDDY Protocol

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TEDDY Protocol

1. Introduction

OBJECTIVE:

The primary objective(s) of this multi-center, multi-national, epidemiological study will be identification of infectious agents, dietary factors, or other environmental exposures that are associated with increased risk of autoimmunity and T1DM. Factors affecting specific phenotypic manifestations such as early age of onset or rate of progression, or with protection from the development of T1DM will also be identified.

ORGANIZATION:

A network of collaborating investigators following common protocol(s) was created to allow for a coordinated, multi-disciplinary approach to this complex problem.

- **Clinical Centers (6 participating centers):**
  - **Colorado** Barbara Davis Center, Univ. CO, Denver, CO
  - **Finland** University of Turku, Turku, Finland
  - **Georgia/Florida** Augusta University, Augusta, GA
  - **Germany** Diabetes Research Institute, Munich, Germany
  - **Sweden** Lund University, Malmö, Sweden
  - **Washington** Pacific Northwest Diabetes Research Institute, Seattle, WA
- **Data Coordinating Center -** University of South Florida, Tampa, FL

DESIGN:

- **Type:** Observational cohort study
- **Inclusion criteria:**
  - Newborns with high risk HLA in the general population or having a first-degree relative affected with T1DM.
  - Newborns are less than 4 months of age
- **Randomization and masking:** None
- **Stratification:** Clinical Centers enroll newborns from both the general population and families already affected by T1DM.
- **Sample size:** Projected - 361,588 cases screened, 17,804 eligible, 7,801 followed; Actual – 424,788 cases screened, 21,589 eligible, 8,677 followed
- **Duration:** 20 years (5 years of recruitment and follow-up of subjects to age 15)

SCHEDULED VISITS:

- **Pregnant mothers (optional):** blood sample is obtained at 12-14 and 25-28 weeks gestation, and at birth.
- **Newborns:** cord blood or the newborn blood sample is obtained for HLA typing. Parents of newborns with genetic high-risk of T1DM are approached for consent to participate in follow-up.
- **Follow-up visits:** visits 1-16 are scheduled at 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, and 48 months of age. At 4 years of age those children who have been deemed persistent autoantibody positive will remain on the three month visit schedule; subsequent visits for all other subjects are every six months. Blood samples are taken at each visit.
- **Infectious agents sampling:** parents collect monthly stool samples up until 48 months of age, after this stool samples are collected every three months until 10 years of age and then every six months after 10 years of age; it was decided to stop all stool sample collections from all subjects in August 2018. Beginning at 9 months of age a minimally invasive nasal swab sample will be collected from each TEDDY subject and will continue to be collected at each visit thereafter.
- **Questionnaires, charts and the TEDDY Book:** parents fill out questionnaires at regular intervals in connection with visits. TEDDY study personnel complete charts at each visit. Parents record events in the child's TEDDY Book.

OUTCOME MEASURES:

- **First Primary Outcome:** appearance of one or more islet cell autoantibodies: GADA, IAA, or IA-2A confirmed at two consecutive visits.
- **Second Primary Outcome:** development of T1DM

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REPOSITORY:
The NIDDK central repository will be used for data and biologic samples saved for subsequent hypothesis based research

TIMETABLE:

- **Recruitment:** 1-5 years (2004-2009)
- **Data collection:** 20 years (2004-2024)
- **Close out:** at age 15 (2019-2024)

2. Background of the Study

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute of Allergy and Infectious Disease (NIAID), National Institute of Child Health and Human Development (NICHD), National Institute of Environmental Health Sciences (NIEHS), Juvenile Diabetes Research Foundation (JDRF), and Centers for Disease Control and Prevention (CDC) have established a consortium of six Clinical Centers (CC) and a Data Coordinating Center (DCC) to develop and carry out studies to identify environmental causes of Type 1 Diabetes Mellitus (T1DM) in genetically susceptible individuals.

T1DM is one of the most common and serious chronic diseases in children and is also often diagnosed in adults, affecting up to 1% of the general population during their lifespan (Green, Patterson, 2004; Onkamo, 1999). The incidence of T1DM is highest in Scandinavia (30-50/100,000), intermediate in the U.S. (15-25/100,000 in 1998) and somewhat lower in Central and Eastern Europe (5-15/100,000). These geographic differences may reflect variation in the genetic susceptibility pool, in prevalence of causal environmental factors, or both. The etiology of T1DM remains unknown and the incidence is increasing by 3-5% per year, particularly in young children (Onkamo et al., 1999). While there is a strong familiar clustering of the cases, approximately 90% of the patients have no first-degree relative with T1DM (Dahlquist et al., 1989). Genetic variability in the HLA region explains ~50% of the familiar clustering (Risch, 1989; Davies et al., 1994); other genes have also been identified as pro
viding more modest contributions to risk (Davies et al., 1994; Cox et al., 2001). Additional factors are important, because only 1/15 people in the general population with the highest risk HLA genotypes develops T1DM. The cause(s) of T1DM have not been definitively identified.

Several studies have shown that gestational events contribute to an increased risk of T1DM. The most prominent example is exposure to rubella during pregnancy. About 20 % of children born with congenital rubella develop type 1 diabetes (Menser et al., 1978; Ginsberg-Fellner et al., 1985). More recent studies have shown an increased risk for childhood T1DM if the mother has had an infection with enteroviruses during pregnancy (Dahlquist et al., 1995; Hyoty et al. 1995). Other events during pregnancy or at delivery such as preeclampsia also confer T1DM risk (Dahlquist et al., 1989; Jones et al., 1998; Dahlquist et al., 1999). High birth weight and children born large for gestational age have a higher risk of T1DM than controls (Dahlquist et al., 1996; Stene et al., 2001). Neither of these phenomena are understood and it may be necessary to analyze them in relation to genetic incompatibilities between mother and child such as non-inherited maternal haplotypes (Kockum et al., 1994; Hampe et al., 2002) or blood incompatibility such as ABO-incompatibility was related to an increased risk for T1DM (Dahlquist and Kallen, 1992).

The risk that a child will develop T1DM is increased when born to a T1DM mother compared to the general population. However, the risk of the child developing the disease is higher when born to a T1DM father. Thorough studies have been carried out in the BABYDIAB project to investigate neonatal factors that are associated with the appearance of islet autoantibodies and sometimes T1DM during postnatal follow-up (Ziegler et al., 1999). However, little is known about the consequences for islet autoimmunity or T1DM development when born to a mother with subclinical organ-specific autoimmunity such as a mother with thyroid or islet autoantibodies but not necessarily an autoimmune disease.

In most cases, overt diabetes is preceded by the presence of autoantibodies to islet antigens such as GADA, IAA, and IA-2A. This pre-clinical period, ranging from months to years, provides an opportunity for prevention. However, interventions applied after development of autoimmunity have been so far unsuccessful (Canadian-European Randomized Control Trial Group, 1988; DPT-1, 2002). Interventions initiated before the onset of autoimmunity and significant loss of insulin secretory capacity may be more effective, but may have to be applied in the first few years of life when pre-diabetic autoimmunity develops (Gale, 1996).

Current understanding of T1DM etiology and the preliminary intervention data originate almost exclusively from studies of first-degree relatives of T1DM patients. These data may not be directly applicable to the causes and prevention of T1DM in the general population where 85-90% of the patients occur (Dahlquist et al., 1989). The presence of gene-environment interactions may explain the observed weak effects of candidate environmental agents and genes on T1DM risk. Without accounting for these interactions, we may not detect the true main effects of either the environmental agent or gene.

Approximately 90% of all T1DM patients have either the DRB1*03,DQB1*0201 or the DRB1*04,DQB1*0302 haplotype. While the DRB1*0301,DQB1*0201/ DRB1*04,DQB1*0302 heterozygotes account for only about 3% of the general population, this genotype is present in 30-40% of T1DM patients and in up to 52% of those who develop diabetes in the first 10 years of life (Veijola et al., 1996; Sanjeevi et al., 1995; Baisch et al., 1990). Thus, a great deal could be learned about the causes of T1DM by studying the interactions between plausible environmental causes and the HLA-DR, DQ genotypes.

T1DM has been associated with enteroviral infections (Gamble et al., 1969; Yoon, et al., 1975; Roivainen et al., 2000; Wagenkneckt et al., 1991; Graves et al., 1997; Hyoty and Taylor, 2002) rotavirus (Honeyman et al., 2000) and herpes viruses (Banatvala et al., 1985; Pak et al., 1988; Ivarsson et al., 1993). However, there is lack of consistency in previous reports and it is plausible that non-diabetogenic strains of a virus may induce immunity to antigenetically similar diabetogenic strains and protect from T1DM. To test these hypotheses, large groups of young children at risk for T1DM need to be followed prospectively with collection of appropriate samples at frequent intervals. In addition, state-of-the-art techniques must be used for sensitive and specific detection of both microbial nucleic acids (to demonstrate current acute or persistent infection) and antibodies (to document past infection).

No specific bacterial agent has been linked with onset of T1DM or with diabetes-associated autoimmunity. However, bacterial superantigens have been suggested as possible non-specific immune stimuli that could play a role in development of prediabetic autoimmunity (Lan et al., 1998; Conrad et al., 1994). A number of bacteria have been proposed as sources of superantigens that might be relevant to onset of T1DM, including *Mycobacterium tuberculosis*, *Mycoplasma* species, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, and *Yersinia enterocolitica* (Lernmark et al., 1987).

Lack of breast-feeding (Virtanen et al., 1992; Borch-Johnsen et al., 1984) and early exposure to cow's milk (Akerblom et al., 1994) or wheat have been associated with T1DM. However, the findings from prospective studies have been inconsistent (Kimpimaki et al., 2001). Dietary vitamin D (EURODIAB, 1999) or cod liver oil (omega-3 fatty acid) supplementation (Stene et al., 2000) may be relevant, but needs to be evaluated prospectively using both intake information and biomarkers. According to a nested case-control observation, vitamin E may protect from T1DM (Knekt et al., 1999). N-nitroso compounds may increase the risk of diabetes (Dahlquist et al., 1990; Ferner, 1992), but the effect on human T1DM risk is less clear (Dahlquist, 1997). Exposure to mycotoxin has been recently suggested as another candidate environmental cause of T1DM (Myers et al., 2001).

Psychosocial factors may also contribute to appearance of T1DM. Stress has long been considered a potential trigger for TIDM (Danowski, 1963). Unfortunately, retrospective studies reporting an association between stressful life events and TIDM (Slawson et al., 1963; Stein, 1971; Kisch, 1985; Robinson, 1985; Robinson et al., 1989; Vialettes, 1989; Dahlquist et al., 1991; Hagglof et al., 1991; Thernlund et al., 1995) are methodologically flawed. Prospective studies are needed to more effectively address this issue. Further, screening for high-risk genes associated with T1DM could induce anxiety and distress in family members (Johnson, 2001). As the children grow older, they too may become concerned about their vulnerability to TIDM. It is important that we assess the psychological impact of genetic screening and long-term follow-up of at-risk children on both the children and their families.

Results from previous studies have been confounded by imprecise assessment of exposure, recall bias, failure to account for genetic susceptibility, failure to assess exposures at very early ages or the inability to follow a sufficient sample of children long-term with high intensity. The present multi-center study will provide an opportunity to fill important gaps in our understanding of the events leading to T1DM by studying from birth high-risk general population children and relatives and by systematic screening of candidate environmental and genetic factors. We will apply "cutting edge" molecular immunologic and genetic techniques to samples collected in six cohorts of high-risk children. In addition, samples collected by TEDDY will create a valuable resource for investigators proposing innovative hypotheses concerning candidate environmental and genetic factors.

The long-term goal of the TEDDY study is the identification of infectious agents, dietary factors, or other environmental agents, including psychosocial factors which trigger T1DM in genetically susceptible individuals or which protect against the disease. Identification of such factors will lead to a better understanding of disease pathogenesis and result in new strategies to prevent, delay or reverse T1DM.

3. Study Organization

The TEDDY Consortium will allow for a coordinated, multi-disciplinary approach to this complex disease. Collection of information and samples in a standardized manner will achieve greater statistical power than smaller independent investigations. The TEDDY study will establish a central repository of data and biologic samples for subsequent hypothesis based research.

The DCC is responsible for support of the study protocol and manual of operations, for communication and coordination among the CCs, and for managing the collection and analysis of genetic, immunologic, pathogen, and clinical data. The DCC will establish the data acquisition, transfer, and management system; develop procedures for ensuring subject confidentiality and safety; develop procedures for quality control, training, and certification; develop and produce a manual of operations and other study materials; and supervise the orderly collection and transmission of data.

The CCs will recruit and enroll subjects, including obtaining informed consent from parents prior to or shortly after birth, obtaining genetic and other samples from neonates and parents, and prospectively following selected neonates throughout childhood or until development of islet autoimmunity or T1DM. The CCs will collect and transmit genetic and other samples and familial and clinical data as delineated in the manual of operations.

3.1. Site Description

3.1.1. Denver/Colorado

The Colorado Center includes investigators located in two departments of the School of Medicine: Barbara Davis Center for Childhood Diabetes/Pediatrics (Rewers, Eisenbarth, Gottlieb, and Fiallo-Scharer) and the Preventive Medicine and Biometrics Department (Norris, Baxter). Since 1993, these investigators have worked together on the Diabetes Autoimmunity Study in the Young (DAISY) – a large epidemiological study similar in principle to TEDDY.

General Population recruitment: Blood and informed consent will be obtained for HLA typing from 66,844 newborns from the St. Joseph Hospital (previously the site of DAISY) and 7 to 10 additional Denver hospitals. The newborns screened will be representative of the general population of the Denver Metropolitan Area. Approximately 3,300 high-risk newborns will be eligible for follow-up and an estimated 42% of those will enroll.

First-degree Relatives recruitment: Young siblings and offspring of persons with T1DM will be recruited from families of diabetic children seen in the Barbara Davis Center or The Children's Hospital in Denver; other practice sites where diabetic parents may seek care (e.g. high risk OB and adult endocrinology practices, families of diabetic

children identified by the Colorado IDDM Registry and more recently the SEARCH project, and media publicity. In addition, some (0.6%) of the newborns screened at the three area hospitals will have a parent or sibling with T1DM and will be enrolled in this cohort. Currently over 3,000 children and 2,000 young adults with T1DM receive their diabetes care at the Barbara Davis Center - the only specialized diabetes care facility for children in the Rocky Mountain Region. Over the five years, these screening efforts are expected to yield 824 FDR babies screened and approximately 113 enrolled.

3.1.2. Finland

The Finnish Clinical Center includes investigators located in Turku (J. Toppari, J. Ilonen), Oulu (R. Veijola), Tampere (H. Hyoty), and Helsinki (M. Knip, S. Virtanen) who have collaborated since 1994 on DIPP - a large on-going cohort study similar to TEDDY.

General Population recruitment: Following the model of the DIPP study, investigators will continue to screen all babies born in the cities of Turku, Oulu, and Tampere (approximately 11,000 births annually) using HLA Class II screening modified to reflect TEDDY eligibility criteria. Approximately 3,135 high-risk newborns without a first-degree T1DM relative will be eligible for follow-up and an estimated 44% of those will enroll.

First-degree Relatives recruitment: About 3% of the newborns (300 among 10,000 screened annually) will have a first-degree relative with diabetes. However, only those from Turku will be eligible for TEDDY from its beginning. Newborns with a T1DM first-degree relative in Oulu and Tampere will be enrolled into the TRIGR study for the first two years and thereafter into TEDDY. We expect that, over the TEDDY five year screening period, at least 230 of the newborns with a T1DM first-degree relative will be eligible for follow-up based on their high-risk HLA genotypes and that nearly 60% of them will enroll into prospective follow-up.

3.1.3. Georgia and Florida

This Clinical Center includes investigators located at Augusta University in Augusta (She, Muir, McIndoe) and at the University of Florida in Gainesville (Schatz). Since 1995, these investigators have worked together on the PANDA Study – a large epidemiological study similar in principle to TEDDY.

General Population recruitment: Blood and informed consent will be obtained for HLA typing from 68,000 newborns from the hospitals located in Augusta and Atlanta in Georgia and Gainesville, Florida. Approximately 2,320 high-risk newborns will be eligible for follow-up and an estimated 33% of those will enroll.

First-degree Relatives recruitment: Over the initial five years of TEDDY, we aim to screen 740 neonates who are first-degree relatives of patients with T1DM. Of those, 16.4% are expected to carry one of the ten T1DM high-risk genotypes detailed in the

Genetics section below. We anticipate that 50% of high-risk FDR families will participate in the TEDDY study.

3.1.4. Germany

This Clinical Center includes investigators located in Munich (Ziegler, Hummel) and in Milan (Bonifacio). Since 1989, the BABY-DIAB study initiated by Dr. Ziegler has prospectively followed offspring of persons with T1DM with objectives similar to those of TEDDY.

General Population recruitment: Over the five year screening period approximately 28,500 newborns will be recruited for TEDDY-screening and an estimated 1,100 of those will be eligible according to the TEDDY inclusion criteria and 30% of those are expected to agree to participate in the TEDDY study.

First-degree Relatives recruitment: Over the five years of screening approximately 300 neonates will be eligible for the study, who are first-degree relatives of patients with type 1 diabetes mellitus and who carry the T1DM high risk genotype defined by the TEDDY inclusion criteria.. This will require genetic screening of about 1600 newborns of mothers, fathers or siblings with T1DM from all regions of Germany (total population >70 million). Recruitment of first-degree relatives is through 63 obstetric departments, 135 pediatricians, and through patients themselves throughout Germany. This network of collaborating centers was generated for recruitment of relatives at birth into BABYDIAB; it is currently used for the recently initiated BABYDIET study. Around 300 offspring from mothers of fathers with T1DM were included per year into BABYDIAB through this network. TEDDY will include both offspring and siblings, and we estimate that a figure of 1,600 recruited for genetic screening over the five year period is achievable.

3.1.5. Sweden

The Swedish Clinical Center includes investigators located at the Clinical Research Center (CRC) at Lund University hospital MAS in Malmö (Lernmark Å, Lernmark B, Larsson H, Cilio, Ivarsson, Agardh, Andren Aronsson, Bianconi Svensson, Hansson A, Törn), and at the hospitals in Lund (Carlsson), Helsingborg (Neiderud), Kristianstad (Larsson), and Ystad (Jönsson). Recruitment is through almost 200 Maternity Health Care clinics and all five Maternity Clinics in the Skåne region. The Swedish Clinical Center has three TEDDY Clinics located in Malmö, Helsingborg and Kristianstad.

General Population recruitment: Each year approximately 9,000 newborns will be screened representing about 75% of all children born into a population of 1.18 million. Over the five years approximately 3,300 high-risk newborns without T1DM in a first-degree relative will be eligible for follow-up and an estimated 65% of those will enroll.

First-degree Relatives recruitment: About 2% of the newborns (200 among 9,200 screened annually) will have a first-degree relative with diabetes. We expect that

approximately 40 of these newborns with a T1DM first-degree relative will be eligible each year for follow-up based on their high-risk HLA genotypes. Over the period of five years, approximately 180 children with a T1DM first-degree relative will be identified and at least 65% of those are expected to enroll.

3.1.6. Washington State

This Clinical Center includes investigators located at the Pacific Northwest Diabetes Research Institute in Seattle. The DEWIT Study, a general population newborn screening effort based on the state-wide newborn screening program in place since 1999, has formed the basis for this center's effort.

General Population recruitment: This site plans to screen over five years, about 91,250 neonates from the general population. Recruitment will take place at the obstetrics wards of 18 Puget Sound area hospitals. It is estimated that more than 80% of families approached will consent to screening, resulting in the 91,250 total. About 3.9% will be eligible for intensive follow-up, based on the TEDDY eligibility criteria. We estimate that 37% of families with an infant who screened as eligible will agree to participate.

First-degree Relatives recruitment: Over five years, at least 750 infants within the first 3.99 months of life, who are first-degree relatives of patients with T1DM, will be screened. Recruitment is through obstetric departments, pediatricians, and through patients themselves throughout the Pacific Northwest and neighbouring states. A network of collaborating centers will be generated for recruitment of relatives at birth into TEDDY. This center has sampled approximately 1,000 mostly adult T1DM patients over the last 10 years, and actively identifies young children with new T1DM via a general population newborn screening efforts. By expansion of all these efforts, the center expects to screen a total of 750 FDR neonates. Of those, 26.7% are expected to carry one of the ten T1DM high-risk genotypes detailed in the Genetics section below, making them eligible for follow-up. About 75% of FDR families with a neonate deemed high risk after screening are expected to participate.

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4. Objectives

The primary objective of this study is to identify environmental factors that predispose to or protect from beta-cell autoimmunity and T1DM.

The secondary objectives include:

1. Identification of potential differences in the environmental determinants of T1DM across diverse populations and ethnic groups.
2. Identification of potential differences in the environmental determinants of T1DM between children with and without first-degree T1DM relatives.
3. Establishment of a central repository for data and biologic samples for subsequent hypothesis based research.
4. Exploration of psychosocial corollaries of the ascertainment of risk status for autoimmunity and T1DM in newborns.
5. Exploration of gene-environmental interactions.
6. Better understanding of the natural history of the disease.

5. Hypothesis

1. Initiation of persistent beta-cell autoimmunity and progression from beta-cell autoimmunity to diabetes is increased with:
   a. Exposure to a trigger factor during pregnancy, such as infections, preeclampsia, blood incompatibility, or birth weight.
   b. Differences in the timing of the introduction and/or the type of dietary constituents that include exposure to cereals or gluten, exposure to cow's milk during infancy and/or childhood, and short duration of breast-feeding;
   c. Lower intake of serum 25 hydroxyvitamin D in early infancy, vitamin E, anti-oxidants (e.g., carotenoids, ascorbic acid, selenium, or omega-3 fatty acids);
   d. Higher frequency of specific (e.g., enterovirus, rotavirus, or bacterial) infections, or non-specific childhood infections including those that exhibit molecular mimicry;
   e. Increased exposure to routine childhood immunizations and their timing;
   f. Environmental factors that may be contained in drinking water (e.g., low concentrations of zinc or high concentrations of nitrates, or lower pH levels);
   g. Exposure to household pets, and various allergies;
   h. Excessive weight gain;
   i. Increased psychological stress.

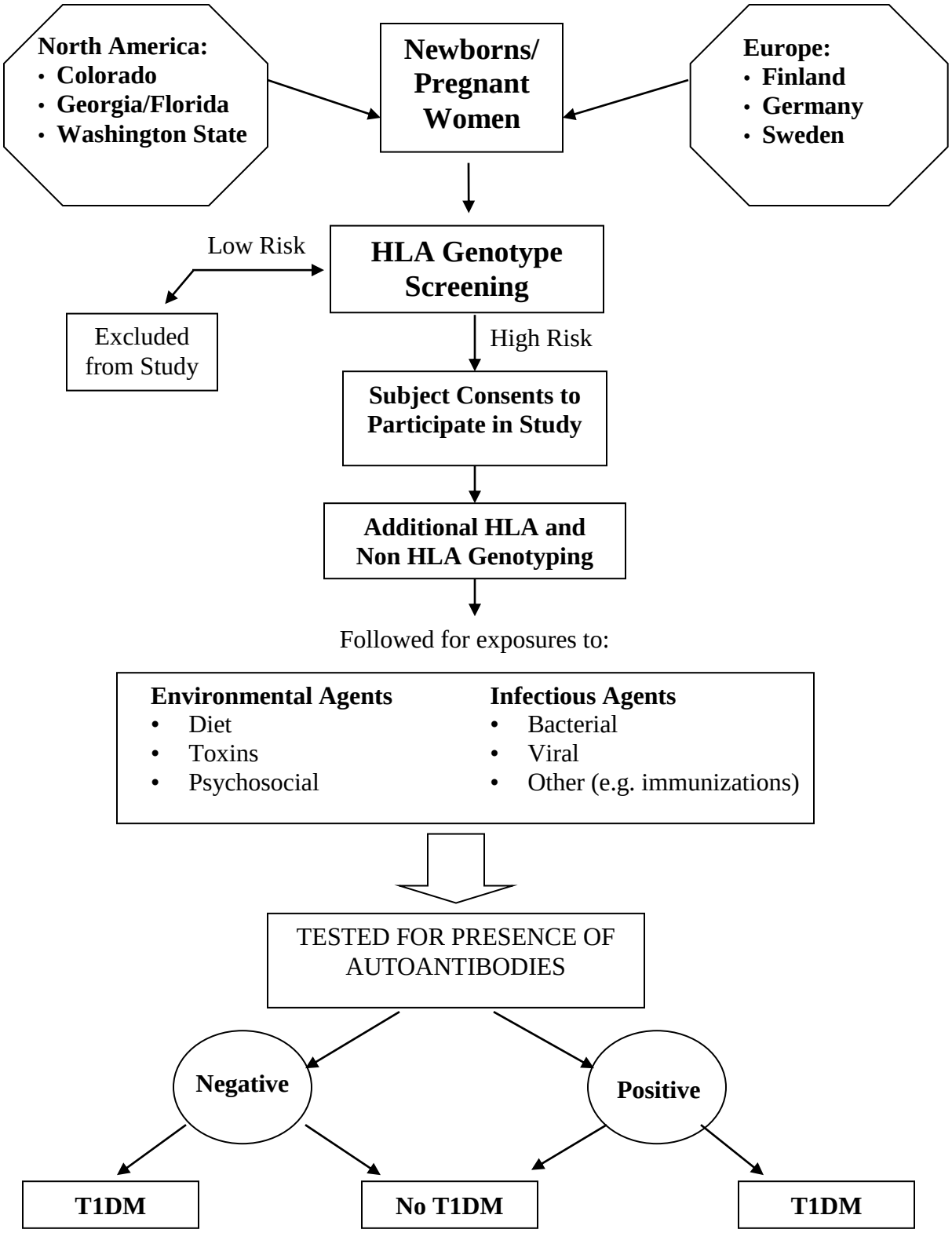
2. The risk of persistent beta-cell autoimmunity is lower in children from the general population than in offspring or siblings of T1DM patients when stratifying for the HLA DR-DQ genotype and exposure to environmental triggers.

3. The interaction of HLA DR-DQ genotype with exposure to dietary or infectious factors leads to increased incidence of beta-cell autoimmunity and T1DM.

4. We expect that in some families study participation will be associated with affective (anxiety, depression) and behavioral responses (e.g. actions to prevent possible T1DM).

6. Study Design

Schema 1: The Environmental Determinants of Diabetes in the Young (TEDDY) Study



6.1. Study population

A cohort of children with elevated genetic risk for T1DM will be established by screening newborns from the general population and from families with first-degree relatives diagnosed with T1DM.

6.1.1. Infants are eligible for screening if they:

- Are less than 4 months of age.
- Have a parent or primary caretaker who has given informed consent for screening.

Infants are excluded if:

- They have an illness or birth defect that precludes long-term follow-up or involves use of treatment that may alter the natural history of diabetes (e.g. steroids or insulin).

6.1.2. Infants from the general population are eligible for enrollment and long term follow-up if they:

- Have any one of the following HLA genotypes:
  a. DR4[&]-DQA1*030X-DQB1*0302[@] / DR3-DQA1*0501-DQB1*0201
  b. DR4[&]-DQA1*030X-DQB1*0302[@] / DR4[&]-DQA1*030X-DQB1*0302[@]
  c. DR4[&]-DQA1*030X-DQB1*0302[@] / DR8-DQA1*0401-DQB1*0402
  d. DR3-DQA1*0501-DQB1*0201 / DR3-DQA1*0501-DQB1*0201

  [@]Acceptable alleles in this haplotype include both DQB1*0302 and *0304
  [&]For general population subjects, DR4 subtyping must exclude DRB1*0403

  Each extended haplotype listed above must be accurately identified, which usually requires allele typing at two or more of the three genes. For subjects indicated as DR4 above, DR4 subtyping must be used to identify DRB1*0403 for exclusion. Screening centers may use methods to identify DRB1*0403 for General Population exclusion which do or do not separately identify DRB1*0407. TEDDY will allow but not require DRB1*0407 subjects to be excluded from the General Population follow-up.

- Have a parent or primary caretaker who has given informed consent for surveillance enrollment.

Infants are excluded if:

- They have an illness or birth defect that precludes long-term follow-up or involves use of treatment that may alter the natural history of diabetes (e.g. steroids or insulin).
- The parent or primary caretaker refuses to have the child's samples stored at the NIDDK Repository.

6.1.3. Infants who are first-degree relatives (FDR) are eligible for enrollment and long-term follow-up if they:

- Have any one of the following HLA genotypes:
  a. DR4- DQA1*030X-DQB1*0302[@] / DR3- DQA1*0501-DQB1*0201
  b. DR4- DQA1*030X-DQB1*0302[@] / DR4- DQA1*030X-DQB1*0302[@]
  c. DR4- DQA1*030X-DQB1*0302[@] / DR8- DQA1*0401-DQB1*0402
  d. DR3-DQA1*0501-DQB1*0201 / DR3-DQA1*0501-DQB1*0201
  e. DR4- DQA1*030X-DQB1*0302[@] / DR4- DQA1*030X-DQB1*020X
  f. DR4- DQA1*030X-DQB1*0302[@] / DR1[#]- DQA1*0101-DQB1*0501
  g. DR4- DQA1*030X-DQB1*0302[@] /DR13-DQA1*0102-DQB1*0604
  h. DR4- DQA1*030X-DQB1*0302 / DR4- DQA1*030X-DQB1*0304
  i. DR4- DQA1*030X-DQB1*0302[@] / DR9- DQA1*030X-DQB1*0303
  j. DR3- DQA1*0501-DQB1*0201 / DR9- DQA1*030X-DQB1*0303

  [@]Acceptable alleles in this haplotype include both DQB1*0302 and *0304
  [#]In this DQB1*0501haplotype, DR10 must be excluded. Only DR1 is eligible

- Each extended haplotype listed above must be accurately identified, which usually requires allele typing at 2 or more of the 3 genes. DR4 subtyping is not required for relatives.

- Have a parent or primary caretaker who has given informed consent for surveillance enrollment.

Infants are excluded if:

- They have an illness or birth defect that precludes long-term follow-up or involves use of treatment that may alter the natural history of diabetes (e.g. steroids or insulin).
- The parent or primary caretaker refuses to have the child's samples stored at the NIDDK Repository.

7. Screening procedures

7.1. HLA typing

7.1.1. HLA screening

Genotype screening will be performed using either a dried blood spot (DBS) punch or a small volume whole blood lysate (WBL) specimen format. Screening blood sample will be obtained generally at birth as a cord blood sample, but potential participants, especially first-degree relatives of T1DM patients can be screened using heel stick capillary sample up to the age of 4 months. This exception is made to maximize the number of newborn relatives participating in this study. The experience of the ongoing studies, such as DAISY and BABY-DIAB is that some families with a diabetic proband learn about those studies after

the baby is born, but are extremely interested in participation. These studies have also found that relatives are at a higher risk of T1DM than high-risk newborns from the general population, even stratifying for HLA-DR,DQ genotypes, and that these families are much less likely to drop out from prospective follow-up, thus particularly valuable to TEDDY. After PCR amplification of exon 2 of the HLA Class II gene (DRB1, DQA1 or DQB1), alleles will be identified either by direct sequencing, oligonucleotide probe hybridization, or other genotyping techniques. Additional typing to sufficiently identify certain DR-DQ haplotypes is as specified in Section 6.1 above.

7.1.2. HLA additional genotyping

Better definition of the HLA genotypes will be performed by the central HLA Reference Laboratory on 100% of the positive samples from the clinical centers. Additional high resolution HLA genotyping will be performed by the central HLA Reference Laboratory, on subjects who meet eligibility criteria for enrollment and follow-up, and who consent to participate in the study. High resolution HLA genotyping will occur at DRB1, DQA1, DQB1 and may also occur at DPB1, HLA-A, HLA-B, MIC-A and/or other MHC loci determined by the Steering Committee. The insulin 5'VNTR using the –23 Hph SNP will be typed by the HLA Central Laboratory on subjects who are enrolled in the study and consent to participate. A whole blood sample will be drawn from subjects at the 6, 9 12 or 15 month clinic visit for better definition and additional genotyping. Sites are encouraged to complete this collection by the earliest visit with a full volume blood draw, but in all cases by the 15 month visit. If the HLA confirmation sample is collected at the 6 month visit, only 0.5 mL of blood is required to be collected for this sample. If the HLA confirmation sample is collected at the 9, 12 or 15 month visit 1 mL of blood should be collected for this sample.

7.1.3. Screening Results

Before the HLA screening test, information about T1DM and the TEDDY study will be provided to families in an IRB-approved brochure. Added information is contained in the IRB-approved screening consent form, a copy of which will be given to each family. A study coordinator will explain these materials and answer any questions prior to the primary caregiver signing the screening consent. A similar but separate brochure will be used for families known in advance to have a T1DM family member. Patient materials for each site will be edited and approved by a professional diabetes genetic counselor hired by the TEDDY study.

Optional long term storage of cord blood samples from screened population: Clinical sites may opt to store the cord blood that remains after the HLA sample has been taken and shipped to the lab in order to make use of this sample for screening for other immunologic disorders and other studies. For those sites that will be storing the remaining cord blood sample additional informed consent

language is required in the screening informed consent that specifically consents participant's for this use and provides guidance to the study staff should the participant want to be kept informed of such testing. The information from this portion of the consent will be kept only at the local clinical center, which is also responsible for any tracking, storage, and subsequent use of this sample. Any future use of these samples is subject to approval by the TEDDY Ancillary Study Committee.

After the HLA screening test, families are not given genetic results as such, but instead a diabetes risk assessment, in many ways similar to discovering a family history of a disease. HLA-eligible general population subjects will be termed "high risk" (about 1 in 20) and HLA-ineligible general population subjects termed "not high risk" (same or lower than the average child, which is about 1 in 300). Risk levels for FDR families will be given as about 1 in 12 for HLA-eligible FDR subjects and about 1 in 50 for HLA-ineligible FDR subjects, respectively. Appropriate site specific risk estimates may be used depending upon local circumstances.

Information about T1DM and the TEDDY study will be provided to families of infants eligible for follow-up as an IRB-approved follow-up brochure. Additional information will be contained in the IRB-approved follow-up consent forms, a copy of which will be given to each family. Study personnel will be trained to provide additional education, to answer questions, and to refer families with continuing concerns to a senior study coordinator who can provide additional advice and counseling as needed. The Clinical Center Principal Investigator will be available to speak with parents who request this, or whom the senior clinical coordinator recommends this. All sites and Principal Investigators have many years of experience and expertise in clinic and research settings to counsel participants on disease risk. The genetic counseling provided to subjects will be done by individuals at the clinical centers with experience in diabetes counseling, who are acquainted with the aims of the study, and who have received uniform training. Details on the most recent information of the genetics of diabetes with a special emphasis on T1DM will be provided to study personnel as a short pamphlet so that all sites of the study have the same information. Finally, parents whose concerns are not fully addressed by the above steps, and who request further clarification, will be referred to a professional genetic counselor or medical geneticist. These diabetes genetic counselors will receive uniform training on the TEDDY study.

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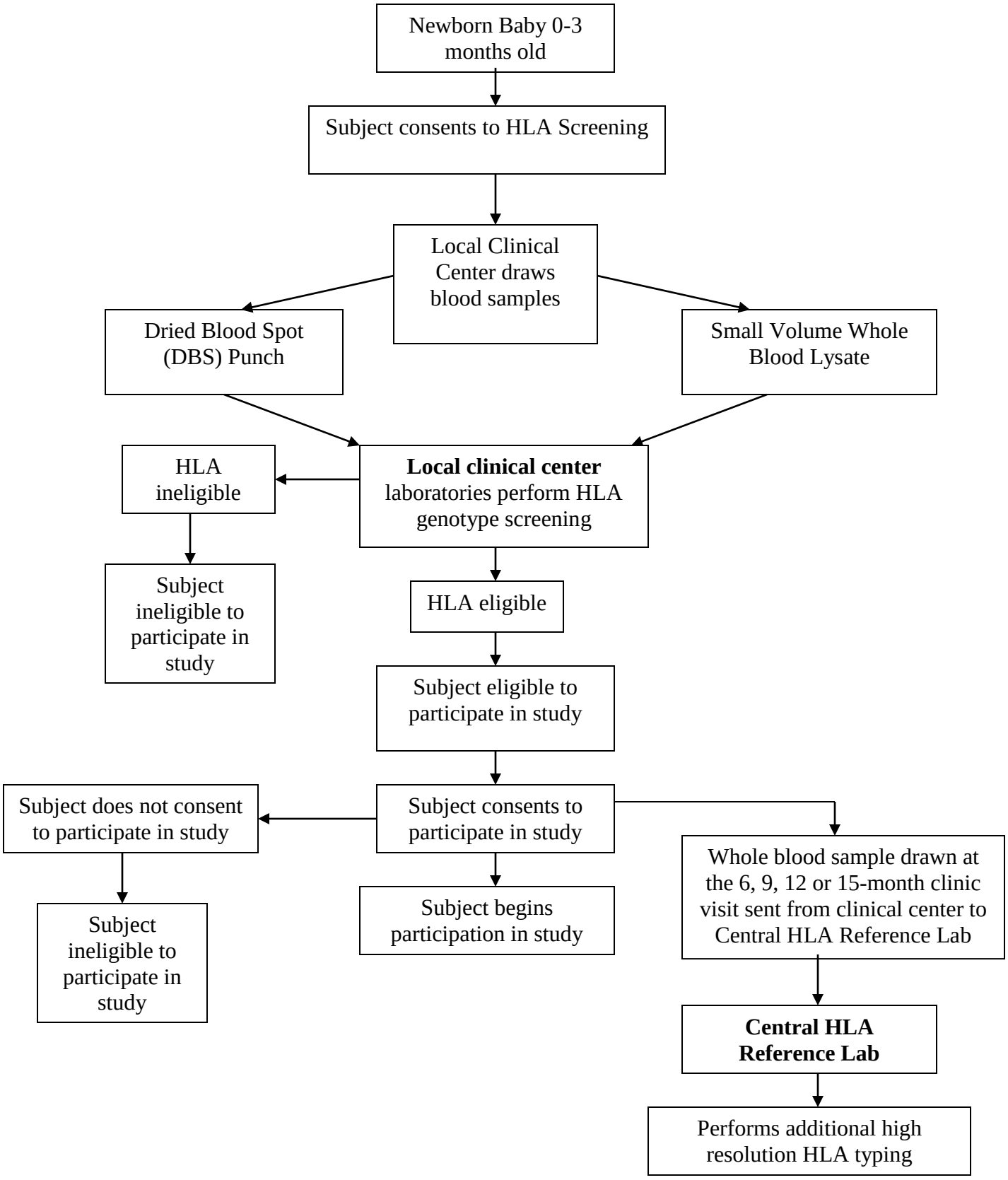
Schema 2: HLA Screening to be Completed Before the Child is 4 Months of Age



8. Enrollment/Follow-Up

8.1. Maternal Enrollment (Optional)

Pregnant women, will be approached to obtain a blood sample at 12-14 weeks and 25-28 weeks of the pregnancy and again at delivery (Table 1). These blood samples will be available for retrospective analysis of mothers of children who develop islet autoimmunity, T1DM, or both. The whole blood will be processed into serum, as described in Section 8.3.1 and assayed for, HLA, islet cell autoantibodies, infectious agent antibodies, and infectious agent nucleic acid, as described in Section 8.13.

Table 1. Maternal Enrollment and Analysis

	Pregnancy Week 12-14	Pregnancy Week 25-28	Delivery
Information about TEDDY	X		
Capillary or Venous Blood	X	X	X

8.2. Follow-up schedule for children with increased genetic risk

Once the results of the genetic screening are available, families are notified. If the child is found to meet the HLA eligibility criteria for enrollment and follow-up then the family is contacted by phone or by mail. The research nurse or other qualified research staff person explains the implications of carrying these gene markers and answers any of the questions the family may have regarding the genetic screening result. At this time, the family is informed that the infant is eligible for the follow-up protocol. The follow-up protocol is explained to the family in greater detail and if the family is interested in participating, the appropriate informed consent documents are mailed to them. The family is then invited to schedule an in-person meeting, or a subsequent phone call with the research nurse to go over the informed consent and have any questions answered. The research nurse and/or the Study Investigator co-sign the consent forms.

Each subject will participate in the more intensive follow-up phase until reaching the age of 4 years. At 4 years of age and beyond those children who have been deemed persistent autoantibody positive will be reinstated on the three month visit schedule and all other subjects will revert to a less intensive follow-up protocol until the age of 15.

8.2.1. Follow-up schedule

Children with increased genetic risk will be followed for environmental exposures and diet with a clinic visit every three months for the first 4 years of life. At 4 years of age and beyond those children who have been deemed persistent autoantibody positive will follow a three month visit schedule (confirmation results from the confirmatory Autoantibody lab will not be taken into

consideration for determining the subject's visit schedule, only the local lab's results will be used for this); all other subjects will attend biannual clinic visits beginning at 4 years of age until age 15. For subjects who become autoantibody positive at 4 years of age or older, the subject will be reinstated on the three month visit schedule at the first indication of autoantibody positivity and will stay on if persistent. If the next available sample is negative, thus the subject is not deemed persistent autoantibody positive, the subject will be placed on the biannual visit schedule from that point on. Subjects who have been persistently single autoantibody positive, but who become negative to all antibodies for 1 year or more will be placed on the biannual visit schedule after 4 years of age. Subjects who have been persistently multiple autoantibody positive, but who become negative to all antibodies for 1 year or more will remain on the three month visit schedule. Stool samples will be collected to assess viral exposures at monthly intervals for the first 4 years of life and then every three months until 10 years of age and then biannually after 10 years of age; it was decided to stop all stool sample collections on all subjects in August 2018 (Stool sample collection was less than 20% in Europe and 15% in the US. The small numbers did not warrant the cost of collection, processing, nor the burden on the families).

The following provides specifications for the implementation:

Before and leading up to the age of 4 years, if a child has been:

- Persistent autoantibody positive before the age of 4 years and is still autoantibody positive he/she will remain on a three month visit schedule after 4 years of age.
- Persistent single autoantibody positive before the age of 4 years, but has been autoantibody negative for 1 year leading up to the age of 4 years, the subject will switch to the biannual schedule at 4 years of age.
- Persistent multiple autoantibody positive before the age of 4 years, but has been autoantibody negative for 1 year leading up to the age of 4 years, the subject will remain on the three month visit schedule at 4 years of age and beyond.

Children who become autoantibody positive at 4 years of age or older:

- Will go on the 3 month visit schedule at the first indication of autoantibody positivity. If the next available sample is negative, thus the subject is not deemed persistent autoantibody positive, the subject will be placed on the biannual visit schedule from that point on. If the subject is deemed persistent autoantibody positive the subject will follow a 3 month visit schedule from that point on.
- As above, a child with prior persistent single autoantibody positive status that has been negative for 1 year (for all antibodies) will switch to the biannual visit schedule from that point on.

- As above, a child with prior persistent multiple autoantibody positive status that has been negative for 1 year (for all antibodies) will remain on the three month visit schedule.

The follow-up schedule for samples/visits/questionnaires is described in Table 2.

8.2.2. Clinic Visits

8.2.2.1. Interviews

The study nurse or equivalent will conduct interviews with the child's mother or primary caretaker at each clinical visit when the child is 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45 and 48 months of age. At 4 years of age and beyond those children who have been deemed persistent autoantibody positive will follow a three month interview schedule; interviews for all other subjects will be conducted on a biannual basis beginning at 4 years of age.

8.2.2.1.1. Demographic and family history

Abbreviated demographic and tracking questionnaire will be completed at the first visit at the age of 3 months. However, comprehensive demographic and family history questionnaires will be filled out at the clinical visit when the child is 9 months of age and the study has earned the family's trust and confidence. The demographic data will be updated with the family every 2 years thereafter and the family history data will be updated with the family every 4 years thereafter.

8.2.2.1.2. Medical

Medical information will be obtained by interview or questionnaires at each of the clinical visits when the child is 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45 and 48 months of age. At 4 years of age and beyond medical information will be collected every three months on those children who have been deemed persistent autoantibody positive; medical information on all other subjects will be collected on a biannual basis beginning at 4 years of age. In addition, the parents will be asked to consent to allow TEDDY personnel to access the child's medical record in the event that the child has been hospitalized, or has any outpatient treatments.

8.2.2.1.3. Clinical Measurements

Weight and length/height measurements will be taken at each clinic visit. Weight will be measured in grams (g). The infant will be weighted lying on his/her back without clothes and diaper. Children old enough to stand on a scale will be measured in light clothing on a scale. Length/height

will be measured in centimeters (cm). Length will be measured on all children up to two years of age. It is measured with the child lying on his/her back from the bare heels to the top of the head avoiding toe pointing. After the child is 2 years old the standing height will be measured with the child standing barefoot. A wall-mounted stadiometer is to be preferred for measuring height.

Starting in May 2017, in addition to height and weight, body fat will also be measured on some subjects. Fat distribution may be important in the development of type 1 diabetes (T1D). There is evidence that increased height and/or weight gain may have a role in the etiology of T1D, but no information is available whether the amount or distribution of body fat could play a role in the etiology of T1D. Availability of body fat measurement will increase the usability of the rich data on dietary, psychosocial and other covariates of obesity that is being collected in TEDDY. By measuring body fat in TEDDY, we can better analyze factors related to being overweight or obese. Body composition (weight and kilograms of body fat) will be measured at every TEDDY visit (on some subjects) using the TANITA® DC-430U Dual Frequency Total Body Composition Analyzer. The data will be recorded on the TEDDY Physical Exam Form by the Clinical Centers.

8.2.2.2. Medical record review

The medical record of the child will be accessed following parental consent to extract specific medical information in the event that the child has been hospitalized, or has any outpatient treatments. The information will be entered in the database.

8.2.2.3. Specimen Collection

To the extent possible, specimens will be collected, processed, and stored in such a manner as to be compatible with both immediate and future testing requirements. In general, most specimens will be stored at – 70°C. Since future testing may include new analyses and technologies, it may not be possible to prepare for all possibilities.

Unique laboratory identification numbers will be generated by the DCC and used to label each specimen aliquot. The DCC will also supply each CC with the appropriate identification labels for each specimen container. Aliquots will be mailed in batches on dry ice from the clinical centers to the reference laboratories. Additional aliquots will be sent in batches to the NIDDK repository.

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Table 2. Follow-up Schedule

	Age in Months																							
	Screening		Follow-Up																					
Sampling Frequency	**Birth**	**<4**	**2**		**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**15**	**18**	**21**	**24**	**27**	**12-48 mo Monthly Test**	**24-48 mo Every 3 mo Tests**	**24-48 mo Every 6 mo Tests**	**>48 mo Every 3 mo Tests**	**>48 mo Every 6 mo Tests**	**>48 mo Annual Tests**
			Inform Parents of child's HLA risk	Mail initial enrollment and questionnaire packet																					
Blood**	**X***	**X***			**X+**			**X+**			**X+**			**X+**	**X+**	**X+**	**X+**	**X+**	**X+**		**X+#**			**X+#**	
Stool					**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**			**X (until 10 years)**	**X (at 10 years); Collection stopped August 2018**	
Tap Water											**X**									**Collected every 2 years beginning at the 36 month visit**					
Toenail Clippings																		**X**		**Collected every 2 years beginning at the 24 month visit; Starting May 2017 collected every 1 year**					
Salivary Cortisol																				**Collected when child is 3.5, 4.5 and 5.5 years of age**					
Nasal Swab											**X**			**X**	**X**	**X**	**X**	**X**	**X**		**X#**			**X#**	
Urine																						**X (begins at 3 years)**		**X**	
Primary Tooth	**Collect when tooth naturally falls out - ages will vary**																								
Weight and Length/Height Measurements; Body composition on some subjects					**X**			**X**			**X**			**X**	**X**	**X**	**X**	**X**	**X**		**X#**			**X#**	
Diet Questionnaires																									
-maternal pregnancy diet				**X**																					
-3 day diet record					**X**			**X**			**X**			**X**		**X**		**X**				**X**		**X^**	
Environmental Exposure Questionnaires																									
-maternal pregnancy/birth questionnaire				**X**																					
- parent questionnaire				**X**				**X**							**X**				**X**			**Annually after 27 mos**			
- child questionnaire																									**X (begins at 10 years)**
Demographic/Family History/Other questionnaire											**X**									**Demographic data will be updated every 2 years thereafter; Family History data will be updated every 4 years thereafter**					
TEDDY Book Extraction					**X**			**X**			**X**			**X**	**X**	**X**	**X**	**X**	**X**		**X#**			**X#**	
Child Behavior Checklist/ Strengths and Difficulties Questionnaire																				**CBCL completed when child is 3.5, 4.5 and 5.5 years of age; SDQ completed by both parent and child when child is 11.5 and 13.5 years of age**					
Physical Activity Assessment																									**X (begins at 5 years)%**
Pubertal Status Assessment																								**X (begins at 8 years)**	

***If cord blood is not available at birth for HLA typing then capillary blood should be drawn.**

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+ If venous blood is not available at every three month office visit, then capillary blood should be taken.
**** A blood sample will be obtained by the 24 month visit from mothers who have type 1 or 2 diabetes or gestational diabetes as well as from a mother whose child is shown to be autoantibody positive at three or six months of age. An optional venous blood draw of the mother is obtained at 12-14 weeks of pregnancy, and at the birth of the baby.**
#Children four years of age and older who have been deemed persistent autoantibody positive will remain on the three month visit schedule; this sample/form will be collected/completed at these visits.
^ Continue to collect 3 day diet records every 6 months from subjects who are single or multiple persistent confirmed autoantibody positive (even if the subject reverts to autoantibody negativity), stop 3 day diet record collections on all other subjects after the 10 year visit. Should a subject be deemed single or multiple persistent confirmed autoantibody positive after the 10 year visit, the 3 day diet record collection will be restarted at the next visit.
% Continue to collect physical activity assessments annually from subjects who are single or multiple persistent confirmed autoantibody positive (even if the subject reverts to autoantibody negativity), stop physical activity assessments on all other subjects after the 10 year visit. Should a subject be deemed single or multiple persistent confirmed autoantibody positive after the 10 year visit, the physical activity assessment will be restarted at the next visit.

8.3. Venous/Capillary Blood draws

Venous blood will be drawn for processing into serum, plasma, erythrocytes, buffy coats, and mRNA (Table 3). If venous blood is not available, capillary blood will be drawn. Blood will be processed and aliquoted by the clinical centers, as described below.

8.3.1. Maternal samples (Optional)

The maternal blood sample during pregnancy at weeks 12-14, 25-28, and at delivery is taken as 5-7.5mL EDTA plasma or serum. Clinical sites will draw the samples into serum tubes and process it as described in Section 8.3.4.1. The plasma or serum will then be aliquoted, and stored frozen.

8.3.2. Maternal sample

A blood sample will be obtained by the 24 month visit from mothers who have type 1 or 2 diabetes or gestational diabetes as well as from a mother whose child is shown to be autoantibody positive at three or six months of age.

8.3.3. Parental and sibling DNA collection for heritability analyses

One 5 mL blood sample will be obtained from each parent and sibling of the TEDDY child for heritability analyses. The sample will be drawn into an EDTA tube then transferred to an externally threaded cryovial. The sample will then be sent to the DNA Reference Laboratory for processing and they will send the extracted DNA to the NIDDK repository for storage. An optional 2 ml blood sample will be obtained at the parent's request from each parent and sibling of the TEDDY child for a one time screening for islet autoantibodies. The sample will be drawn into an SST tube and will be tested at the Denver Reference Lab for the US sites and the Bristol Reference Lab for European sites. Confirmation at the other Reference Laboratory will not be performed for any samples, including samples testing positive for islet autoantibodies. Local sites will determine the format for relaying islet antibody results to family members from their site.

8.3.4. Children samples

The optimal volumes of blood to be drawn are shown in Table 3 (Total Blood Volume, last row). These volumes are much smaller than those allowed per IRB and NIH rules for younger children; as children get older additional blood volume will be collected based upon local IRB/Ethics Board approval and the weight of the child. At no time will the blood draw volume exceed what is allowable according to the subject's body weight - 3 mL/kg per visit. The volumes reflect both the scientific needs of the study and the experience of the TEDDY clinical centers from Colorado, Finland, Germany and Florida that have been carrying out similar studies in infants and toddlers over the past 8-14 years. Study personnel

training will place special emphasis on expert pediatric venipuncture skills and sensitivity to potential parental concerns that are rare at the proposed venipuncture volumes but should be immediately addressed. Typically, both antecubital areas are prepared with topical EMLA anesthetic left in place for 30-40 min to provide a painless experience. Only one attempt is allowed at each side and no repeated attempts are allowed if a working venipuncture was "lost". 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.	serum	0.1-0.5 mL whole blood volume
2.	plasma, RBC, PBMC	0.6-1.0 mL whole blood volume
3.	mRNA	2.5 mL whole blood volume
4.	Any additional storage	

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Table 3. Blood Sampling Frequency and Volumes (all volumes are shown in milliliters)

		Age in Months																			
Sample Type		**Screening: Birth**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**15**	**18**	**21**	**24**	**24-48 mo Every 3 mo Tests**	**>48 mo Every 6 mo Tests**	**>24 mo Yearly Tests**	**4 Years**	**8 Years; 14 Years**
Cord Blood		X																			
Venous/capillary Blood (ml)		X*	X			X			X			X	X	X	X	X	X#	X#			
Additional HLA	Whole blood					0.5^			1^			1^	1^								
Autoantibodies	Serum		0.2			0.2			0.2			0.2	0.2	0.4	0.4	0.42	0.4	0.4	0.02**		
Serum cytokines/ inflammation markers	Serum		0.1			0.1			0.1			0.1	0.1	0.1	0.1	0.1	0.1	0.1			
Additional serum aliquots	Serum																0.2§	0.2			
Thyroid Autoantibodies	Serum																				0.1
Entero- & rotavirus PCR	Plasma		0.3			0.3			0.3			0.3	0.3	0.3	0.3	0.3	0.3	0.3			
Antibodies	Plasma		0.1			0.1			0.1			0.1	0.1	0.1	0.1	0.1	0.1	0.1			
Additional infectious agents	Plasma		0.4			0.4			0.4			0.4	0.4	0.4	0.4	0.4	0.4	0.4			
Vitamin D	Plasma		0.05			0.05			0.05			0.05				0.05			0.05		
Alpha-tocopherol, gamma-tocopherol	Plasma					0.07						0.07				0.07			0.07		
Carotenoids	Plasma					0.06						0.06				0.06			0.06		
Ascorbic acid	Plasma					0.05						0.05				0.05			0.05		
Additional plasma aliquots	Plasma																0.8§	0.8			
RBC Membrane Fatty Acid	RBC		0.5			0.5						0.5				0.5			0.5		
MRNA	Whole blood		2.5			2.5			2.5			2.5	2.5	2.5	2.5	2.5	2.5	2.5			
Non-HLA genotyping	Whole blood																			5	
All above samples	Serum	-	0.3			0.3			0.3			0.3	0.3	0.5	0.5	0.52	0.7	0.7	0.02		
	Plasma	-	0.85			1.03			0.85			1.03	0.8	0.8	0.8	1.04	1.6	1.6	0.23		
Repository samples	Serum	-	0.5			0.5			1.0			2.5	2.5	2.3	2.3	2.3	3.03§	3.03			
	Plasma	-	0.15			0.9			1.65			2.9	3.2	3.2	5.2	4.9	6.15§	6.15			
Local laboratory backup	Serum/ Plasma	-	0.2			0.2			0.2			0.2	0.2	0.2	0.2	0.2	0.2	0.2			
Total serum		-	1.0			1.0			1.5			3.0	3.0	3.0	3.0	3.0	3.75§	3.75			
Total plasma		-	1.0			2.0			2.5			4.0	4.0	4.0	6.0	6.0	7.75§	7.75			
Total whole blood		-	2.5			2.5			3.5			2.5	2.5	2.5	2.5	2.5	2.5	2.5			
PBMC			X			X			X			X	X	X	X	X	X	X			
Total RBC		-	1.0			2.0			4.0			4.0	4.0	4.0	4.0	4.0	4.0	4.0			
Serum tube			2.0			2.0			3.0			6.0	6.0	6.0	6.0	6.0	7.5§	7.5			
Plasma tube			2.0			4.0			5.0			8.0	8.0	8.0	12	12	15.5§	15.5			
ABI tube			2.5			2.5			2.5			2.5	2.5	2.5	2.5	2.5	2.5	2.5			
HLA confirmation						0.5^			1^			1^	1^								
Total Blood Volume		-	**6.5**			**8.5 or 9.0^**			**10.5 or 11.5^**			**16.5 or 17.5^**	**16.5 or 17.5^**	**16.5**	**20.5**	**20.5**	**25.5**	**25.5+**			
Blood Glucose		**At every visit once subject tests positive for any autoantibody**																			
OGTT		**Every six months once subject tests positive for two autoantibodies, regardless of autoantibody positivity confirmation or persistence, at any pervious visit and is three years of age or older**																			

HbA1c	**0.25 mL sample taken at every visit from children who are positive at the 9 month visit or later for at least one autoantibody (regardless of autoantibody positivity confirmation or persistence)**		

***If cord blood is not available for HLA typing then capillary blood should be drawn.**
****Additional 0.02 ml for tissue transglutaminase antibodies measurement added to the islet antibody sample sent to the Autoantibody Reference Lab.**
#Children four years of age and older who have been deemed persistent autoantibody positive will remain on the three month visit schedule.
^ Only one HLA confirmation sample is needed from the earliest visit with a full volume blood draw.
§ Beginning at the 36 month visit
+ As children get older additional blood volume will be collected based upon local IRB/Ethics Board approval and the weight of the child. At no time will the blood draw volume exceed what is allowable according to the subject's body weight - 3 mL/kg per visit

8.3.4.1. Serum

A sample of whole blood will be drawn into a syringe and transferred into a serum separation tube of appropriate size. The tube will be allowed to clot at room temperature and then centrifuged (800 x g at 4°C for 20 minutes) to separate the serum from the clot. Serum will be aliquoted into freezer storage tubes for autoantibodies, serum cytokines/inflammation markers, tissue transglutaminase antibodies and thyroid autoantibodies as shown in Table 3 and stored frozen at -70°C in externally threaded plastic cryovials. Serum volume over and above that required for these tests will be stored for confirmatory testing and for additional infectious agent testing.

8.3.4.2. Plasma, peripheral blood mononuclear cells (PBMC) and erythrocytes

A sample of whole blood will be drawn into a syringe and transferred into a CPT tube. Plasma, peripheral blood mononuclear cells and erythrocytes will be separated by centrifugation according to the manufacturer's instructions. After centrifugation, plasma will be removed under sterile conditions in a laminar flow hood, aliquoted into externally threaded plastic cryovials, and stored frozen at -70°C. This plasma will be used for analysis of enterovirus and rotavirus, additional infectious agents, vitamin D, alpha-tocopherol, gamma-tocopherol, carotenoids and ascorbic acid. Plasma volume over and above that needed for planned infectious agent and dietary marker testing will be stored for confirmatory testing and additional infectious agent/dietary marker testing.

Peripheral blood mononuclear cell layer will then be removed in sterile fashion from above the gel plug, and transferred into a sterile tube. Using sterile technique in a laminar flow hood, PBMC will be washed, counted by hemocytometer, resuspended in storage buffer, transferred to cryovials, and carefully frozen down using the PBMC procedure specified in the TEDDY MOO.

It is expected that each Clinical Center will be able to isolate and freeze sterile PBMC from 8 or more TEDDY subjects per day. Occasionally, the available samples will exceed the local capacity to process them, in which case the following priority order will be used:

1) first degree relatives;
2) general population subjects who are Genotype Category A (HLA DR3/4);

3) general population subjects who were positive for any islet autoantibody at the last TEDDY visit;
4) all other general population subjects.

For subjects where it is not possible to isolate and freeze living PBMC, the cells will be harvested and frozen as a buffy coat sample at all clinic visits at all TEDDY child ages. The buffy coat will be harvested and placed in externally threaded cryovials and stored at -70°C. It will then be batch shipped to the NIDDK repository for storage.

Finally, 500 µL of erythrocytes will be removed from under the plug material and then stored frozen at -70°C in externally threaded plastic cryovials for use in RBC membrane fatty acid assays.

All processed materials will be batch-shipped to the NIDDK repository for storage.

8.3.4.3. Messenger RNA

A 2.5 mL sample will be collected into an ABI tube for preparation of total RNA from subjects. Samples will be sent to the RNA Reference laboratory for processing and they will send the extracted RNA to the NIDDK repository for storage. The RNA samples will be used to identify novel disease markers and environmental triggers. RNA can also be used to study gene expression of inflammation, infection, immunity, and molecular mechanism arising from TEDDY findings. These studies will be performed using a nested case-control study design as well as longitudinal studies with subjects who have progressed to autoantibody-positive and/or diabetes. The genes to be analyzed will be determined by the steering and appropriate advisory committees.

8.3.4.4. Whole Blood

A whole blood sample will be drawn into an EDTA tube at the 6, 9, 12 or 15 month visit. Sites are encouraged to complete this collection by the earliest visit with a full volume blood draw, but in all cases by the 15 month visit. If the HLA confirmation sample is collected at the 6 month visit, only 0.5 mL of blood is required to be collected for this sample. If the HLA confirmation sample is collected at the 9, 12 or 15 month visit 1 mL of blood should be collected for this sample. This blood will be transferred to an internally threaded cryovial and stored at -70°C. The sample will be batched and sent monthly to the central genetics lab for additional HLA genotyping.

A 5 mL sample of whole blood will be drawn into an EDTA tube when the child is 4 years old. This blood will be transferred to an externally threaded cryovial and sent to the NIDDK DNA Repository for non-HLA genotyping.

Additional whole blood samples, not to exceed the maximum blood volume, will be sent to the Repository for storage.

8.3.4.5. HbA1c

A 0.25 mL sample of whole blood will be drawn into a 0.5 mL EDTA bullet tube, for an HbA1c test, at the next TEDDY visit and every visit thereafter from children who are positive at the 9 month visit or later for at least one autoantibody (regardless of autoantibody positivity confirmation or persistence). Following this logic, the first possible visit that the HbA1c sample could be collected at is the 12 month visit. This blood will be stored at -70°C in the EDTA tube. The sample will be batch shipped to the central HbA1c measurement laboratory. Collection of the sample may be discontinued if the child, previously positive for islet autoantibodies, has been negative for at least 12 months. Special consideration will be given to children with known hemoglobinopathy (e.g. HbSS, persistent HbF) or hemolytic condition (e.g. hereditary spherocytosis) that is known to affect HbA1c results; HbA1c still provide useful information in some of these children, especially change within an individual over time.

8.4. Stool

The child's parent(s) will collect at least 5g of the child's stool each month up until 48 months of age, then every three months until the age of 10 years and then biannually thereafter into the three plastic stool containers provided by the clinical center. In August 2018 all stool sample collections were stopped on all subjects. Stool sample collection compliance was less than 20% in Europe and 15% in the US. The small numbers did not warrant the cost of collection, processing, nor the burden on the families.

The TEDDY study group has adopted a compromise position that *promotes* stool sample collection 4 times a year for children who are antibody positive and *encourages* stool sample collection 4 times a year for children who are antibody negative, after age 4. The difference in approach is that children who are antibody positive are, according to the current protocol, on a 4 times per year follow up schedule which makes the increased frequency of stool sample collection consistent with their increased surveillance schedule (visits and blood draws). For antibody negative children, those who are willing to submit stool samples will be asked to continue on the more frequent schedule (which may actually increase compliance) while others who do not will not be considered as non compliant. Rectal swab collection will be an optional collection method for non-compliant subjects who are less than four years of age; the rectal swab collection will occur in the TEDDY clinic by the study nurse.

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.

8.5. Nasal Swab Samples

Beginning at 9 months of age a minimally invasive nasal swab sample will be collected from each TEDDY subject and will continue to be collected at each visit thereafter. The nasal swabs will be collected for the purpose of detection of respiratory infections that may trigger development of islet autoimmunity or progression to T1D. The aim is to cover respiratory viruses and other agents which are difficult to detect from stool or plasma samples. Samples will be collected using commercially available swabs designed for taking nasal swabs from young children (Pediatric Flocked Swabs from Copan Diagnostics Inc). Samples will be taken by the study nurse from one nostril of the child using a minimally invasive method (no deeper than 2 cm inside the nostril in children less than 2 years of age and approximately 3 cm inside the nostril of older children). The sample will be eluted in 1 ml of special Universal Transport Medium in a bar-coded tube (Copan Diagnostics Inc.) and frozen in this tube at -70ºC as soon as possible after the sample has been collected. Frozen samples will be shipped from clinical centers to the NIDDK repository along with plasma samples. Boxes containing nasal swab samples will be stored and shipped in separate plastic bags to ensure that they will not contaminate serum samples.

8.6. Toenail Clippings

Toenails clippings from all 10 toes of the child will be collected first at the age of 2 years and then every one year until the age of 15 years to measure selenium, an antioxidant that is expected to play a role in the development of chronic diseases, including diabetes (Fairweather-Tait et al, 2010) and to measure cortisol stress levels in order to test: (1) psychological stress as a possible trigger of persistent beta-cell autoimmunity and progression to TID, and (2) psychological stress and increased susceptibility to illness which, in turn, may increase the child's risk for autoimmunity and T1D.

8.7. Drinking Water

Tap water samples will be tested in all households. The TEDDY family will be asked to bring in six 2 mL cryovials (filled to the 1.8 mL mark) at the 9 month clinic visit (supplies and instructions will be given to the family at the 6 month clinic visit), for a total of 10.8 mL of water. From the tap water samples, zinc and nitrate concentrations and the water's pH will be tested. The extra aliquots will be used for additional tests or for quality control checks.

Subsequent water samples will be collected every two years at the annual visit for ages 3 years, 5 years, 7 years, etc. through the life of the study. No additional collections will be requested in the event a family changes residences.

8.8. Salivary Cortisol

Salivary cortisol will be used as a biomarker of the child's overall stress level and the child's reaction to a standardized stressor (the TEDDY visit's blood draw) to the TEDDY protocol. This biomarker will permit a more definitive test of: (1) psychological stress as a possible trigger of persistent beta-cell autoimmunity and progression to TID, and (2) psychological stress and increased susceptibility to illness which, in turn, may increase the child's risk for autoimmunity and T1D.

There will be three annual salivary cortisol collections from each subject at 3.5 years, 4.5 years, and 5.5 years of age. Each of the annual collections will consist of collecting three salivary samples. The first of the three samples should be collected by the child's parent at home 30 minutes after the child wakes on the morning the TEDDY child comes into the clinic (at the previous visit, parents will be provided with a salivary cortisol kit and instructions on how to collect the cortisol sample). Parents may collect the sample by having the child spit into a collection device or they may use the Sorbette (cotton pad on a stick). They will bring the morning saliva collection with them to the TEDDY visit.

When the child comes to the TEDDY visit, two salivary cortisol samples will be collected: a "baseline" assessment immediately prior to the blood draw and a "post-stress" assessment, 20 minutes after the blood draw. Salivary samples will be collected with a Sorbette (cotton pad on a stick). Immediately after the blood draw, during the 20 minute waiting period between saliva collections, it is recommended that the child be given a coloring book or some other play activity.

Prior to the pre-blood draw saliva collection, parents should provide information on the time the child woke up in the morning. Study staff should note the time of the saliva collections and blood draw. Parents should also confirm the child is not on oral steroids and has NOT had caffeinated drinks before the clinic visit, milk or food within 30 minutes before the pre-blood draw saliva collection. If food or drink has been consumed within these time intervals, the site may wait the necessary time interval before conducting the saliva collection, or re-schedule the visit.

The child should rinse his/her mouth out with water before the pre-blood draw saliva collection. If the child needs more than one stick to get the blood sample, this should be noted. The second, post-blood draw saliva collection should occur 20 minutes after the second attempt.

The first 100 children at each participating site will have their salivary cortisol analyzed at a central laboratory. The remaining samples should be sent to the NIDDK Central Repository for storage.

8.9. Urine

Urine samples are a valuable source of information regarding footprints of certain systemic infections (CMV), exposures to environmental toxins, metabolites and proteins/peptides. Further, collection of urine is non-invasive and generally well tolerated by children. Urine sample collection in TEDDY is both robust in terms of future applications and adds little burden to study participants and staff.

A 6-15 mL sample of urine should be collected directly into a standard clinical sterile specimen cup from all TEDDY subjects beginning at 3 years of age and will continue to be collected every 6 months. All TEDDY subjects are eligible as long as the sample can be processed within 24 hours of collection and can be kept refrigerated until processing; the sample should be frozen as soon as possible after processing, but within 24 hours of collection. The preferred collection location is the TEDDY clinic, with specimen cup placed immediately on ice or refrigerated. If clinic collection is not possible, then the sample may be collected offsite. If collected offsite, the sample should be refrigerated continuously and must be processed and frozen at the TEDDY clinic within 24 hours of collection. The "clean catch" technique is not required (including girls). No preservative and no centrifugation are needed at the TEDDY clinic. Urine should be thoroughly mixed and then divided/transferred equally into three 8-ml screw-cap etched cryovials and frozen at -80°C until shipment to the NIDDK Repository.

8.10. Primary Tooth

The intent is to collect at least one tooth from each child when they naturally fall out. Parents will be asked to save the tooth and bring it to their next TEDDY clinic visit. The site will store the tooth in an etched vial and record the date it fell out. Planned analyses of the teeth provide a record of environmental exposures throughout the child's life since teeth form daily growth rings. The technology to measure these exposures which extend to both prenatal and post-natal periods include metal exposures as well as bone constituents. This technology is developing and may provide measures of other exposures that might be informative to TEDDY.

It is clear that this is a convenience sample in that there is no expectation that teeth will be collected on every child, or that the teeth which are collected will come from children of the same age. The availability of the teeth will determine which analyses are feasible. At a minimum, the available teeth can verify the parent reported record of exposure and can be used to correlate with measures obtained from serum and/or plasma. Should more than one tooth be available from a TEDDY child, the study will accept all available which would provide more material to analyze.

8.11. Physical Activity Assessment

Measurement of physical activity will be used to examine the effect of physical activity and test the accelerator and overload hypotheses on T1D development in the TEDDY cohort. Assessment of physical activity will be used to test the following hypotheses: (1) low rates of physical activity, high body mass index, a pattern of high caloric intake in a single meal, and high consumption of foods with a high glycemic index are associated with the development of persistent anti-islet autoantibodies in genetically at-risk children, and (2) low rates of physical activity, high body mass index, a pattern of high caloric intake in a single meal, and high consumption of foods with a high glycemic index are associated with more rapid progression to T1D in children who have developed persistent anti-islet autoantibodies.

On an annual basis, beginning at 5 years of age, TEDDY participants will wear the Actigraph GT3X accelerometer for 7 consecutive days (including 2 weekend days) in order to generate adequate valid data. Accelerometers are small light-weight devices that measure change in velocity over time. When worn on the hip, lower back, or ankle, they are used to quantify the volume and intensity of movement in 1-, 2-, or 3-planes. They can also be worn on the wrist to assess sleep patterns and efficiency (Oliver 2007).

In August 2018, the collection protocol was changed so as to continue to collect physical activity assessments annually from subjects who are single or multiple persistent confirmed autoantibody positive (even if the subject reverts to autoantibody negativity) and to stop physical activity assessments on all other subjects after the 10 year visit. Should a subject be deemed single or multiple persistent confirmed autoantibody positive after the 10 year visit, the physical activity assessment will be restarted at the next visit. This will provide a complete dataset on all subjects up through 10 years of age and continued collection on persistent confirmed autoantibody positive subjects through the end of the study. Continued data collection on persistent confirmed autoantibody positive individuals will enable the TEDDY study to explore the role of energy expenditure changes through early adolescent years, on progression to T1D. These data will enable TEDDY to include physical activity patterns in assessments of T1D risk along with other exposures and changes occurring during the peri-pubertal period to include energy intake (diet), growth, hormonal changes and glucose demand. It has been recognized that glycaemia can be influenced by activity levels. As well, epidemiological data points to increased T1D incidence during this period, TEDDY has also observed a declining rate of conversion from autoantibody negative to autoantibody positive (i.e., lower incidence of seroconversion during this age range). This reduces the statistical power to see an effect of activity level changes during the 10-15 year age range. Coupled with a lower compliance rate, as compared to children willing to wear the actigraphs among children who are persistent confirmed autoantibody positive, it seems prudent to reduce the burden on children, families and clinic staff by discontinuing data collection after 10 years of age for families whose child is autoantibody negative. TEDDY will have a complete data set on this population through age 10 years so it

will be able to address the contribution of activity levels in the cumulative incidence of islet cell autoimmunity up to this age.

To maximize compliance, families will receive in-person demonstrations on how to wear and use the accelerometer during the TEDDY visit immediately prior to the specific TEDDY visit targeted for accelerometer data collection. One month prior to the TEDDY visit targeted for the accelerometer data collection, the accelerometer will be mailed to families with instructions to begin using the device two days before the three day diet recording period and for two days after. A follow-up reminder telephone call will be used to prompt device use and to answer any questions. All families will be asked to return the accelerometer at their next TEDDY visit and will be given a pedometer as a "thank you" for the prompt return of the device. For those who forget to bring the accelerometer to the TEDDY visit, mailing envelopes and reminder phone calls will be used to prompt return of the accelerometer.

When the children reach 8 years of age, TEDDY will reference reported epidemiological surveys (Sallis 1006, Kimm 2000, CFC YRBSS) and develop a comprehensive questionnaire on child activities to use in conjunction with the objective accelerometer data collection.

8.12. Questionnaires and Structured Interviews

8.12.1. Dietary Questionnaires, Records, and Interviews

8.12.1.1. Maternal Nutrition

Measurement of maternal diet will be collected by means of a short food frequency questionnaire (FFQ), which concentrates on the intakes of fish and fish products, milk and milk products, and cereal and cereal products during the eighth month of pregnancy (for Finland and Germany) (Erkkola et al., 2001). The eighth month is considered the appropriate reference month in these countries because mothers start their maternity leave in the ninth month. Therefore, the eighth month might more accurately reflect the pregnancy diet than the ninth month because of the change in lifestyle that would occur when one went on maternity leave. In the United States and Sweden, this type of maternity leave does not occur, and therefore the reference month will be the ninth month for the collection of the food frequency questionnaire. The use of dietary supplements is asked as well as source of drinking water. The height of the mother is inquired as well as the weight before pregnancy and the weight gain during pregnancy.

8.12.1.2. Dietary study in children

In addition to food consumption, dietary habits of the participating infants (e.g. feeding pattern) will be assessed by mailed questionnaire that is to be completed prior to the first clinic visit, a structured interview at each clinic

visit, and records kept by the mother in the TEDDY Book (see Table 2 and section 8.11.2). The duration of total and exclusive breastfeeding, age at introduction of various foods during the first 2 years of life, type of infant formulas used, source of drinking water (local waterworks, bottled water, private wells), elimination diets, and use of dietary supplements will be recorded.

Primary caretakers (usually mothers) will be trained during the three-month clinic visit to keep 3-day food diaries of the child's dietary intake at 3 month intervals during the first year of life and biannually thereafter. A 24-hour recall of the child's diet will be obtained at the first (3-month) visit. The collection of this 24-hour recall will have two purposes: 1) to assist in *training* the primary caretakers in what types of food items they will need to record when they complete the 3-day diet records; and 2) the dietary data from the 24-hour recall will be used to reflect the infant's diet at 3 months of age. The first 3-day food diary will be given to the primary caretaker at the three-month visit after training. The primary caretaker will be instructed to fill out the 3-day food diary directly prior to the next clinic visit (in this case the 6 month clinic visit), so that they can bring the completed record with them (Gregory et al., 1995). At each clinic visit, the diet records will be reviewed by trained study personnel with the primary caretaker.

In August 2018, the collection protocol was changed so as to continue to collect 3 day diet records every 6 months from subjects who are single or multiple persistent confirmed autoantibody positive (even if the subject reverts to autoantibody negativity) and to stop 3 day diet record collections on all other subjects after the 10 year visit. Should a subject be deemed single or multiple persistent confirmed autoantibody positive after the 10 year visit, the 3 day diet record collection will be restarted at the next visit. This will provide a complete dataset on all subjects up through 10 years of age and continued collection on persistent confirmed autoantibody positive subjects through the end of the study. Continued data collection on persistent confirmed autoantibody positive individuals will enable the TEDDY study to explore the role of diet, and dietary changes, through early adolescent years, on progression to T1D. It has been noted that the diet of individuals in this 10-15 year age interval is changed as compared to the diet at younger ages. These data will enable TEDDY to include these dietary patterns in assessments of T1D risk along with other exposures and changes occurring during the peri-pubertal period to include energy intake, energy expenditure, growth, hormonal changes and glucose demand. Epidemiological data points to increased T1D incidence during this period, TEDDY has also observed a declining rate of conversion from autoantibody negative to autoantibody positive (i.e., lower incidence of seroconversion during this age range). This reduces the statistical power to see an effect of dietary changes during the 10-15 year age range. Coupled with a lower compliance rate, as compared to families completing the 3 day diet record whose child is persistent confirmed

autoantibody positive, it seems prudent to reduce the burden on families and clinic staff by discontinuing data collection after 10 years of age for families whose child is autoantibody negative. TEDDY will have a complete data set on this population through age 10 years so it will be able to address the contribution of diet in the cumulative incidence of islet cell autoimmunity up to this age.

Research personnel of all TEDDY countries need continuous training for checking of food diaries. Standardization will be done at all levels of the study: training of research personnel, advice material, checking of diaries, food data entry, food composition databases, and food and nutrient calculations. Food databases to be used in the TEDDY countries will be compared to assess which dietary factors are comparable (De Henauw et al., 2002). All food diaries will be entered continuously at each respective center/country.

Table 4. Nutritional Factors of Interest in the TEDDY Study

Foods	Nutrients and Energy	Other Nutritional Factors
Cows milk	Energy intake	Nitrates, nitrites and N-nitroso compounds
Cereals, wheat (gluten)	Proteins	Patulin
Soy	Vitamins C, D and E	Bafilomycin
Meat	Nicotinamide	Increased weight and/or height gain (fetal period, infancy, childhood)
Coffee and tea	n-3 fatty acids	
Breast milk	Zinc	
Cod liver oil	Carotenoids and selenium	

8.12.2. TEDDY Book

At the 3 month clinic visit, primary caretakers (usually mothers) will be introduced to the TEDDY Book. This is a notebook that is to be used by the primary caretaker to record events in their child's life that are of interest to the study. Primary caretakers are instructed to write down things such as when foods are introduced in their child's diet, use of food and vitamin supplements, medications, vaccinations, length and weight history of the child, illnesses and symptoms of the child, doctor's visits and hospitalizations, and life events of the child. The primary caretakers will be asked to bring in the TEDDY book to each clinic visit. At each visit, study personnel will go over the book with the primary caretaker and extract pertinent information using standardized study forms. The TEDDY book the primary caretaker first receives will be used up until the age of 2 years. After that, a more age-appropriate book will be distributed.

8.12.3. Infectious/Immunization Questionnaires and Interviews

At each clinic visit when the child is 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45 and 48 months of age information on infectious illnesses and immunizations that occurred since birth or the last visit will be recorded. At 4 years of age and beyond information on infectious illnesses and immunizations will be collected every three months from those children who have been deemed persistent autoantibody positive; information on infectious illnesses and immunizations will be collected from all other subjects on a biannual basis beginning at 4 years of age. At 3 months, this will be done by a standardized interview. At subsequent visits, this will be done by extracting the information from the TEDDY book (see above).

8.12.4. Psychosocial Questionnaires and Interviews

8.12.4.1. Background

Genetic screening for disease risk raises a number of psychosocial and ethical issues. Genetic screening of children is particularly controversial when there is no available intervention to prevent the disease. (Weber, 1997; American Academy of Pediatrics, 2001; Johnson, 2001; Roth, 2001; Roth, 2002; Ross, 2003). Human subject consent procedures also require participants to be informed of the costs and benefits, including the psychosocial costs and benefits, of any study protocol. Since the TEDDY protocol will require the longitudinal study of infants at increased genetic risk for diabetes, in the absence of any known effective intervention to prevent the disease, the psychosocial impact on families who agree to participate must be addressed.

The psychosocial components of the TEDDY protocol are designed to: (1) assure participants are appropriately informed and supported during their study participation; (2) assess the psychosocial impact of study participation on families; (3) examine the role of psychosocial stress as a potential trigger for type 1 diabetes; and (4) identify family characteristics that discriminate study completers from study drop-outs.

8.12.4.2. Informing and Supporting Families During the Study

The literature suggests that individuals understand risk information best if both a categorical descriptor or label and a numerical risk estimate are provided (Kong et al., 1986; Shaw and Dear, 1990). In a previous study with mothers of infants at increased genetic risk for TIDM, we provided both a label and a numerical estimate of an infant's diabetes risk. We then examined the accuracy of mothers' estimates of infant risk approximately 4 weeks and 4 months after risk notification. Mothers who recalled both the label and a numerical estimate were more likely to be accurate about their infant's actual risk (Carmichael et al., 2003).

Based on these findings, TEDDY parents will be informed about the implications of their child's positive genetic test result-using a standard protocol: both a label (e.g., increased risk for diabetes) and a numerical estimate (e.g., 2 in 100 infants) will be used. If the child shows evidence of persistent autoimmunity, a standard protocol will be used to inform parents of the presence of persistent autoimmunity and its associated increased risk for T1DM in the child.

Each study site will specify procedures to promote participant retention as part of its study protocol. Since sites may differ in terms of participant needs (e.g., proximity of study assessment sites) and human subjects study requirements, these issues will be addressed in a site-specific manner. Each site's procedures will include, but not be limited to: contact information of friends or family members who are likely to have contact information for the participating families in years to come; evidence that the parents understand the nature of the study procedures and the length of time commitment required; procedures designed to minimize study procedure burden (e.g., use of distraction procedures and lidocaine-prilocaine – EMLA - cream prior to blood draws in the infant; reimbursing travel costs); procedures designed to maximize study procedure comfort and convenience (e.g. food record forms designed for ease of use; availability of study assessments as part of usual well-baby visits or in the evenings, on weekends, or at home); and procedures designed to thank and support participants for their efforts (e.g., birthday cards for infant and parents, small thank you baby gifts, educational or health information re the child's growth and development).

Since the nature and availability of psychological resources will vary across sites, these procedures will be specified by each site and will include a protocol for assessing whether a family wishes psychosocial support and if needed, how this support will be provided.

8.12.4.3. Assessing the Psychological Impact of Study Participation

The psychological impact of study participation will be assessed by a mailed questionnaire prior to the 3 month clinical visit and by self-completed questionnaire at the 6, 15, 27 month study visits, and annually thereafter. If questionnaire data cannot be obtained due to missed study visits, or insufficient time to complete the questionnaire during a study visit, the information may be obtained by mail, telephone interview, or at the next study visit.

Should anyone drop out of the study or develop Type 1 Diabetes, the Parent Experiences Questionnaire will be given in clinic, mailed or completed online to assess participant experiences in the TEDDY study and reasons for dropout. These data will be obtained from the child's primary caretaker, usually the

mother. On an elective basis, fathers/partners will be invited to participate. They will be given the same questionnaires as given to the primary caretakers.

Once the child is 10 years of age, the psychological impact of study participation on the child will be added to the protocol as part of the annual assessment. Data collection from children below the age of 10 may be conducted on an elective basis.

Children who withdraw from the study or who develop Type 1 Diabetes will complete a Child Experiences Questionnaire if he/she is at least 10 years of age. The questionnaire will be given in clinic, mailed or completed online.

8.12.4.3.1. Parents' distress (anxiety and depression) in response to infant's at-risk status

The 20-item State portion of the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970) is a reliable assessment instrument for assessing situation-specific anxiety in the U.S. and internationally. It has been used in several studies assessing anxiety in islet cell antibody positive (ICA+) children and adults and their family members (Johnson et al., 1990; Roth et al., 1994; Johnson and Tercyack, 1995; Roth et al., 1996; Johnson and Carmichael, 2000; Hummel et al., submitted). It has also been used to assess anxiety in mothers after they were told their infant was genetically at-risk for type 1 diabetes in PANDA (Prospective Assessment in Newborns of Diabetes Autoimmunity) (Carmichaelet al., 2003; Johnson et al., 2004) and BABY DIAB (Hummel et al., submitted). We propose to use a 6-item short form of this instrument in the self-completed questionnaire filled out prior to the initial 3-month clinic visit as well as at the 6-, 15-, and 27-month visits. In a sample of over 400 PANDA mothers, whose infants were genetically at-risk for diabetes, the six-item short form correlated highly with the STAI full scale ($r = .95$) and showed excellent internal consistency ($\alpha = .92$).

In addition, at any time during the study, should a child show evidence of persistent autoimmunity (a positive autoantibody test result on two consecutive occasions), parental reactions to the news of the child's increased diabetes risk will be assessed using the 6 item short form STAI at the child's next clinic visit. Although there is a small literature assessing the initial impact of telling a mother that her child is at increased risk for TIDM, there are no longitudinal studies assessing the impact of learning a genetically at-risk child is showing evidence of further progression toward diabetes.

At the 6-month clinic visit, parental depression will be assessed by the Edinburgh Postnatal Depression Scale administered as part of the self-completed questionnaire. In a recent study of 192 PANDA mothers with

at-risk infants, Edinburgh Postnatal Depression Scale scores were reliable ($\alpha = .89$) and predictive of the mother's understanding of risk (higher depression scores were associated with underestimating risk), anxiety (higher scores were associated with higher anxiety) and study drop out (higher scores were associated with early study drop-out) (Hood, 2003). A brief 6-item Depression Scale from Bradley's Well-Being Questionnaire will be included in the self-administered parent questionnaire completed at the 15 - and 27-month study visits. This scale has been previously used in World Health Organization (WHO) studies and has been translated into several languages.

After 27 months, we propose to collect measures of parental psychological stress/function annually. We will conduct an interim analysis of data collected from the primary caretaker through 27 months to guide us in terms of retaining the psychological stress/function measures or adding new ones.

At 39 months, we will add questions to the parental/primary caretaker's annual assessment that address the parent's or primary caretaker's perceptions of the child's function and well-being (e.g., does the caretaker overprotect, stigmatize or treat the child differently because the child is at-risk for diabetes). Under consideration are Parsons et al's (2002) rejection/protection index and adjective checklist approach used with four year old boys with Duchenne muscular dystrophy identified through newborn screening. However, since it will be more than three years before these assessments will be conducted, in two years we will conduct a through review of the available literature to identify the best available instrument for this purpose.

8.12.4.3.2. Child reactions to study participation and at-risk status

We expect families to differ considerably in terms of when they choose to inform a child of his or her increased risk status. Parents will be encouraged to make their own decision in this regard. However, appropriate expertise will be available at each site to assist parents in determining at what age the child should be informed and what language should be used to assure adequate understanding. TEDDY will develop written materials appropriate for various ages as well as suggested age-appropriate language to use when explaining the child's increased TIDM risk.

Children who are so informed and who are at least 10 years of age will be assessed for their reactions to their increased risk status, general psychological function, and reactions to study participation. Reactions to their increased risk status will be assessed using strategies previously employed with children in the DPT-1 and with children who are ICA+

(Johnson 1990, 1995, 2000, 2002). General functioning will be assessed by Harter's Self Perception Profile for Children or Adolescents. Assessments will be conducted annually. Given the demands on staff to conduct assessments of children less than 10 years of age, such assessments will be considered elective. Since assessments of the child will begin 5-10 years after the child enters TEDDY, we will consider alternative child assessment measures 5 years post-TEDDY inception. For example, should the National Children's Study be under way, we may want to select some measures used in that large-scale longitudinal study to assess functioning of TEDDY children.

8.12.4.3.3. Behavior changes the family may make in an effort to prevent the disease in the child

Data from several sources suggest that individuals who believe themselves to be at-risk for diabetes report behavior changes in an effort to prevent the disease. In Belgium, a large majority (73%) of adults with a first degree diabetic relative indicated that they would engage in life style changes if the results of a screening program indicated they were at high risk for the disease (Hendrieckx et al., 2002). Over half of the U.S. participants in the insulin injection arm of the Diabetes Prevention Trial – Type 1 Diabetes (DPT-1) indicated they made some sort of behavior change in an effort to prevent the disease; this was true for both the experimental (insulin injections) and control (watchful waiting) arms of the trial (Johnson et al., 2002). Among ICA+ children, half reported making behavior changes in an effort to prevent the disease; behavior change was associated with greater initial anxiety in response to the news of ICA+ status (Johnson and Tercyack, 1995). Over 60% of mothers of genetically at-risk infants, who were interviewed 2-4 years after learning of their infant's increased risk, indicated that they made behavior changes in an effort to prevent the disease in the child. The most common changes reported were: changes in diet (33%) and exercise (12%), efforts to reduce risk of illness of infection (9%), and increased monitoring of the child for signs and symptoms of diabetes (57%) (Baughcum et al., 2003). Across studies, dietary changes were the most often reported although increased monitoring by parents of at-risk children has been commonly reported as well.

In TEDDY, behavior changes initiated by parents in an effort to prevent diabetes in the child will be assessed by two questions as part of the self-completed questionnaire assessment at the 6-, 15-, 27-month study visits and annually thereafter. A TEDDY Participant Survey to be administered at the completion of TEDDY or at the time the parent leaves the study also assesses behavior changes made in an effort to prevent diabetes in the child.

8.12.4.4. Family satisfaction with participation in the study protocol

Parental satisfaction with study participation will be assessed by 3 items on the self-completed questionnaire administered at 6, 15, 27 months and annually thereafter. At the end of the study, all parents will be given a detailed participant survey evaluating all components of the study. Dropouts will be given this survey, by telephone if necessary, at the time they leave the study. Child satisfaction with study participation will be assessed on an annual basis with youngsters 10 years or older who have been informed of their at-risk status. At the end of the study, all children will also be given a detailed participant survey evaluating all components of the study.

8.12.4.5. Psychosocial Stress as a Potential Trigger for Type 1 Diabetes

Stress has long been considered a potential trigger for TIDM (Danowski 1963). Psychological stress may affect the immune system in a variety of complex ways (Leclere 1989; Saravia-Fernandez et al., 1996). A number of retrospective studies have found stressful life events to be associated with disease onset (Slawson et al., 1963; Stein, 1971; Kisch, 1985; Robinson, 1985; Robinson et al., 1989; Vialettes, 1989; Dahlquist et al., 1991; Hagglof et al., 1991; Thernlund et al., 1995). However, not all investigators have found a link between stress and diabetes onset (Littorin et al., 2001). Further, most previous studies have used retrospective reports of stress, which are methodologically flawed.

In a prospective study of individuals screened for islet cell autoantibodies, Roth and her colleagues found a greater number of loss experiences during the year before the screening procedure in ICA+ families compared to ICA- families (Roth et al., 1994; Roth et al., 1996). In the DPT-1, negative life events were also assessed at the time of ICA screening, before the results were known. Negative life events were not predictive of ICA+ status in children or adults. However, negative life events were associated with increased illness which, in turn, was associated with ICA+ status (La Greca et al., 2000). Others have argued that psychological mechanisms serve as mediating variables between a number of disparate risk factors and the development of type 1 diabetes (Sepa et al., 2002).

Procedures

In TEDDY, psychological stress will initially be prospectively measured in two ways: (1) negative life events documented in the study's baby book by the parent and updated at each study visit, and (2) paternal or primary caretaker anxiety and depression measured by self-completed questionnaire. Once the child reaches 10 years of age and is informed of his/her at-risk status, measures of stressful life events and child functioning will be obtained from the child.

Measures

8.12.4.5.1. Number, nature and timing of negative life events

The number, nature, and timing of negative life events affecting the parent and the child will be assessed by a checklist in the TEDDY Book, filled out by the parent at home, and reviewed and updated at each study visit. Items on the checklist were selected from previously used life events checklists, including those used in the DPT-1 and in ongoing European studies with this population (DiPiS; BABYDIAB).

Once the child reaches 10 years of age and is informed of his/her at-risk status, the child will be invited to complete a standardized life events checklist developed specifically for children and adolescents.

8.12.4.5.2. Parental anxiety and depression

Parents or primary caretakers who are anxious and depressed create a stressful environment for the child. As stated previously, we plan to measure parental anxiety using the 6-item short form of the State Trait Anxiety Inventory. We also plan to measure post-partum depression at 6 months using the Edinburgh Postnatal Depression Scale and general depression at 15 and 27 months using the 6-item Depression scale of the Well-Being Questionnaire. After 27 months, parental or primary caretaker psychosocial functioning will be assessed on an annual basis. Our interim analysis of data collected from the parents or primary caretakers through 27 months will guide us in terms of retaining the 6-item State Trait Anxiety Inventory and the Depression scale of the Well-Being Questionnaire on the annual assessments or selecting alternative measures of parental/primary caretaker psychological stress and functioning.

8.12.4.5.3. Child psychosocial functioning

As discussed previously, once the child is 10 years of age and has been informed of his/her at-risk status, the child's reactions to his/her at-risk status, study participation, as well as general psychosocial functioning will be assessed on an annual basis. Assessment in youngsters younger than 10 years of age may be conducted on an elective basis. These measures may be used to assess whether highly stressed children progress to diabetes sooner than non-stressed children.

8.12.4.6. Internalizing Behavior and Psychosocial Stress as a Potential Trigger for Type 1 Diabetes

The Child Behavior Checklist (CBCL) is a well-validated instrument originally developed by Dr. Thomas Achenbach that has been used extensively worldwide for over 25 years. It is a measure of internalizing behavior (affective and anxiety problems) and externalizing behaviors (attention deficit/hyperactivity problems and oppositional defiant problems) and will be completed by one of the child's parents when the child is 3.5, 4.5 and 5.5 years of age. The internalizing scales are most directly related to the psychological stress hypothesis of TEDDY. Children with high internalizing scale scores are considered particularly stress-reactive (Calkins et al, 2007; Lengua et al, 2006; Smider et al, 2002).

The CBCL will be used together with the Stressful Life Event data to test whether children who develop persistent beta-cell autoimmunity experienced greater life stresses and higher internalizing scores on the CBCL than those who do not develop autoimmunity. We are particularly interested in the interaction between a child's internalizing scores and the number of stressful experiences in the child's life. We expect the high frequency of negative life events will have the greatest impact on beta-cell autoimmunity on children with high internalizing scores.

Because TEDDY collects data on illness episodes, we can also test the link between life stress, a child's internalizing scores, and the child's susceptibility to illness. The link between stress and illness has been well-established (Cohen, 2005; Miller and Cohen, 2005; Wright et al., 2005). It is possible that stress has an indirect effect on the development of T1DM through this mechanism; children who have high internalizing scores and who experience more stress may be more susceptible to illness. Illness in turn, may be linked to the development of autoimmunity and T1DM – one of the primary study hypotheses.

Because the CBCL is so well normed, it can provide important information about the impact of TEDDY on the child. Rates of externalizing and internalizing behavior comparable to normative samples would suggest that TEDDY children are not suffering unnecessarily from their identification of increased T1DM risk in the absence of any means to prevent the disease.

As with any normal sample of children, we expect some children to exhibit elevated internalizing and externalizing scores. Children with elevated scores may be at increased risk for study drop-out. Those with high internalizing scores may show heightened distress to the blood draws, leading parents to drop-out of the study. Those with high externalizing behaviors are often oppositional and exhibit significant behavior problems. Parenting such children is particularly challenging (Paley et al, 2006) and the demands of the

TEDDY protocol may be more than a parent of such a child can manage, leading to study drop-out.

As children age, it will be important to gather self-report data on their psychological functioning. Given the lengthy nature of the CBCL, TEDDY will move from the CBCL (parent-report only) to the Strengths and Difficulties Questionnaire (SDQ) (parent- and child-report) at 11.5 and 13.5 years of age (Goodman, 1997; Goodman et al., 1998). The SDQ is a well-validated screening instrument used in the US National Children's Study as well as internationally in TEDDY countries (Hintermair, 2006; Koskelainen et al., 2000; Malmberg, et al., 2003) that assesses child psychological functioning across five behavioral and emotional domains. The SDQ has 25-items and will allow a determination of whether TEDDY children are comparable to children from the general population in terms of their psychological and behavioral functioning. The SDQ has been cross-validated with the CBCL, thus past TEDDY data using the CBCL can be easily compared (Goodman et al., 1999).

CBCL assessments in the TEDDY protocol

The parent (primary caretaker) should complete the CBCL on an annual basis when the child is 3.5, 4.5 and 5.5 years of age. The CBCL should be completed by the parent at home.

Due to restrictions from the scale constructor the Pervasive Developmental Problems Scale of the CBCL will be administered as well. This scale might show psychological problems not classified as internalizing or externalizing.

It is important that each site has prepared a plan for referral or counselling of children who score high on any of the scales.

8.12.4.7. Identifying Family Characteristics that Discriminate Study Completers from Study Drop-Outs

To improve study efficiency and participant retention, it will be important to identify family characteristics predictive of study completion and drop out. Both demographic and psychosocial measures will be assessed for this purpose. These data will be collected by questionnaire or other study procedures, as described previously.

Demographic predictors. Likely candidates for demographic predictors of study retention and drop out include: family history of diabetes; convenience to the study site (including travel distance and time); expenses associated with study visits; availability of transportation to study visits; parental employment and whether work must be missed for study visits; extent of other child-care or family-care demands; child birth order; child gender; parental age, parental

education, parental income; single-parent status, child and parent race, and study site.

Psychosocial predictors. Likely candidates for psychosocial predictors of retention and drop-out include: parental anxiety and diabetes worry in response to the child's at-risk status (with high anxiety and diabetes worry associated with study retention); parental beliefs about the likelihood the child will develop diabetes (with parental beliefs that the child will never develop diabetes associated with drop-out); parental depression (with high depression more likely to be associated with drop out); negative life events in the parent or child (high number associated with drop-out); parental concerns with confidentiality or possible loss of medical insurance (higher concern associated with drop-out); parental satisfaction (low satisfaction associated with drop-out). Certainly the relative contribution of father vs. mother assessments on these variables is of interest, and may differ across cultures. Once the child reaches the age of assent, child variables may become predictive of study retention or drop-out.

8.12.5. Self-assessment pubertal status instruments

Many children progress to type 1 diabetes (T1D) during puberty but little is known about the potential effects of puberty on autoantibody seroconversion. The TEDDY cohort provides a unique opportunity to prospectively follow children with known genetic risk for T1D through the development of puberty in order to definitively answer questions regarding the effects of androgens and estrogens on diabetes risk.

Specific Aim: To determine if pubertal progression is associated with augmentation in risk for autoantibody seroconversion or development of T1D amongst children in the TEDDY cohort.

Hypothesis 1: Initiation of puberty is associated with an increased risk of seroconversion from negative to positive.

Hypothesis 2: Initiation of puberty is associated with an increased risk of transitioning from single antibody to multiple antibody positivity.

Hypothesis 3: Initiation of puberty is associated with an increased risk of developing T1D.

Therefore TEDDY will use self-assessment pubertal status instruments every 6 months for TEDDY visits beginning at age 8 years and until pubertal status is assessed as Stage 5 for both pubic hair and breast development/genitalia or the child reaches 15 years of age. Self-assessment may be done at the TEDDY clinic during the visit or at home before the visit. The self-assessment can be made by the parent or by the child.

8.13. Specimen and data collection between clinic visits

8.13.1. Stool

Stool specimens will be collected monthly by the family in the period between clinic visits; monthly stool collection will occur up until 48 months of age, every three months until 10 years of age and then biannually thereafter. In August 2018 all stool sample collections were stopped on all subjects. Stool sample collection compliance was less than 20% in Europe and 15% in the US. The small numbers did not warrant the cost of collection, processing, nor the burden on the families. Stool will be collected and shipped as described above. (Section 8.4)

8.14. Specimen testing

Islet cell autoantibodies will be assayed as specimens are obtained to determine whether a subject has reached the study's primary endpoint (see Section 9.1). Metabolic testing will be performed as described in Section 8.15.to diagnose diabetes in subjects who are persistently autoantibody-positive. The remaining testing will be performed on a case-control basis. For case subjects, all specimens obtained prior to the subject reaching the study's primary or secondary endpoints will be tested as described below and in Table 3. Control subjects will be matched to cases as described in Section 10.5.2. The testing procedures described below reflect the current state of knowledge and technology. As new hypotheses are developed and improved methodologies become available, they will be incorporated into the study to the extent possible.

8.14.1. Autoantibodies

8.14.1.1. Background

Autoantibodies against islet antigens are markers of T1DM. Autoantibodies that have been repeatedly shown to be markers of T1DM are islet cell antibodies and the biochemically defined antibodies against insulin, glutamic acid decarboxylase antibodies (GADA) and IAA or IA-2A. The biochemically defined antibodies to GADA, IA-2A, and to IAA will be measured as outcome markers in the TEDDY study. Antibodies will be measured on samples obtained from each scheduled clinic visit. ZnT8A will also be measured on samples that are found to be positive for at least one of the three islet autoantibodies (GADA, IA-2A and/or IAA), on all future samples of subjects who have had ZnT8A measured on any past sample (unless subject becomes antibody negative for all antibodies for one year – at which point ZnT8A would be stopped until autoantibody positivity reappears for GADA, IA-2A and/or IAA) and on samples of subjects who are deemed persistent confirmed single or multiple autoantibody positive.

8.14.1.2. Reference Laboratories

Measurements will be performed in two central laboratories, one located in the US and another in Europe. The US central laboratory will measure samples from Washington State, Florida/Georgia, and Colorado clinical centers, and the European central laboratory will measure samples from Finland, Germany and Sweden clinical centers. All samples identified as positive and a subset of negative samples will be tested in both central laboratories. Autoantibodies in samples will be classified as positive only if reported positive in both central laboratories.

> Note: ZnT8A measurement will only be performed at the US central laboratory and the lab will perform the measurements for both the US and European sites.

8.14.1.3. Sampling

Two aliquots of 200 μL each of serum will be obtained every three months for the first four years of life at each clinic visit for determination of autoantibodies. At 4 years of age and beyond those children who have been deemed persistent autoantibody positive will continue to have two aliquots of 200 μL each of serum collected every three months for determination of autoantibodies while all other subjects will have two aliquots of 200 μL each of serum collected every six months for determination of autoantibodies. One of the samples will be sent to the Autoantibody Reference Laboratory for testing, and the other sample will be sent to the NIDDK repository for storage.

A single aliquot of sample will be utilized by the reference laboratory to determine GADA, IAA, IA-2A autoantibodies and ZnT8A on samples that are found to be positive for at least one of the three islet autoantibodies (GADA, IA-2A and/or IAA), on all future samples of subjects who have had ZnT8A measured on any past sample (unless subject becomes antibody negative for all antibodies for one year – at which point ZnT8A would be stopped until autoantibody positivity reappears for GADA, IA-2A and/or IAA) and on samples of subjects who are deemed persistent confirmed single or multiple autoantibody positive. It is envisioned that the reference laboratory will repeat all positive samples internally prior to reporting positive or negative, and will measure twice, if specific autoantibodies positive are confirmed, and up to three determinations if there is discrepancy between initial positive and second determination (2/3 internal lab reported as positive, with mean of consensus positives or negatives reported in WHO units). Results will then be sent electronically to the Data Coordinating Center. The Data Coordinating Center will then send the NIDDK repository the ID for all positive samples and a subset of negative samples (5%), and the repository will send the second aliquot of serum to the alternate Reference laboratory for confirmatory testing.

8.14.2. Infectious Agents

8.14.2.1. Background

Viral infections during pregnancy and childhood have been associated with increased risk of T1DM in both human and animal studies. The most well known example is congenital rubella, which seems to lead to diabetes in 10-20 % of infected individuals (Hyöty and Taylor, 2002). Routine immunizations have greatly decreased the number of rubella virus infections making it difficult to evaluate this question in TEDDY.

Currently the strongest candidate for diabetogenic viruses is the group of enteroviruses. These viruses consist of more than 60 different serotypes and are common in all age groups, particularly in young children, who experience several serial infections by different serotypes. Enterovirus infections have been linked to T1DM in several cross-sectional case-control studies during the past 30 years (Graves et al., 1997; Hyöty and Taylor, 2002; Hyöty 2002). Certain virus strains can also cause diabetes in mice and damage beta cells in vitro. In addition, their risk effect has been documented in many prospective studies suggesting that enterovirus infections could play a role in the initiation of the process. However, these prospective studies have been based on relatively small series, and no association was found in two of them. Accordingly, there is a clear need to confirm the risk effect of enterovirus infections in larger prospective series using standardized protocols and methods.

Several other viruses have also been connected to the pathogenesis of T1DM, even though the evidence is less convincing than that for enteroviruses. Among the most interesting candidates are rotaviruses, which have been linked to T1DM in a previous prospective study (Honeyman et al., 2000). However, another prospective study failed to show any risk effect (Blomqvist et al., 2002). Rotavirus can also infect beta cells in vitro (Coulson et al., 2002) and share mimicry epitopes with beta cell autoantigens (Honeyman et al., 1998). Other potentially interesting viruses include cytomegalovirus, mumps virus, parvovirus and retroviruses (Hyöty and Taylor, 2002). Practically nothing is known about possible role of other microbes, such as bacterial infections and intestinal microbiota.

Due to the large amount of potentially interesting microbes TEDDY will first focus on the two most likely candidates, enteroviruses and rotaviruses (Tier 1 agents), but leaving open all options to evaluate the role of any other agents. Therefore, a wide range of samples, including plasma, stool and nasal swab samples, will be collected for a long-term storage in a way, which allows the screening of a wide range of microbial agents.

Diagnosis of Tier 1 agents

Diagnosis of enterovirus infections in prospective studies is more demanding than that of many other virus infections. A great majority of infections (90%) are subclinical and diagnosis is based on the detection of the virus (virus isolation) or viral nucleic acids (RT-PCR) in clinical samples or on the measurement of enterovirus-specific antibodies in serum. The sensitivity of these methods depends on technical performance of the assay as well as the timing of sampling (acute vs. convalescent sample) and the test's ability to detect all enterovirus serotypes.

Current RT-PCR assays can detect practically all enterovirus serotypes, but the virus is detectable only during a limited period following the infection, usually from some days up to two weeks in blood and up to 2-6 weeks in stools. Antibodies can be detected longer after the infection, and in prospective studies significant increases in IgG, IgM or IgA class antibodies between serial samples can be used to diagnose infection. However, the sensitivity of antibody assays is not very good, mainly because the large number of enterovirus serotypes makes it difficult to cover them all.

In the TEDDY study serial samples will be collected on a regular basis during the follow-up and the length of sample intervals greatly influences the sensitivity to detect enterovirus infections. The investigators of the TEDDY consortium have a long experience from the diagnosis of entrovirus infections in prospective series, and the decision to keep the sample intervals relatively short (monthly) is based on this experience.

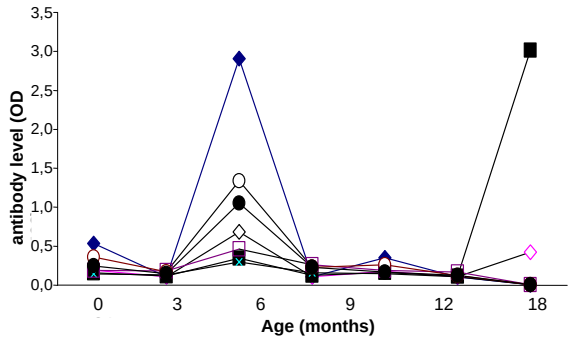
For example, in the Finnish DIPP study, where serum samples have been collected every 3-6 months and stool samples every month, altogether 5.7 % of the follow-up serum samples from the case subjects (children who developed clinical diabetes) and 2.2 % of serum samples from control subjects were positive for enterovirus RNA (unpublished findings). The corresponding figures for stool samples were 9.4 vs. 7.9 %, respectively (Salminen et al., 2004). In most cases the child was enterovirus positive in serum only once during the follow-up while the virus was often detected repeatedly in stools as the child experienced serial infections. However, only a single stool sample was usually positive during each infectious episode, even though in some cases the same virus was detected in two consecutive samples taken one month apart.

It was also observed that the ability of antibody assays to detect enterovirus infections depends on the length of sample intervals: Some infections could only be diagnosed if samples were taken every 3 months but not if the sample interval would have been longer (Figure 1). According to this type of experience from previous prospective studies, it can be estimated that by increasing the serum sample intervals from the planned 3 months to 6 months

at least 40 % of enterovirus infections would be missed. The same would be true if stool sample intervals would be increased from one to two months. Taking extra samples during acute infectious episodes could not compensate this loss, because most enterovirus infections are subclinical. Diagnosis of rotavirus infections is not this sensitive to the length of sample intervals, but even this would be hampered if sample intervals would be longer (particularly the diagnosis of re-infections, which may only be reflected by transient increases in antibody levels).

Short sample intervals have also another advantage - they make it possible to analyze time-relationships between infections and the appearance of autoantibodies. This has been demonstrated in previous prospective studies where enterovirus infections were clustered to the time period immediately preceding the appearance of autoantibodies (Salminen et al., 2003).

Figure 1



◆ CBV4 IgG, ◊ CBV4 IgA, ■ EV11 IgG, □ EV11 IgA, ● Peptide IgG, O Peptide IgA

Legend to Figure1.

Antibody levels and viral RNA in serum during the follow-up of a child of the second pilot study of TRIGR trial. He had an IgG response of short duration to the CBV4 antigen, which would have been missed if longer sample intervals would have been used. This type of transient antibody responses are seen when the serotype of the enterovirus antigen used in the EIA test does not match with the serotype causing the infection (which was CBV3 in this child).

8.14.2.2. Serology for infectious agents

A 100 µL aliquot of plasma will be used for enterovirus and rotavirus serology. Specific antibodies will be measured by ELISA. Specific methods will be determined by the testing laboratories once they are chosen by NIH. Additional aliquots of plasma will be stored at –70°C to allow for future testing for other infectious agents.

8.14.3. Serum Cytokines/Inflammation Markers

Serum proteins that are markers of inflammation or that can distinguish infection or inflammation ‘type’ will be considered for measurement on a case-control basis. These include CRP measured with highly sensitive assays, and cytokines and chemokines that are relatively stable (i.e. can be measured in blood samples kept at room temperature for up to 24 hours). The steering and appropriate advisory committees will select the markers to be measured. One aliquot of 100

μL of serum will be obtained for the TEDDY study for measurement of inflammatory markers.

8.14.4. Tissue Transglutaminase antibodies

T1DM and celiac disease share HLA susceptibility alleles. Tissue transglutaminase antibodies are markers of celiac disease and are a result of an abnormal immune response to dietary gluten. Diabetes-associated autoimmunity may also be influenced by dietary gluten intake. The TEDDY study cohort therefore provides an opportunity to investigate the environmental determinants of celiac disease and determine whether an abnormal response to gluten in the form of tissue transglutaminase antibodies modifies T1DM risk. Measurement will be performed on all subjects at age 2 years and annually thereafter. This will be performed on serum aliquots used for islet autoantibody measurements; the islet antibody sample blood volume will be increased to 220 μl. The measurements of the transglutaminase antibodies samples will be performed in the two Autoantibody Reference Laboratories. If the annual transglutaminase antibodies sample, which starts at 2-years, is positive, the antibodies are analyzed again after 3 months (if subject is on 6 month visit schedule, the antibodies are analyzed again after 6 months); if negative the antibodies are analyzed again after one year. If confirmed positive, the child will have attained the TEDDY study endpoint for transglutaminase antibodies. Children positive and those negative for transglutaminase antibodies will continue to be screened annually. Persistently positive children will be referred to their pediatricians for confirmation of CD diagnosis outside of the study protocol by an intestinal biopsy and possible initiation of gluten free diet, if clinically indicated. These aliquots will be stored by the central laboratories at –20°C for the duration of the study.

8.14.5. Thyroid autoantibodies and TSH

Thyroid disease is common in children diagnosed with type 1 diabetes (De Block et al, 2001). In the Swedish BDD study, 12 % of newly diagnosed children were positive for TPOA and or ThGA (Jonsdottir et al, 2013). Similarly, in newly diagnosed type 1 diabetes (T1D) children in the DAISY study almost 25% had TPOA (Triolo et al, 2011). In children to parents with T1D followed in the BABYDIAB study, TPOA showed an accumulated risk of 20% by 14 years of age and occurred more often in GADA positive children (Bonifacio et al, 2009). Cross-sectional preliminary data from children at genetic risk for T1D without family history of diabetes, followed prospectively in the DiPiS study, revealed that 6 % were positive for TPOA and 10 % for ThGA at 10 years of age (analyses are ongoing). Additionally, preliminary data from children with multiple islet autoantibodies including GADA, indicated that 14 % have persistent autoimmunity for thyroid disease, some developing thyroid autoimmunity already at age 5 or 6. Autoantibodies against TPO are found in 2-5% of the general population, although this prevalence is greater with increasing age, and less in children. HLA DR3 is related to both thyroid and islet autoimmunity, and in islet

autoimmunity especially to GADA. Additionally, polymorphisms at other non-HLA loci such as CTLA4 are known to influence both T1D and thyroid disease (Ueda et al, 2003). Although thyroid disease is common among newly diagnosed type 1 diabetes patients, it is important to determine the relationship between early thyroid autoimmunity and islet autoimmunity, their temporal relationship, and genetic influences on these.

Hypotheses:

1. Incidence of Islet and thyroid autoantibodies are statistically related in individuals in the TEDDY cohort.
2. The increased co-occurance of GADA and thyroid autoantibodies is not explained solely by the presence of the HLA DR3 haplotype.
3. The increased co-occurance of islet and thyroid autoantibodies is not explained solely by non-HLA loci known to be associated with both T1D and thyroid disease.
4. Islet antibodies precede thyroid antibodies in individuals in the TEDDY cohort.

TEDDY will test autoantibodies to thyroid peroxidase (TPOA) and thyroglobulin (ThGA):

1. In all children at 8 years of age or at current visit for those older than age 8. Samples from children positive for either thyroid antibody will also be tested for TSH in the same sample.
2. In all children at 14 years of age. Samples from children positive for either thyroid antibody will also be tested for TSH in the same sample.
3. Children positive for TPOA and/or ThGA at the 8 year visit and/or the 14 year visit will have a confirmatory sample draw at the next TEDDY visit, which will be analysed for TPOA and ThGA.
4. In children positive for either thyroid autoantibody at a given sample from step 1 or step 2, additional samples previously collected on that individual will be tested for TPO and ThGA autoantibodies sequentially backwards in sampling age to determine the first sample with either thyroid autoantibody.
5. All TEDDY children who have developed diabetes should be analysed for TPO and ThGA autoantibodies at the time of clinical diagnosis or at the last TEDDY visit prior to diagnosis.

Children who are found to have thyroid autoimmunity with or without elevated TSH will be informed by TEDDY staff and will be referred for medical care outside of TEDDY based on the normal site-specific protocol.

8.14.6. Dietary Biomarkers

An aliquot of 230 μL of plasma will be used for biomarker assays.

8.14.6.1. Background

The goals of the dietary collection component of TEDDY are 1) to identify dietary factors that predispose to or protect from islet autoimmunity and T1DM; and 2) to identify potential differences in dietary determinants of islet autoimmunity and T1DM across diverse populations and ethnic groups. TEDDY is designed to test and confirm existing dietary hypotheses as well as explore new, less well-documented hypotheses. Examples of the former include: initiation of persistent islet autoimmunity is associated with 1) early and late exposure to cereals or gluten in the infant diet and/or short duration of breast-feeding; 2) exposure to cow milk in infancy and later in childhood; and 3) lower intake of vitamin D or omega-3 fatty acids, an example of the latter is the collection of maternal diet during pregnancy. Studies from DAISY and BABYDIAB investigators recently suggested a strong association between timing of first exposure to cereals and risk of islet autoimmunity (Ziegler et al, 2003; Norris et al, 2003). These two studies, while remarkably consistent, did not agree on whether the exposure in question was to all cereals or to only gluten-containing cereals; and whether late first exposure (after 6 months) increased risk of islet autoimmunity in addition to early exposure. In order to investigate this issue further and resolve these discrepancies across studies, it is necessary to use common data collection protocols, the same recruitment criteria and the same follow-up protocols. Questions remain as to whether this association is driven by dose or quantity of exposure, or whether it is related to a proportional measure, such as percent energy from carbohydrates. In order to quantify exposure, one needs to collect information on the entire diet, which would allow one to get absolute intake (as opposed to frequency) and to adjust for energy intake. This requires the collection of a food record. There are no adequate biomarkers to measure the intake of cereals or specifically gluten. The findings of both case-control and cohort studies are inconsistent regarding the putative effects of cow milk intake on beta-cell autoimmunity and type 1 diabetes (reviewed in Virtanen and Knip 2003).

One prospective cohort study and a retrospective case-control study have reported a reduced risk of type 1 diabetes after vitamin D supplementation in infancy (Hypponen et al 2001, EURODIAB 1999). A case-control study suggested that cases of diabetes were less likely to have been given cod liver oil, which contains, in addition to vitamin D, vitamin A and the omega-3 fatty acids, DHA and EPA, in infancy compared with controls (Stene et al 2003). The next step in investigating the role of vitamin D (Norris et al, 2001) and fish oil is to use a prospective study design with complete dietary assessment and biomarkers. Therefore, in addition to the previously mentioned diet records, TEDDY will measure the biomarkers, 25,hydroxyvitamin D and erythrocyte membrane fatty acid composition in blood samples drawn from study subjects.

TEDDY also proposes to study more exploratory hypotheses. In a nested case-control study higher serum alpha-tocopherol levels were related to lower risk of type 1 diabetes in adults (Knekt et al. 1999). Serum alpha-tocopherol is a measure of anti-oxidant status in individuals. We hypothesize that low levels of alpha-tocopherol is associated with the development of islet autoimmunity. Other anti-oxidants, such as the carotenoids, ascorbic acid and selenium could work independently or in concert with alpha-tocopherol in preventing or reversing islet autoimmunity. Therefore, in order to investigate these exploratory hypotheses, we will collect intake of these micronutrients via the diet records as well as measure plasma levels of alpha-tocopherol, the carotenoids, and ascorbic acid. We will measure the anti-oxidant, selenium, via toe nail clippings in order to preserve the blood samples for other biomarkers that can only be measured in the blood.

Haglund et al (1996), Zhao et al (2001) and Stene et al (2002) suggested that zinc concentration in water was inversely associated with diabetes risk. Kostraba et al (1992) and Parslow et al (1997) suggested that higher nitrate concentrations in the water were associated with diabetes risk. Stene et al (2002) suggested that a lower pH level of drinking water is associated with increased diabetes risk. We will investigate these hypotheses in TEDDY by collecting a sample of tap water, which will be tested for zinc, pH and nitrate.

8.14.6.2. 25, hydroxyvitamin D

25,hydroxyvitamin D will be assayed using 50 μL of plasma collected from the infant at the age of 3 months, 6 months, 9 months and 12 months, and annually thereafter. The assay is an ELISA.

8.14.6.3. Alpha tocopherol, gamma tocopherol

Alpha tocopherol, and gamma tocopherol will be assayed using 70 μL of plasma collected from the infant at 6 months of age, 12 months and annually thereafter. The collection tubes should be amber or foil-covered in order to avoid exposing the sample to light. The plasma should be separated immediately and the plasma sample should be frozen at –70°C. Alpha- and gamma-tocopherol will be measured by HPLC. Total lipids will be measured using a colorimetric assay (Knight et al., 1972).

8.14.6.4. Carotenoids (beta-carotene, alpha-carotene, lycopene) and Ascorbic Acid

Carotenoids and ascorbic acid will be measured in plasma on samples taken at 6 months, 12 months and then annually. The blood sample should be kept from light using foil covered blood collection tubes and amber storage tubes. For the ascorbic acid assay, the plasma will be put in trichloride acetic acid for

storage. The assays will be HPLC. The carotenoid assays require 60 µL of plasma, and the ascorbic acid assay requires 50µL of plasma.

A paired-ion, reversed-phase, high-performance liquid chromatography procedure using electrochemical detection and internal standard quantitation with isoascorbic acid is used for the determination of ascorbic acid.

8.14.7. Erythrocyte Membrane Fatty Acid

Erythrocyte membrane fatty acids will be assays using 500 µL of red blood cells. The samples of red blood cells will be initially extracted for lipids. A neutral-polar separation utilizing a dry column, based on the method of Marmer and Maxwell (Marmer and Maxwell, 1981) will be performed. The fatty acids present will be methylated using the base-catalyzed procedures in preparation for analysis by gas chromatography with mass spectral detection.

8.15. Whole Genome Sequencing and Epigenetics Studies

To fully examine the genetic basis of islet autoimmunity in the TEDDY study, a comprehensive determination of genomic variation, through whole genome sequencing (WGS) will be conducted. TEDDY also plans to characterize the occurrence and potential functions of epigenetic modifications, using both global, unbiased approaches as well as the study of smaller chromosomal regions when appropriate, in order to understand how epigenetic regulatory mechanisms contribute to beta cell autoimmunity and T1D. Deep whole genome sequencing and epigenetic analyses will be completed on DNA samples from TEDDY subjects positive for islet autoantibodies or who have progressed to the clinical onset of diabetes and matched autoantibody negative TEDDY control subjects.

DNA samples from TEDDY parents will be used for estimating Mendelian inheritance errors (as one of the quality control criteria from the whole genome sequencing data set). By including the parents' samples, we will be able to compare the genotypes of the children to the parents; hence we will be able to provide estimation of Mendelian inheritance error rates. Moreover, whole genome sequences of a family of four, consisting of two siblings and their parents, will allow us to delineate recombination sites precisely and identify very rare single nucleotide variants.

DNA samples will be received at the University of Virginia for initial processing and quality control, followed by receipt at Macrogen USA for whole genome sequencing. DNA samples for epigenetic analyses will be sent to a TBD laboratory.

Whole genome sequencing data that are to be made available with specific data-sharing restrictions (consent) will need to be registered with the appropriate

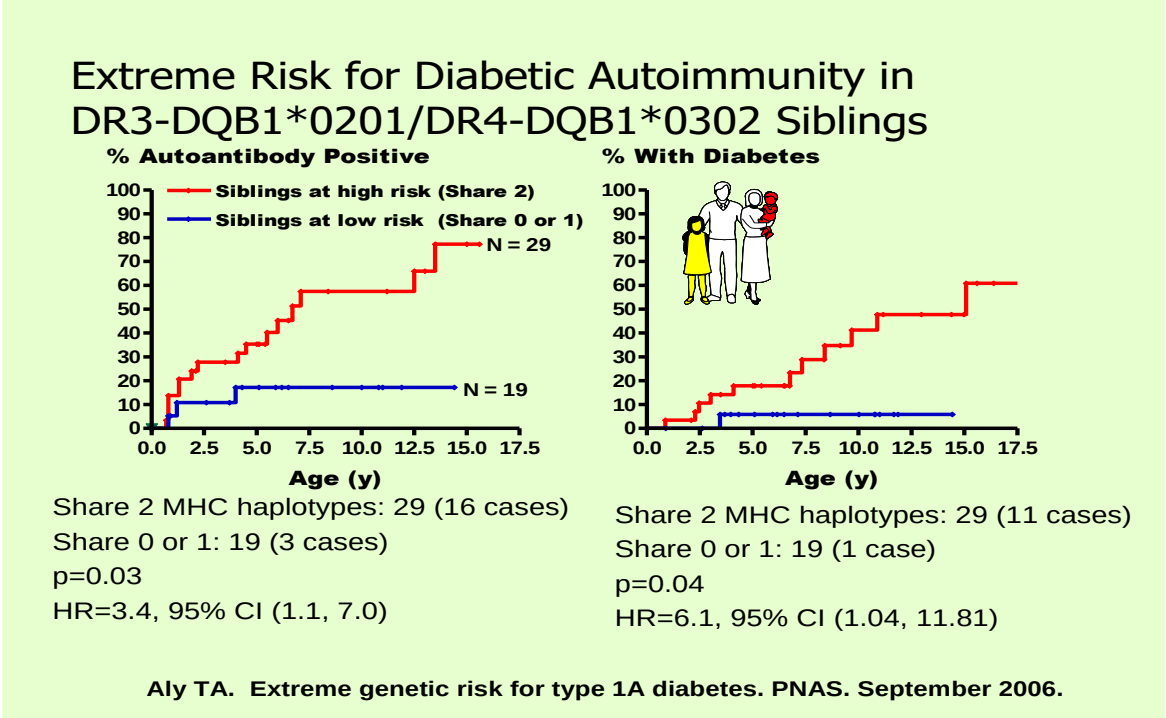
consent/data sharing information at the dbGaP, through the dbGaP Registration Portal/System. Access to those sequence data will be only through the dbGaP, according to the data sharing specification signed by participants.

8.16. Parental and sibling DNA collection

The HLA region encompasses many hundreds of genes that are more or less in complete linkage disequilibrium within extended haplotypes. In TEDDY we designate major immune response gene haplotypes by their DRB1-DQA1-DQB1 allele associations, but they are in fact much more extensive. Even for DR and DQ our definitions of haplotypes with only typing of the TEDDY child is probabilistic for the haplotypic association (alleles of these different genes on the same chromosome). In some cases, even probabilistic definition is not possible (e.g. specific DRB1*04 alleles with individual having two DR4 haplotypes with alleles DQB1*0301 and DQB1*0302 it is not possible to assign which DR4 allele (e.g. 0401 or 0404) goes with which DQ allele). In addition, for families in which there is an affected sibling, without genetic analysis of that sibling, we have no information as to inheritance of haplotypes identical by descent (IBD) for the TEDDY child. For families in which one child is DR3/4 and has diabetes and the sibling followed in TEDDY is also DR3/4, there is only an approximate 2/3 chance that the DR3/4 haplotypes (one haplotype from mother and one from father) were inherited identical by descent. This lack of identity by descent occurs for instance when one parent is homozygous for DR3 and passes on a different DR3-containing chromosome to each child. Though HLA haplotypes are emphasized here, the same general concepts apply to haplotype definition anywhere in the genome.

There is substantial evidence in the literature that inherited haplotypes that are identical by descent with an affected family member can confer very high risk of T1D to other family members. Analysis of siblings of patients with T1D (with parents) from the DAISY study has been particularly interesting as it was possible to define inheritance of both HLA regions identical by descent with their proband. For DR3/4-DQ2/DQ8 sibling identical by descent for the HLA, a risk of activating islet autoimmunity by age 12 as high as 80% was observed (Figure 2).

Figure 2



In order to discover additional genes within those extended haplotypes that contribute to T1D development in the child, one must determine which extended haplotypes were (and were not) passed to ultimately affected children. The most practical way to do this is to collect genomic DNA from each parent and sibling of each TEDDY child via one 5 ml venous blood sample. The parents and sibling must each undergo standard informed consent and sign a consent/assent form prior to sample collection. Collection can occur via TEDDY phlebotomists at the Clinical Centers at the same visit where blood is collected on the TEDDY child. Each center will obtain local IRB approval for sampling these additional subjects as part of TEDDY.

Genotyping of these samples is planned using a nested case-control design similar to that approved for other analyses of reposited TEDDY samples. This will not occur for a number of years until the endpoint status of the subjects (islet antibodies and/or diabetes) is known. The extensive genotyping required will likely be available in a more complete fashion at lower cost at that time. Samples chosen for genotyping will be analyzed at a large number of polymorphisms (e.g. SNPs, microsatellites or specific alleleic probes) across a wide area of the human HLA region and potentially other regions as the architecture of the genome is better defined. Analyses will then determine which inherited extended haplotypes appeared to confer elevated T1D risk and which did not.

While there are many possible genetic variations among the extended haplotypes, it is clear that this large TEDDY study will have sufficient samples to identify

features in common to either high-risk or low-risk extended HLA haplotypes. Such information is likely to contribute to analysis of gene-environment interactions affecting the development of T1D in the affected TEDDY children. A very simple first analysis would be to analyze families with a DR3/4 "high risk" TEDDY child who has an affected sibling, for analysis of development of anti-islet autoantibodies and T1D stratifying the TEDDY children by whether they inherit both HLA haplotypes identical by descent (versus not) to their proband sibling. It would take less than 82 siblings by simple proportions analysis of autoantibody cases (21 per group) to have power of 0.8 (alpha .05), based on autoantibody frequency estimates from the DAISY study. In some cases, interactions of HLA risk with binary environmental factors (yes or no exposure) will be best revealed via analyses of TEDDY children that are not IBD at both haplotypes with an affected first degree relative, but rather are at a somewhat lower genetic risk. In this case the effect of a specific binary environmental factor would presumably be more apparent. On the other hand, if very high risk (e.g. 80% risk by age 12) is confirmed in TEDDY children identity by descent at both haplotypes with an affected relative, this would be an ideal population to evaluate for timed influence of environmental factors on activating autoimmunity. In addition to analysis of identity by descent where the affected sibling is obviously crucial, for the determination of TEDDY participant haplotypes, non-affected siblings are also important. as many families will be non-informative for defining haplotypes of the TEDDY participant unless information on additional siblings is available. This is simply a result of increased informativeness of the family depending on inheritance of different haplotypes among siblings. In summary, we believe this genetic information, only available if DNA is obtained from parents and siblings, will be highly useful in TEDDY analyses.

As noted above, genotyping on these samples will not occur for a number of years, after the highest-risk period for development of T1D in the siblings is likely to have already passed. Additionally, the greatest risk to the child and family from this type of genetic analysis is the discovery of non-paternity. Therefore, families will not be told the identity of any specific alleles or haplotypes based on genotyping TEDDY parent or sibling samples. This policy is consistent with the longstanding TEDDY policy prohibiting any release of specific allele or haplotype information on the TEDDY child. At the time of consent, families will be informed that genetic typing results will not be available for many years on TEDDY siblings or parents, and that only general T1D HLA risk levels will ever be available. In the future, when those general risk levels are conveyed to the family, the same genetic counseling procedure and features that are used in TEDDY children will be followed. To ensure adherence to the above policy, specific parent and sibling genotyping allele or haplotype information will never be available to TEDDY clinic staff, but only to TEDDY investigators performing genetic or gene-environment analyses on this data. It should be noted that many TEDDY family members (e.g. First Degree Relatives) are eligible for risk marker testing as part of other diabetes studies approved by local IRBs at many of the

Clinical Centers, and centers are encouraged to make this testing available to all eligible TEDDY family members.

8.17. Metabolic markers

Random plasma/blood glucose and OGTT tests will be used for the diagnosis of diabetes according to the recommendations by WHO and the American Diabetes Association.

8.17.1. Random plasma/blood glucose

Random plasma/blood glucose tests will be conducted on every visit for every child that has tested positive for any islet cell autoantibodies.

A clinical center may stop doing random glucose measurements on a subject who meets the following criteria:

There has been only 1 positive antibody sample in the child's life (excluding maternal transfer of autoantibodies)

AND

There have been 2 consecutive negative antibody samples after the positive.

8.17.2. OGTT (Oral Glucose Tolerance Test)

Oral Glucose tolerance test (OGTT) tests will be performed every six months on every child who has tested positive for two or more autoantibodies (GADA, IAA, IA-2A, ZnT8A), regardless of autoantibody positivity confirmation or persistence, at any previous visit (but both antibodies must be positive at the same visit) and is three years of age or older. Oral glucose is administered in a dose of 1.75 g/kg body weight to a maximum of 75 grams in children, as a solution in flavored water, consumed within 5 minutes. A six-time point OGTT is performed with venous samples at -10, 0, 30, 60, 90, 120 minutes, which includes sampling for glucose, insulin and C-peptide at all time-points. Glucose will be measured locally. All samples will be shipped to the OGTT laboratory for processing.

Children who are not willing to participate in a six-time point OGTT can complete the original two-time point protocol instead: A two-time point OGTT is performed with venous glucose at time 0 minutes (for rare cases when it is not possible to obtain a venous sample from the subject, a capillary glucose at 0 minutes is acceptable) and capillary glucose at 120 minutes (if venous blood is available at 120 minutes then venous blood should be used instead of capillary blood). Glucose will be measured locally. Samples for glucose, insulin and C-peptide will be collected at time 0 minutes and if a venous sample is collected

glucose, insulin and C-peptide samples will also be collected at time 120 minutes; all samples will be shipped to the OGTT laboratory for processing.

9. Outcome measures

9.1. Autoantibodies

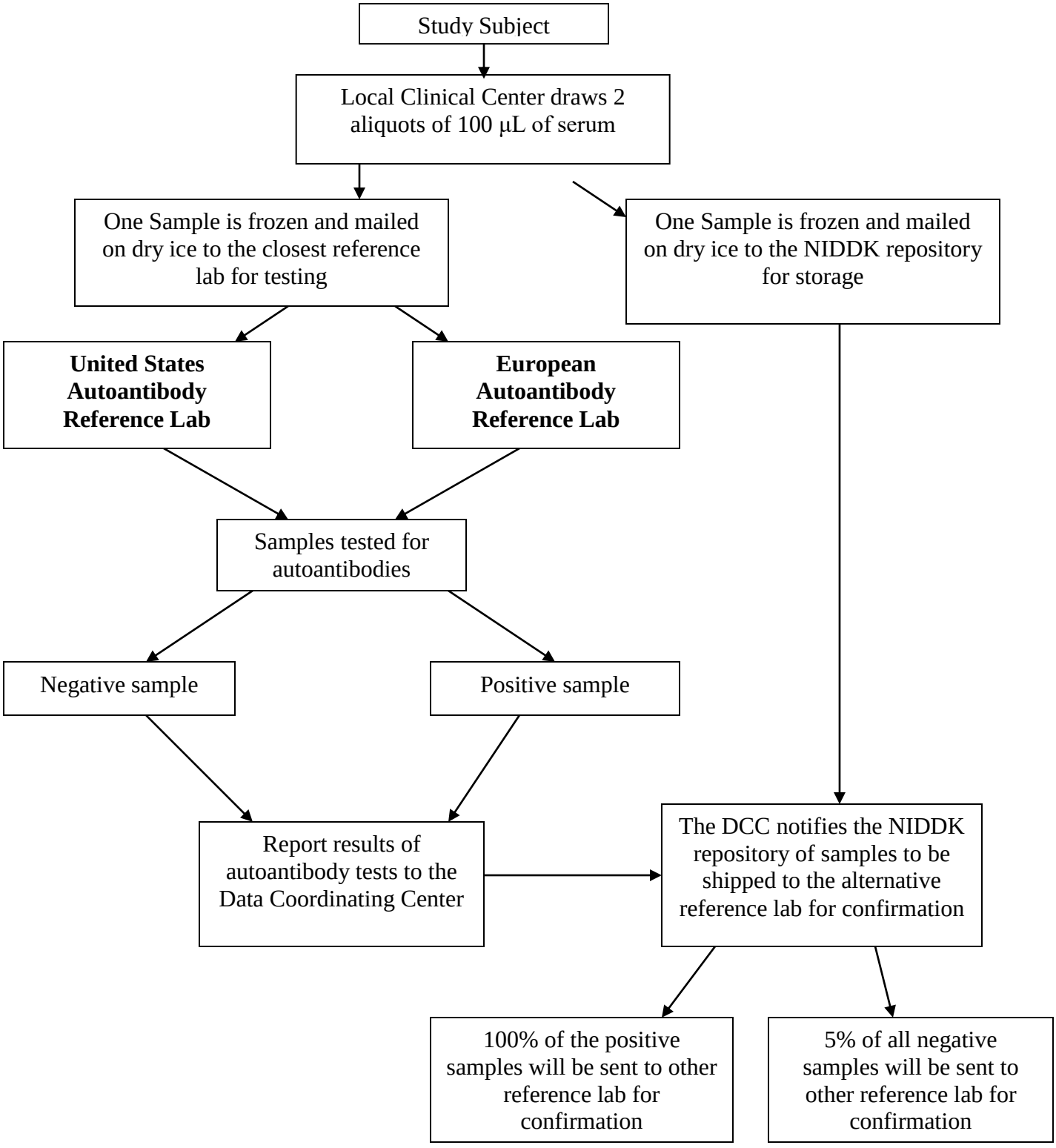
The first primary endpoint of TEDDY will be the first appearance of **persistent**, **confirmed positive** anti-islet autoantibodies. It is likely that this endpoint will be relatively uncommon (many more individuals negative) and important to be rigorously defined for all the environmental evaluations and thus the following classification errors on side of rigidly defining positivity (indeterminants count as negative). An additional complication will be the potential for transplacental autoantibodies in the study cohort.

There will be two TEDDY Central Autoantibody Laboratories, one in the United States and one in Europe. All samples identified as positive in one central laboratory will be sent to the alternative central laboratory and the following criteria used for classification of samples:
Pos/Pos: If both laboratories report the sample as positive it will be classified as **confirmed positive**.
Pos/Neg discrepant samples will be classified as **indeterminate**.
Samples reported as negative in the central laboratory will be classified as **negative**.

A proportion (5%) of samples reported as negative in the central laboratory will be randomly identified by the Data Coordinating Center and also sent to the alternate central laboratory for quality control purposes only. If there are significant discrepancies between the two reference laboratories, an ad hoc investigatory committee will determine what measures will be taken to improve concordance.

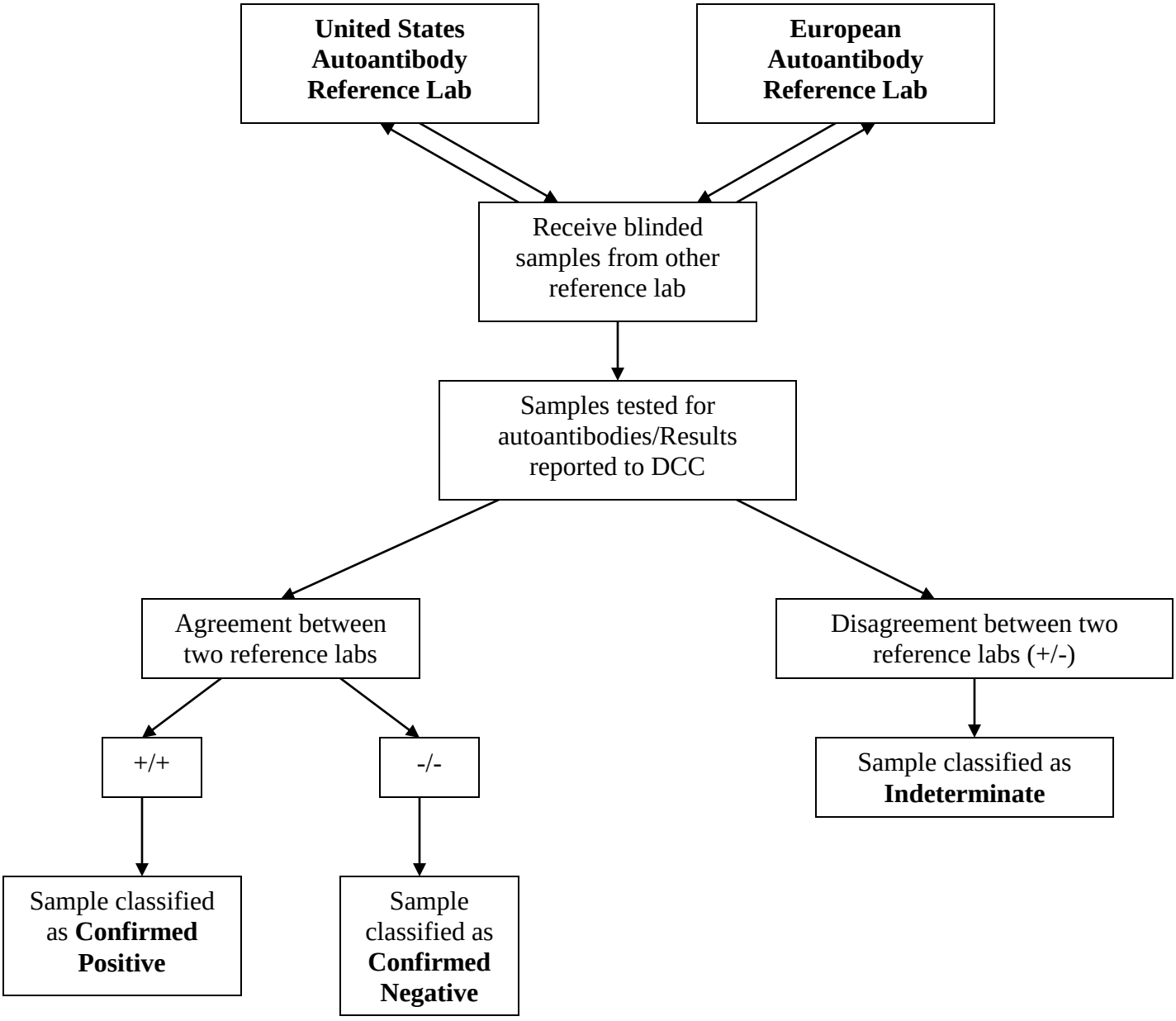
TEDDY Protocol

Schema 3: Autoantibody Testing



TEDDY Protocol

Schema 4: Autoantibody Confirmation Testing



For classification of individuals, **Persistent confirmed positive** is the major endpoint.

Persistent confirmed positive is defined as:

a. >=1 Autoantibody **Confirmed Positive** Autoantibody reacting with GADA, IAA , or IA-2A referring to a single sample and same specific autoantibodies have to be "confirmed" in the reference laboratories.

And **Persistent** (b, and c below) – refers to time sequence and the specific autoantibodies not important (for b), rather the presence of >=1 confirmed autoantibody on subsequent samples.

b. >=1 Confirmed Autoantibody positive on the next **sequential** serum sample or **diabetes** diagnosed

And

c. If sample drawn at less than 18 months of age there must be a prior sample negative for one or more autoantibodies confirmed positive or the cord blood/ mother negative for this autoantibody.

Individuals with confirmed positive autoantibodies who do not fulfill b, c and d will be classified as:

1. **Transient confirmed positive** if autoantibodies negative on follow-up and diabetes has not developed.
2. **Confirmed positive/not retested** for those without a follow-up sample.
3. **Potential Transplacental autoantibody** if cord blood or mother at time of birth has only autoantibody detected as confirmed positive (and no negative preceding sample in child)

9.2. Diabetes

The second primary outcome of the TEDDY study will be the development of T1DM. Diabetes will be defined using the ADA Expert committee classification (Expert Committee on the Diagnosis of Diabetes Mellitus, 1997). This classification is based on pathogenesis rather than the requirement for insulin therapy.

The most common form of childhood diabetes is type 1A diabetes, caused by the autoimmune destruction of pancreatic islet beta cells leading to an absolute deficiency of insulin and marked by the presence of at least one of the islet autoantibodies described in the previous section. It is expected that by age 15 years, type 1A diabetes will develop in up to 380 children enrolled in the TEDDY follow-up study.

The HLA eligibility criteria and family history of type 1 diabetes make it unlikely that other forms of diabetes will be observed. However, it is possible that some children will progress to diabetes without an identifiable period of islet autoantibody positivity.

TEDDY Protocol

Recently, increased numbers of children with diabetes presenting with clinical characteristics of type 2 diabetes (typically considered a disease of adults) have been reported, especially in minority populations (American Diabetes Association, 2000). Other categories of specific disorders, (onset usually during childhood) include mitochondrial mutations[6-8] and various forms of Maturity Onset Diabetes of Youth (MODY), characterized by mild to severe insulin deficiency (Hattersley, 2000;Owen et al., 2003). In addition, a form of diabetes termed "atypical diabetes mellitus in adolescents" is being reported to occur in approximately 10% of African Americans and is associated with episodes of ketoacidosis followed by disease remissions where insulin therapy is not required to prevent ketoacidosis (Winter et al., 1987).

The issue of "double diabetes", i.e. coexistence of islet autoimmunity, insulin resistance and beta cell failure will likely be more important in TEDDY population than rare forms of diabetes, such as MODY. While presence of islet autoantibodies in TEDDY participants diagnosed with diabetes will be sufficient to classify those cases as type 1A diabetes, markers of insulin resistance (e.g., higher BMI, higher fasting insulin or C-peptide levels or HOMA (Matthews et al., 1985) will have to be included as potential confounders in the analyses of time to progression to diabetes. The "Accelerator Hypothesis" (Wilkin, 2001) argues that autoimmune destruction of beta cells is accelerated by weight gain and insulin resistance, thus type 1A diabetes may develop faster in children who are overweight. In addition, some suggested that higher BMI may be associated with increased prevalence and titer of islet autoantibodies, especially GAD (Weets et al., 2001; Rolandsson et al., 1999).

To diagnose diabetes, the following ADA Criteria must be met on two occasions (unless criteria 4 is present):

1. Casual (any time of day without regard to time since last meal) plasma glucose >= 200 mg/dL, if accompanied by unequivocal symptoms (i.e. polyuria, polydipsia, polyphagia, and/or weight loss.)

Or

2. Fasting (no caloric intake for at least 8 hours) plasma glucose >= 126 mg/dL

Or

3. 2-hour plasma glucose >=200 mg./dL oral glucose tolerance test (OGTT). Glucose dose is determinant on body weight to a maximum of 75 grams.

Or

4. Unequivocal hyperglycemia with acute metabolic decompensation (i.e. ketoacidosis)

Unless criterion 4 is present or the fasting glucose is >=250 mg/dL (at the bedside or in the local laboratory on the day of testing), it is preferred that at least one of the two testing occasions involve an oral glucose tolerance test (OGTT). If the first criterion met is #3, i.e. by the 2-hour OGTT value, the OGTT should be repeated within 60 days. It is essential that every effort be made to obtain the necessary tests to establish the diagnosis of diabetes. Subjects will be instructed to eat a balanced diet in the days leading up to the OGTT.

9.2.1. Additional TEDDY Clinic Visit after Diagnosis of Type 1 Diabetes in order to Collect Data and Biological Samples at the Final End-point

TEDDY families will be informed that they should contact a TEDDY staff member to arrange an additional TEDDY visit should the TEDDY child develop T1DM in between TEDDY visits. This visit should be organized as soon as possible (within 6 weeks) after the diagnosis of T1DM and include the same age-appropriate procedures as a regular TEDDY visit would (e.g. clinical sample collection, extraction of data from the TEDDY book, administration of TEDDY Study questionnaires), if the 4 year non-HLA genotyping sample has not yet been collected from the child, if possible this sample should also be collected, if possible a 7 time-point MMTT should be completed, if possible a Diabetes Management Form should also be completed and if possible Quality of Life Questionnaires should also be completed. The families will also be asked to collect an additional stool sample as soon as possible (within 7 days) after the diagnosis of T1DM using the standard TEDDY stool sample collection and shipment protocols. An additional stool sample will also be collected within 7 days after the post-diagnosis visit. In August 2018 all stool sample collections were stopped from all subjects.

If diabetes is diagnosed by abnormal glucose values or OGTT results at a routine TEDDY visit an additional visit will be scheduled within 6 weeks of the diagnosis. This additional visit will include the same age-appropriate procedures as a regular TEDDY visit would (e.g. clinical sample collection, extraction of data from the TEDDY book, administration of TEDDY Study questionnaires), if the 4 year non-HLA genotyping sample has not yet been collected from the child, if possible this sample should also be collected, if possible a 7 time-point MMTT should be completed, if possible a Diabetes Management Form should also be completed and if possible Quality of Life Questionnaires should also be completed. The families will be asked to collect an additional stool sample as soon as possible (within 7 days) after the diagnosis of T1DM using the standard TEDDY stool sample collection and shipment protocols and an additional stool sample will also be collected within 7 days after the post-diagnosis visit. In August 2018 all stool sample collections were stopped from all subjects.

This final visit will be the “official” end-point for the child’s participation in the TEDDY Study and will offer an opportunity for psychological support to the family.

10. Statistical analyses

The goal of TEDDY is to carry out studies to identify environmental causes of autoantibodies and T1DM in genetically susceptible children. While some environmental exposures can be measured via questionnaires and interviews, other exposures can only be measured via markers in biological samples. We are proposing to do all biomarker tests in a nested case-control fashion. This would be an efficient way to test hypotheses

with regard to dietary and toxin exposures, by only needing to assay a small sample of the entire longitudinal cohort. However, in order for this to be successful, appropriate samples would have to be collected and stored for all visits on all participants (or as indicated).

10.1. Master Plan of Analysis

The primary goal of the statistical analyses to be performed as part of this study will be the identification of factors associated with the development of autoimmunity and T1DM. In order to accomplish this, we will employ two different types of analysis, depending on the nature of the factor being studied.

1) For those factors whose values will be known to us without additional costs, we will perform log rank tests and Cox Proportional Hazards Regressions. For these analyses, the dependent variable will be the age at which the event being studied (development of autoimmunity or diagnosis of T1DM) occurred. Those not achieving the event when the analyses are being performed and those lost to follow-up without achieving the event will be considered censored as of the date last known to be event-free. The log rank test will be used to study factors with a few discrete values. For example:
    (a) Family History
    (b) Haplotype
    (c) Breast-feeding

    For factors with continuous values, we will test for conformity of those values with the proportional hazards assumption by examining the significance of the interaction of the time variable (treated as a time-dependent covariate) with the factor. If there is an indication of a non-zero interaction coefficient, ($p < 0.10$) we will dichotomize the variable and use the log rank test instead of proportional hazards regression. Otherwise, we will use proportional hazards regression to test for significance at the .01 significance level. The following are examples of factors to be studied in this manner:
    (a) Amounts of certain nutrients consumed
    (b) Duration of breast feeding
    (c) Maternal anxiety and depression as measured by the State Trait Anxiety Inventory and the Edinburgh Postnatal Depression Scale

    Proportional hazards regression will be used in both a univariate and multivariate manner and reported both ways. For each factor studied, estimated hazard ratios and their 95% confidence intervals will be computed. When a set of factors that predict conversion to T1DM or autoimmunity are determined as described above, we will compute estimated "survival" curves for subjects having specific factor profiles.

2) For those factors whose determination is costly we will employ a nested case-control design. At the time at which a cohort subject converts to autoimmunity or

T1DM (referred to as cases) we will randomly select k subjects who did not convert to autoimmunity or T1DM (controls). The sampling for the selection of matched controls is based upon incidence density sampling which allows the comparison of cases with a subset of the cohort at risk of being cases at the time when each case occurs, or equivalently matches cases and controls for the duration of follow-up. Since controls selected in this way may become cases over time, we will employ over sampling of controls and may select different controls at different points in time.

We will then compare these cases to controls as a matched case control study using conditional logistic regression. The value of k will be determined in order to have at least 80% power based on tables 14 -16, taking into consideration the number of cases available for the analysis. Thus, these expensive determinations will be made only on the cases and their chosen matched controls. Matching will be based on HLA type, study center, duration of follow-up, and completeness of data including serial biological samples. Every effort will be made to use the same controls for multiple case-control studies. This will allow inclusion of all key exposures simultaneously in the analytical model and exploration of confounding, effect modification, and interactions between exposures. The standard set of controls will include only those with complete data/samples for all components of the protocol. Since we do not need to have the same number of controls for each case, we will strive for an average of k controls for each case – not exactly k for each case. These analyses will also be performed in both a univariate and multivariate fashion. Odds ratios for each factor will be computed, as will their 95% confidence intervals. ROC curves for sensitivity vs. 1 – specificity based on combinations of these factors will be computed. Here sensitivity refers to the ability of the factors to predict conversion to the study endpoint among those who do convert and specificity refers to ability to predict conversion free survival among those who are.

Similar analyses will be performed for development of autoimmunity and T1DM. Since the cases for T1DM will be a subset of the cases for autoimmunity, we will attempt to use the same controls. However, since there will be fewer cases, additional controls will be needed. Again, the (average) number of controls per case will be guided by tables 14 – 16.

The following is a non-exhaustive listing of planned statistical analyses for the prospective part of the project:

1. We will classify infants as to whether or not they had early exposure to cereals or gluten in the diet (vs. those receiving only breast milk for the first 3 months). Those exposed will be compared to those not exposed using the Log Rank Test.
2. Subjects will be classified, based on diet questionnaires, as consuming low or high levels of anti-oxidants such as carotenoids, ascorbic acid, and selenium. Those with low levels will be compared to those with high levels with respect to the development of T1DM and autoimmunity using Log Rank Tests.

3. Subjects' drinking water will be analyzed and subjects classified as drinking water with high or low concentrations of zinc, and nitrate and as low or high pH. Groups will be compared using the Log Rank Test.
4. Cox Regression will be used to study the association of the level of psychosocial stress with the development of T1DM and autoimmunity.

The following analyses will be performed in the nested case control portion of the study:

1. The association between the number of enterovirus infections a child has and the development of T1DM and islet autoimmunity will be studied with Cox Regression. Using zero as the reference, dummy variables will be created to represent 1, 2, etc infections. The hazard ratios of these values relative to zero will be estimated and p values of the estimates computed. The same type of analyses will be done for the number of rotavirus infections.
2. Low levels of omega-3 fatty acids, EPA, and DHA in children's erythrocyte membranes have been associated with increased risk of islet autoimmunity. The odds ratio for each of these exposures will be estimated.
3. Low levels of alpha-tocopherol has been associated with increased risk of islet autoimmunity. The odds ratio for each of this exposure will be estimated

All analyses will be performed using SAS (version 9 or later). Among the procedures to be used will be Proc Logistic, Proc Lifetest, and Proc Phreg, and Proc Lifereg. Observations with missing values of a variable will be omitted from those analyses using that variable, but not from analyses not using those variables. No data imputation methods will be applied to missing values. Programs will be written to check all data for values that are out of range or inconsistent. Reports of such data will be sent to the P.I., discussed, and corrected and the data corrected as appropriate. Indications of confusing data forms or data entry problems will be remediated.

10.2. Plan and Timeline of Proposed Analyses

In general the study is designed to have 80% power or greater for detecting hazard ratios of 2 or greater for exposures 10% or greater, based on the expectation of being able to enroll 7,013 subjects from the general population and 788 relatives in five years, with 15 years of post-accrual follow-up. The actual study experience may be different and it is prudent to provisionally plan for interim analyses. In doing so, we consider the following caveats: 1) Laboratory determinations made for interim analysis of stored samples need to be identical to the laboratory methods of the same determinations to be made at the end of the study if the data are to be aggregated, and 2) Some longitudinal testing of stored samples is prudent to ensure sample integrity and quality.

10.2.1. Protocol Monitoring

Protocol compliance in terms of screening, recruitment, and collection of protocol defined biological samples, questionnaires, and diaries will be monitored on a

monthly basis. This report will summarize the accrual to the study (including maternal registrations), the demographic distribution of the subjects on study, the HLA distribution, and other baseline variables available. Accrual rates will be contrasted with expected or planned rates for Steering Committee review. Monthly reports will also be prepared from the data accumulating on laboratory monitoring quality assurance programs as specified by the Laboratory Monitoring Committee. Calculations of protocol compliance rates are based upon the tests/samples/forms due as a function of the age of enrolled subjects.

Also, we plan to monitor monthly parameters that provide estimates of psychological impact of study participation and parental satisfaction with study participation.

10.2.2. Exposure Monitoring

Since most exposures are measured from the analysis of biological samples, little will be available in the interim to assess exposure rates. Yet, we believe it is prudent to try to obtain some measures to ensure that sample collection procedures are adequate. To this end, we will target samples in which we may have more than enough volume. For example, current sample collection procedures suggest that volume will not be a problem for stool specimens and we may periodically sample the cohort to conduct interim analyses of viral exposures. In that we project collecting in excess of 26,000 stools samples in the first year, we would plan to sample the 1-year cohort to conduct these preliminary analyses. We no not expect to have surplus blood volumes to conduct interim analyses since all blood volume study requirements were based upon the minimal amounts needed for the study analyses.

Where exposure rates can be estimated from diaries, questionnaires, psychological assessments (e.g., anxiety, depression, behavior changes, stress) and food frequency instruments will be tabulated cumulatively and as a function of subjects' age. These tabulations will also be made monthly. Risk factors with unanticipated exposure rates will be discussed and adjustments to power calculations made as appropriate.

Retention of study participants is a high priority and we will provide continuous assessment of study dropouts. These analyses will include baseline demographics and environmental exposures for comparison with subjects continuing the study cohort.

10.2.3. Outcome analysis

After five and ten years of accrual, interim analyses of the prospective portion of the study will be performed and reported to the participating investigators. These analyses will address the relationships of the triggers being studied to the development of T1DM using the same Cox Proportional Hazards Regressions

planned for final analyses. Tables 12 and 13 present power calculations for these interim analyses for the general population. Note that a 4% conversion rate at 15 years for those unexposed implies conversion rates of 1.4% and 2.7% at 5 and 10 years respectively, assuming exponential conversion.

As the study progresses, we will have a more accurate picture of accrual and follow-up rates and estimates of some exposure rates based upon the diaries and questionnaires. As well, we will be able to observe cases of autoimmunity and T1DM and base our planned analyses on these rather than projections. For some environmental triggers, assessed from the entire prospective cohort, we will be able to schedule interim analyses. Our first priority will be to confirm, or not, previous reports of risk factors that have been reported in the literature. For example, the study will have an early picture of diet. Literature reports of risk factors with hazard ratios of 3 or greater can be tested in interim analyses with reasonable power. Examples of these might be:

- Food supplementation with gluten containing foods before age 3 months (reported hazard ratio 4.0, 95% CI 1.4-11.5).
- Children initially exposed to cereals between ages 0 and 3 months (reported hazard ratio 4.3, 95% CI 2.0-13.8)
- Vitamin D supplementation (2000 IU daily) compared to those who regularly received less (relative risk 0.22, 95% CI 0.05-0.89)

And from the maternal study:

- CVB5 in maternal sera collected in the first trimester of pregnancy (OR 10, 95% CI 1.4-43.4)

For most of these, the literature report includes a minimum of 4-6 years of follow-up. For other hypotheses that can be tested in the interim, the duration of follow-up will be the rate-limiting factor as well as many of the other studies in the literature report calculated risk in children up to 15 years of age. TEDDY investigators will propose additional hypotheses to be tested and, prior to conducting interim analyses, each will be evaluated with respect to the detectable hazard ratio at a minimum of 80% power. We recognize that this leads to increased chances of finding associations as a result of increasing studywide type

1 error, but as a hypothesis forming epidemiological study we will balance this against the calculated hazard ratios and exercise caution in our interpretations.

10.3. Sample size and power determination

Subjects will be recruited over a five-year period and followed until they reach the age of 15 years old. Thus all subjects will have 15 years of follow-up.

Table 5

	Screening/year for remaining 3 years of accrual		Eligible/year for remaining 3 years of accrual				Enroll/year for remaining 3 years of accrual			
Center	**General Population**	**FDR**	**Eligibility Rate GP**	**General Population**	**Eligibility Rate FDR**	**FDR**	**Enrollment Rate GP**	**General Population**	**Enrollment Rate FDR**	**FDR**
Colorado	16,316	176	5.1%	832	23.5%	41	42%	350	64%	26
Finland	11,204	132	5.6%	627	35.1%	46	44%	276	60%	28
Georgia/Florida	14,272	172	3.5%	500	16.4%	28	33%	165	50%	14
Germany	7,296	356	3.9%	285	19.5%	69	30%	85	80%	56
Sweden	8,956	220	7.6%	681	18.3%	40	65%	442	65%	26
Washington State	23,628	204	3.9%	921	26.7%	54	37%	341	75%	41
TOTAL:	81,672	1,260		3,846		278		1659		191

	Actual total screened by site for first 2 years of accrual		Projected total screened by site for remaining 3 years of accrual		Total screened by site for 5 years of accrual	
Center	**General Population**	**FDR**	**General Population**	**FDR**	**General Population**	**FDR**
Colorado	17,896	296	48,948	528	66,844	824
Finland	22,827	271	33,612	396	56,439	667
Georgia/Florida	25,204	226	42,816	516	68,020	742
Germany	6,695	526	21,888	1,068	28,583	1,594
Sweden	17,991	358	26,868	660	44,859	1,018
Washington State	20,363	139	70,884	612	91,247	751
TOTAL:	110,976	1,816	245,016	3,780	355,992	5,596

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	Actual total eligible by site for first 2 years of accrual		Projected total eligible by site for remaining 3 years of accrual		Total eligible by site for 5 years of accrual	
Center	**General Population**	**FDR**	**General Population**	**FDR**	**General Population**	**FDR**
Colorado	811	66	2,496	123	3,307	189
Finland	1,254	94	1,881	138	3,135	232
Georgia/Florida	824	34	1,500	84	2,324	118
Germany	244	99	855	207	1,099	306
Sweden	1,282	61	2,043	120	3,325	181
Washington State	635	28	2,763	162	3,398	190
TOTAL:	5,050	382	11,538	834	16,588	1,216

	Actual total enrolled by site for first 2 years of accrual		Projected total enrolled by site for remaining 3 years of accrual		Total enrolled by site for 5 years of accrual	
Center	**General Population**	**FDR**	**General Population**	**FDR**	**General Population**	**FDR**
Colorado	262	35	1,050	78	1,312	113
Finland	556	43	828	84	1,384	127
Georgia/Florida	204	15	495	42	699	57
Germany	72	69	255	167	327	236
Sweden	796	40	1,326	78	2,122	118
Washington State	146	14	1,023	123	1,169	137
TOTAL:	2,036	216	4,977	572	7,013	788

Over 5 years	General Population	FDR
Enroll	7,013	788
Cases - autoantibodies by age 6	281 (4%)	105 (13.3%)
Cases - T1DM by age 15	281 (4%)	105 (13.3%)

Based on submissions by the participating institutions, we expect to be able to enroll 7,013 subjects from the general population and 788 relatives in five years. Those from the general population and the relatives are expected to have about 4% and 13.3% autoimmune conversion at 6 years respectively. Tables 6-13 below give the power for a Log Rank Test at a two-sided 0.01 significance level for the general population subjects, the relatives, and the pooled sample accrued over 5 years with 15 years of post-accrual follow-up for dichotomous pre-birth triggers of frequencies 1%, 5%, 10%, and 20% and hazard ratios of 1.5 and 2.0 and losses to follow-up at rates of 0%, 2%, 5%, and 10% per year. These calculations are based on the method of Lakatos (Lakatos, 1998) using a computer program described by Cantor (Cantor, 2003).

Table 6: Power for Prospective Study- General Population –Diabetes End Point,
Alpha = .01, N = 7013, 5 Years Accrual, Follow-Up to age 15,
4% Conversion among Unexposed at 15 Years

Exposed %	LTFU %/year	Power (HR = 1.5)	Power (HR = 2.0)
1	0	0.05	0.21
5	0	0.26	0.89
10	0	0.52	0.99
20	0	0.81	0.99
1	2	0.04	0.17
5	2	0.21	0.82
10	2	0.44	0.98
20	2	0.72	0.99
1	5	0.03	0.13
5	5	0.16	0.71
10	5	0.34	0.95
20	5	0.59	0.99
1	10	0.03	0.09
5	10	0.11	0.52
10	10	0.23	0.83
20	10	0.42	0.98

Table 7: Power for Prospective Study- Relatives – Diabetes End Point
Alpha = .01, N = 788, 5 Years Accrual, Follow-Up to age 15,
13.3% Conversion among Unexposed at 15 Years

Exposed %	LTFU %/year	Power (HR = 1.5)	Power (HR = 2.0)
1	0	0.02	0.06
5	0	0.07	0.33
10	0	0.14	0.62
20	0	0.27	0.87
1	2	0.02	0.05
5	2	0.06	0.27
10	2	0.12	0.53
20	2	0.22	0.80
1	5	0.02	0.04
5	5	0.05	0.21
10	5	0.09	0.42
20	5	0.17	0.68
1	10	0.02	0.03
5	10	0.04	0.14
10	10	0.06	0.29
20	10	0.11	0.51

Table 8: Power for Prospective Study- Pooled Sample –Diabetes End Point,
Alpha = .01, N = 7801, 5 Years Accrual, Follow-Up to age 15,
5.5% Conversion among Unexposed at 15 Years

Exposed %	LTFU %/year	Power (HR = 1.5)	Power (HR = 2.0)
1	0	0.07	0.35
5	0	0.43	0.98
10	0	0.75	0.99
20	0	0.95	0.99
1	2	0.06	0.29
5	2	0.35	0.96
10	2	0.66	0.99
20	2	0.91	0.99
1	5	0.05	0.22
5	5	0.27	0.90
10	5	0.54	0.99
20	5	0.82	0.99
1	10	0.04	0.14
5	10	0.18	0.75
10	10	0.37	0.96
20	10	0.64	0.99

Table 9: Power for Prospective Study – General Population – Autoantibodies End Point,
Alpha = .01, N = 7013, 5 Years Accrual, Follow-Up to age 15,
4% Conversion among Unexposed at 6 Years

Exposed %	LTFU %/year	Power (HR = 1.5)	Power (HR = 2.0)
1	0	0.12	0.55
5	0	0.66	0.99
10	0	0.93	0.99
20	0	0.99	0.99
1	2	0.10	0.47
5	2	0.57	0.99
10	2	0.88	0.99
20	2	0.99	0.99
1	5	0.07	0.36
5	5	0.45	0.99
10	5	0.78	0.99
20	5	0.96	0.99
1	10	0.05	0.25
5	10	0.31	0.93
10	10	0.60	0.99
20	10	0.87	0.99

Table 10: Power for Prospective Study –Relatives – Autoantibodies End Point,
Alpha = .01, N = 788, 5 Years Accrual, Follow-Up to age 15,
13.3% Conversion among Unexposed at 6 Years

Exposed %	LTFU %/year	Power (HR = 1.5)	Power (HR = 2.0)
1	0	0.03	0.12
5	0	0.16	0.67
10	0	0.34	0.94
20	0	0.61	0.99
1	2	0.03	0.10
5	2	0.14	0.59
10	2	0.29	0.89
20	2	0.52	0.99
1	5	0.03	0.08
5	5	0.11	0.48
10	5	0.22	0.80
20	5	0.42	0.97
1	10	0.02	0.06
5	10	0.08	0.35
10	10	0.15	0.65
20	10	0.29	0.89

Table 11: Power for Prospective Study – Pooled Sample – Autoantibodies End Point
Alpha = .01, N = 7801, 5 Years Accrual, Follow-Up to age 15,
5.5% Conversion among Unexposed at 6 Years

Exposed %	LTFU %/year	Power (HR = 1.5)	Power (HR = 2.0)
1	0	0.15	0.68
5	0	0.78	0.99
10	0	0.98	0.99
20	0	0.99	0.99
1	2	0.12	0.56
5	2	0.67	0.99
10	2	0.94	0.99
20	2	0.99	0.99
1	5	0.12	0.56
5	5	0.67	0.99
10	5	0.94	0.99
20	5	0.99	0.99
1	10	0.08	0.40
5	10	0.49	0.99
10	10	0.82	0.99
20	10	0.98	0.99

Table 12: Interim Analysis Power for Prospective Study- General Population –Diabetes End Point, Alpha = .01, N = 7013, 5 Years Accrual, Follow-Up to age 5, 1.4% Conversion among Unexposed at 5 Years

Exposed %	LTFU %/year	Power (HR = 2.0)	Power (HR = 3.0)	Power (Hr = 5.0)
1	0	0.08	0.42	0.98
5	0	0.50	0.99	0.99
10	0	0.81	0.99	0.99
20	0	0.97	0.99	0.99
1	2	0.08	0.39	0.97
5	2	0.46	0.99	0.99
10	2	0.78	0.99	0.99
20	2	0.95	0.99	0.99
1	5	0.07	0.34	0.95
5	5	0.41	0.98	0.99
10	5	0.72	0.99	0.99
20	5	0.93	0.99	0.99
1	10	0.06	0.28	0.91
5	10	0.34	0.95	0.99
10	10	0.63	0.99	0.99
20	10	0.87	0.99	0.99

Table 13: Interim Analysis Power for Prospective Study- General Population –Diabetes End Point, Alpha = .01, N = 7013, 5 Years Accrual, Follow-Up to age 10, 2.7% Conversion among Unexposed at 10 Years

Exposed %	LTFU %/year	Power (HR = 2.0)	Power (HR = 3.0)	Power (HR = 5.0)
1	0	0.14	0.66	0.99
5	0	0.75	0.99	0.99
10	0	0.96	0.99	0.99
20	0	0.99	0.99	0.99
1	2	0.12	0.59	0.99
5	2	0.69	0.99	0.99
10	2	0.94	0.99	0.99
20	2	0.99	0.99	0.99
1	5	0.10	0.50	0.99
5	5	0.59	0.99	0.99
10	5	0.89	0.99	0.99
20	5	0.99	0.99	0.99
1	10	0.08	0.38	0.97
5	10	0.46	0.99	0.99
10	10	0.77	0.99	0.99
20	10	0.95	0.99	0.99

The above tables demonstrate that a trigger will have to be associated with a hazard ratio of about 2.0 in order for us to have reasonable power. In that case, we will have adequate power for exposure rates of at least 5% if we have little (not exceeding 2% per year) loss to follow-up. If we have more losses to follow-up, we will need to have at least a 10% exposure rate for a trigger with a hazard ratio of 2.0.

10.4. Risk factor analysis using prospective methods

10.4.1. Exposure variables

- HLA
- Diet
- Psychological Distress
- Clinical events

10.5. Risk factor analysis using case-control methods

10.5.1. Exposure variables

- Viral exposure
- Bacterial exposure
- Dietary Biomarkers
- Inflammatory Markers

10.5.2. Matching Criteria

As part of the study design of the case-control studies controls will be matched to cases on several confounding factors. This will be done to achieve a more statistically efficient analysis. The factors that are being considered as possibilities to match on are:

- HLA type
- Study center
- Duration of follow-up
- Completeness of data including serial biological samples
- Season of birth

10.5.3. Statistical Considerations

For the study of risk factors for the development of autoimmunity, those subjects developing autoimmunity will be cases and those not developing autoimmunity at that time will be controls (population density sampling). For each case, we will attempt to identify k controls (see below for considerations for the determination of k) that are matched to the case on study center, gender, and HLA type. Cost efficiency is achieved by determining the values of the laboratory parameters only for the cases and the chosen matched controls. We will then perform stratified

logistic regression using the matched sets as strata in order to study the effect of various possible risk factors, univariately and multivariately, on the odds of developing autoimmunity. Note that this analytic method does not require that every matched set contain exactly k controls. We can have varying numbers of controls for each case. Thus while we will attempt to have the same number of controls for each case, we will allow for fewer controls for cases with fewer matches and will choose more controls, to compensate, for those cases with more matches.

For the study of risk factors for the development of T1DM, we will consider those who developed T1DM by age 15 to be cases. For those who also developed autoimmunity as well, we will use the same controls as were chosen for the study of autoimmunity. The same analytic methods will be used.

The number of controls needed per case will depend, of course, on the number of cases and thus cannot be fully determined until the end of the planned follow-up periods. One plausible scenario, given in Table 14, assumes that for the pooled sample we would have 5.5% conversion by age 15 to diabetes among those exposed to a risk factor, that 20% would be exposed, that the hazard ratio for the risk factor is 2.0, and that 0% would be lost to follow-up. This would lead to about 460 cases.

The following tables presents the power for a case control study with 1 – 3 controls per case, odds ratio = 1.5 and 2.0, and 380 – 500 cases.

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Table 14: Power Table for 1 Control per Case

Power with 1 Control per Case
Alpha = .01

	Exposure among Controls	Power (OR = 1.5)	Power (OR = 2.0)
380 Cases	.05	.11	.43
	.10	.22	.75
	.15	.33	.89
	.20	.41	.95
400 Cases	.05	.11	.45
	.10	.23	.78
	.15	.34	.91
	.20	.43	.96
420 Cases	.05	.11	.47
	.10	.24	.80
	.15	.36	.93
	.20	.46	.97
440 Cases	.05	.12	.50
	.10	.26	.83
	.15	.38	.94
	.20	.48	.97
460 Cases	.05	.13	.52
	.10	.27	.85
	.15	.40	.95
	.20	.50	.98
480 Cases	.05	.13	.55
	.10	.29	.87
	.15	.42	.96
	.20	.53	.98
500 Cases	.05	.14	.57
	.10	.30	.88
	.15	.44	.97
	.20	.55	.99

Table 15: Power Table for 2 Controls per Case

Power with 2 Controls per Case
Alpha = .01

	Exposure among Controls	Power (OR = 1.5)	Power (OR = 2.0)
380 Cases	.05	.17	.62
	.10	.34	.89
	.15	.48	.97
	.20	.58	.99
400 Cases	.05	.18	.65
	.10	.36	.91
	.15	.50	.98
	.20	.60	.99
420 Cases	.05	.19	.68
	.10	.38	.93
	.15	.52	.98
	.20	.63	.99
440 Cases	.05	.20	.70
	.10	.40	.94
	.15	.55	.99
	.20	.65	1.0
460 Cases	.05	.21	.73
	.10	.42	.95
	.15	.57	.99
	.20	.68	1.0
480 Cases	.05	.22	.75
	.10	.44	.96
	.15	.59	.99
	.20	.70	1.0
500 Cases	.05	.23	.77
	.10	.45	.97
	.15	.62	.99
	.20	.72	1.0

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Table 16: Power Table for 3 Control per Case

Power with 3 Controls per Case
Alpha = .01

	Exposure among Controls	Power (OR = 1.5)	Power (OR = 2.0)
380 Cases	.05	.21	.70
	.10	.40	.93
	.15	.55	.98
	.20	.65	1.0
400 Cases	.05	.22	.73
	.10	.42	.95
	.15	.57	.99
	.20	.67	1.0
420 Cases	.05	.23	.76
	.10	.44	.96
	.15	.60	.99
	.20	.70	1.0
440 Cases	.05	.25	.78
	.10	.46	.97
	.15	.62	.99
	.20	.72	1.0
460 Cases	.05	.26	.80
	.10	.48	.97
	.15	.64	1.0
	.20	.75	1.0
480 Cases	.05	.27	.82
	.10	.51	.98
	.15	.67	1.0
	.20	.77	1.0
500 Cases	.05	.28	.83
	.10	.53	.98
	.15	.69	1.0
	.20	.79	1.0

10.6. Gene-environment interactions

While the description of the analytic plan addresses univariate analyses of possible triggers of autoimmunity and T1DM, clearly the interactions of environmental exposures and genetic variability are of keen interest. Multivariate models, including time-dependent covariates, are planned. A variety of models are posited to include Cox Proportional Hazards, generalized estimating equations for longitudinal analyses and general linear models.

10.7. Criteria for the termination of study

Criteria for termination of the study would fall into the following categories: lack of feasibility, achievement of planned accrual and follow-up, analytic results that demonstrate a clear and unequivocal environmental and/or genetic basis for autoimmunity or T1DM, or successful attainment of planned study end points. Subject accrual and protocol compliance with respect to collecting the planned samples and study information will be monitored on a continuing basis. It would be anticipated that start-up will be gradual and that estimates of the projected accrual rates may not become stable for the first year after the study begins. Should these estimates differ from planned, then discussion will be held with the Principal Investigators from the clinical centers to address ways in which to improve or augment accrual rates. Similarly, if the estimates of protocol non-compliance exceed those identified in the tables, an action plan will be developed to make necessary improvements and corrections. If the combination of these efforts still result in projections that the study will not have adequate statistical power to detect a risk factor with odds ratios of 5 or less, then the feasibility of the study becomes questionable and both the study group and the External Advisory Board will be presented with the information for possible action.

10.8. Procedure for accounting for missing, unused, and spurious data

Every effort will be made to obtain complete ascertainment of study data as defined by the protocol. All data will be subject to careful error checking (ranges and consistency) to identify possible spurious data for correction. Efforts to account for missing data will be based upon accepted techniques so as to not introduce bias into the study. An important component is the ability to ascertain study end points from subjects who may not wish to continue participation with planned study follow-up. The study benefits in this regard from diabetes surveillance programs available in four of the clinical sites: the SEARCH for Diabetes in Young (covering all Colorado and part of the state of Washington population) as will national diabetes registries in Finland and Sweden. These registries are ongoing sources to obtain data on children developing diabetes who were either not followed or may have dropped out from the study.

10.9. Deviation from the original statistical plan

The design parameters for the TEDDY study assume an accrual over four years and follow-up until age 15 for each subject accrued. These parameters, augmented with

site specific accrual rates, rates of persistent autoantibodies by age 6, and T1DM by age 15 give rise to a specific sample size projection to have adequate statistical power to address the study hypotheses and end points. As there are a number of exposures, haplotypes, and gene-environment interactions to be evaluated as possible triggers of autoimmunity or T1DM, the calculated sample size appears as series of tables in which effect of the prevalence of the exposure and the rate of withdrawals are taken into account. Deviations in the statistical plan can occur within the designated ranges and the impact on the study is the ability to detect a certain odds ratio or relative risk with adequate statistical power as shown in the tables. Because deviations in the estimates of study parameters are expected, the TEDDY Steering Committee will be presented these data on an ongoing basis to evaluate their impact on study design. In addition, a number of study endpoints are being evaluated in a case-control setting. The plan includes a projection of the number of cases (subjects with persistent autoimmunity) and then the selection of match controls. For these cases and controls stored samples will be sent to the appropriate laboratories for analysis. It is likely, that the study will wait until sufficient numbers (determined on the basis of cost and feasibility) are identified before these analyses are begun.

11. Assessment of Safety

11.1. Observational Study Monitoring Board

The External Evaluation Committee (EEC) members and chairperson are appointed by the NIDDK in consultation with other sponsors (National Institute of Allergy and Infectious Diseases (NIAID), National Institute of Child Health and Human Development (NICHD), National Institute of Environmental Health Sciences (NIEHS) and Centers for Disease Control and Prevention (CDC) and Juvenile Diabetes Research Foundation International (JDRF)) and will reflect the scientific disciplines and medical expertise necessary to evaluate the study design, aid in interpreting the data, and ensure protection of human subjects in the studies performed by TEDDY. Ad hoc members may be appointed for specific protocols, as circumstances require. Such appointments will be made by the NIDDK in consultation with other sponsors.

The EEC will act as the observational study monitoring board for TEDDY. Members will be completely independent of the studies being reviewed. They shall not be actively involved with any TEDDY Study Unit. They must have no financial interest in the outcomes of any studies reviewed by the EEC.

EEC members will:

- Review all protocols for studies in type 1 diabetes to be performed within The Environmental Determinants of Diabetes in the Young (TEDDY) study prior to enrollment of subjects, and to advise the sponsors of TEDDY of any concerns.

- Examine recruitment and data from TEDDY protocols, including safety data and adverse events, and make recommendations to the TEDDY and the sponsors of any concerns and/or recommendations regarding continuation, termination or other modification of studies.

- Review the general progress of the studies and to assist the TEDDY and the sponsors in resolving any problems which arise

- Provide scientific advice to the TEDDY and the sponsors on developments and opportunities that may facilitate or accelerate research in the TEDDY.

- Consider the pilots recommended by the Steering Committee and to help the sponsors make decisions about the allocation of resources to the TEDDY

- Provide feedback to the sponsors regarding the future plans of the TEDDY.

11.2. Specifications of Safety Parameters

The physical risks of participation in this protocol are those associated with venipuncture and adverse effects arising from ingestion of oral glucose. Patients may feel brief pain at the time of the needle stick during 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. Some subjects may experience minor and transient symptoms (nausea) during an OGTT.

Screening for genetic markers associated with (but not diagnostic for) a severe and currently incurable disease, such as T1DM, raises important ethical issues. Our study placed special emphasis on: 1) voluntary participation ensured by the informed consent process; 2) disclosure of the screening results to parents, combined with education about T1DM and genetic risk counseling; and, 3) confidentiality of genetic information that cannot be disclosed to health providers or other parties without parental consent. The informed consent involves genetic counseling. Since the prognostic significance of these markers is currently uncertain, psychological support of the families will be made available as needed, so that undue anxiety about developing T1DM is not invoked. Children who become positive will be followed closely and counseled concerning the best treatment. Thus, the likelihood that diabetes will develop abruptly, leading to significant morbidity or mortality, will be lessened.

11.3. Recording and Reporting Adverse Event/Intercurrent Illnesses

A standardized case report form will be completed by TEDDY personnel as needed to report possible adverse events and serious adverse events that may occur related to phlebotomy or other study procedures. Summaries of adverse events will be provided to the IRB annually.

11.4. Benefits

The detection of increased risk for future T1DM through screening and risk assessment could lead to earlier diagnosis of T1DM than would otherwise be the case. To avoid more fulminant presentations of new-onset T1DM, participants and their caregivers will be advised of the symptoms that suggest a diagnosis of T1DM. They will be advised that they should seek medical attention in the event that these symptoms develop. The TEDDY research program might eventually increase knowledge regarding the environmental factors that participate in the development of T1DM, and support development of prevention of T1DM.

12. Quality Control

12.1. HLA Quality Control for the Clinical Centers

QC will consist of two excellent and complimentary programs. First, the CDC Newborn Screening Proficiency Testing program will send a 50-sample set to each Clinical Center for Proficiency Testing. The Center must score at least 98% accuracy to qualify to begin screening. One repeat test per year will be allowed if the first test is failed. To continue to screen, the center must continue to pass annual Proficiency Testing by the same mechanism.

The second level of quality control will be the TEDDY Central Genotyping Laboratory, which will confirm eligibility on 100% of positive samples and a small % of negative samples, sent in quarterly batches. The Clinical Center must demonstrate a 98% or greater accuracy for eligibility compared to the Central Lab, to continue screening during the interval between CDC proficiency tests.

12.2. Autoantibody Quality Control

Each central laboratory will be responsible for its internal quality control program. This is expected to include daily testing of quality control preparations, blind replicates, participation in the DASP proficiency program, and repeat testing. Analysis of quality control data will be by standard means that includes the use of Shewart plots and monthly means. Data for QC preparations will be sent to the DCC on a monthly basis.

Under coordination of the DCC, each laboratory will also receive and test blinded samples and split samples, and a panel of QC samples bi-monthly. These will be generated at the clinical centers under direction of the DCC.

In order to control for and minimize between laboratory variation, all samples identified as positive in one central laboratory and 5% of negative samples randomly

selected by the DCC will be tested by the second central laboratory. Discrepancies will be identified and if necessary action taken to adjust thresholds for positivity if systematic differences are found, or to determine causes of discrepancies if sporadic discrepancies are found.

12.3. Infectious Agent Quality Control

All laboratories will participate in the European Union "Quality Control for Molecular Diagnostics" (QCMD; http://www.qcmd.org) quality control program for the TEDDY agents for which they will test, insofar as QC panels are available through the QCMD program. Proficiency panel scores and internal quality control data will be provided to the TEDDY Steering Committee.

12.4. Questionnaire studies (diet and psychosocial factors)

Questionnaire studies will be monitored by the DCC through analyses of internal consistency of questions that should yield the same answer, repeat interviews by two individuals or similar approaches.

12.5. Immunization records

Parent reported data will be verified in random samples by independent analysis of hospital or clinical records of immunizations.

12.6. Family history data

Recent studies on the way parents report data on their family history has shown that self reported pedigrees are accurate (Bratt et al., 1999). The Data Coordinating Center will not monitor.

13. Ethical Issues

13.1. Institutional Review Board

All TEDDY studies must be approved by the local IRB, prior to their initiation as required by the national statutes and good clinical practice. If requested, the IRB will be given the opportunity to monitor the progress of the studies.

13.2. Informed Consent

A two-step consent process will be used. The first consent will be specific for screening newborns for high-risk genotypes at the HLA and other loci in the general population or in families having a first-degree relative affected with T1DM (Phase 1). The second consent will cover procedures that will be used in the follow-up of the

risk for T1DM (Phase 2). The assent process for the TEDDY child will be completed at an appropriate age as determined by the local IRB/Ethics Board.

TEDDY study coordinators or investigators at each site will administer the informed consent forms. Each study participants' parents/primary caretakers will have sufficient time to fully read the consent forms and have any questions answered. They will be told that they can take the consent forms home and request consultation with other individuals. It will be explained to them that there will be separate consent for each phase of the study and that consent for Phase 1 of TEDDY does not mean consent for further participation in other TEDDY studies, ancillary studies or potential intervention trials. Additional consent will be required.

13.3. Gender and Ethnic Diversity

Both boys and girls, and members of all racial and ethnic populations of the United States, Finland, Germany and Sweden will be screened. The distributions of gender, race, and ethnic group will be monitored and reported annually to the TEDDY Steering Committee. If the study population does not reflect recruitment targets, corrective actions will be taken.

Table 17.

Estimated Screening Numbers by Each Minority Group per Year

Ethnic Category	Colorado	Finland	Georgia/Florida	Germany	Sweden	Washington	Total
Hispanic or Latino	3,789	0	573	0	184	1,380	5,926
Not Hispanic or Latino	9,744	11,421	13,179	6,035	8,992	17,020	66,391
Total	13,534	11,421	13,752	6,035	9,175	18,400	72,317

Racial Category	Colorado	Finland	Georgia/Florida	Germany	Sweden	Washington	Total
American Indian	136	0	0	0	0	361	497
Asian	406	0	229	0	18	1,315	1,968
Native Hawaiian/ Pacific Islander	136	0	0	0	9	435	580
Black or African American	1,488	0	5,157	0	18	890	7,553
White	11,368	11,421	8,366	6,035	9,130	15,399	61,719
Total	13,534	11,421	13,752	6,035	9,175	18,400	72,317

13.4. Disclosure of Results to Participants

There will be several times during the course of the study when results of testing will be disclosed to participants' parents/primary caretakers. Such information will be provided in a standardized fashion. The TEDDY investigators fully recognize the potential impact of this information on study participants and their parents/primary caretakers. Therefore, this information will only be made by qualified individuals who will receive specific training. Study coordinators and investigators from each Center will share the responsibility of disclosing results to subjects. The procedures for these disclosures are presented below.

Results of Genetic Screening (Phase 1)

A letter will be sent to participants who did not have an increased genetic risk indicating that they do not qualify for further participation in the TEDDY study. However, it will also indicate that those children could still develop T1DM.

TEDDY research staff will attempt to contact participants' parents/primary caretakers who tested positive for genetic risk either by phone or will mail a letter or postcard to the parents asking them to call in for their child's test results. The research staff should provide the initial explanation of the child's risk by telephone or in-person. (If parents, who were sent a mailed request to call in for the child's test results, do not call in, study staff will make every effort to contact these parents by telephone.) If the TEDDY staff is unable to reach the participant's parents/primary caretakers by phone or in-person, it is up to the individual clinical centers as to how they will communicate these results (subject to local IRB approval). At the time the parent is notified of the child's increased TIDM risk, Phase 2 of the study will be described. Before any child can proceed to Phase 2, the parent/primary caretaker must fully review and sign the informed consent form. All parents/primary caretakers will understand that Phase 2 requires an initial period of visits to a clinic at 3-month intervals, and that the children will be followed until age 15 years unless they develop diabetes.

Results of Autoantibody Screening (Phase 2)

Autoantibodies will be measured at each scheduled follow-up visit. Parents will be informed after each follow-up visit about the result of islet autoantibody testing. If islet autoantibody levels are within the normal range, parents will receive written notification of islet antibody titers, the upper limit of normal range of each islet antibody marker, and a statement explaining the results (see below). If islet autoantibody levels are above normal range, parents will be contacted by phone by the Teddy Research Team and the risk status will be explained before a written result notification of islet antibody titers, the upper limit of normal range of each islet antibody marker, and a statement explaining the results (see below) will be sent by mail.

The following explanation of islet antibody results have a 6.8 grade readability level.

Example #1: All islet antibodies within the normal range
Your child's last islet antibody tests were normal. Your child's risk for type 1 diabetes has not changed. We will continue to test your child's islet antibodies at each study visit.

Example #2: One islet antibody elevated for the first time.
One of your child's islet antibody tests was high. One high test result does not change your child's risk for type 1 diabetes. We will continue to test your child's islet antibodies at each study visit.

Example #3: One islet antibody elevated and confirmed in a second sample.
One of your child's islet antibody test was high for a second time. Your child's risk for type 1 diabetes may be slightly increased. We will continue to test your child's islet antibodies at the next study visit (in three months).

Example #4: More than one islet antibody elevated and confirmed in second sample.
Two/three of your child's islet antibody tests were high for a second time. This means your child's risk for type 1 diabetes has increased. Not all children with high test results get diabetes. If there are two children with your child's test results, one is likely to get type 1 diabetes within 5 years. We will continue to test your child's islet antibodies at each study visit.

Follow-up Risk Assessment (Phase 2)

Should a child develop persistent positive antibody test results, parents will be informed of the TEDDY, TrialNet and other prevention trials that are available for which they might qualify. Should testing reveal the presence of T1DM, participants' parents/primary caretakers will be informed immediately and guided to proper treatment; they will be informed of any TEDDY, TrialNet or other available studies recruiting subjects with new-onset T1DM.

13.5. Confidentiality

Personal information that is obtained for TEDDY will be maintained in distinct databases at each TEDDY Clinical Center. The personal data will be kept separate from study data obtained during the TEDDY Study at the local TEDDY Clinical Center. All information obtained from this study will be identified with a unique study number, and will not be kept with the participant's name. Data from TEDDY examinations and procedures will be sent to the TEDDY Data Coordinating Center. This information will be entered into a database that will be used for statistical analysis. The Data Coordinating Center will not receive any personal information on study participants.

Samples collected will be primarily stored at the local TEDDY Clinical Centers or at the NIDDK central repository. The stored samples may be used by TEDDY investigators to further characterize factors predicting risk of developing T1DM. All samples will be coded with a unique study number. Linkage of the unique study number to the names of the participants will be maintained at the local TEDDY Clinical Center. However, the names of participants will not be disclosed to any of the TEDDY investigators or to any other individuals except for informing participants' parents/primary caretakers of test results or possible participation in future studies. If such disclosure is requested for specific research or other purposes, approval by the TEDDY Steering Committee will be required. To further ensure privacy, a Certificate of Confidentiality will be obtained for the study by the TEDDY Data Coordinating Center. An explanation of the Certificate of Confidentiality is included in the consent forms.

13.6. Clinical Alerts

13.6.1. Diabetes mellitus

Random blood glucose (RBG) alert:
All study participants found to be positive for islet autoantibodies will have random blood glucose (RBG) measured at each TEDDY visit. Details for these procedures are outlined in the study Manual of Operations.

OGTT alert:
All study participants found to be positive for two or more islet autoantibodies will be asked to undergo standard OGTT every six months at the time of their regular TEDDY visit. Details for these procedures are outlined in the study Manual of Operations.

13.6.2. Possible celiac disease

Children positive for autoantibodies to tissue transglutaminase (TG) - see section 8.14.4 - will be referred to their pediatricians for confirmation or rule out of celiac disease outside of the study protocol by an intestinal biopsy and possible initiation of gluten free diet, if clinically indicated.

13.6.3. Thyroid autoimmunity

Children who are found to have thyroid autoimmunity with or without elevated TSH – see section 8.13.5 - will be informed by TEDDY staff and will be referred for medical care outside of TEDDY based on the normal site-specific protocol.

13.6.4. Possible depression

The prevalence of postpartum depression is roughly 10 to 15 % (e.g., Carothers & Murray, 1995; O'Hara, Neunaber, & Zekoski, 1984; O'Hara & Swain, 1996). The Edinburgh Postnatal Depression Scale (Cox, Holden, & Sagorsky, 1987) is the most widely used measure of postpartum depression and is the instrument to be used with TEDDY mothers at the 6-month study assessment. The questionnaire consists of 10 items that are each rated on a four point scale (0 to 3). There is an empirically derived cutoff score of 13 and those scoring above this cutoff score are likely to meet diagnostic criteria for a major depressive disorder (Cox et al., 1987). Item 10 of the questionnaire asks specifically if the mother has thought about harming herself. If the mother responds in the affirmative to item 10 or gets a total score of ≥13, the mother will be questioned further and provided an appropriate referral if warranted.

Below is the interviewer protocol used in a previous study on mothers of newborns at risk for diabetes who showed evidence of postpartum depression. Each site will develop a similar protocol for use with mothers having elevated scores on this measure.

If the mother answers anything other than NEVER to item 10, or has a total score ≥13, ask:
Have you told your doctor or anyone else about your feelings of feel blue or hurting or harming yourself? (YES/NO).

Are you currently receiving treatment for these feelings? (YES or NO).

IF NO: Would you like information about doctors in your area that you may be able to see about these feelings? (YES or NO).

IF YES: Provide mother with names of providers in her county or related area. You may initially provide her with the crisis hotline number for her county, obtain insurance information, and call her back with the names of providers.

IF NO: We feel it may be highly beneficial for you to speak with someone regarding this matter. It would be advisable to see your general physician or go to the local health department. It may be a good idea for us to contact the crisis center in your area and have them call you to further speak with you about your feelings. Would this be OK with you? Should you continue to feel blue or think about harming yourself, please contact either your primary care physician, therapist, or call us at (xxx) xxx-xxx if you would like the names of specific doctors in your area. Ask for_____.

13.7. Qualification for Additional TEDDY Studies

All individuals participating in the TEDDY Study will be eligible for consideration for participation in other studies as those studies become available. These studies

may include prevention trials in individuals who have not progressed to T1DM, intervention trials in individuals who progress to T1DM while in the TEDDY Study, and ancillary studies requiring additional data beyond that to be collected for this protocol. Participants of the TEDDY Study, who subsequently enter intervention trials, will be advised that they continue to contribute data toward the TEDDY study if they do not specifically wish to be withdrawn from the TEDDY study. In all cases of new studies, eligibility will require that the inclusion and exclusion criteria specific to those studies be met.

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