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# First trimester enterovirus IgM and beta cell autoantibodies in mothers to children affected by type 1 diabetes autoimmunity before 7 years of age

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ABSTRACT

*Background:* Autoimmune (type 1) diabetes (T1D) is a frequent chronic disease in children and adolescents globally. Gestational enterovirus (EV) infections have been associated with an increased risk for T1D in the offspring. We test the hypothesis that EV infections during the first trimester were associated with beta cell autoantibodies in mothers of children who developed islet autoantibodies before 7 years of age.

*Materials and methods:* Local registries were used to identify mothers to children born 2000–2007 who developed either beta cell autoantibodies or T1D during follow up. Serum samples from the first trimester were located in the Biobank. A total of 448 index mothers were identified and compared to 891 matched control mothers. EV-IgM was determined in a capture enzyme immunoassay. Beta cell autoantibodies were analyzed in standard radio binding assays.

*Results:* The frequency of EV-IgM in index mothers was 20% (89/448), which did not differ from the control mothers 20% (175/891) ($p = 0.922$). Index mothers had multiple beta cell autoantibodies more often than control mothers ($p = 0.037$).

Beta cell autoantibodies were increased during the November–April winter months in index compared to control mothers ($p = 0.022$). The observed difference was possibly explained by the months of February-April ($p = 0.014$). Concomitant EV-IgM and beta cell autoantibodies tended to be more common among index compared to control mothers ($p = 0.039$).

*Conclusion:* EV-IgM during the first trimester may be associated with beta cell autoantibodies in mothers to children who developed either beta cell autoantibodies or T1D before 7 years of age.

## 1. Introduction

Type 1 diabetes (T1D) is the result of autoimmune destruction of pancreatic beta cells. The disease is one of the most frequent chronic diseases in children and adolescents globally. The etiology of the disease has not been clarified but viruses have been considered an environmental factor that may induce a first beta cell autoantibody directed against either insulin (IAA), GAD65 (GADA), or both (Cabrera-Rode et al., 2003; Sarmiento et al., 2013). Following the appearance of a first autoantibody, multiple autoantibodies may appear as strong biomarkers of an immune pathogenic process leading to clinical onset of diabetes (Baekkeskov et al., 1987; Barker et al., 2004; Eisenbarth, 2004). The appearance of a first beta cell autoantibody in the child may follow an EV infection (Sadeharju et al., 2001; Salminen et al., 2003). However, it has also been reported that EV infection may accelerate the pathogenesis resulting in a more rapid progress to clinical onset of diabetes (Beyerlein et al., 2013; Hober and Sane, 2010; Hober and Sauter, 2010). Whether gestational EV infections also can be associated with an increased risk for beta cell autoimmunity and type 1 diabetes in the offspring is controversial (Dahlquist et al., 1995; Lindehammer et al., 2011b; Tauriainen et al., 2007). However, mothers with HLA-DQ 2/2 or 2/X genotypes showed an increased risk for beta cell autoantibodies at delivery. After adjusting for parity, maternal age, year of birth, and season of early pregnancy, early pregnancy EV-IgM combined with DQ2/2 or 2/X increased the risk for beta cell autoantibodies (Resic Lindehammer et al., 2012). It is unclear if the risk for T1D in the offspring differs if the mother already had beta cell autoantibodies in early pregnancy or acquired them during pregnancy (Lernmark et al., 2006; Lindehammer et al., 2011b).

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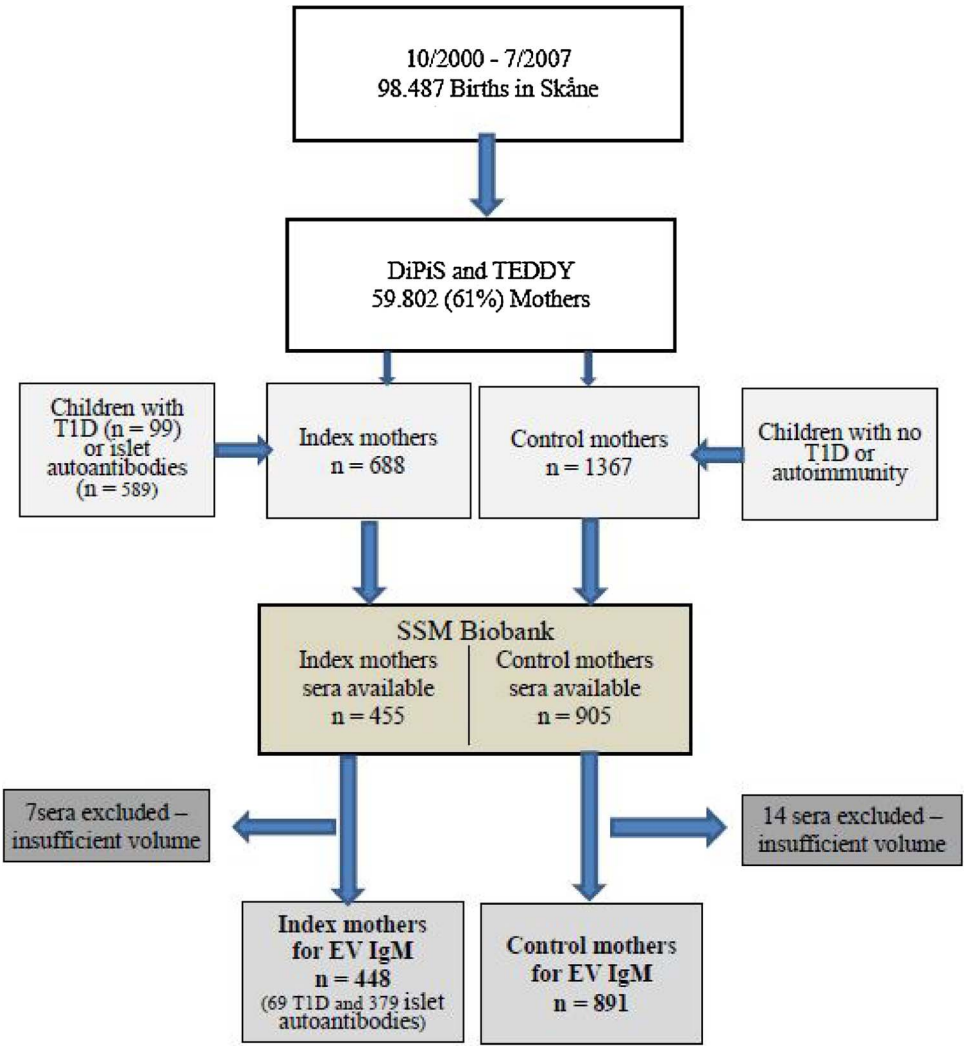


**Fig. 1.** Study flowchart of 448 mothers who gave birth to a child developing beta cell autoantibodies, Type 1 diabetes, or both (index mothers) along with 891 (control mothers) matched for age (+/− 2 years), gender of the child, birth date of the child (+/− 2 months) and birth place (hospital).

Our objective was to analyze EV-IgM along with GADA, IA-2A, and all three variants (amino acid R, W or Q at position 325) of ZnT8A in serum samples obtained from pregnant women during their first trimester. We test the overall hypothesis that concomitant exposure to EV and beta cell autoimmunity during the first trimester increased the risk for postpartum beta cell autoantibodies, T1D, or both.

## 2. Materials and methods

### 2.1. Study population

The number of live births in the Skåne County between year 2000 and 2007 was 98,487, median per year: 12,277 and range between 11 151 and 13 837 per year (Fig. 1). Between October 2000 and August 2004, 35,758 children were screened at birth in the Diabetes Prediction in Skåne (DiPiS) study (Larsson et al., 2004). Between September 2004 and July 2007, 24,951 children were screened for The Environmental Determinants of Diabetes in the Young (TEDDY) study (Group, 2007). Taken the two studies together, more than 5 000 children were followed either annually from two years of age in DiPiS or quarterly from 3 months of age in TEDDY(Group, 2007; Larsson et al., 2004). In 2014, 688 children born 2000–2007 who had developed a beta cell autoantibody or T1D were identified.

The Southern Sweden Microbiological Biobank (SSM-Biobank) has been collecting serum samples during the first trimester (gestational weeks 10–16) in > 98% of all pregnant women in Skåne (Lindehammer et al., 2011b; Ryding et al., 2008). After confirmed pregnancy at the first visit to the Maternity Care Clinic, a blood sample was taken for the analysis of Rubella, HIV, Hepatitis B and Syphilis. The leftover sera were stored at − 20 °C for up to 20 years in the SSM-Biobank for research purposes (Lindehammer et al., 2011b; Ryding et al., 2008).

It was possible to obtain the first trimester sample from 448/688 (65%) mothers (index mothers) giving birth to a child who during 2000–2014 had developed either a beta cell autoantibody or T1D (Fig. 1). Of the 448 index mothers, 7 healthy mothers had beta cell autoantibodies, 21 had T1D and 19 were diagnosed with gestational diabetes (Table 1). There were seven samples with insufficient volumes (Fig. 1).

The control mothers were matched for age (+2 years), gender of the child, birth date of the child (+2 months) and birth place (hospital). Five control mothers for each index mother were first identified in the database of the screening for DiPiS and TEDDY, respectively. The next step was to find these mothers in the registry of SSM-Biobank. Here it was possible to select two control mothers for each index mother and in the final step we located samples from 905 control mothers in the actual sample repository. Of 891 available control mothers, 17 healthy mothers had beta cell autoantibodies, 9 were diagnosed with T1D, 2 were diagnosed with type 2 diabetes and 51 were diagnosed with gestational diabetes (Table 1). Insufficient serum volume made it necessary to exclude 14 control mothers (Fig. 1).