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

TEDDY Study Group

Abstract

The etiology of type 1 diabetes (T1D) remains unknown, but a growing body of evidence points to infectious agents and/or components of early childhood diet. The National Institutes of Health has established the TEDDY Study consortium of six clinical centers in the United States and Europe and a data coordinating center to identify environmental factors predisposing to, or protective against, islet autoimmunity and T1D. From 2004–2009, TEDDY will screen more than 360,000 newborns from both the general population and families already affected by T1D to identify an estimated 17,804 children with high-risk HLA-DR, DQ genotypes. Of those, 7,801 (788 first-degree relatives and 7,013 newborns with no family history of T1D) will be enrolled in prospective follow-up beginning before the age of 4.5 months. As of May 2008, TEDDY has screened more than 250,000 newborns and enrolled nearly 5,000 infants—approximately 70% of the final cohort. Participants are seen every 3 months up to 4 years of age, with subsequent visits every 6 months until the subject is 15 years of age. Blood samples are collected at each visit for detection of candidate infectious agents and nutritional biomarkers; monthly stool samples are collected for infectious agents. These samples are saved in a central repository. Primary endpoints include (1) appearance of one or more islet autoantibodies (to insulin, GAD65 or IA-2) confirmed at two consecutive visits; (2) development of T1D. By age 15, an estimated 800 children will develop islet autoimmunity and 400 will progress to T1D; 67 and 27 children have already reached these endpoints.

Keywords

type 1 diabetes; islet autoimmunity; environmental triggers; epidemiologic study; HLA; infectious agents; dietary factors; psychosocial factors

Background

Over the past 60 years, the incidence of type 1 diabetes (T1D) worldwide has been increasing by 3–5% per year,[1–3] (Fig. 1) doubling approximately every 20 years.[4] While several T1D susceptibility genes are known, such a rapid increase can only be explained by a powerful influence in the environment interacting with a relatively common genetic background. In some populations, the incidence has increased most markedly in the very youngest children,[3] suggesting a role for very early exposures. The disease also appears to spread to children who carry lower-risk HLA-DR, DQ genotypes,[5,6] consistent with an increase in the penetrance of the environmental exposure(s). Population-based cohort studies that preceded TEDDY[7–9] as well as rapid advances in immunology and genetics have provided new insights into the pathogenesis of T1D. On the other hand, none of the candidate environmental exposures has

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Conflicts of Interest

The authors declare no conflicts of interest.

been shown beyond reasonable doubt to cause a significant number of the cases. The role of the TEDDY study is to accelerate progress towards preventing T1D prevention through a large-scale sustained international effort to clearly define the causes of T1D.

Islet autoimmunity, marked by the presence of autoantibodies to pancreatic β cell antigens such as GAD65, insulin, or IA-2, precedes clinical T1D in most cases by a few years (Fig. 2). This preclinical period provides a theoretical opportunity for prevention. However, two large randomized trials in relatives of T1D patients—the European Nicotinamide Diabetes Intervention Trial[10] and the Diabetes Prevention Trial-1 (using parenteral[11] and oral insulin[12])—failed to prevent or delay progression from autoimmunity to diabetes. Significant β cell damage present at trial entry could also play a role. In contrast, TRIGR (the Trial to Reduce IDDM in the Genetically at Risk)[13] is attempting T1D prevention by eliminating cow's milk in infant nutrition before the onset of islet autoimmunity. Pilot studies using omega-3 fatty acids (NIP) or human oral insulin (Pre-Point) are under way in genetically susceptible young children to prevent islet autoimmunity and T1D. While these approaches may be effective, we lack convincing evidence concerning the initiators of islet autoimmunity to design optimal primary prevention trials. Of importance, our current understanding of T1D etiology originates predominantly from studies of first-degree relatives (FDRs) of T1D patients. These data may not be directly applicable to the causes and prevention of T1D in the general population, in which 90% of the cases occur. TEDDY is filling important gaps in our understanding of the natural history of T1D by studying from birth high-risk general population children and relatives followed systematically for environmental determinants of T1D.

A number of environmental exposures have been proposed to contribute to T1D risk. These include exposures taking place during pregnancy, infancy, childhood, and beyond. Not all islet autoantibody–positive subjects progress to diabetes,[11] and hence the importance of distinguishing whether an environmental agent triggers development of islet autoimmunity or promotes disease progression. This can only be determined by prospective follow-up of large numbers of genetically at-risk children from a very young age.

Exposure to rubella during pregnancy has resulted in diabetes in about 20% of children.[14] Similarly, the risk for T1D in childhood is reported to be increased in children born to mothers with enterovirus infections during pregnancy.[15,16] Other potential risk factors include ABO incompatibility and hyperbiliru-binemia,[17,18] preeclampsia,[19] mother's age,[20] and high birth weight for gestational age.[20,21] Further evidence of fetal programming of T1D risk comes from the still unexplained decreased T1D risk in children of mothers with T1D as compared to children of fathers with T1D.[22,23] Finally, the HLA type of the child appears to affect fetal growth, suggesting potential genetic programming that goes beyond the immune repertoire.[24,25] There is a gap in understanding to what extent gestational factors, including genetic interactions, may trigger islet autoimmunity or merely increase T1D susceptibility in the offspring. It cannot be excluded that gestational infection may induce immunologic tolerance to the virus.[26,27] An ability of the offspring immune system to regard a virus as self may have consequences for latency and reinfection.

Seroconversion to positivity for islet autoantibodies, the earliest measure of islet autoimmunity which may lead to clinical T1D, may occur already 3–6 months after birth.[20,28] Candidate autoimmunity and T1D risk factors operating in infancy include those related to exposure to infectious agents, improved hygiene,[29] mucosal exposure to dietary constituents,[30,31] and requirement for increased beta cell functioning.[32,33]

Previous virus studies have sought to provide a direct evidence for virus-induced T1D. However, in some of these patients developing T1D, it was found that either the insulitis was chronic or that the patients already had islet cell autoantibodies. It could therefore not be

excluded that the virus infection accelerated an already ongoing process of islet autoimmunity. [34] Further studies of these phenomena as well as of other microbial agents are therefore warranted to take into account that subjects with an increased T1D risk may show responses that lead to islet autoimmunity or affect ongoing islet autoimmunity.

Enteroviruses, and in particular coxsackie B viruses, remain the prime candidate by nature of their tropism for beta cells,[34,35] possible molecular mimicry,[36] and early and more recent reports of their presence in beta cells of patients with T1D.[37,38] Data from Finland showing a relationship between enterovirus infection and the appearance of islet autoantibodies as well as a seasonal fluctuation in the appearance of islet autoantibodies supports a role early in the disease.[39] However, substantially more evidence is required to establish a causal role for enterovirus in T1D, especially as a trigger of the islet autoimmunity, because the association could not be demonstrated in children outside Scandinavia, including those in Colorado[40] and Germany.[41] Rotavirus has also been shown to infect beta cells and to have a link to islet autoimmunity by way of molecular mimicry,[42,43] but evidence for a causal role is lacking.[44]

Seemingly in contrast to the infectious hypotheses is the notion that improved hygiene is responsible for upward trends in T1D incidence as well as incidences of other hyperimmune response diseases such as allergy.[29] There are epidemiologic studies indicating that crowding and exposure to others in day care are associated with reduced T1D risk, supporting the hygiene hypothesis. Few studies have examined the relationship of hygiene to the development of islet autoimmunity. Related to hygiene is a potential role of vaccinations in the development of islet autoantibodies or progression to T1D. Some have suggested that vaccination increases T1D risk, but well-designed studies have found no evidence for this.[45,46] The temporal relationship of vaccinations to the development of islet autoantibodies or T1D has never been examined. Prospective analyses of children from 3 months of age through the entire period of mandatory or voluntary vaccination are needed to establish effects of vaccinations on islet autoimmunity and progression to T1D.

Substantial data have been generated on the role of breast-feeding and early exposure to cow's milk[47] or cereals.[30,31] In addition to these there are reports of associations of T1D development with low intakes of vitamin D,[48] tocopherols,[49] ascorbic acid,[50] vitamin E,[51] and omega-3 fatty acids.[52] Other suspected exposures include drinking water, with an increased risk if water is from a local well compared to water-plant drinking water, possibly related to the amount of zinc.[53] Moreover, *N*-nitroso compounds[54] and mycotoxins[55] have been associated with an increased risk of T1D.

The TEDDY study is uniquely positioned to elucidate the association between T1D and celiac disease because the study eligibility HLA genotypes confer susceptibility to both diseases. [56–58] TEDDY is measuring autoantibodies against tissue transglutaminase (tTG), which is a very sensitive and specific marker of celiac disease.

Psychosocial factors may also contribute to appearance of T1D. Stress has long been considered a potential trigger for TID.[59] Screening for high-risk genes associated with T1D could induce anxiety and distress in family members.[60] Prospective studies utilizing detailed psychosocial evaluation of participating parents as well as children as they grow older will be necessary to effectively determine whether life events or stress may increase the risk for islet autoimmunity. Experiences in studies of children at genetic risk for T1D who have gone on to develop T1D have identified benefits such as absence of severe ketoacidosis and a reduction in hospitalization.[61]

While there are preliminary data and intriguing hypotheses as to the etiology of T1D, the data are often 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. Most of the few studies that have attempted to look at exposure from an early age and in relation to the development of islet autoantibodies were underpowered. TEDDY will fill important gaps in our understanding of the events leading to T1D. In addition, samples collected by TEDDY will create a valuable resource for investigators proposing innovative hypotheses concerning candidate environmental and genetic factors.

TEDDY Study: Organization and Goals

The Environmental Determinants of Diabetes in the Young (TEDDY), a multicenter prospective cohort study, was initiated in 2003 to identify environmental factors that trigger or protect against the development of islet autoimmunity and T1D. The details of the TEDDY study's organization and protocol have been previously published.[62] In brief: the consortium comprises 6 clinical centers located in Denver (Colorado), Augusta (Georgia)/Gainesville (Florida), and Seattle (Washington) in the United States, and in Finland (Turku), Sweden (Malmo), and Germany (Munich). The Data Coordinating Center is in Tampa, Florida. Autoantibody Reference Laboratories are located in Denver (serving the U.S. Clinical Centers) and Bristol (serving the European Clinical Centers). The Central Genetics Reference Laboratory is in Oakland, California, and the Central mRNA Laboratory is in Augusta, Georgia. The NIDDK Bio-sample and Genetics Repositories store samples. For more details, see the Appendix.

The primary objectives of this study are:

1. To identify environmental factors that trigger or protect against the development of islet autoantibodies or T1D.

     a. *Infectious agents*:

     Blood and stool samples as well as other bodily fluids are collected and analyzed for infectious agents to test the hypothesis that specific virus(es) may trigger islet autoimmunity or promote progression to T1D. In addition, the hypothesis that certain infections may reduce the risk of islet autoimmunity or T1D will also be tested.

     b. *Dietary factors*:

     Primary caretakers provide 3-day food diaries and 24-hour-recall dietary records, and blood samples are used to analyze vitamin D, alpha-tocopherol, gamma-tocopherol, cartenoids, ascorbic acid, and red blood cell membrane fatty acids to test the hypothesis that dietary factors may trigger/accelerate/ reduce islet autoimmunity or promote progression to T1D.

     c. *Psychosocial factors*:

     Stressful life events and other indicators of stress in the child and family are monitored to assess their contribution, if any, to the development of islet autoimmunity or T1D. In addition, participating families provide structured information that may help to identify factors affecting retention and study participation.

     d. *Other*:

     TEDDY will evaluate other factors such as toxins, immunizations, pets, and allergies in the triggering of and/or protection against islet autoimmunity or T1D.

2. Genes both within and outside the HLA region are typed to identify gene–environment interactions. It is expected that the joint analyses of genetic and environmental data will improve the identification of both genetic and environmental factors influencing development of islet autoimmunity and T1D, and may explain mechanisms of these interactive effects.

3. The prospectively collected specimens from TEDDY subjects (DNA, RNA, serum and plasma, cells and other samples) provide a unique opportunity for scientists within and outside the TEDDY consortium to test novel hypotheses.

Study Progress

Consent for genetic screening is obtained from parents of babies born in area hospitals or identified after birth. This blood sample is analyzed at approved and monitored laboratories for the HLA haplotypes that qualify a child for TEDDY. Those results are returned to the DCC and local clinical centers, who notify all subjects of the genetic screening outcome. Among those who are HLA-eligible, trained local staff (e.g., study nurses) contact the subject's parent to explain genetic risk and introduce in some detail the follow-up phase of TEDDY for which their child is now eligible to join. Enrollment, informed consent, and completion of the first visit must occur before the child reaches the age of 4.5 months. Once this visit has been completed the subject is then on the follow-up visit schedule for data collection described below. A portion of the 9-month blood sample is used to confirm HLA eligibility at a central reference laboratory, where additional high-resolution typing is done to confirm eligibility. Table 1 summarizes current TEDDY accruals by center and cohort (FDRs versus infants without a history of T1D in a FDR). Table 2 compares current accruals with the overall study goals for the number of infants to complete screening, found eligible, and enrolled into follow-up. As of May 2008, TEDDY has screened more than 250,000 newborns and enrolled nearly 5,000 infants—approximately 70% of the study goals. As shown in Figure 3, TEDDY is ahead of the enrollment goals by several months and on track to complete the screening and enrollment by the end of 2009.

Eligible children are enrolled into intensive prospective follow-up before the age of 4.5 months.

The childrens' exposure to dietary and other environmental factors is recorded at clinic visits every three months for the first 4 years of life and then biannually until age 15. Stool samples are collected to assess viral exposures at monthly intervals for the first 4 years and then biannually until age 15. The prospective data collection protocol is summarized in Table 3. Compliance rates to specific data collection components of the protocol are at high levels (Table 4).

Figure 4 indicates that there is a small early loss to follow-up after the initial couple of visits that is consistent with observations from previous similar cohort studies (DAISY in Colorado and DIPP in Finland). The loss seems to plateau after the subjects reach 1 year of age and is noticeably lower among the FDRs. The overall rate of disenrollment is 9% over 2 years, and 12% by the age of 30 months, well within the planning parameters of the study. Single missed visits range from 2% (month 27) to 7% (month 18). Data beyond 30 months are considered too recent to provide reliable estimates.

Standardization of Assays for Islet Autoantibodies

TEDDY is unique among the NIDDK-sponsored studies in having two core autoantibody laboratories with confirmation of positive samples in both laboratories before samples are classified as positive. This strategy was agreed on (i) to have a high degree of certainty in positive results, an important consideration in view of the numerous samples tested for three

autoantibodies from each subject, and (ii) to provide an internal mechanism for checking and improving assay performance. We believe that the strategy has proved highly successful in both of these areas. Having two laboratories has posed additional requirements with respect to establishing concordance and the TEDDY study has performed several tasks addressing this point.

Prior to inception of the study protocol, and in order to determine and improve concordance of measurement during TEDDY, both laboratories measured autoantibodies to insulin, GAD65, and IA-2 in serum samples from 496 nondiabetic high-risk children (from the DAISY study) and from 60 patients with new-onset disease. Analysis of these data showed that there were differences between calling sample positives caused by different relative thresholds in the two laboratories. The data were used to simulate performance (ROC analysis) and concordance at different thresholds and to determine the thresholds from each laboratory that were expected to be equivalent on a common sample set. These thresholds were selected for the TEDDY protocol.

Because of the number of studies using islet autoantibodies as an outcome or selection criteria and the observations made with respect to two laboratories in TEDDY, the NIDDK decided to endorse this proposal and to embark on developing a common method of measurement (Islet Autoantibody Measurement Harmonization Project).

Children are born with maternal IgG, including maternal islet autoantibodies, especially if the child has a mother with diabetes. It is necessary to exclude positive results that are due to this maternal IgG transmission when defining subject outcome. An algorithm was developed that considered islet autoantibody status of the mother (measured when the child was aged 6 or 9 months), whether a child had a negative sample prior to their first positive sample, and whether the islet autoantibody titer increased or decreased in subsequent samples.

TEDDY participants are considered persistently islet autoantibody–positive (major study end point) if they had at least two confirmed positive samples that were not due to maternal islet autoantibody transfer or if they had one confirmed positive sample and developed diabetes prior to the next sample collection. The study protocol requires that all positive samples and 5% of negative samples be tested in the second central laboratory. As of March 31, 2008, 53 children from the general population and 16 FDRs have developed persistent islet autoantibody. The cumulative incidence of this endpoint by the age of 40 months was 4.6% in FDRs and 2.8% in the general population children. However, some young FDRs may later be reclassified as having had maternal autoantibodies as data mature.

Development of Type 1 Diabetes

As of March 31, 2008, 21 children from the general population and 6 FDRs have developed T1D. The cumulative incidence of this endpoint by the age of 40 months was 2.5% in FDRs and 1.8% in the general population children. Preliminary observations suggest that the participation in TEDDY significantly reduced the severity of clinical presentation and has eliminated most of the expected hospitalizations and DKA in these very young patients.

Other Accomplishments

TEDDY has mapped the frequencies of T1D susceptibility genotypes in diverse populations including African Americans, Asian Americans, and Hispanics in the United States. Preliminary results have been presented elsewhere.[63]

The study developed novel comparisons and standardization between the four national TEDDY food databases (U.S., German, Swedish, and Finnish). A comprehensive nutrient data

dictionary has been compiled of different nutrients that are calculated in TEDDY: units of measurement, methods of analysis, and derivations and descriptions of each nutrient in each TEDDY food database. Most nutrients are comparable between the databases, whereas some need to be recalculated (protein, energy) and some are not comparable between all of the four databases (fiber, folate), and all the databases do not contain all TEDDY nutrients (e.g., some of the fatty acids). The Finnish and Swedish national food databases (which both are used in TEDDY) take part in an EU program (Eurofir), which aims at harmonization of algorithms that will result in more uniform procedures (especially at the food level) and will, in the long run, benefit TEDDY. TEDDY has already discovered significant differences in infant feeding practices between the United States and Europe[64] and is exploring variability in infant nutrition within the U.S. population.

The TEDDY protocol includes studies focused on identification of psychosocial factors that predispose to or protect from β cell autoimmunity and T1D. A secondary objective is to explore the psychosocial corollaries of the ascertainment of risk status for autoimmunity and T1D in newborns. In addition, the TEDDY Psychosocial Committee assists with developing appropriate procedures and identifying resources to assure adequate informed consent, minimize study burden, maximize procedure convenience/comfort, thank and support participants, and provide psychosocial support, as needed. Parent anxiety in response to the infants' increased risk is assessed using a 6-item short form of Spielberger's State Anxiety Inventory (SAI) administered when the child was 3–4.5 months old and again when the child is 6-months old. Postpartum depression is measured when the child is 6 months of age by the Edinburgh Post-natal Depression scale. Families that failed to enroll in TEDDY are asked to provide reasons for their decision. We also examined those who dropped out of TEDDY within 1 year of recruitment and found several important predictors. TEDDY has also uncovered significant differences between the levels of parental stress and postpartum depression between the U.S. and European societies.[65]

In conclusion, the TEDDY Consortium is nearing the final stages of the initial phase of the project, with more than 70% of the screening and 63% of the enrollment goals already achieved. The retention of study participants and compliance remain high despite the very demanding protocol.

Preliminary findings point to major inter-population differences in genetic susceptibility and candidate environmental risk factors for T1D.

Acknowledgments

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Appendix

TEDDY Study Group

[*Note:* Superscript numbers refer to the Committees listed below.]

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Committees—[1]Ancillary Studies, [2]Diet, [3]Genetics [4]Human Subjects/Publicity/ Publications, [5]Immune Markers, [6]Infectious Agents, [7]Laboratory Implementation, [8]Maternal Studies, [9]Psychosocial, [10]Quality Assurance, [11]Steering, [12]Study Coordinators, [13]Celiac Disease, and [14]Clinical Implementation.

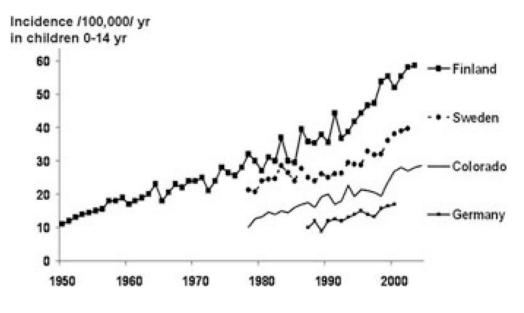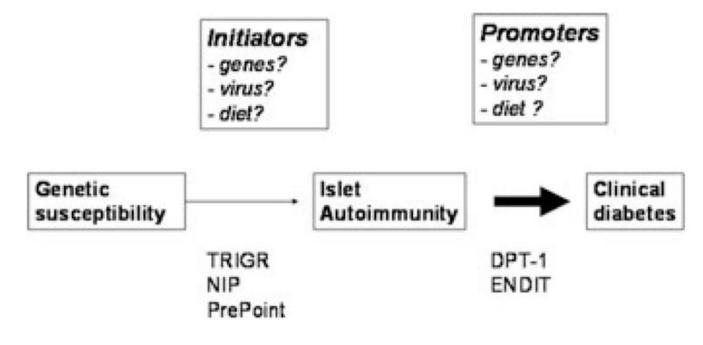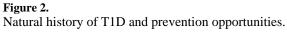Incidence /100,000/ yr
in children 0-14 yr

Figure 1.
T1D incidence has doubled every 20 years. Data for Finland are from the Finnish National Public Health Institute (V. Harjutsalo and J. Tuomilehto); data for Sweden are from the Swedish Childhood Diabetes Registry;[66] data for Germany are a compilation of two reports; [67,68] data for Colorado are from the Colorado IDDM Registry, the Barbara Davis Center for Childhood Diabetes, and SEARCH for Diabetes in Youth.[4]
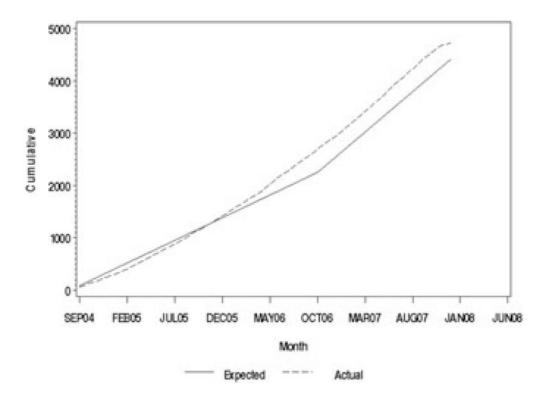
Figure 2.
Natural history of T1D and prevention opportunities.

Figure 3.
Cumulative number of enrolled subjects (data as of 3/31/08).

Figure 4.
Early indicators of future retention (data as of 3/31/08).

TABLE 1

Current TEDDY Accruals (as of May 22, 2008)

Clinical Center	Infants Screened			Infants HLA-Eligible			Infants Enrolled		
	FDRs	Gen Pop	Total	FDRs	Gen Pop	Total	FDRs	Gen Pop	Total
Colorado	575	41,900	42,475	123	2212	2335	79	649	728
Finland	571	39,813	40,384	191	2221	2412	103	1039	1142
Georgia/Florida	621	55,903	56,524	113	1814	1927	50	480	530
Germany	1011	18,277	19,288	187	687	874	140	166	306
Sweden	674	31,433	32,107	128	2303	2431	91	1526	1617
Washington	526	63,733	64,259	116	2437	2553	62	584	646
Total	4011[a]	251,059	255,070[a]	866	11,674	12,540	531[a]	4444	4975[a]

FDRs = first-degree relatives newborn of a person with T1D; Gen Pop = newborn without a first-degree relative with T1D.

[a]Including 43 FDRs screened and 6 enrolled by the Pittsburgh and New York TRIGR sites.

TABLE 2

Expected Number of Infants to Complete Screening, Eligible, Enrolled and Endpoints Ascertained

	General population	% of goal attained as of May 22, 2008	FDRs	% of goal attained as of May 22, 2008	Total
Screened	355,992	71%	5,596	72%	361,588
Follow-up eligible	16,588	70%	1,216	71%	17,804
Enrolled	7,013	63%[a]	788	67%[a]	7,801
With persistent islet autoantibodies by 6 years of age	281	53[b]	105	16[b]	386
With type 1 diabetes by 15 years of age	281	21[b]	105	6[b]	386

[a] Enrollment is completed on average 3–4 months after screening, hence lower % of goal attained than for screening;

[b] As of March 31, 2008.

TABLE 3

Follow-up Protocol

	Birth	3	6	9	12	15	18	21	24	>24 q3 mo	>48 q6 mo
						Age (months)					
Cord blood (mL)	0.3–7.0										
Venous blood (mL)											
Serum		2.0	2.0	3.0	6.0	6.0	6.0	6.0	6.0	6.0	
Plasma		2.0	4.0	5.0	8.0	8.0	8.0	12.0	12.0	12.0	
mRNA (ABI)		2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	
DNA				1.0							
Total blood volume (mL)		6.5	8.5	11.5	16.5	16.5	16.5	20.5	20.5	20.5	TBD

Note: Follow-up included: stool samples (monthly); TEDDY Book (parental records of nutrition, infections, medications, and immunization); maternal pregnancy diet, child's 24-hour recall, and 3-day food record; heights/weights (q 3 mo.); negative life events, parental distress, anxiety, and depression; family history; tap water, toenail clippings.

TABLE 4

Percentage of Forms and Samples That Have Been Collected of TEDDY Study Visits with a Physical Exam[a]

(Forms/labs)	3 mos. (N = 4418/4252)	6 mos. (N = 3934/3763)	9 mos. (N = 3432/3288)	12 mos. (N = 2974/2853)	15 mos. (N = 2555/2445)	18 mos. (N = 2173/2031)	21 mos. (N = 1768/1649)	24 mos. (N = 1415/1300)	27 mos. (N = 1103/991)	30 mos. (N = 795/689)	33 mos. (N = 500/413)
Physical exam	100	100	100	100	100	100	100	100	100	100	100
Diet recall	97	96	94	94		89		86		86	
Serum	99	99	99	100	99	99	98	95	97	99	99
Plasma	74	80	81	84	86	85	87	85	83	86	85
mRNA	62	71	73	76	79	79	81	79	79	83	82
TEDDY book	100	100	100	100	100	100	100	100	100	100	100

[a] Percentages are based upon expected numbers. Data as of January 31, 2008.