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Cord blood IA-2 autoantibodies are associated with increased risk of type 1 diabetes in the population based Diabetes Prediction in Skåne study

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Abstract

Aims/hypothesis:

To examine effect of cord blood autoantibodies on the risk for type 1 diabetes (T1D) in children followed prospectively from birth.

Methods

The Diabetes Prediction in Skåne (DiPiS) study consists of 35,853 children from the general population born during 2000-2004. Samples were collected at birth and analysed for HLA-genotypes and autoantibodies to glutamate decarboxylase 65 (GADA), insulin (IAA) and insulinoma-associated protein 2 (IA-2A). After adjusting for HLA, gender, maternal age and parental T1D, independent association with risk of diabetes were assessed using multivariate Cox proportional hazards models.

Results

A total of 151 children (0.4%) had developed T1D by the end of 2013 at a median age of 5.8 years (0.8-12.2 years). In the multivariate analysis, IA-2A in cord blood (HR 6.88; 95% CI 1.46-32.4; p=0.003), but not maternal diabetes (HR 1.38; 95% CI 0.24-7.84; p=0.71), was associated with risk of developing T1D. No increased risk could be seen for GADA or IAA.

Conclusions/interpretation

Our study indicates that the presence of cord blood autoantibodies to IA-2 superimposes maternal diabetes and other cord blood islet autoantibodies as a predictor of T1D development in the child. These findings may be of significance for future screening and study protocols on T1D prediction.

Keywords: Type 1 diabetes, GAD65 Autoantibodies, IA-2 Autoantibodies, Insulin Autoantibodies, prediction, Paediatric

Abbreviations:
BDD Better Diagnosis of Diabetes, DiPiS Diabetes Prediction i Skåne, GADA Autoantibodies to glutamic acid decarboxylase, IAA Insulin autoantibodies, IA-2A Insulinoma-associated protein 2 autoantibodies, T1D Type 1 diabetes

Introduction

Early prediction of type 1 diabetes (T1D) is crucial in the attempts at preventing or stalling the disease. Identifying risk factors for islet autoimmunity and factors accelerating the autoimmune process will enable more successful study enrolment and treatment attempts. Islet autoantibodies are found in 3 to 5% of cord blood samples from newborns in the general population[1], with an even higher proportion found, as expected, in offspring to mothers with T1D[2]. Previous studies, mainly recruiting children with first-degree relatives with T1D, have investigated the significance of cord blood autoantibodies with contradictory results[3, 4].

As only 10-13% of newly diagnosed T1D children and young adults have a first-degree relative with the disease it is difficult to make predictions on the general population based on analysis of these subgroups. In the Diabetes Prediction in Skåne (DiPiS) study, children from the general population are followed from birth with the aim of identifying risk factors and predictive markers of T1D. In this study we had the opportunity to examine the impact on risk for T1D of cord blood autoantibodies.

Research design and methods

Study Population and participants

In the southernmost part of Sweden, cord blood of children born between September 2000 and August 2004, was analysed for HLA-DQ genotypes, autoantibodies to glutamate decarboxylase 65 (GADA), insulin (IAA) and insulinoma-associated protein 2 (IA-2A) in the DiPiS study[5]. When the child reached two months of age the parents were asked to fill out a written consent form and a questionnaire regarding family history of diabetes, birth weight, gestational age and perinatal infections[5]. The study protocol and collection of data are illustrated in supplementary figure 1.

By the end of 2013, the DiPiS cohort had reached 9-13 years of age and 151 children had been diagnosed with T1D. New patients are registered via the Better Diabetes Diagnosis study (BDD), covering an overwhelming majority of new diabetes cases in Sweden.

The regional ethics review board in Lund, Sweden approved the study.

HLA-genotyping

HLA was analysed on dried blood spot (DBS) filters as described in detail elsewhere[6]. In this analysis, HLA was classified as HLA-DQA1*0501-DQB1*0201 (DQ2) or HLA-DQA*0301-DQB1*0302 (DQ8) and stratified into 4 risk groups: 1) DQ 2/8, 2) DQ8/8 or 8/X, 3) DQ2/2 or 2/X and 4) DQ X/X (X is neither DQ2 nor DQ8).

Cord blood autoantibodies

DBS eluates were incubated with labelled antigen to GAD65 and IA-2 and autoantibody-bound labelled antigen separated from free with Protein A-Sepharose. Positive samples (combined GADA and IA-2A analysis >99th percentile) were reanalysed in separate assays for GADA and IA-2A. IAA was screened in serum in a micro assay. All samples above the

99th percentile were reanalysed to correct for nonspecific binding. The exact procedure has been described elsewhere[7].

Statistical methods

Statistical analyses were performed using SPSS (version 21, SPSS, Chicago, IL) and R version 3.03, using the survival package (R Core Team (2014) R Foundation for Statistical Computing, Vienna, Austria). Hazard ratios (HR) with corresponding 95% confidence intervals, as estimated by univariate Cox proportional hazards models, were used to identify factors with significant influence on risk of T1D in the child. A multivariate Cox proportional hazards model tested for independent association of each autoantibody on the child's diabetes risk while also adjusting for HLA-DQ, gender, maternal age and parental T1D. A separate baseline hazard was fitted for each HLA risk group. Hazard ratios for HLA genotype were calculated in a multivariate model without stratification. Multivariate analysis included the following variables: Cord blood IA-2A, IAA and GADA (categorized as positive if above the 99th percentile, treated as categorical variables), gestational week, relative birth weight percentile, infection during pregnancy, HLA risk group, gender, maternal T1D, paternal T1D and mothers age at delivery.

Results

Baseline characteristics

A total 35,683 cord blood samples were collected in 48,058 children born during the study period. Parents of 25,392 children returned the 2-month questionnaire. Among the responders to the 2-month questionnaire 190 children had a mother with T1D and 985 had gestational diabetes. Paternal T1D was reported in 285 of the children (Supplementary Table 1). As of 31 December 2013, 151 children had been diagnosed with T1D with a median age at diagnosis of 5.8 years (0.8-12.2 years). In total 12 children had a parent with T1D (8%), 8 fathers and 4 mothers. Autoantibody status and demographics at diagnosis can be seen in Supplementary Table 1.

Autoantibodies in cord blood and their influence on T1D risk

Autoantibodies against GAD65, IA-2 and insulin were found in 295 (0.8%), 78 (0.2%) and 509 (1.4%) cord blood samples, respectively. A total of 44 samples (0.1%) were positive for both IA-2A and GADA and 25 samples (0.07%) were triple positive. Contingency tables with autoantibody status and overlap with maternal T1D is shown in supplementary table 2-4. In children to diabetic mothers, the presence of IA-2A in cord blood was associated with increased risk for the child to develop of T1D (Fisher's exact test p=0.037) (Supplementary Table 2). However, in children with IA-2A present in cord blood, mother with diabetes was not associated with development of T1D in the child (Fisher's exact test p=1) (Supplementary Table 3).

In a multivariate model with autoantibodies and parental T1D included, only IA-2A was a predictor of the child's risk for T1D. The risk was significant in both a univariate model (HR 14; 95% CI 5.0-37; p <0.001) and a multivariate model (HR 6.4; 95% CI 1.8-22; p =0.003).

GADA and IAA alone show evidence of association with risk of T1D, but not after adjusting for maternal T1D status at birth (GADA HR 1.5; 95% CI 0.5-4.8; p=0.48, IAA HR 1.1; 95% CI 0.4-3.4; p =0.82) (Table 1).

HLA-DQ derived risk and parental diabetes

The risk profile with HLA_DQ genotypes was similar to previous studies (Table 1).

Having a father with T1D was associated with an increased risk for T1D (HR 3.4; 1.6-7.0; p <0.001). Maternal T1D was associated with the child's risk of T1D (HR 3.5; 95% CI 1.3-9.5; p=0.014) but not after adjusting for IA-2A (HR 1.6; 95% CI 0.3-7.0; p=0.60) (Table 1).

Discussion

In this population-based prospective study, we report that the presence of IA-2A in cord blood increase the risk for the child to develop T1D, and that this risk seem to be unrelated to the diabetes status of the mother.

It has been well established that islet autoantibodies, and specifically IA-2 autoantibodies, measured during childhood predict the onset of T1D. Few studies have, however, been made regarding diabetes risk and cord blood autoantibodies and the existing results are somewhat conflicting. In several studies of children born to T1D mothers, the origin of the cord blood autoantibodies has been studied, primarily concluding them to be of maternal origin[1, 8, 9]. Also in a study of non-diabetic mothers, the maternal origin of the cord blood autoantibodies was confirmed, but further studies on this matter are needed[7]. In offspring to T1D mothers, cord blood autoantibodies have been reported to increase the risk for the child to develop the disease[10], but also to be of no effect[4] or even protective[3]. In a retrospective case-control study of cord blood sera from children diagnosed before the age of 15, cord blood autoantibodies was reported to increase the risk for the child to develop T1D, also after excluding T1D mothers. Unfortunately, no analysis of IA-2 had been performed and instead ICA was used[10]. It cannot be excluded that IA-2A may have contributed risk of cord blood autoantibodies for T1D in this study. In a recent Danish study, a T1D risk increase for children born with cord blood autoantibodies was reported, but no separate analysis was performed for IA-2A and GADA[9].

One of the major strengths of our analysis is that the data originates from a large scale screening program of the general population with 70% of all children born in the Skåne Region sampled at birth. Our data regarding prevalence of T1D in this cohort, now 9-13 years of age, is considered complete. The well-covered study area in Sweden where the study took

place, with five pediatric clinics, combined with the validation against the Better Diabetes Diagnosis (BDD) study makes the risk of having missed new cases small.

Our study is limited by relying on questionnaire data regarding some pre- and perinatal factors. We can therefore assume that the data suffer from recall. Missing data from the two-year questionnaire affects the number of patients eligible for multivariate analysis, However, we were still able to use data from 17,287 children and 89 T1D patients. The number of children who were autoantibody positive at birth is small as well as the number of children with mothers with T1D. This introduces some uncertainty to the statistical analysis.

In conclusion, our study indicates that the presence of cord blood autoantibodies to IA-2, but not GADA or IAA, increases the risk of developing T1D compared to the general population. The increased risk of maternal diabetes disappears after adjusting for IA-2A in cord blood, suggesting the possibility that IA-2A is the primary risk factor. Further studies are needed to confirm this finding.

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Conflict of interest

The authors declare that there is no duality of interest associated with this manuscript

Contribution statement

ML analysed the data and wrote the manuscript. KL were involved in study design and critically revising the manuscript for important intellectual content. CL provided statistical support and database management. HEL designed the study, was involved in data collection, interpreted data and contributed to and edited the manuscript. All authors gave final approval of the version to be published.

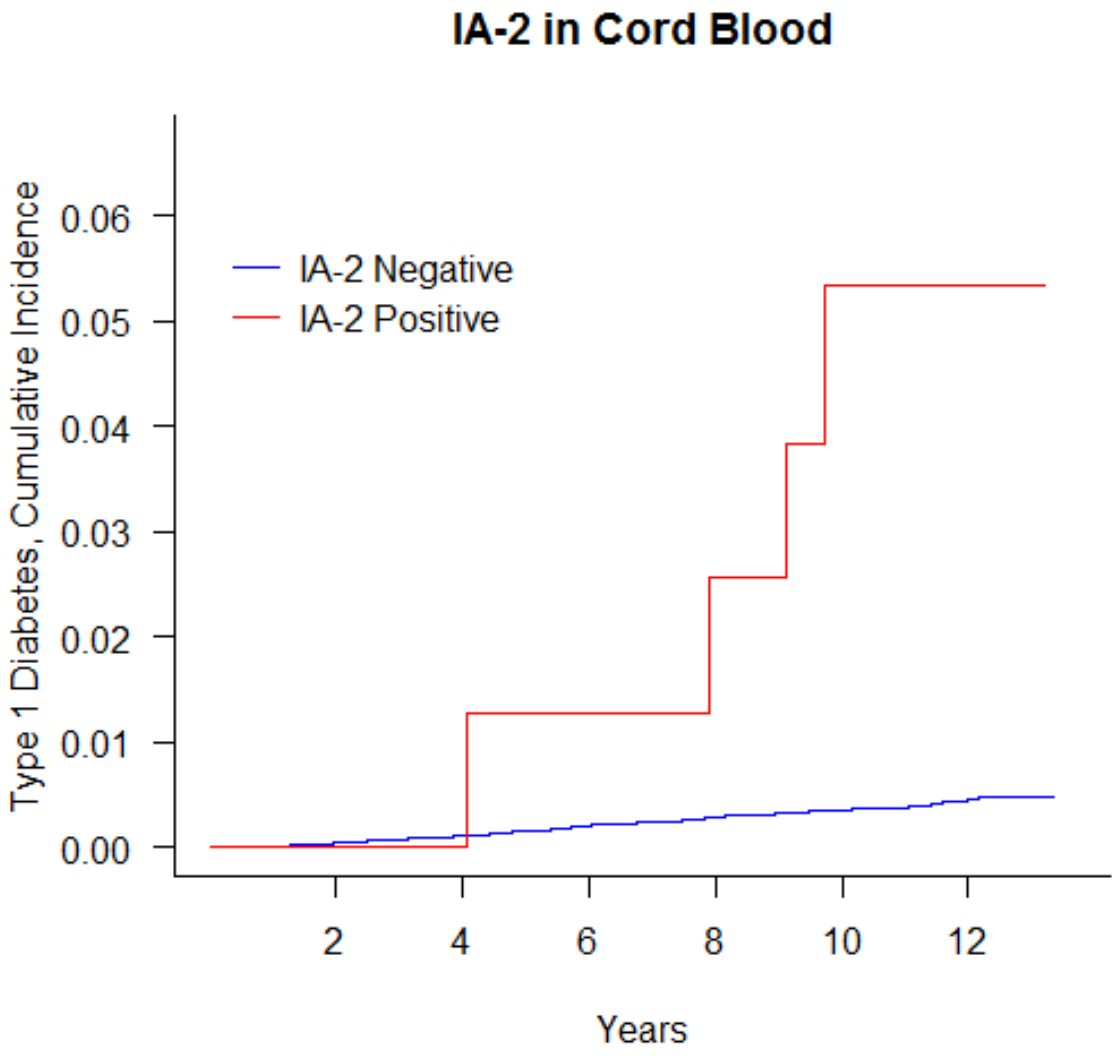
Table 1: Association between selected factors and diagnosis of type 1 diabetes in the DiPiS cohort.

	Univariate Hazard Ratio (95% CI)	p value	Multivariate [a] Hazard Ratio (95% CI)	p value	Multivariate [b] Hazard Ratio (95% CI)	p value
Parental type 1 diabetes:						
-Mother	3.50 (1.30-9.47)	0.014	1.51 (0.32-7.03)	0.60	1.38 (0.24-7.84)	0.71
-Father	6.21 (3.02-12.8)	<0.001	3.39 (1.64-7.02)	<0.001	3.70 (1.77-7.77)	<0.001
IA-2A positive	13.5 (5.02-36.6)	<0.001	7.73 (1.94-30.7)	0.003	6.88 (1.46-32.4)	0.015
IAA positive	2.41 (0.98-5.88)	0.053	0.75 (0.19-3.00)	0.82	0.65 (0.14-3.08)	0.58
GADA positive	3.44 (1.27-9.29)	0.015	1.15 (0.33-4.01)	0.48	1.11 (0.27-4.50)	0.88
Relative birth weight, quartile	1.08 (0.91-1.29)	0.38			1.02 (0.85-1.23)	0.82
Infection during pregnancy	0.85 (0.52-1.39)	0.51			0.82 (0.47-1.43)	0.48
Age of mother	1.01 (0.98-1.04)	0.63			1.00 (0.95-1.04)	0.94
Gestational length	0.86 (0.80-0.93)	<0.001			0.85 (0.73-0.99)	0.04
Gender	0.89 (0.65-1.23)	0.48			0.89 (0.58-1.34)	0.57
HLA risk-group						
-DQ 2/8	18.2 (13.1-25-3)	<0.001	42.4 (23.3-77.2)	<0.001		
-DQ 8/8 or 8/X	3.48 (2.42-5.01)	<0.001	10.9 (5.74-20.7)	<0.001		
-DQ 2/2 or 2/X	2.02 (1.30-3.16)	0.002	8.51 (4.20-17.2)	<0.001		
-DQ X/X (ref)	1.0 (ref)	ref	1.0 (ref)	ref		

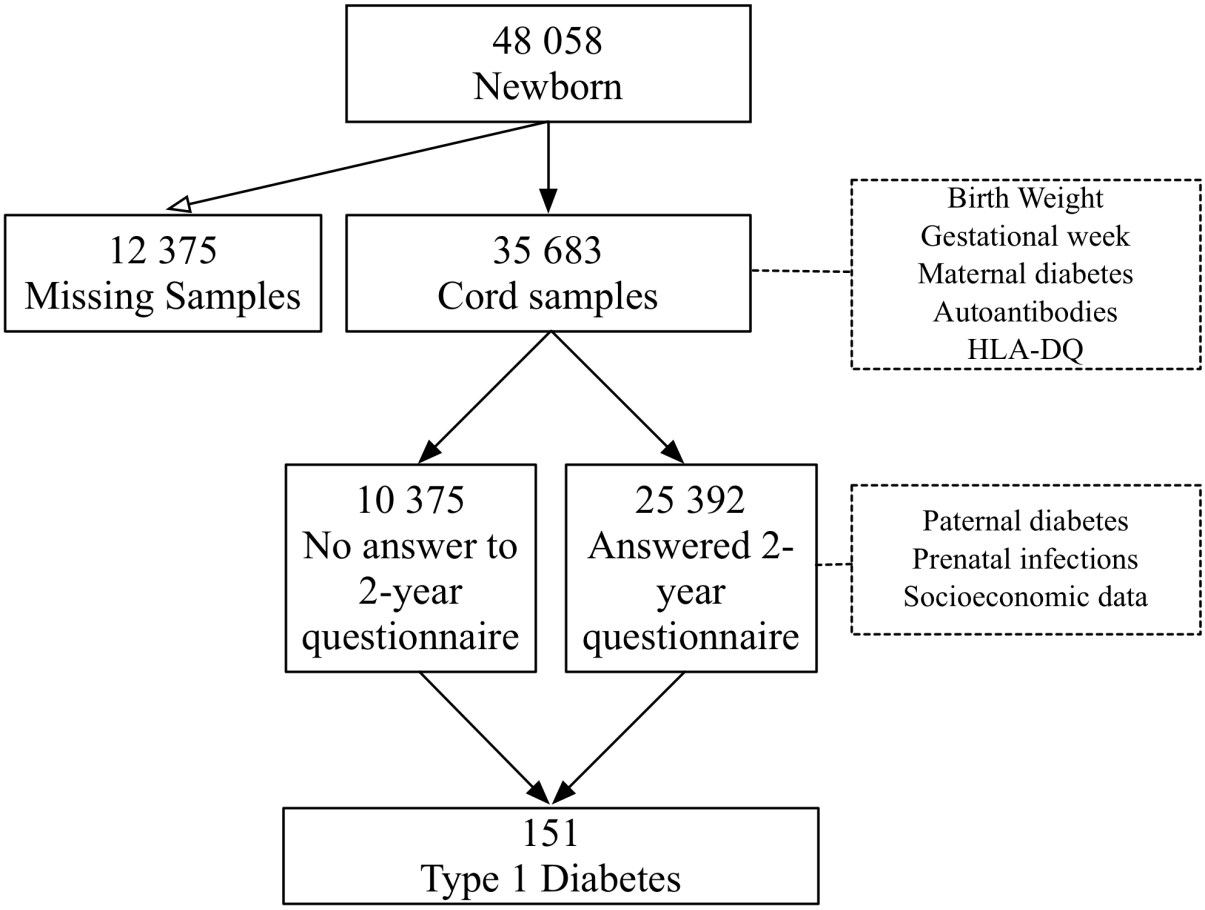
[a] Multivariate model including: Maternal T1D, Paternal T1D, IA-2A, GADA and IAA and stratified for HLA-DQ. HLA hazard ratio calculated in maltivariate model without stratification.

[b] Multivariate model including all available variables and stratified for HLA-DQ

Figure 1: Progression to type 1 diabetes related to IA-2 autoantibody cord blood positivity



Supplementary Figure 1: DiPiS study design and data collection



Supplementary Table 1: Baseline characteristics and clinical characteristics at diagnosis of the children in the DiPiS study with cord blood samples

Variable	Baseline			Diagnosed with T1D		
	n	(%)	Missing data (%)	n	(%)	Missing data(%)
Gender						
Female	16695	49	3,5	80	53	0
Male	17722	51		71	47	
HLA-DQ genotype						
2/8	1259	3.5	0.2	59	40	0
8/8, 8/X (X is not 2)	3276	9.2		52	34	
2/2, 2/X (X is not 8)	2932	8.2		25	17	
X/X (X is neither 2 nor 8)	28153	79		15	9	
Autoantibody positive						
GADA	295	0.8	<0.1	95	63	5.3
IAA	509	1.4	0.9	60	40	11
IA-2A	78	0.2	<0.1	113	75	5.3
Autoantibody, count						
Single	656	1.9	1.0	42	28	11
Double	75	0.2		48	32	
Triple	25	0.1		40	27	
Infection during pregnancy	4566	13	35	20	18	27
Maternal T1D	190	0.9	40	4	2.9	9.9
Paternal T1D	285	1.3	36	8	7.2	27

Supplementary Table 2: Contingency table of children with diabetic mother in relation to IA-2A present in cord blood and development of type 1 diabetes in the child

	Child without type 1 diabetes	Child with type 1 diabetes	Total
Negative IA-2A	146	1	147
Positive IA-2A	40	3	43
Total	186	4	190

In children to diabetic mothers, the presence of IA-2A in the cord blood was associated with development of type 1 diabetes in the child (Fisher´s exact test p=0.037).

Supplementary Table 3: Contingency table of all children with IA-2A present in cord blood in relation to type 1 diabetic mother and development of type 1 diabetes in the child.

	Child without type 1 diabetes	Child with type 1 diabetes	Total
Mother without diabetes	18	1	19
Mother with diabetes	40	3	43
Total	58	4	62

In children with IA-2A present in cord blood, mother with diabetes was not associated with development of type 1 diabetes in the child (Fisher´s exact test p=1)

Supplementary Table 4a. Contingency table of GADA and mother's diabetes status.

	Negative GADA in cord blood	Positive GADA in cord blood	Total
Mother without type 1 diabetes	21269	137	21406
Mother with type 1 diabetes	130	60	190
Total	21399	197	21596

Supplementary Table 4b. Contingency table of IA-2A and mother's diabetes status.

	Negative IA-2A in cord blood	Positive IA-2A in cord blood	Total
Mother without type 1 diabetes	21387	19	21406
Mother with type 1 diabetes	147	43	190
Total	21534	62	21596

Supplementary Table 4c. Contingency table of IAA and mother's diabetes status.

	Negative IAA in cord blood	Positive IAA in cord blood	Total
Mother without type 1 diabetes	21001	214	21215
Mother with type 1 diabetes	80	105	185
Total	21081	319	21400