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REVIEW

# Type 1 Diabetes: Prospective Cohort Studies for Identification of the Environmental Trigger

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**Abstract** Type 1 diabetes (T1D) is one of the most common chronic diseases with childhood onset, and the disease incidence has increased two to fivefold over the past half century by as yet unknown means. T1D occurs when the body's immune system turns against itself, destroying in a very specific and targeted way—the pancreatic β-cells. T1D results from poorly defined interactions between susceptibility genes and environmental determinants. In contrast to the rapid progress in finding T1D genes, identification and confirmation of environmental determinants remain a formidable challenge. This review article will give an overview of ongoing prospective cohort studies aiming to identify the environmental trigger(s) causing T1D.

**Keywords** Autoimmunity · Cohort studies · Environmental factors · Genetic factors · Type 1 diabetes

**Abbreviations**

| | |
|---|---|
| BMI | Body mass index |
| DAISY | Diabetes Autoimmunity Study in the Young |
| DIPP | Diabetes Prediction and Prevention study |
| FDR | First-degree relatives |
| HLA | Human leukocyte antigen |
| HR | Hazard ratio |
| MIDIA | Norwegian acronym for: Environmental Triggers of T1D |
| TEDDY | The Environmental Determinants of Diabetes in the Young |
| T1D | Type 1 diabetes |

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## Introduction

Type 1 Diabetes (T1D) is one of the most common chronic diseases with childhood onset. Its incidence has increased two to fivefold over the past half century by as yet unknown means (DIAMOND Project Group 2006; Harjutsalo et al. 2008). It was interpreted that if the present trend continues, the prevalence of children with the disease in Europe will increase by 50 % before year 2020, and by 70 % in children younger than 5 years of age (Patterson et al. 2009). Most recently European data were compared between the periods 1989–1998 and 1999–2008, and it was shown that the increase in T1D is not necessarily uniform; it has periods of less rapid and more rapid increase in incidence in different countries (Patterson et al. 2012). These patterns of change suggest that important risk exposures may differ over time in different European countries.

T1D occurs when the body's immune system turns against itself, destroying—in a very specific and targeted way—the pancreatic islet β cells, the only cells in the body that produce the vital hormone, insulin (Atkinson and Eisenbarth 2001; Eizirik et al. 2009). This autoimmune destruction is irreversible and the disease presently seems to be incurable. If pancreas or islets are transplanted they too are destroyed, unless heavy immunosuppression is applied (Natasha et al. 2011).

T1D results from poorly defined interactions between susceptibility genes and environmental determinants. T1D susceptibility is primary defined by genetic factors within

the human leukocyte antigen (HLA) on chromosome 6. The main disease factors are the HLA-DQ molecule encoded by DQA1 and DQB1 genes and the HLA-DR molecule defined by DRB1 alleles (Rønningen et al. 1991, 1993; Rønningen 1997; She 1996; Undlien et al. 1997). In addition, recent genome-wide studies have identified genetic association in 40 other intervals that may harbor T1D susceptibility genes (Barrett et al. 2009).

In contrast to the rapid progress in finding T1D genes, identification and confirmation of environmental determinants remain a formidable challenge (Knip et al. 2005; Rønningen 2011). The reason underlying the lack of progress is multi-faceted. First, large numbers of different environmental determinants could contribute to the triggering or protection of T1D (Benson et al. 2008; Clements et al. 1995; Dahlquist et al. 1990, 1991; Elfving et al. 2008; EURODIAB Substudy 2 Study Group 1999, 2002; Frisk et al. 1992; Gibbon et al. 1997; Honeyman et al. 1998, Honeyman 2005; Horwitz et al. 1998; Hyppönen et al. 2001; Jun and Yoon 2003; Knekt et al. 1999; Lamb et al. 2008; Osame et al. 2007; Parslow et al. 2001; Pundziute-Lycka et al. 2004; Richer and Horwitz 2008, 2009; Stene et al. 2000, 2001, 2003). Although many candidates have been suggested in the past, few have been definitively proven beyond reasonable doubt. Second, exposures may occur any time before the onset of disease, from in uteri to T1D onset (Dahlquist et al. 1995a, 1995b; Hyöty et al. 1993, 1995; Menser et al. 1978). Third, environmental determinants may differ among populations, possible partly depending on their genetic architecture—which is, rather understandably, also quite variable among populations.

The autoimmune process of islet destruction is accompanied by development of various autoantibodies against various components of the β cell. These autoantibodies are not regarded as destructive, yet they have proven to be an invaluable marker of β cell autoimmunity. Islet cell antibodies were the first marker that was possible to test for by using indirect immunofluorescence on a section of frozen human pancreas from a blood group O donor (Botazzo et al. 1974). More recently recombinant antigens are being used to test for specific antibodies (Bingley et al. 1997; Savola et al. 1998; Wenzlau et al. 2007; William et al. 1997).

Islet autoantibodies precede the development of T1D, and can appear throughout childhood (Hummel and Ziegler 2011). In prospective studies of offspring of parents with T1D, the peak of islet autoantibodies appearance was observed at around 1 year of age, followed by a decline through 2–5 years and a subsequent rise in incidence towards puberty (Ziegler et al. 2012). Children with increased HLA-associated risk for T1D followed up from the general population got positive for autoantibodies at all ages. Early seroconversion occurs in children who progress fast to T1D (Parikka et al. 2012). It has been observed that of the children who progress to T1D during a follow-up at 13 years, 64 % became autoantibody positive before the age of 2, and 82 % before the age of 3 (Parikka et al. 2012). Early antibody positivity frequently starts with insulin autoantibodies (IAA) followed by glutamic acid decarboxylase antibodies (GADA) or positivity for both IAA and GADA in the same sample. Usually insulinoma-associated protein 2 (IA-2) and zinc transporter 8 (ZnT8) antibodies develop closer to onset of T1D (Ziegler et al. 2012).

Identification of environmental determinants requires frequent follow-up for autoantibody testing in a large number of individuals observed from early life until disease onset. To accomplish such ambitious goals, long-term prospective studies on cohorts of children at increased risk of developing the disease, are necessary. This review describes four such prospective cohort studies and their results.

## The German BABYDIAB/BABYDIET Studies

BABYDIAB is a study from birth in 1,650 children born to a mother or father with T1D. Its aim is to identify environmental trigger(s) in first-degree relatives (FDR). Recruitment began in 1989 and ended in 2000. All children (840 boys, 810 girls) were recruited in Germany, and 97 % of the families are of German or of European descent; the cohort is not population based (Winker et al. 2008; Ziegler et al. 1999, 2003, 2011). Islet autoantibodies directed against insulin (IAA), glutamic acid decarboxylase, IA-2 and ZnT8 are tested at all scheduled visits, and every 6 months in children positive for islet autoantibodies, see Table 1. The median follow-up from birth to last sample in BABYDIAB is 11.7 years (Ziegler et al. 2012).

HLA genotyping not an inclusion criterion, but was performed later for scientific purposes. Early seroconversion has been shown to be most evident in children with the HLA-DR3/4-DQ8 or DR4/DR4-DQ8 genotypes.

Islet autoantibody incidence was examined also in a confirmatory cohort of 150 children who participated in the BABYDIET study (Schmid et al. 2004). These children

**Table 1** HLA typing and time points for follow-up in BABYDIAB

(a) No HLA typing required for recruitment and to be found eligible for follow-up

(b) Time points for blood samples: 9 months, 2, 5, 8, 11, 14, 17, 20 years

(c) Questionnaires at 9 months and at 2 years with respect to breastfeeding

(d) Stool samples not asked for

**Table 2** HLA typing and time points for follow-up in BABYDIET

| | |
|---|---|
| (a) Eligible HLA types | DRB1*03-DQA1*0501-DQB1*0201/DRB1*04-DQA1*0301-DQB1*0302 |
| | DRB1*03-DQA1*0501-DQB1*0201/DRB1*03-DQA1*0501-DQB1*0201 |
| | DRB1*04-DQA1*0301-DQB1*0302/DRB1*04-DQA1*0301-DQB1*0302 |
| | DRB1*04-DQA1*0301-DQB1*0302/DRB1*08-DQA1*0401-DQB1*0402 |
| | DRB1*04-DQA1*0301-DQB1*0302/DRB1*01-DQA1*0101-DQB1*0501 |
| (b) Time points for blood samples | Followed from the age of 3 months with 3-monthly blood samples until age 3 years and subsequently with yearly blood samples |
| (c) Questionnaires: | At 6 and 12 months |
| (d) Stool samples: | From birth to 3 years (Simonen-Tikka et al. 2011) |

**Table 3** HLA typing and time points for follow-up in DIPP

| | |
|---|---|
| (a) Eligible HLA types | DQB1*02/DQB1*0302 |
| | DQB1*0302/X (X # DQB1*02, 0301, 0602/0603) |
| (b) Time points for blood samples | Autoantibodies are measured in Tampere and Oulu at 3, 6, 18 and 24 months and then annually. In Turku every 3 months until 2 years, and then every 6 months until the age of 14 years |
| (c) Questionnaires | Filled out at the clinical visits at 3, 6, 18 and 24 months, and then annually |
| (d) Stool samples | Parents are asked to send it from 3 months to 3 years |

were selected for having both a first-degree relative with T1D, and T1D risk HLA genotypes, see Table 2. The median follow-up time from birth to last sample was 5.2 years (interquartile range: 3.2–6.6) (Hummel and Ziegler 2011).

### The Finnish Diabetes Prediction and Prevention Study

In the Finnish population-based Diabetes Prediction and Prevention (DIPP) study, children with HLA-conferred susceptibility to T1D have been monitored prospectively from birth. The aim of the DIPP study was to identify the environmental trigger(s) of T1D in a population with a very high incidence of the disease. DIPP was launched in Turku in November 1994, and 1 and 3 years later at the University Hospitals of Oulu and Tampere, respectively. Newborns from the general population as well as FDR have been screened for genetic risk, and families with a baby testing positive were then invited to a prospective follow-up at 3–12 months intervals until the age of 15 (Table 3) (Elfaitouri et al. 2007; Hermann et al. 2003; Kimpimaki et al. 2001; Nejentsev et al. 1999; Salminen et al. 2004; Tauriainen et al. 2007; Uusitalo et al. 2008; Virtanen et al. 2006; Viskari et al. 2005).

Children with the HLA*02/0302 genotype were categorized into the high-risk group (8 % T1D risk before age 15 years), and those with the *0302/x genotype to the moderate-risk group (3 % T1D risk). In the general population cohort, 13.7 % were identified for prospective follow-up, and 3.4 % of these are expected to develop T1D before the age of 15 years. Between November 1994 and December 2007, 122,636 newborn infants were screened for HLA-conferred susceptibility to T1D. Of these, 11,689 with genetic susceptibility were enrolled in the DIPP study, and 7,165 were followed up (Parikka et al. 2012). Since screening and recruitment are ongoing, the age of the children in the study, ranges at the moment from 3 months to 19 years (Hyöty, personal communication).

### The Diabetes Autoimmunity Study in the Young

Between December 1993 and October 2004, the Diabetes Autoimmunity Study in the Young (DAISY) screened for T1D susceptibility the HLA-DR, DQ genotypes and tested over 33,000 newborns from the general population of Denver, Colorado (Table 4). The aim of the study was to identify environmental trigger(s) for both T1D and celiac disease (Rewers et al. 1996a). Of all children born at the St. Joseph's Hospital and eligible for the screening, 90 % participated. The study population was representative of the general population of the Denver Metropolitan Area and included children classified by their mothers as non-Hispanic white (58 %), Hispanic (28 %), African American (7 %), Asian American (2 %) or biracial/other (5 %) (Rewers et al. 1996b). Newborns were categorized into four risk groups: (1) high diabetes risk, 20 times higher than in the general population (HLA-DRB1*03/04,DQB1*0201/0302 genotype and negative for DRB1*0403); (2) moderate diabetes risk, 3–7 times higher than in the general population (HLA-DR,DQ 4/4, 1/4, 8/4, and 9/4 (the DR4 haplotype carrying DQB1*0302), and DR3/3); (3) average diabetes risk, similar to that for the general population

**Table 4** HLA typing and time points for follow-up in DAISY (general population)

| | |
|---|---|
| (a) Eligible HLA types[a] | DRB1*03-DQB1*02/DRB1*04-DQB1*0302, and negative for DRB1*0403 |
| | DRB1*04-DQB1*0302/DRB1*04-DQB1*0302, and negative for DRB1*04034 |
| | DRB1*03-DQB1*02/DRB1*03-DQB1*02 |
| | DRB1*04-DQB1*0302/DRB1*01-DQB1*0501, and negative for DRB1*0403 |
| | DRB1*04-DQB1*0302/DRB1*08-DQB1*0402 |
| | DRB1*04-DQB1*0302/DRB1*09-DQB1*0303 |
| (b) Time points for blood samples | 9, 15, 24 and thereafter annually |
| (c) Questionnaires | Filled out at the clinical visits at 9, 15, and 24 months of age, and thereafter annually |
| (d) Rectal swabs and saliva | At 9, 15, and 24 months of age, and annually thereafter |

[a] No HLA typing for siblings of a child with T1D

(HLA-DRB1*03/x or *04/x), and (4) low diabetes risk—all others. The combination of high- (2.1 %) and moderate-risk genotypes (7.5 %), present in 9.6 % of the general population would identify 71 % of the future cases of T1D developing in the initial 5 years of life in Colorado children. All high-risk children and those at moderate or low risk were invited to participate in the follow-up (Graves et al. 2003; Norris and Scott 1996; Norris et al. 2003, Simpson et al. 2011; Stanley et al. 2004).

Families with a member having T1D were identified using The Barbara Davis Center for Childhood Diabetes in Denver, other diabetes care clinics, the Colorado T1D Registry, and newspaper publicity. Non-diabetic children within these families who were under the age of 4 were eligible; of those who were contacted, 86 % participated.

## The Norwegian MIDIA Study

The MIDIA (a Norwegian acronym for Environmental Triggers of T1D) study recruited newborns from the general population with the highest genetic risk for developing T1D (those carrying the HLA-DRB1*0401-DQA1*03-DQB1*0302/DRB1*03-DQA1*05-DQB1*02 genotype), and follows them for 15 years (Table 5). The high-risk HLA genotype is present in 2.1 % of Norwegian newborns (Cinek et al. 2000), and confers a calculated 7 % risk for getting T1D before 15 years of age and a life time risk of 20 % (Joner and Søvik 1989, 1991; Mølbak et al. 1994; Patterson et al. 2009; Rønningen et al. 1991; Undlien et al. 1997). Inclusion of only the high-risk genotype makes the cohort exceptionally homogeneous while identifying about 30 % of future cases of T1D (Rønningen et al. 1991; Undlien et al. 1997). The recruitment was done by public health care nurses (Stene et al. 2007).

It started in small scale in the summer of 2001, and gradually spread, covering the whole country of Norway from March 2006 (60,000 births per year). All recruitment to MIDIA was stopped effectively from December 10, 2007 due to problems with previously issued approvals. A legal opinion emerged that screening was against the Norwegian Biotechnology law of 1994, which prohibits genotyping children younger than 16 years of age for those medical conditions where no effective clinical treatment can be offered.

Altogether, 48,000 children were recruited to MIDIA. One thousand and forty-seven were identified to carry of whom 96.8 % took part in the follow-up (Aas et al. 2010; Stene et al. 2007), and about 95 % of scheduled blood samples, stool samples and questionnaires have been received.

At the end of 2012, 23 of MIDIA children had T1D, 40 are confirmed positive for two or three autoantibodies, and 30 for one autoantibody. A total of 5,356 blood samples, 18,275 stool samples and 4,791 questionnaires are presently available for analysis in the cohort (Bøås et al. 2012; Cinek et al. 2006, 2012; de Muinck et al. 2011; Rasmussen et al. 2009, 2011; Stene et al. 2007; Tapia et al. 2008, 2010, 2011a, 2011b; Witsø et al. 2006, 2011). The MIDIA cohort has a large overlap with the Norwegian Mother and Child Cohort study: approximately 50 % of the participants in MIDIA have mothers who have consented for usage of their pregnancy samples and questionnaires in MIDIA. Questionnaires have been asked for at 17th, 22nd and 30th week of pregnancy, when the child is 6 and 18 months, and at 3, 5, 7, 10 and 12 years of age (Magnus et al. 2006). Blood samples were asked for at 17th week of pregnancy and at the time of delivery from the mother, and cord blood was taken from the baby (Rønningen et al. 2006).

## Results from the DIPP, BABYDIAB/BABYDIET, DAISY and MIDIA Studies

### Viral Infections

Enteroviruses have been studied both in DIPP, BABYDIAB, DAISY and MIDIA as promising candidates in the etiology of T1D (Stene et al. 2010). In the Finnish DIPP

**Table 5** HLA typing and time points for follow-up with blood samples in MIDIA

| | |
|---|---|
| (a) Eligible HLA type | DRB1*03-DQA1*05-DQB1*02/DRB1*0401-DQA1*03-DQB1*0302 |
| (b) Time points for blood samples | 3, 6, 9, 12 months, thereafter annually |
| | If positivity for one autoantibody, a new blood sample after 6 months. If positivity for two or three autoantibodies, a new sample after 3 months |
| (c) Questionnaires | Collected at 3, 6, 9 and 12 months and thereafter annually |
| (d) Stool samples | Collected each month from 3 months to 3 years of age |

study, a higher proportion of enterovirus was observed in serum of children with islet autoimmunity compared with their age-matched controls (Lönnrot et al. 2000; Oikarinen et al. 2011; Stene et al. 2010) or a combination of serological analysis and stool samples (Salminen et al. 2004). Other studies done outside of Finland do not seem to yield such clear results: the DAISY study, conducted in USA, indicated the same enterovirus frequency in autoantibody positive children and controls (Graves et al. 2003), and no differences were found in the German BABYDIET study, either (Simonen-Tikka et al. 2011). In contrast to blood, islet autoimmunity has not been associated with enterovirus in stool samples (Salminen et al. 2004; Tapia et al. 2011a, 2011b).

### Nutritional Factors

Reduced total or exclusive breastfeeding did not significantly increase risk of developing autoantibodies in the BABYDIAB, DAISY or the DIPP study (Norris et al. 2003; Virtanen et al. 2006; Ziegler et al. 2003). No report has been published from the MIDIA study on breastfeeding. Whether or not breastfeeding protects against T1D is still controversial (Cardell et al. 2012; Gerstein 1994).

Infant feeding with gluten-containing food before 3 months was associated with a significant autoantibody risk both in the BABYDIAB and the DAISY studies (Norris et al. 2003; Ziegler et al. 2003). In the DAISY study, solid food in the form of cereals increased the risk for autoantibodies also when it was introduced after 7 months of age. (Norris et al. 2003). In the DIPP study, an early introduction of gluten-containing cereals seemed to increase the risk of advanced β cell autoimmunity, but only under the age of 3 years (Virtanen et al. 2011). The same study showed also that early introduction of root vegetables was associated with advanced β cell autoimmunity in young children. A recent report from the DIPP study indicates that also the intake of cow milk, fruit and berries was related to the development of advanced β cell autoimmunity (Virtanen et al. 2012).

In the DAISY study, investigators reported that higher intake of ω-3 fatty acids was associated with a lower risk of islet autoimmunity (hazard ratio [HR]: 0.45), and likewise, that higher amount of ω-3 fatty acids in erythrocyte membranes was associated with a lower risk of autoimmunity (HR: 0.63) (Norris et al. 2003). The natation has not been confirmed by any of the other cohort studies, but supportive evidence was found by two case–control studies from Norway. The first paper reported that maternal use of cod liver oil during pregnancy reduced the risk of TD1 in the offspring, whereas infant use did not (Stene et al. 2000). The other study concluded that the use of cod liver oil during infancy decreased marginally the risk of TD1 in the child, while maternal use during pregnancy showed no significant effect (Stene et al. 2003).

The relation of body mass index (BMI) to risk of islet autoimmunity was investigated in the MIDIA and the DIPP studies. In MIDIA, both the maternal BMI $\geq$30 kg per square meter before pregnancy, and the weight gain $\leq$15 kg predicted increased risk for islet autoimmunity, HR at 2.5 for both situations (Rasmussen et al. 2009). In a bigger cohort as part of the DIPP study, neither initial BMI nor weight gain during pregnancy was associated with advanced β cell autoimmunity in the offspring (Arkkola et al. 2011). Both the MIDIA and the DIPP data were based on self-reported weight and the time point for the initial weight may vary between the two cohorts as well as within the cohorts.

### Wheezing in Early Infancy

When a cohort of 42 cases and 843 non-cases in MIDIA was studied, self-reported "pneumonia, bronchitis or RS-virus" had HR at 3.5, $p = 0.001$ for developing islet autoimmunity before 4 years of age (Rasmussen et al. 2011). In addition, a Swedish study with data collected at the age of 2½ years found that wheezing during the first year of life was significantly associated with islet autoimmunity (Wahlberg et al. 2011).

## The Diversity of the Data Obtained in the Different National Cohort Studies

It is apparent from what is summarized above that different factors have been reported to confer T1D susceptibility in the different national prospective cohorts, leaving a number of holes and a troubling lack of consistency. It is likely that the results have been confounded by imprecise assessment of dietary exposure, recall bias, failure to assess exposures at very early ages, different definitions of exposure, and

small sample sizes. While many of these issues will be worked out with time as the scientific process takes place, the collaborative study, The Environmental Determinants of Diabetes in the Young (TEDDY) will facilitate all research in this field.

## The TEDDY Consortium

In 2003, the TEDDY study with a multicenter design was initiated. The consortium comprises six clinical centers located in Denver (Colorado), Augusta (Georgia)/Gainesville (Florida), and Seattle (Washington) in the United States, and in Finland (Turku, Tampere and Oulu), Sweden (Malmø), and Germany (Munich). The Data Coordinating Center is in Tampa, Florida. The Autoantibody Reference Laboratories are located in Denver (serving the US 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 role of the TEDDY study is "to accelerate progress towards preventing T1D through a large-scale sustained international effort to clearly define the causes of T1D". The aim of the TEDDY study is very similar to the national ongoing cohort studies (TEDDY Study Group 2007, 2008):

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 body 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 to analyze vitamin D, α-tocopherol, γ-tocopherol, carotenoids, ascorbic acid, and red blood cell membrane fatty acids to test the hypothesis that dietary factors may trigger/accelerate/reduce islet autoimmunity or progression to T1D.

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

**Table 6** HLA typing and time points for follow-up with blood samples in TEDDY

| (a) Eligible HLA types | Code | Genotype | Abbreviation | FDR | GP |
|---|---|---|---|---|---|
| | A | DRB1*03-DQA1*05-DQB1*02/DRB1*04-DQB1*DQA1*03-0302 | DR3/4 | Y | Y |
| | B | DRB1*04-DQA1*03-DQB1*0302/DRB1*04-DQA1*03-DQB1*0302 | DR4/4 | Y | Y |
| | C | DRB1*04-DQA*03-DQB1*0302/DRB1*08-DQA1*04-DQB1*0402 | DR4/8 | Y | Y |
| | D | DRB1*03-DQA1*05-DQB1*02/DRB1*03-DQA1*05-DQB1*02 | DR3/3 | Y | Y |
| | E | DRB1*04-DQA1*03-DQB1*0302/DRB1*04-DQA1*03-DQB1*02 | DR4/4b | Y | N |
| | F | DRB1*04-DQA1*03-DQB1*0302/DRB1*01-DQA1*0101-DQB1*0501 | DR4/1 | Y | N |
| | G | DRB1*04-DQA1*03-DQB1*0302/DRB1*13-DQA1*0102-DQB1*0604 | DR4/13 | Y | N |
| | H | DRB1*04-DQA1*03-DQB1*0302/DRB1*09-DQA1*03-DQB1*0303 | DR4/9 | Y | N |
| | I | DRB1*03-DQA1*05-DQB1*02/DRB1*09-DQA1*03-DQB1*0303 | DR3/9 | Y | N |
| (b) Time points for blood samples | | First sample at 3 months of age, every 3 months until 4 years of age, thereafter every 6 months until the age of 15 years | | | |
| (c) Questionnaires | | The children's exposure to dietary and other environmental factors is recorded at clinical visits every 3 months for the first 4 years of life and then biannually until age 15 | | | |
| (d) Stool samples | | At monthly intervals until the age of 4 years, and then biannually until age 15 | | | |

Although DQB1*0302 is shown above, DQB1*0304 is acceptable in its place for TEDDY inclusion. Subtyping was not required for further characterization of DQB1*02 and DQA1*03 genotypes. DR4 subtyping was required to exclude GP newborns with DRB1*0403, but no other DRB1*04 subtyping was required

*FDR* first-degree relative, *GP* general population, *TEDDY* The Environmental Determinants of Diabetes in the Young, *Y* eligible, *N* not eligible for TEDDY inclusion

2. Genes both within and outside the HLA region will be typed to identify gene-environment interaction. It is expected that the joint analysis 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 hypothesis.

Both general population and first-degree relative newborns were recruited to TEDDY. To develop a HLA screening strategy for TEDDY, HLA data on the healthy background population, T1D patients and their FDR were assembled from the six TEDDY clinical centers based in Colorado, Washington State, Georgia/Florida, Finland, Germany and Sweden. All centers provided historical data on their background population that were used to develop the TEDDY HLA strategy for general population subjects. Three centers (Finland, Germany and Sweden) also provided first-degree relative data. Odds ratios (ORs) for association with T1D were calculated for each genotype in each population. Genotypes were then ranked by OR in each of the study populations. Interestingly, the rank order for the top five increased risk genotypes was identical for all six data sets. From the combined data set, it was possible to identify nine increased risk genotypes which had an estimated OR >3 in all six data sets.

The four genotypes found eligible for the general population and the nine for FDR are shown in Table 6. Using these inclusion criteria, 4.8 % of the general population infants and 31 % of FDR were eligible for follow-up (Hagopian et al. 2011).

From September 2004 to February 2010, TEDDY screened a total of 414,714 general population newborns. Of these newborns, 19,906 were found to be eligible for follow-up, 6,333 FDR were screened, and 1,415 of them eligible for follow-up. The enrolment rate for the eligible children was higher at the European centers than in the US and varied from 67 % in Sweden to 25 % in Washington. There is a small loss to follow-up after the initial couple of visits. The loss seems to plateau after the subject reaches 1 year of age. The overall rate of disenrollment is 9 % over 2 years, and 12 % by the age of 30 months (The TEDDY Study Group 2008).

## Concluding Remarks

The prospectively collected specimens from TEDDY subjects (DNA, RNA, serum and plasma, cells and other samples) should be a unique opportunity also for scientists outside the TEDDY consortium to test novel hypothesis. In Finland recruitment to the DIPP study has happened in parallel with the recruitment to the TEDDY study, and recruitment to DIPP is still ongoing. The possibility for identification of the environmental trigger(s) of TD1 is therefore increasing.

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**Conflict of interests** The author declares no conflict of interest.

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