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Link to publication

Seroconversion to Islet Autoantibodies After Enterovirus Infection in Early Pregnancy

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Abstract

Gestational enterovirus (EV) infections have been associated with an increased risk for type 1 diabetes in the offspring. We therefore analyzed non-diabetic mothers for EV exposure in early pregnancy in relation to type 1 diabetes HLA-DQ risk genotypes and seroconversion to islet autoantibodies during pregnancy. Non-diabetic mothers who had islet autoantibodies (n = 365) against glutamic acid decarboxylase (GADA), islet antigen-2 autoantibodies (IA-2A), or insulin autoantibodies (IAA), in early pregnancy and at delivery were compared to islet autoantibody-negative mothers (n = 1457) matched for age and sampling date. Mothers were genotyped for HLA-DQ and analyzed for both EV-RNA and EV-IgM. EV-IgM, but not EV-RNA, was detected during early pregnancy in 12% of islet autoantibody-positive mothers compared to 11% of the controls. In early pregnancy, mothers with HLA-DQ 2/2 or 2/X genotypes showed an increased risk for islet autoantibodies at delivery (OR 1.85; $p = 0.001$). 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 islet autoantibodies (OR 3.10; 95% CI 1; $p = 0.008$). EV-IgM in early pregnancy increased the risk for islet autoantibodies at delivery in non-diabetic mothers with HLA-DQ 2/2 or 2/X type 1 diabetes risk genotypes.

Introduction

Type 1 diabetes is a chronic autoimmune disorder of unknown etiology that is strongly associated with islet autoimmunity and progressive loss of pancreatic islet beta cells (1). Islet autoantibodies such as glutamic acid decarboxylase (GADA), islet antigen-2 autoantibodies (IA-2A), and insulin autoantibodies (IAA), are strong predictive markers for type 1 diabetes. Islet autoantibodies serve as warning signals and identify a symptomless phase of varying length prior to the clinical diagnosis (5). The genetic etiology is strongly associated with human leukocyte antigen (HLA) class II HLA-DQ on chromosome 6 (1,5,8). The genetic predisposition is mainly associated with the HLA-DQA1-B1 haplotypes DQA1*03-B1*03:02 (DQ8) and DQA1*05-B1*02:01 (DQ2) (1,5). The highest risk is conferred by the DQ2/8 genotype, followed by homozygosity for DQ8, as well as some but not all combinations of DQ8 or DQ2 and other HLA-DQ haplotypes, including homozygosity for DQ2 (18). The risk for type 1 diabetes is reduced by the presence of the DQA1*01-B1*06:02 (DQ6.2) haplotype, even in combination with both DQ8 and DQ2.

The incidence of type 1 diabetes varies between countries but seems to be increasing worldwide (16). After Finland, Sweden has the second highest incidence rate in the world (27). It is generally accepted that both genetic and environmental factors contribute to the etiology of type 1 diabetes. Enterovirus (EV) infection has received particular attention, and numerous studies have suggested associations not only between EV infections and initiation of islet autoimmunity, as EV infections appeared to coincide with seroconversion to islet autoantibodies (13), but also infections prior to manifestation of clinical type 1 diabetes in islet autoantibody-positive children (33,39). However, EV infections remain controversial, as others have reported no association between EV infections and either initiation of the autoimmune process (11) or manifestations of clinical type 1 diabetes (23).

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Gestational virus infections have been related to the risk of diabetes in the offspring since the first report on congenital rubella (9). In 1995, two independent reports suggested that gestational EV infection increased the risk for type 1 diabetes in the offspring (3,14). In a larger cohort of mothers who gave birth to children developing diabetes at 7 or 15 y of age, the results suggested that EV infection during the first trimester of pregnancy was not associated with increased risk for type 1 diabetes in the child (38). On the other hand, mothers giving birth to children, boys in particular, who developed type 1 diabetes between 15 and 30 y of age were more often EV-IgM-positive at delivery (7). In a study with limited numbers of children, both enteroviral RNA and IgM antibodies were increased in early pregnancy samples of mothers whose children developed type 1 diabetes after birth (4). It was unclear from this preliminary investigation if any of the mothers developed islet autoantibodies during pregnancy as a possible outcome of their EV infection in early pregnancy. Islet autoantibody positivity in non-diabetic mothers who give birth to children with these autoantibodies detectable in either the cord blood (21), or in serum eluted from the Guthrie Phenylketonuria (PKU) papers (6), increases the risk for the child to develop type 1 diabetes. It is therefore critical to explore whether exposure to EV infection in early pregnancy may be associated with islet autoantibody seroconversion prior to delivery.

The objective was to answer the question whether EV exposure in early pregnancy of non-diabetic mothers was associated with seroconversion to islet autoantibodies during pregnancy. The secondary objective was to ask if the seroconversion was associated with the human leukocyte antigen (HLA). To answer these questions we combined real-time RT-PCR with IgM-capture ELISA to test whether non-diabetic mothers who gave birth to children with cord blood islet autoantibodies have had an ongoing or recent EV infection in early pregnancy. Enteroviral RNA was detected using rRT-PCR (25), with specificity for essentially all human EV serotypes. EV-IgM was identified using an IgM-capture enzyme immunoassay (EIA) against purified coxsackievirus B3 (CVB3), CVA16, and echovirus 11 (38).

Materials and Methods

Study population

Diabetes prediction in Skåne. The Diabetes Prediction in Skåne (DiPiS) study was initiated in September 2000 with the aim to screen all newborns in the Skåne region of Sweden for type 1 diabetes HLA risk genotypes, for inclusion in prospective follow-up for the development of islet autoantibodies and type 1 diabetes (20). Blood samples were obtained until August 2004 at the time of delivery from 35,683 mothers and their children out of the 48,058 recorded live births in the region of Skåne (Fig. 1) (24). Delivery samples were also analyzed for GADA, IA-2A, and IAA (19). A total of 2001 mothers were excluded since they had diabetes during pregnancy (gestational, type 1, type 2, unknown type, or uncertain diagnosis). The final number of mothers (n = 33,682) was next divided into islet autoantibody-positive (n = 537) and islet autoantibody-negative (n = 33,154) mothers (22). Four islet autoantibody-negative mothers per islet autoantibody-positive mother were selected as controls matched for age, date of birth, and sampling date during early pregnancy ± 1 wk (Fig. 1).

The Southern Sweden Microbiological Biobank. Early pregnancy serum samples (gestational weeks 10–16) were retrieved from the Southern Sweden Microbiological Biobank (SSM-Biobank) (22). The SSM-Biobank stores more than 120,000 public health test samples at −20°C, which were obtained between 1986 and 2006 from all pregnant mothers at their first visit to the prenatal care clinics in Skåne (29). The blood sample taken at this occasion is mandatory, as every pregnant mother in Sweden is tested for syphilis, HIV, hepatitis B, and rubella virus antibodies. After these assays were completed, the early pregnancy sample was typically discarded. However, since the introduction of quality-controlled biobanking, the samples have been stored for research purposes.

Early pregnancy study population. Of the 537 autoantibody-positive mothers, early pregnancy serum samples were available from 365 and analyzed for islet autoantibodies and EV-specific RNA and IgM. Four matched controls per subject, three matched by sampling date and one matched by maternal age, were selected randomly from the population of islet autoantibody-negative mothers. Among the resulting 1460 matched controls, it was not possible to determine EV-IgM status for three mothers, and thus they were excluded from the rest of the analysis. Missing HLA-DQ data (n = 86) was not associated with the mother's status for either islet autoantibodies or EV-IgM.

The Regional Ethical Review Board in Lund, Sweden approved this study. All mothers gave informed consent to participate in both the SSM-Biobank and in the DiPiS study.

HLA genotyping

HLA genotyping was carried out by a validated, quality-controlled (34), PCR-based method, as previously described (15). In brief, HLA was analyzed on dried blood spots (DBS) on 3-mm-diameter filters that were punched into 96-well PCR plates. A venous blood sample from the mother taken in conjunction with delivery was used to prepare the DBS. Following PCR, the amplified biotin-conjugated product was transferred to DELFIA streptavidin-coated microtiter plates (Perkin-Elmer, Boston, MA). HLA-DQ alleles were analyzed by hybridization with sequence-specific oligonucleotide probes, and conjugated with samarium, europium, or terbium. The fluorescence results were then read with a VICTOR2 MultiLabel Counter (Perkin-Elmer).

Islet autoantibody assays

GADA and IA-2A antibody levels were determined in standardized radioligand binding assays as previously described (12). The titers of islet autoantibodies in the early pregnancy samples were determined from the international WHO standard for GADA and IA-2A, which defines titers in units/mL (U/mL). The standard was diluted in serum from GADA- and IA-2A-negative healthy subjects. The cut-off limit for positivity at delivery was previously reported (19). The early pregnancy samples of the control mothers were used to define a cut-off at the 99th percentile for GADA, and 99.8th percentile for IA-2A. Our laboratory is participating in the Diabetes Autoantibody Standardization Program (DASP) (35). In the DASP 2009 workshop, our laboratory's specificity and sensitivity for GADA were 99% and 68%, respectively,
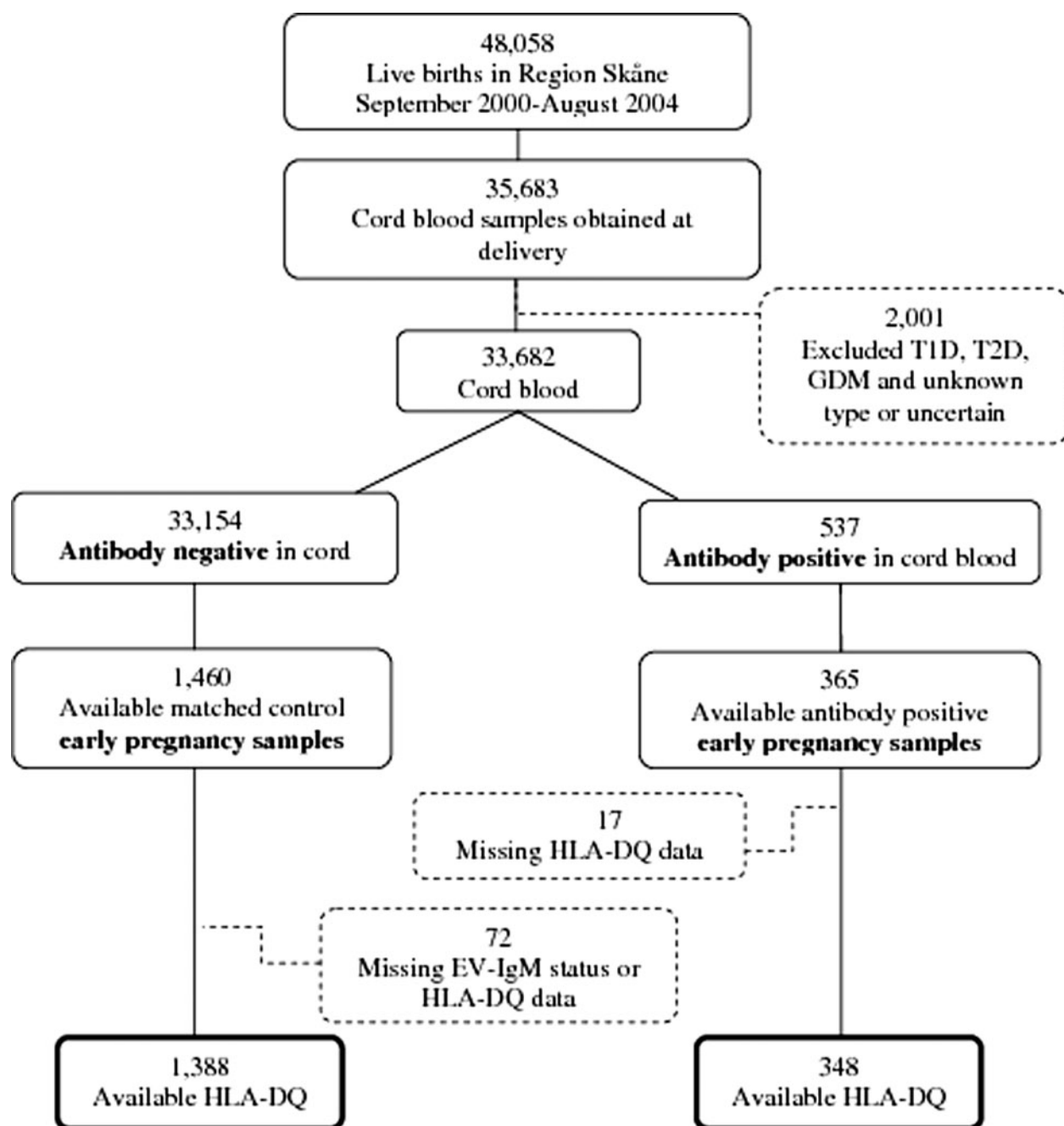
FIG. 1. Study design of participating mothers (T1D, type 1 diabetes; T2D, type 2 diabetes; GDM, gestational diabetes mellitus).

and for IA-2A were 99% and 60%, respectively, well within the range of top-performing laboratories.

IAA antibody levels were determined by an established competitive radioligand assay as previously described (36). The early pregnancy and the corresponding delivery sample of each mother were run on the same microtiter plate to minimize inter-plate variability. All samples above the 99th percentile were re-analyzed in duplicate wells using 8 U/mL unlabeled insulin to block non-specific binding (36). The results were expressed in arbitrary units. The early pregnancy samples of the control mothers were used to define a cut-off at the 99.8th percentile for IAA (19). In the competitive assay,

the inter-assay and intra-assay coefficients of variation were 8% and 6%, respectively. In the DASP 2009 workshop (35), our laboratory's specificity and sensitivity for IAA were 89% and 20%, respectively, again within the range of top-performing laboratories.

Enterovirus PCR

RNA was extracted from 100-μL serum samples using a High Performance MagNA Pure LC RNA Isolation Kit (Roche Applied Science, Indianapolis, IN) (10). The use of automated MagNA Pure sample processing reduces the

hands-on time required to process specimens, and minimizes specimen-to-specimen contamination during processing.

The presence of enteroviral RNA was detected by an rRT-PCR assay established at the Picornavirus Laboratory, Centers for Disease Control and Prevention (Atlanta, GA) with a sensitivity of as few as 10 RNA copies per reaction (26). The rRT-PCR primers and probes (GGCCCCTGAATGCGGCTA ATCC, nt 458–480; GCGATTGTCACCATWAGCAGYCA, nt 603–581; FAMCCGACTACTTTGGGWGTCCGTGT-BHQ1, nt 546–568) target sites in the 5'-non-translated region, which is fully conserved among all EVs, and allows amplification of all members of the genus (17).

Capture IgM ELISA

EV IgM was detected in a capture ELISA assay as previously described in detail (31). Briefly, IgM-class anti-EV antibodies were measured against a mixture of three EV antigens (CVB3, CVA16, and echovirus 11) using a capture EIA method. The detection antibodies were produced by immunizing rabbits by highly purified heat-treated CVB3, CVA16, and echovirus 11. The IgG fraction of rabbit hyperimmune sera was purified by fast protein liquid chromatography using a protein A column (Pharmacia Fine Chemicals, Uppsala, Sweden), and biotinylated to facilitate detection.

Microtiter plates (Nunc-Immuno™ MaxiSorp; Nunc, Glostrup, Denmark) were coated with monoclonal anti-human IgM antibodies (Medix Biochemica, Kauniainen, Finland) overnight at 4°C. Sera at 1:200 dilution were incubated for 1 h at 37°C in PBS supplemented with 1% (w/v) BSA, 2% (w/v) NaCl, and 0.05% (v/v) Tween-20. The mixture of heat-treated EV antigens (10 μg/mL each) was incubated for 30 min at 37°C, after which a comparable mixture of biotinylated detection antibodies (10 μg/mL each) was added and incubated for 30 min at room temperature. Streptavidin-horseradish peroxidase conjugate (Life Technologies, Gaithersburg, MD) was incubated for 30 min at room temperature in the dark. Finally, ortho-phenylenediamine-dihydrochloride substrate was added and incubated at room temperature in dark, and the color reaction was measured at 490 nm. The cut-off level of seropositivity was determined to be three times the level of conjugate controls included in each assay.

Statistical analysis

Odds ratios (OR) with 95% confidence intervals (95% CI) was used to explore the likelihood of HLA risk types in islet autoantibody-positive subjects and controls. The differences in proportions were tested using chi-square tests. The association between HLA-DQ risk genotypes and EV-IgM for the presence of islet autoantibodies in the cord blood was tested using multiple logistic regression analysis. Statistical analysis and graphs were performed using SPSS (www.spss.com), and PRISM GraphPad (www.graphpad.com/prism/prism.htm), respectively. p Values < 0.05 were considered statistically significant.

Results

The frequency in early pregnancy of islet autoantibody-positive mothers with EV-IgM (44/365; 12%) was no different from controls (156/1457; 11%; p = n.s.). There was no difference in the frequency of EV-IgM between the group of mothers with islet autoantibodies already present in early pregnancy (22/191; 12%), and mothers who had seroconverted by delivery (22/174; 13%). None of the samples were positive for EV-RNA.

We next examined the distribution of HLA-DQ genotypes available from 348/365 of the islet autoantibody-positive mothers, and 1388/1457 of the islet autoantibody-negative control mothers, to determine the risk for type 1 diabetes among the group of non-diabetic mothers at delivery. It was found that the DQ2/2, 2/X (X is any haplotype except 2, 8, or 6.2), moderate-risk genotypes were more frequent among islet autoantibody-positive mothers (83/348; 23.9%) compared to the controls (208/1388; 15.0%; p < 0.001; Table 1).

We therefore analyzed whether the frequency of mothers with EV-IgM in early pregnancy differed by type 1 diabetes risk (Fig. 2a and b). Among the mothers who were islet autoantibody-negative at delivery, the frequency of EV-IgM did not differ between the type 1 diabetes risk genotypes (Fig. 2a). However, among the mothers positive for islet autoantibodies at delivery, the percentage with EV-IgM differed significantly across HLA-DQ risk groups (p = 0.022; Fig. 2b).

Our further analysis demonstrated that mothers with HLA-DQ2/2, 2/X were significantly more likely to have had EV-IgM (18/83; 22%) in early pregnancy compared to the remaining islet autoantibody-positive mothers with other HLA-DQ risk groups (24/265; 9%; p < 0.001; Table 2). Among mothers who seroconverted during pregnancy (n = 166), it was found that 25% (9/36) HLA-DQ2/2, 2/X mothers were EV-IgM positive, compared to 9% (12/130) among the mothers with other HLA-DQ risk groups (p = 0.012). Among mothers who were already islet autoantibody-positive in early pregnancy (n = 182), we also observed that EV-IgM was

TABLE 1. HLA-DQ TYPE 1 DIABETES RISK GENOTYPE DISTRIBUTION AMONG MOTHERS POSITIVE FOR AUTOANTIBODIES AT DELIVERY AND CONTROLS

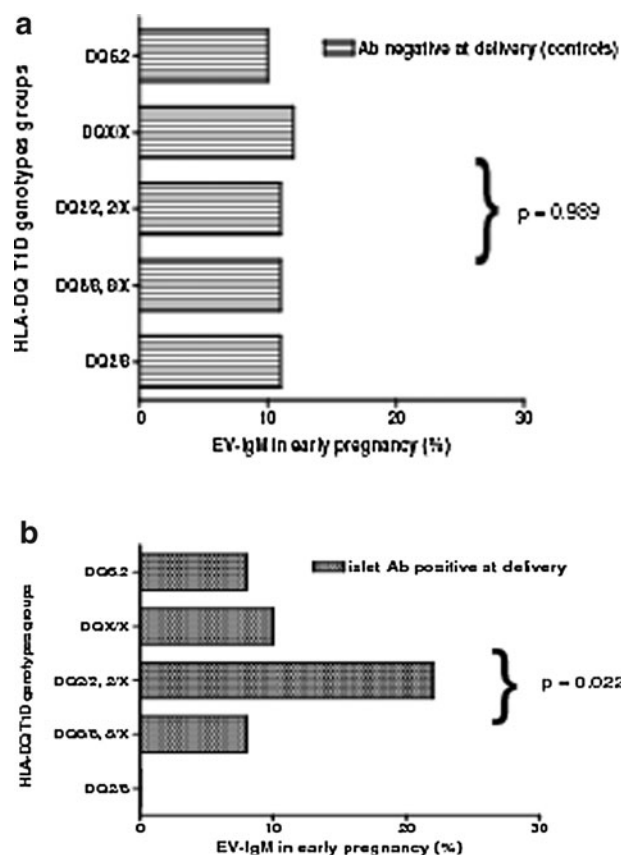|	Controls	Islet antibody positive	Controls versus islet antibody-positive		
	n (%)	n (%)	OR	95% CI	p Value
HLA-DQ risk genotypes					
Non type 1 diabetes risk (DQX/X)	592 (42.7)	128 (36.8)	1.00	Ref	
Very high risk (DQ2/8)	44 (3.2)	12 (3.4)	1.26	0.65–2.45	0.495
High risk (DQ8/8, 8/X)	223 (16.1)	63 (18.1)	1.31	0.93–1.83	0.122
Moderate risk (DQ2/2, 2/X)	208 (15.0)	83 (23.9)	1.85	1.34–2.54	< 0.001
Low risk (DQ6.2)	321 (23.1)	62 (17.8)	0.89	0.64–1.25	0.506

FIG. 2. (**a**) Percent of controls in each HLA-DQ group with EV-IgM in early pregnancy. (**b**) Percent of autoantibody-positive mothers at delivery in each HLA-DQ group.

more frequent among the HLA-DQ2/2, 2/X mothers (9/47; 19%) than the mothers with other HLA-DQ risk groups (11/135; 8%; p=0.038; Table 2).

We next used multiple logistic regression analysis to determine the OR to be positive for islet autoantibodies at delivery for a mother with either HLA-DQ2/2, 2/X or with EV-IgM in early pregnancy (Table 3). The data suggest that mothers with HLA-DQ2/2, 2/X were 52% more likely to have islet autoimmunity (p<0.009), but not EV-IgM (p=0.249) at delivery, whether adjusted for parity, maternal

TABLE 2. ENTEROVIRUS IGM IN EARLY PREGNANCY
IN MOTHERS WHO SEROCONVERTED OR NOT TO ISLET
AUTOANTIBODIES IN RELATION TO HLA-DQ GENOTYPE

	EV-IgM	p Value
Mothers with islet autoantibodies at delivery (n=348)		
HLA-DQ2/2, 2/X	18/83 (22%)	
Other HLA-DQ genotypes	24/265 (9%)	<0.001
Mothers who seroconverted during pregnancy (n=166)		
HLA-DQ2/2, 2/X	9/36 (25%)	
Other HLA-DQ genotypes	12/130 (9%)	0.012
Mothers who were islet autoantibody-positive in early pregnancy (n=182)		
HLA-DQ2/2, 2/X	9/47 (19%)	0.038
Other HLA-DQ genotypes	11/135 (8%)	

age, year of birth, and season of early pregnancy, or not (Table 3). However, mothers positive for both HLA-DQ2/2, 2/X and EV-IgM were 2.93 times more likely to have islet autoantibodies at delivery (p<0.010). After adjusting for parity, maternal age, year of birth, and season of early pregnancy, this association remained statistically significant (p=0.008; Table 3).

Discussion

The present study provides novel data on the possible relationship between EV infection and mechanisms that may trigger islet autoimmunity. Although the design of the study was a retrospective cohort study of data between 2000–2004 utilizing stored samples in the SSM-Biobank, it was still possible in 2010 to prospectively analyze the data.

The early pregnancy sample was obtained at a first visit to the prenatal care center. The blood sample taken at this occasion is mandatory, as every pregnant mother in Sweden is tested for syphilis, HIV, hepatitis B, and rubella virus antibodies. After these assays have been completed, the early pregnancy sample was typically discarded. However, since the introduction of quality-controlled biobanking, the samples have been stored at −20°C in the SSM-Biobank (29).

Our analysis of EV-IgM and islet autoantibodies in early pregnancy revealed the presence of EV-IgM in 12% of the early pregnancy samples, which is consistent with previous reports (14,3). We also noted that the mothers who had islet autoantibodies at delivery could be divided into two groups at the first visit to the prenatal care center. One group already had islet autoantibodies at the first visit. The other group was negative for islet autoantibodies at the first visit but positive at delivery, and thus we concluded that they seroconverted sometime during pregnancy. It was therefore interesting to note that islet autoantibodies, but not EV-IgM, in early pregnancy was associated with HLA-DQ 2/2 or 2/X. This finding supports previous data in healthy adults that islet autoantibodies may be associated with HLA-DQ 2/2 or 2/X (28). EV-IgM was not associated with islet autoantibodies in early pregnancy. In contrast, and this is the key observation of our study, EV-IgM in early pregnancy was associated with seroconversion to islet autoantibody positivity during pregnancy. To our knowledge this is the first observation suggesting that EV infection in early pregnancy may induce islet autoantibodies during pregnancy, primarily in mothers with the HLA-DQ 2/2 and 2/X genotypes.

Our study focused on events during pregnancy. In earlier studies, EV infections in relation to the risk for type 1 diabetes were analyzed without information about whether or not the mother seroconverted to islet autoimmunity. EV infections during pregnancy were reported in children who had developed diabetes before 15 y of age (3,14). These two studies analyzed serum samples collected at birth from mothers. Control subjects were selected from the same hospital and same birth month. Four years later, Dahlquist et al. published a study (4), in which both enteroviral RNA and IgM antibodies were analyzed in first trimester samples of mothers whose children developed type 1 diabetes. In that study they showed that 6 of 85 mothers of diabetic children had signs of an ongoing or recent EV infection in early pregnancy, compared with 1 of 172 control mothers. Three of these 6 mothers were EV-RNA positive. Despite the small

TABLE 3. ASSOCIATION OF HAVING AN HLA-DQ2 GENOTYPE AND ENTEROVIRUS IgM
IN EARLY PREGNANCY WITH THE PRESENCE OF ISLET AUTOANTIBODIES AT DELIVERY

Factors	Unadjusted Odds ratios of islet autoantibodies in cord blood			Adjusted[a] Odds ratios of islet autoantibodies in cord blood		
	OR	95% CI	p Value	OR	95% CI	p Value
HLA-DQ2 risk genotype						
No	1.00	Ref		1.00	Ref	
Yes (DQ2/2, 2/X)	1.52	1.11–2.09	0.009	1.55	1.13–2.13	0.009
EV-IgM positive						
No	1.00	Ref		1.00	Ref	
Yes	0.76	0.48–1.21	0.249	0.74	0.46–1.18	0.206
HLA-DQ2 + EV-IgM positive interaction						
DQ2/2, 2/X and EV-IgM positive	2.93	1.29–6.66	0.010	3.10	1.35–7.15	0.008

[a]Multiple logistic regression analysis adjusting for parity, maternal age, year of birth, and season of early pregnancy (quarter).

number of subjects, the authors concluded that the study was the first to report detection of EV RNA in first trimester samples in mothers of children who later become diabetic. Much larger numbers of mothers analyzed for EV-IgG and EV-IgM at the end of the first trimester in cord blood at delivery was recently reported (37). Neither seroconversion of islet autoantibodies nor HLA associations were studied, as the controls were matched for HLA-DQ. Regardless, only a slight increase in EV antibodies was found in the mothers, which led the authors to conclude that an EV infection during pregnancy was not a major risk factor for type 1 diabetes in the offspring. This report and our findings are not comparable, especially as seroconversion in relation to HLA-DQ was not studied, and that our study was not designed based on type 1 diabetes in the offspring, but on mothers with islet autoantibodies at the time of delivery (37).

Associations between HLA and virus responsiveness have been reported in a number of studies. It has been shown that 96% of type 1 diabetes patients positive for coxsackievirus B-specific IgM at onset had the HLA-DR3, DR4, or both haplotypes (32). A recent study suggests that diabetes-associated HLA-DR alleles were associated with a strong immune responsiveness, and protective alleles with a weak responsiveness against EV antigens (30). Another study demonstrated an increased frequency in the ability of T lymphocytes to respond to mumps and coxsackievirus B4 (CVB4) when presented together with HLA-DR4, as compared with other DR determinants. In contrast, a decreased frequency was found in T lymphocytes responding to mumps or CVB4 together with HLA-DR3, compared with other DR determinants (2). However, the exact mechanisms by which HLA genes modulate diabetes risk are not known.

The DiMe (Childhood Diabetes in Finland) study followed siblings of type 1 diabetes patients who seroconverted to islet cell antibodies (ICA) during a prospective follow-up (13). The cohort consisted of 765 initially ICA-negative and non-diabetic siblings, and comprised blood sampling every 6 mo. IgG, IgM, and IgA class antibodies were analyzed for a panel of EV antigens. An increase in EV antibody levels was significantly more frequent at sample intervals during which ICA first appeared than at sample intervals in the ICA-negative control sibling. The children who converted to ICA during an EV infection had the DQ2/8 genotype more often than children who remained ICA-negative. These data are consistent with our finding of an increased frequency of islet autoantibodies in mothers with HLA-DQ 2/2 or 2/X genotypes, and that such mothers more often had EV-IgM in early pregnancy. We speculate therefore that there is a link between EV infection in early pregnancy and the presence of islet autoantibodies at the time of delivery.

The strength of the present study was the unusual access to blood samples from the SSM-Biobank, which allowed us to retrace the path of blood samples from mothers who had islet autoantibodies at the time of delivery (24). The availability of samples from early pregnancy as well as from the time of delivery made it possible to evaluate islet autoantibody seroconversion in relation to EV-IgM during early pregnancy. The main finding that high levels of EV-IgM in early pregnancy were associated with seroconversion for islet autoantibodies during pregnancy in HLA-DQ2/2, 2/X mothers warrants a population-based prospective study. The weakness of the present investigation was the lack of multiple sampling during pregnancy, which should be done in future studies. Also, the timing of sampling in the present study precluded direct identification of specific EV serotypes.

Conclusions

In this study, there is evidence supporting an interaction between maternal HLA-DQ 2/2 or 2/X genotype and EV infection, as detected by EV-IgM during pregnancy, as a factor contributing to the increased risk for islet autoantibodies at delivery. Longitudinal studies are needed to establish to what extent seroconversion to islet autoantibodies during pregnancy increases the risk for type 1 diabetes in the offspring.

Acknowledgments

expert assistance in enterovirus antibody analyses, and Malin Fex and Ahmed Delli for advice and for reviewing the manuscript.

Author Disclosure Statement

No competing financial interests exist.

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