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Maternal respiratory infections in early pregnancy increases the risk of type 1 diabetes

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Abstract

Background/Objective: Is exposure to maternal infections and use of antibiotics in the prenatal period associated with increased risk of T1D, regardless of genetic risk?

Methods: Data on infections and use of antibiotics during pregnancy were collected from questionnaires at birth from parents to 16 292 children in the All Babies in Southeast Sweden (ABIS) cohort and validated against national diagnosis registers. As of November 2017, 137 ABIS children had developed T1D, 72 boys and 65 girls (0.8% of the original cohort).

Results: More cases were born in spring and summer than fall and winter. However, onset of T1D appeared to be more common in either summer or winter. In univariate analyses, respiratory tract infection in the first trimester ($P = .002$) and gastroenteritis during pregnancy ($P = .04$) were associated with later risk of T1D in the offspring. Other types of infection or antibiotic treatment were not associated with an increased risk. In a multiple logistic regression model, a mother with an autoimmune disease ($P < .001$), father with T1D ($P < .001$) and respiratory tract infection during the first trimester ($P = .005$) remained as risk factors for T1D in the offspring. In children with neutral HLA alleles antibiotic treatment may increase the risk of T1D ($P = .01$, OR 3.46, 95% CI 1.25-9.55).

Conclusions: In the general population there seems to be an association between seasonal maternal respiratory tract infection in the first trimester of pregnancy and later risk of T1D in the offspring. HLA may play a role for the effect of exposure to infections and antibiotics.

KEYWORDS

child, diabetes mellitus, infections, pregnancy, type 1

1 | INTRODUCTION

The etiology of type 1 diabetes (T1D) is unknown. Even though there is a strong genetic component mainly associated with certain HLA class II genotypes,[1,2] the current evidence supports one or more additional, environmental exposures in the etiology of T1D, that is, frequent infections, weight gain or serious life events.[3]

Onset of T1D is common during childhood and the increased incidence during recent decades may partly be explained by a raised proportion of cases diagnosed at younger ages than before.[4,5] This shift

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towards diagnosis at a younger age is of importance since especially patients with a diabetes diagnosis before the age of 10 have a risk of shorter life.[6] As no treatment exists to stop the disease process, we need to identify modifiable environmental risk factors for T1D in population-based cohort studies, and then develop intervention strategies to prevent or postpone the disease.[3]

Infections are one of the main candidate triggers of islet autoimmunity, and mainly perinatal maternal- or childhood viral infections have been suspected to trigger the disease process leading to T1D.[7-9] The main candidates are enteroviruses, which have shown a tropism for islet cells.[10,11] Involvement of other viruses, including mumps, CMV, rotavirus, EBV, influenza and congenital rubella has also been suggested.[8] A clustering effect of T1D, that is, several new cases appearing together geographically shortly after each other, has been reported.[12] There is also a clear seasonal variability, with fewer cases usually diagnosed during summer,[13] consistent with a viral etiology.[14]

An infection in a genetically predisposed individual could activate the immune system to attack the islet-cells of the pancreas.[3] The infection itself also increases insulin resistance, thus increasing the insulin demand and β-cell stress.[15]

Different exposures or environmental factors may be important in different geographical areas or during different periods of development, and the trigger to islet autoimmunity will not necessarily promote the progress to overt T1D without other mediating factors, for example, rapid weight gain, puberty or other states of increased insulin demand.[15]

Other large, multi-center, prospective studies investigating environmental risk factors of T1D are based on populations of genetically at-risk children, that is, the TEDDY study[16] or the DIPP study in Finland.[17] These studies are restricted to populations with high genetic (HLA) risk, while the incidence also may increase in children with lower HLA risk.[18] Several other population-based cohorts, either from multi-center collaborations like the EURODIAB study,[19] or recruited from several clinics and covering a large part of the population[20,21] use either retrospective data which is vulnerable to recall bias, or data from medical records which might underestimate exposures that would not need medical attention. The Norwegian Mother and Child cohort study,[22] like ABIS, uses prospective data from a population-based cohort followed until 18 months of age. Using data from the ABIS cohort allows for a prospective analysis of possible risk factors from pregnancy through adolescence in a non-selected population of children born and raised within a certain geographical area.[23]

The aim of this study was to investigate whether infections in the prenatal period are associated with an increased risk of T1D in the general population, and if these associations are affected by HLA risk in children with T1D.

2 | METHODS

2.1 | Subjects

The study is based on the ABIS cohort (All Babies in Southeast Sweden), a prospective population-based cohort study including all children born in southeast Sweden during the period October first 1997 to October first 1999. Informed consent was given for 17 055 children out of 21 700 (78.6%).

Data on hereditary factors and exposures, including maternal infections and parental life situation, were collected from questionnaires answered by 16 292 mothers within 3 days after the birth of their child (75.7% response rate). Details about the study layout have been published elsewhere.[23]

Cases with T1D were identified via the Swedish National Patient Register and the Swedish pediatric diabetes quality register SWEDIABKIDS. The diagnosis was validated by inter-linking to the Swedish National Drug Prescription Register.

Data on population statistics for the year 2000 by county, age and sex were collected from the Statistics Sweden website.

2.2 | Procedure

The birth questionnaire was aimed at the child's guardian and was completed during the child's first week of life. Questions included environmental exposures in utero. Of importance to this study were questions regarding infections and vaccination status in the mother. Two main questions were used for the analyses: 1. "Did you have an episode of gastroenteritis during pregnancy (yes/no/don´t know) and if yes, during which month of pregnancy?" 2. "Did you have any other type of infection during pregnancy (yes/no/don´t know) and if yes, what type of infection and during which month of pregnancy?". The responses (eg, "urinary tract infection", "strep throat", "common cold", "ear infection" etc. were then categorized manually according to affected organ system (ie, gastrointestinal, respiratory, urinary tract and skin infection). Note, urinary tract infection also included sexually transmitted infections. By manually categorizing the responses, it was possible to exclude data that was incorrectly reported as infections (eg, "appendicitis" or "pre-eclampsia"). One case of myocarditis was excluded from the analyses as it did not fit into any of the predetermined categories, but all other data were successfully categorized. A follow-up question about medications, including antibiotics, used during pregnancy was used to categorize the data as infections with or without antibiotic treatment. Due to the wording on the questionnaires, total number of infections during pregnancy could not be determined. Therefore, infections were coded as binary, that is, no infections vs one or more infections. Timing of antibiotic prescription or total number of antibiotic courses could not be determined due to lack of data. Additional questions were asked regarding the birth including gestational age at birth, mode of delivery and neonatal complications in the first week of life (including need of neonatal intensive care), as well as relevant medical history from both parents, siblings and extended family. The questionnaire also included questions for example about living situation, maternal and paternal occupation and educational level, diet, alcohol consumption, smoking, pets, exposure to radiation, and self-reported physical and mental health during pregnancy. These areas were not studied further in this paper as they were not

considered relevant to answer the research question, or plausible confounders to the studied exposures.

Blood samples were not collected from the majority of mothers during pregnancy, so analysis of viral antibodies was not possible.

2.3 | Genetic risks

Blood samples have been analyzed for 128 of 137 children diagnosed with T1D during the study period to identify specific HLA haplotypes associated with T1D.[1,2,10] These 128 children are categorized into one of four risk groups:

1. A high genetic risk is defined as the presence of both DR3-DQ2 and DR4-DQ8 haplotypes.
2. An increased genetic risk is defined as the presence of either one of these (DR3-DQ2 or DR4-DQ8) without any protective haplotypes.
3. A neutral genetic risk is defined as either a combination of risk (DR3-DQ2 and DR4-DQ8) and protective haplotypes (DRB1*0602 and DQA1*0102) or presence of only neutral haplotypes.
4. A decreased genetic risk is defined as the presence of only protective haplotypes without any associated risk haplotypes.

Out of 128 children with identified HLA haplotypes associated with risk for T1D, 38 were categorized as high risk, 66 as increased risk, 16 as neutral risk, and 8 as decreased risk. None of the children not analyzed with HLA haplotypes had relatives with T1D. As there is no data on HLA genotype for the control children, effect of HLA risk was analyzed by comparing cases within each defined HLA risk group to the remaining cohort for each exposure studied. Note that cases without a defined HLA risk were excluded (n = 9) from the analyses, and no adjusted regression analyses were performed due to small number of cases in each subgroup, specifically the neutral and decreased risk groups.

2.4 | Early diagnosis

To examine the effect of prenatal exposures on the risk of early disease onset, subgroup analyses of cases with a diagnosis of T1D before 5 years of age were performed.

2.5 | Statistical methods

The data were stored in a common database and statistically analyzed using the SPSS 23.0 program (IBM Corp., Armonk, NY: USA). All variables, except HLA risk groups, were categorized into two categories, and univariate analysis was used to identify individual risk factors predictive of T1D. Categorical variables were analyzed using chi square-analysis. The primary hypothesis tested was whether exposure to

infections or antibiotics during pregnancy affected the risk of T1D in the general population, or if genetic risk defined by HLA alleles affected the outcomes. As a secondary analysis, exposures that were found to be significant at the 5% level were then analyzed to examine potentially sensitive periods during pregnancy, by studying each trimester and gestational month separately for each exposure.

We used the univariate analyses to identify variables individually predictive of the development of T1D (stage 1). To adjust for multiple testing, significance was determined at the 1% level. Those variables found to be significant in the univariate analyses were then included in the multivariate model, adjusting for interaction with gender, heredity of T1D in the father and maternal autoimmune disease (including hypothyroidism, hyperthyroidism, IBD, pernicious anemia, SLE, Addison's disease, T1D, Celiac disease and rheumatism), as well as type 2 diabetes (T2D) and gestational diabetes, using logistic regression (stage 2).

2.6 | Ethical consideration

Participating families received written and oral information about the project, as well as the opportunity to watch a video about the study. Informed consent was given when the parents delivered biological samples and the questionnaire at birth.

The ABIS project was approved by the Research Ethics Committees of the Faculty of Health Science at the University of Linköping, Linköping, Sweden and the Medical Faculty at the University of Lund, Lund, Sweden (Dnr 99 227, Dnr 99 321).

2.7 | Data and resource availability

The datasets generated during and/or analyzed during this study are available from responsible for ABIS, johnny.ludvigsson@liu.se on reasonable request.

3 | RESULTS

3.1 | Characteristics of the ABIS birth cohort

As of November 2017, 137 children included in the ABIS study have been diagnosed with T1D (0.8% of original cohort). Mean age was 9.5 years, SD 4.5 years, range 1-17 years. There were slightly more boys than girls both among cases with T1D (girls n = 65, 47%, boys n = 72, 53%), and control subjects (girls n = 7817, 48%, boys n = 8412, 52%), see Table 1. There was no difference in number of siblings at birth between cases and controls, and no difference in mean maternal age at birth (29.04 years for mothers of cases compared to 29.56 years for mothers of controls). Vaginal birth was associated with a decreased odds ratio for T1D (OR 0.67, 95% CI 0.46-0.98, P = .04), however this association disappeared when mothers with T1D were excluded from the analysis. There was no

TABLE 1 Characteristics of the ABIS birth cohort

	No. Developed T1D n (%)	(%) Did not develop T1D n (%)	P-value	OR (95% CI)
Demographic factors				
Boys	72/137 (52.6)	8412/16229 (51.8)	.87	0.97 (0.69-1.36)
Mean maternal age at birth	29.04	29.56	.19	
One or more siblings at birth	83/131 (63.4)	9835/15842 (62.1)	.76	1.06 (0.74-1.51)
Mother born outside of Sweden	1/134 (0.7)	1060/15886 (6.7)	.006*	0.11 (0.02–0.75)
Family member with T1D	23/137 (16.8)	381/16288 (2.3)	<.001***	8.42 (5.32-13.34)
Mother	11/137 (8.0)	118/16288 (0.7)	<.001***	11.96 (6.29–22.74)
Father	13/137 (9.5)	175/16288 (1.1)	<.001***	9.65 (5.35–17.42)
Sibling	2/137 (1.5)	108/16288 (0.7)	.26	2.22 (0.54-9.08)
Second-degree relative	19/137 (13.9)	1457/16288 (8.9)	.05	1.67 (1.01-2.67)
First-degree relative with T2D	4/137 (2.9)	196/16288 (1.2)	.07	2.47 (0.90-6.74)
Mother	4/137 (2.9)	87/16288 (0.5)	<.001***	5.60 (2.03–15.48)
Father	0/137 (0)	111/16288 (0.7)	.33	—
Sibling	0/137 (0)	14/16288 (0.1)	.73	—
Second-degree relative	25/137 (18.2)	2385 (14.6)	.24	1.30 (0.84-2.01)
Gestational diabetes in mother	2/137 (1.5)	191/16288 (1.2)	.76	1.25 (0.31-5.08)
Maternal autoimmunity	19/137 (13.9)	781/16288 (4.8)	<.001***	3.20 (1.96–5.22)
Delivery				
Vaginal birth	100/137 (73.0)	13 047/16288 (80.1)	.04	0.67 (0.46–0.98)
Perinatal factors				
Neonatal intensive care	22/133 (16.5)	1472/15584 (9.4)	.005*	1.90 (1.20–3.01)
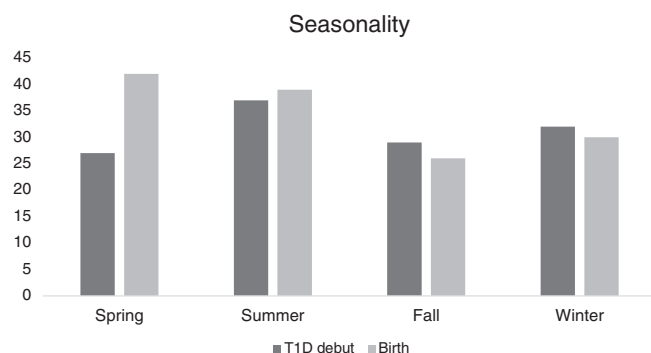
FIGURE 1 Season of birth and diagnosis of type 1 diabetes for cases (n = 137 for season of birth and n = 125 for season of diagnosis)

statistically significant association to either gestational week or birth weight between cases and controls (data not shown).

More cases were born in spring and summer than fall and winter (Figure 1). Onset of T1D appeared to be more common in either summer or winter (Figure 1).

Need of neonatal intensive care was associated with a risk of later development of T1D (OR 1.90, 95% CI 1.20-3.01, P = .005). This association disappeared when mothers with T1D were excluded from the analysis.

Cases more often had a parent with T1D (OR 11.96, 95% CI 6.29-22.74, P < .001 for a mother with T1D, and OR 9.65, 95% CI 5.35-17.42, P < .001 for a father). The absolute number of fathers with T1D in the entire cohort is larger than the absolute number of mothers (188 and 129 respectively), which likely influences the narrower confidence interval and slightly lower OR for fathers with T1D. No significant association could be seen for T1D in a sibling or second-degree family member with T1D. There was also an association with type 2 diabetes (T2D) in the mother and subsequent T1D in the child (OR 5.60, 95% CI 2.03-15.48, P < .001), but no such association was found for T2D in the father, sibling or second-degree relatives or for gestational diabetes in the mother. The majority of cases (n = 114) did not have a family member with T1D. Any type of autoimmunity in the mother (hypothyroidism, hyperthyroidism, IBD, pernicious anemia, SLE, Addison's disease, T1D, Celiac disease, and rheumatism) was also associated with an increased risk of T1D in the child (OR 3.20, 95% CI 1.96-5.22, P < .001).

Mothers to children with T1D were more likely to be born in Sweden (99.3%) compared to mothers of controls (93.3%), and this difference was statistically significant (OR 0.10, 95% CI 0.02-0.75, P = .006). However, there is no other data on maternal ethnicity for mothers born outside of Sweden. Data from Statistics Sweden from the year 2000 describes a rather homogenous population. A majority of the 11.3% of the population born outside of Sweden originate from other Nordic countries (28%), the European Union (19%) or other parts of Europe (17%).[24]

TABLE 2 Exposures during pregnancy

	No. Developed T1D (n = 137)	(%) Did not develop T1D (n = 16 288)	P-value	OR (95% CI)
Any infection during pregnancy	77/137 (56.2)	8029/16288 (49.3)	.11	1.32 (0.94-1.85)
Respiratory tract	33/137 (24.1)	2853/16288 (17.5)	.04	1.49 (1.01–2.22)
First trimester	14/137 (10.2)	764/16288 (4.7)	.002*	2.31 (1.32-4.04)
Gestational month 1	1/137 (0.7)	193/16288 (1.2)	.62	0.61 (0.09-4.41)
Gestational month 2	2/137 (1.5)	336/16288 (2.1)	.62	0.70 (0.17-2.85)
Gestational month 3	12/137 (8.8)	374/16288 (2.3)	<.001*	4.09 (2.24–7.45)
Second trimester	11/137 (8.0)	1200/15288 (7.4)	.77	1.10 (0.59-2.04)
Gestational month 4	4/137 (2.9)	472/16288 (2.9)	.99	1.00 (0.37-2.74)
Gestational month 5	8/137 (5.8)	454/16288 (2.8)	.03*	2.16 (1.05–4.44)
Gestational month 6	1/136 (0.7)	512/16288 (3.1)	.11	0.23 (0.03-1.62)
Third trimester	8/137 (5.8)	836/16288 (5.1)	.71	1.15 (0.56-2.35)
Gestational month 7	6/137 (4.4)	620/16287 (3.8)	.73	1.16 (0.51-2.63)
Gestational month 8	1/137 (0.7)	160/16288 (1.0)	.77	0.74 (0.10-5.33)
Gestational month 9	1/137 (0.7)	139/16288 (0.9)	.88	0.85 (0.12-6.15)
Gastroenteritis	56/137 (40.9)	5285/16288 (32.4)	.04	1.44 (1.02–2.03)
First trimester	20/137 (14.6)	1764/16288 (10.8)	.16	1.41 (0.87-2.27)
Gestational month 1	6/137 (4.4)	586/16288 (3.6)	.63	1.23 (0.54-2.79)
Gestational month 2	9/137 (6.6)	888/16288 (5.5)	.57	1.22 (0.62-2.41)
Gestational month 3	14/137 (10.2)	1038/16288 (6.4)	.07	1.67 (0.96-2.92)
Second trimester	25/137 (18.2)	2231/16288 (13.7)	.12	1.41 (0.91-2.18)
Gestational month 4	10/137 (7.3)	950/16288 (5.8)	.47	1.27 (0.67-2.43)
Gestational month 5	10/137 (7.3)	839/16286 (5.2)	.26	1.45 (0.76-2.77)
Gestational month 6	6/137 (4.4)	807/16288 (5.0)	.76	0.88 (0.39-2.00)
Third trimester	16/137 (11.7)	2087/16288 (12.8)	.69	0.90 (0.53-1.52)
Gestational month 7	7/137 (5.1)	868/16287 (5.3)	.91	0.96 (0.45-2.05)
Gestational month 8	7/137 (5.1)	790/16287 (4.9)	.89	1.06 (0.49-2.68)
Gestational month 9	3/137 (2.2)	765/16288 (4.7)	.17	0.45 (0.14-1.43)
Urinary tract infection	8/137 (5.8)	1336/16288 (8.2)	.32	0.69 (0.34-1.42)
Skin infection	1/137 (0.7)	138/16288 (0.8)	.88	0.86 (0.12-6.20)
Infection treated with antibiotics	31/116 (26.7)	2644/13457 (19.6)	.06	1.49 (0.99-2.56)
Infection not treated with antibiotics	38/116 (32.8)	4812/13457 (35.8)	.50	0.88 (0.59-1.29)
Antibiotics	34/126 (27.0)	2982/14784 (20.2)	.06	1.46 (0.99–2.17)
HLA risk groups				
High risk				
Respiratory tract	8/38 (21.1)	2854/16306 (17.5)	.57	1.26 (0.58-2.74)
Gastroenteritis	12/38 (31.6)	5291/16306 (32.4)	.91	0.96 (0.48-1.91)
Urinary tract infection	3/38 (7.9)	1336/16306 (8.2)	.95	0.96 (0.30-3.13)
Skin infection	1/38 (2.6)	138/16306 (0.8)	.23	3.17 (0.43-23.24)
Antibiotics	9/33 (27.3)	3002/14857 (20.2)	.13	1.48 (0.69-3.19)
Increased risk				
Respiratory tract	18/66 (27.3)	2854/16306 (17.5)	.04*	1.77 (1.03–3.04)
Gastroenteritis	28/66 (42.4)	5291/16306 (32.4)	.08	1.53 (0.94-2.50)
Urinary tract infection	4/66 (6.1)	1336/16306 (8.2)	.53	0.72 (0.26-1.99)
Skin infection	0/66 (0)	138/16306 (0.8)	.45	—
Antibiotics	17/62 (27.4)	2985/14795 (20.2)	.16	1.50 (0.85-2.62)

TABLE 2 (Continued)

HLA risk groups				
Neutral risk				
Respiratory tract	5/16 (31.3)	2854/16306 (17.5)	.15	2.14 (0.74-6.17)
Gastroenteritis	9/16 (56.3)	5291/16306 (32.4)	.04	2.68 (1.0–7.19)
Urinary tract infection	1/16 (6.3)	1336/16306 (8.2)	.78	0.75 (0.10-5.66)
Skin infection	0/16 (0)	138/16306 (0.8)	.71	—
Antibiotics	7/15 (46.7)	2985/14795 (20.2)	.01*	3.46 (1.25–9.55)
Decreased risk				
Respiratory tract	1/8 (12.5)	2854/16306 (17.5)	.71	0.67 (0.08-5.48)
Gastroenteritis	3/8 (37.5)	5291/16306 (32.4)	.76	1.25 (0.30-5.23)
Urinary tract infection	0/8 (0)	1336/16306 (8.2)	.40	—
Skin infection	0/8 (0)	138/16306 (0.8)	.79	—
Antibiotics	0/8 (0)	2992/14810 (20.2)	.16	—
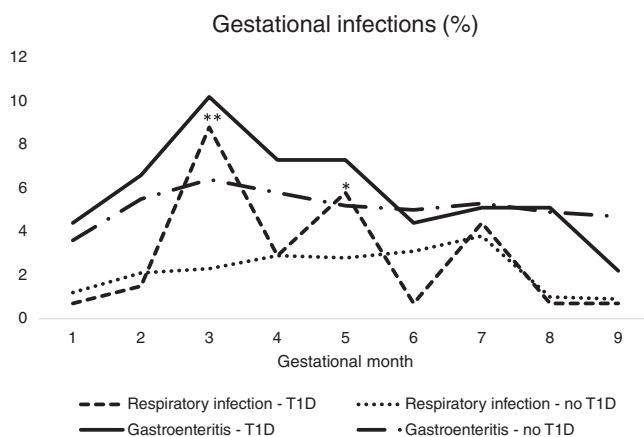
FIGURE 2 Cases of one or more episodes of respiratory tract infections and gastroenteritis per gestational month during pregnancy (% of total population). **P-value <.001; *P-value <.05

3.2 | Maternal infections during pregnancy

We found increased odds ratios for respiratory tract infection (OR 1.49, 95% CI 1.01-2.22, P = .04), and gastroenteritis during pregnancy (OR 1.44, 95% CI 1.02-2.03, P = .04), but not for urinary tract infections or skin infections. Any infection, compared to no infection, was not associated with an increased risk of T1D. See Table 2.

Respiratory tract infection during the third gestational month(OR 4.09, 95% CI 2.24-7.45, P < .001, see Figure 2) or the first trimester (OR 2.31, 95% CI 1.32-4.04, P = .002, see Table 2) was significantly associated with T1D. Respiratory tract infection during gestational month 5 had increased odds ratios (OR 2.16, 95% CI 1.05-4.44, P = .03). No other gestational month with respiratory tract infection had a significant association with T1D, and there was no significant association to any specific trimester or gestational month for gastroenteritis (see Table 2).

When categorizing infections based on prescribed antibiotics, neither infections treated with or without antibiotics during pregnancy were associated with an increased risk of T1D in the general population.

3.3 | Use of antibiotics during pregnancy

The use of antibiotics during pregnancy was not significantly associated to T1D in the offspring OR 1.46, 95% CI 0.99-2.17, (P = .06). The most commonly used antibiotics (self-reported) were penicillin (64.8% of total antibiotic use), followed by nitrofurantoin (16.3%), erythromycin (6.3%), pivmecillinam (3.8%), cefadroxil (3.8%), amoxicillin (2.6%), and clindamycin (1.3%). Of the studied infections, urinary tract infections were most often treated with antibiotics (65.6% of reported UTIs), followed by skin infection (49.6%), respiratory tract infection (47.5%), and gastroenteritis (23.5%).

3.4 | Genetic risk groups

The group with an increased genetic risk (n = 66) had increased odds ratios for respiratory tract infection during pregnancy (OR 1.77, 95% CI 1.03-3.04, P = .04 see Table 2), but there were no other statistically significant associations for this risk group.

Mothers to children with a neutral risk (n = 16) more often received antibiotics during pregnancy (OR 3.46, 95% CI 1.25-9.55, P = .01), and had more episodes of gastroenteritis (OR 2.68, 95% CI 1.0-7.19, P = .04) compared to controls. There was no other association between HLA risk and any other kind of infection.

3.5 | Early diagnosis of type 1 diabetes

Of the 137 children with T1D, 27 were diagnosed before the age of 5 (19.7%). In this group with early diagnosis there was no

TABLE 3 Factors related to development of type 1 diabetes. Result of the logistic regression analysis

Independent variable	Model 1 OR (95% CI)	P-value	Model 2 OR (95% CI)	P-value
Adjusting variables				
Gender	1.15 (0.80-1.64)	.45	1.15 (0.81-1.65)	.43
Vaginal birth	1.21 (0.78-1.88)	.39	1.21 (0.79-1.88)	.38
Mother born outside of Sweden	0.00	.99	0.00	.99
T1D father	10.21 (5.57–18.73)	<.001***	9.89 (5.41–18.10)	<.001***
Maternal autoimmunity	2.16 (1.27–3.67)	.004**	2.22 (1.31–3.76)	.003**
T2D mother	1.66 (0.37-7.54)	.51	1.60 (0.34-7.23)	.54
Neonatal intensive care	1.61 (0.97-2.68)	.06	1.60 (0.97-2.66)	.07
Antibiotic use	1.18 (0.78-1.79	.42	1.20 (0.79-1.82)	.38
Respiratory infection month 3	4.00 (2.14-7.47)	<.001***	—	—
Respiratory infection trimester 1	—	—	2.15 (1.20–3.85)	.01*

Note: Dependent variable is type 1 diabetes, coded as 0 = no diabetes and 1 = diabetes. Maternal autoimmunity includes hypothyroidism, hyperthyroidism, IBD, pernicious anemia, SLE, Addison's disease, type 1 diabetes, Celiac disease and rheumatism.***P-value < .001, **P-value < .01, *P-value < .05.

significant association with any of the studied exposures, that is, neonatal intensive care, respiratory tract infection, gastroenteritis, UTI, skin infection, all infections combined or antibiotic use (data not shown).

3.6 | Gender differences

When dividing the cohort into gender, we found increased odds ratios for boys with either a respiratory tract infection (OR 1.82, 95% CI 1.08-3.05, P = .02) or gastroenteritis (OR 1.75, 95% CI 1.10-2.79, P = .02) during pregnancy. There were no significant associations to any infection during pregnancy for girls (data not shown).

3.7 | Logistic regression

We performed a multiple regression model based on the variables with a significant association in the univariate analyses (maternal autoimmunity, mother born outside of Sweden, father with T1D, vaginal birth, neonatal care, and respiratory tract infection), adjusting for gender. Antibiotic use was included to adjust for confounding between infection and treatment for infection (eg, antibiotics). Because respiratory tract infections during the first trimester and third gestational month are nested in each other, two models were created, see Table 3. Note that T1D in the mother is included in maternal autoimmunity. In the first model, respiratory tract infection during the third gestational month was included. Both maternal autoimmunity (OR 2.16, 95% CI 1.27-3.67, P = .004), a father with T1D (OR 10.21, 95% CI 5.57-18.73, P < .001) and respiratory tract infection during gestational month 3 (OR 4.00, 95% CI 2.14-7.47, P < .001) remained as

risk factors for T1D in the offspring. In the second model, respiratory tract infection during the first trimester was included. Maternal autoimmunity (OR 2.26, 95% CI 1.31-3.76, P = .003), a father with T1D (OR 9.89, 95% CI 5.41-18.10, P < .001) and respiratory tract infection during the first trimester (OR 2.15, 95% CI 1.20-3.85, P = .01) remained as risk factors.

4 | DISCUSSION

In this prospective birth cohort, we found an association with respiratory tract infections, especially early in pregnancy, and later risk of T1D in the offspring. While this association remained significant in the logistic regression analysis, it is important to note that these analyses are based on a small total number of infections, and conclusions should be drawn cautiously. A subgroup analysis of cases with an early onset of T1D (<5 years of age) showed no significant association to any of the studied prenatal infections, possibly due to the small sample size.

Most previous population-based studies on maternal infections as a risk factor for T1D have not found evidence of maternal infection as a risk factor of T1D.[19,21] Some inconsistencies might be due to the fact that in those studies data were collected from medical records where infections during pregnancy might be underestimated, or their retrospective design where recall bias could skew the results. One case-control study from the UK found only associations with non-specific infections in pregnancy,[25] while a Swedish case-control study based on the Swedish childhood diabetes register found no association between maternal infections and T1D.[26] One of the largest prospective cohort studies, the Norwegian MoBa study,[22] found no association between maternal infections and T1D. A previous study performed on a randomly selected subset of the ABIS cohort found an association between maternal gastrointestinal infection and beta-

cell autoantibodies in the offspring at 1 year of age.[27] Maternal gastroenteritis was likewise associated with increased odds ratios of T1D in our study, but not statistically significantly.

Some studies have found evidence of maternal enterovirus infection during pregnancy as a risk factor of T1D in the offspring[28,29] while other studies failed to confirm this finding.[30,31]

As our study was not designed to categorize infections by biological agents, it is not possible to draw any conclusion on enterovirus infections from our results. An attempt to differentiate between the effect of infections with or without antibiotic treatment showed no significant associations. It is possible that the significant association between respiratory tract infections and T1D is driven by viral infections rather than bacterial infections, as antibiotic treatment during pregnancy was not significantly associated with T1D. Very little is known about bacterial infections and development of T1D.[3] Use of antibiotics could be a surrogate marker for bacterial infections. A large Swedish registry-based study showed a significant association between antibiotic treatment in the first year of life and development of T1D before 10 years of age,[32] while the population-based Norwegian MoBa study reported no link between use of antibiotics in childhood and development of T1D,[22] confirming earlier findings.[21] Our results do not support a general association between exposure to antibiotics in utero and later development of T1D. However, we did find a significant association to antibiotics during pregnancy in children with a neutral HLA risk, as well as increased odds ratios after gastroenteritis. This is especially interesting as we have found a significant difference in gut microbiome between children with and without high HLA risk.[33] These observations should be interpreted cautiously and need to be tested in further studies, as comparing a group with a specified genetic risk to a general population with unspecified genetic risks may give inadequate information.

Our results indicate that maternal infections may have some importance for later development of T1D, especially respiratory tract infections during a window around the first trimester. This coincides with the embryological development of the pancreas; where endocrine functions begin around week 10-13 post conception.[34] Of the children who developed T1D, the majority were born in spring and summer. The end of the first trimester in these pregnancies would overlap with fall and winter, coinciding with the infectious season in the northern hemisphere. Moreover, the diagnosis of T1D is usually concentrated to the infectious season. Our results give us an interesting hypothesis with a two-hit infection model: first a congenital infection during early pregnancy primes the immune system or influences the development of the pancreas, and later a second infection triggers seroconversion and manifest T1D. Similar seasonality has been described before.[35,36] These results need to be replicated in other prospective studies before any major conclusions can be drawn.

In the univariate analyses, we found that boys appear to be more sensitive to prenatal exposure to infections, although this association disappeared in the logistic regression possibly due to decreased power. Interestingly, there are gender differences in disease characteristics at onset[37] and it is possible that boys are affected by other environmental triggers of the autoimmune process than girls. In fact,

women are overrepresented in almost all types of autoimmune disease, with T1D as the notable exception.[38]

Major strengths of our study are the prospective design and high response rate, which limits recall bias, as all questionnaires were collected before disease debut.

All data were collected from questionnaires and have not been controlled against hospital records. This lack of validation is a limitation but may also be a strength as it is unlikely that all instances of infections would be included in medical records as many women do not seek medical care for common colds or gastrointestinal infections and the total number of infections would therefore be underestimated. Unfortunately, we lack serological data from the pregnancy that could validate our findings. There is a possibility that our results are still suffering from underreporting, considering the difference in total number of infections during pregnancy between our cohort and similar studies (eg, the Norwegian MoBa study reported 61.3% respiratory tract infections during pregnancy[22]). This underreporting might be due to the amount of time passed between the beginning of pregnancy and birth when the questionnaire was completed, but we do not expect a significant difference in reporting between cases and controls.

Although ABIS itself include a large study population, a limiting factor to our conclusions is the small sample sizes, particularly in the subgroup analyses performed, and the small number of events in each group. Several cases may not be diagnosed yet.[36]

In conclusion, we found an association between seasonal maternal respiratory tract infections during the first trimester of pregnancy and later risk of T1D in the offspring, and in individuals with a neutral HLA risk we found associations between development of T1D and both use of antibiotics and gastroenteritis during pregnancy. The importance of HLA for the effect of exposure to infections need further investigations.

AUTHOR CONTRIBUTIONS

M.B. analyzed the ABIS data, drafted the initial manuscript and revised the manuscript. J.W. did the study analysis plan, contributed to the discussion and reviewed/edited the manuscript. J.L. performed ABIS, conceived and designed the study, contributed to the discussion, reviewed/edited the manuscript and is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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