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La Torre, Daria; Seppänen-Laakso, Tulikki; Larsson, Helena; Hyötyläinen, Tuulia; Ivarsson, Sten; Lernmark, Åke; Oresic, Matej

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LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Decreased cord-blood phospholipids in young age at onset type 1 diabetes

Short title: Cord-blood lipidomics

Authors: Daria La Torre¹, Tulikki Seppänen-Laakso², Helena E. Larsson¹, Tuulia Hyötyläinen², Sten A. Ivarsson¹, Åke Lernmark¹, Matej Orešič² and the DiPiS study group[§].

¹Department of Clinical Sciences, Lund University/ CRC/, Skåne University Hospital SUS, Malmö, Sweden.

²VTT Technical Research Centre of Finland, Espoo, Finland.

§§ Annelie Carlsson, Department of Pediatrics, Lund University Hospital; Elisabeth Cederwall, Department of Pediatrics, Ängelholm Hospital; Björn Jönsson, Department of Pediatrics, Ystad Hospital; Karin Larsson, Department of Pediatrics, Kristianstad Hospital; Jan Neiderud, Department of Pediatrics, Helsingborg Hospital.

Corresponding author

Daria La Torre , MD, PhD

Department of Clinical Sciences

Lund University

Skåne University Hospital SUS

SE-205 02 Malmö, Sweden.

Mobile: +393493967268

Fax: +464039191

e-mail: daria.la_torre@med.lu.se; lаторredaria@yahoo.it

Abstract

Children developing type 1 diabetes may have risk markers already in their umbilical cord blood. It is hypothesized that the risk for type 1 diabetes at an early age may be increased by a pathogenic pregnancy and be reflected in altered cord-blood composition. In this study metabolomics was used to test if the cord-blood lipidome was affected in children diagnosed with type 1 diabetes before eight years of age. The present case-control study of 76 index children diagnosed with type 1 diabetes before eight years of age and 76 healthy controls matched for HLA risk, gender and date of birth as well as mother's age and gestational age revealed that cord-blood phosphatidylcholines and phosphatidylethanolamines were significantly decreased in children diagnosed with type 1 diabetes before four years of age. Reduced levels of triglycerides correlated to gestational age in both index and control children and to age at diagnosis only in the index children. Finally, gestational infection during the first trimester was associated with lower cord blood total lysophosphatidylcholines in both index and control children. In conclusion, metabolomics of umbilical cord blood may identify children at increased risk for type 1 diabetes. Low phospholipid levels at birth may represent key mediators of the immune system and contribute in early induction of islet autoimmunity.

Type 1 diabetes is one of the most common chronic diseases among children and adolescents, with world-wide increase in incidence (1, 2). The clinical onset occurs when insulin secretion decreases due to autoimmune destruction of beta cells. Often overlooked is a prodrome of islet autoimmunity manifested by autoantibodies towards the islet autoantigens insulin, GAD65, IA-2 and ZnT8 (3-6). type 1 diabetes is also strongly associated with specific HLA-DQ haplotypes (7).

Factors triggering the onset of islet autoimmunity and the subsequent transition to overt diabetes are largely unknown, thus making a prevention strategy still a challenge. A critical role of the environment including gestational, perinatal and postnatal factors cannot be excluded (8). It is known that gestational events might increase disease risk for the offspring (9) including type 1 diabetes (10, 11) and celiac disease (12). It is hypothesized that the risk for type 1 diabetes at an early age may be increased by a pathogenic pregnancy, and be reflected in altered cord-blood composition. It is therefore critical to identify biomarkers of type 1 diabetes risk as early as possible in life.

Global profiling of serum metabolites has been used to dissect clinical and pathogenic aspects of the progression to islet autoimmunity and type 1 diabetes (13-15). Metabolomics analysis of samples from children in the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) study diagnosed with type 1 diabetes at 0.5-13.5 years of age (14) showed that serum metabolites may mark progression to islet autoimmunity and from islet autoimmunity to diabetes. In that study (14), umbilical cord-blood levels of phosphatidylcholines were reduced in children who developed diabetes compared to healthy autoantibody-negative children matched for HLA (14). This observation supports the notion that children exposed to gestational events may have increased risk for type 1 diabetes (16). More recently and independent of the present investigation, low cord blood content of choline-containing phospholipids was associated with progression to diabetes (17). In the Diabetes Prediction in Skåne (DiPiS) study, we found that gestational events affect birth weight and length (18), as well as the appearance of islet autoantibodies (19). We therefore analyzed umbilical cord serum by lipidomics (20-21) to test the hypothesis that a specific molecular lipid profile predicts type 1 diabetes.

The DiPiS study is a population-based study on a cohort of more than 35,000 children (17, 22, 23). We compared 76 DiPiS children who developed type 1 diabetes before 8 years of age with 76 controls matched for HLA, gender, date of birth and mother's age. In this group of 152 children we also analyzed the cord-blood lipidomic profile in relation to gestational events reported by the parents in clinical questionnaires (18).

RESEARCH DESIGN AND METHODS

Population and study design.

The DiPiS study was initiated in the Skåne region of Southern Sweden between September 2000 and August 2004, when umbilical cord-blood samples were collected from more than 35,000 newborns (24) and analyzed for HLA genotypes and islet autoantibodies (Figure 1) (17, 22). DiPiS children at increased risk for type 1 diabetes and islet autoantibody appearance have been analyzed every 4-12 months depending on the number of islet autoantibodies. Gestational and perinatal events were reported by about 25,000 parents in a questionnaire administered at the second post-natal visit (18).

Until April 2010, when the first sample selection was initiated, a total of 112 children had developed type 1 diabetes (Figure 1). Twins/triplets, children born to diabetes mothers (any kind) and children positive for islet autoantibodies in the cord blood were not included in this study (Figure 1). The suitable controls had not developed persistent islet autoantibodies or type 1 diabetes by April 2010. The case-control matching was based on date of birth (difference 0-27 days), also to match for sample storage time, in addition to HLA, gender, mother's age and gestational age. The study population consisted of 76 (40 males) index children diagnosed with type 1 diabetes and 76 control children. The age at diagnosis ranged from 10 months to 8 years (median 4.5 years). Index and control children did not differ in birth weight ($p=0.88$) and length ($p=0.76$) while index children (39.0 ± 1.7 weeks) showed slightly shorter gestational age than the controls (39.2 ± 1.3 weeks; $p=0.046$) (Table 1). Retrospective questionnaires were available for a total 101 children including 55

index and 46 controls and reported information on the newborn (birth weight and length, jaundice) and gestational events (infections, vaccinations, diet, drugs, weight gain).

The study was approved by the Regional Ethics Board (2009/244) and parents gave informed consent when the child was two months of age.

Lipidomic analysis.

The samples were blinded, prepared and analyzed in random order in duplicates with ultra-high performance liquid chromatography combined with mass spectrometry (UPLC-MS) (20). Mass spectrometry (MS) analysis was performed with electrospray ionization (ESI) in positive ion mode.

Data processing (detection and alignment of peaks, peak integration and normalization) was performed as already described in detail (14). Lipid identification was carried out through an in house reference compound library or MS/MS spectrum (20). Only identified lipids were included in the final dataset. The established lipidomics platform applied in this study covers the major lipid classes found in serum including phosphatidylcholines, sphingomyelins, phosphatidylethanolamines, ceramides and triglycerides.

Metabolite stability.

All samples were stored in the same place for 6.1- 9.9 years at a temperature -20°C . The strict case/control matching for date of birth implies that each matched case-control pair has been stored for the same time and has undergone approximately the same number of freeze-thaw cycles. The concentration of the most abundant lysophospholipid (lysophosphatidylcholine (LPC) 16:0) was not affected by storage (R^2 linear 0.008, $p=0.1$) while the following phosphatidylcholines (PC(40:5), PC(40:7), PC(38:4), PC(38:5), PC(38:5e), PC(36:4), PC(36:5e)/PE(38:5e)) decreased significantly with increasing sample storage time (data not shown).

Statistical analysis.

The index child 117 was a strong outlier (Grubbs' procedure $p<0.005$ and MV Hotelling's T^2 range plot) and excluded from further analysis with the matched control.

Multivariate data analysis was performed on N=150 samples by SIMCA-P+12.0 statistical software (Umetrics, Umeå, Sweden). Multivariate methods appear a valid tool for the analysis of complex and interdependent variables requiring multiple adjusting. The data was centered and pareto-scaled, to reduce the influence of noisy variables on the modeling results, and analyzed through projections methods as principal component analysis (PCA) and orthogonal partial least squares projections to latent structures Discriminant Analysis (OPLS-DA). PCA reduced the complexity of multidimensional datasets creating low-dimensional coordinates from the principal determinants of the data variance. OPLS divided the data into related (predictive) and unrelated to a preset outcome and was applied to correlated the metabolite pattern with type 1 diabetes development, clinical features and sample storage time. The data was evaluated through S-plots and Shared and Unique Structures (SUS)-like plots for the identification of metabolic biomarkers (25). Additional statistical analysis was carried out with the Standard Statistical Package for Windows version 18.0 (PASW 18 Inc, Chicago, Ill, USA) with $p < 0.05$ considered significant. Case-control differences were calculated with parametric or non-parametric matched-pair tests, as appropriate. To account for false discovery rate in multiple comparisons (26), q values were calculated with the *q-Value Calculator* (statistical software R). The correlation between metabolite levels and storage duration or clinical features was evaluated through Spearman's *rho* non-parametric correlation.

RESULTS

Metabolites detected in cord blood samples.

A total of 106 [lipid metabolites](#) were identified in the cord-blood samples of the 152 children, including phospholipids (PLs) [phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), sphingomyelins (SMs), and lysophosphatidylcholines/ethanolamines (LPCs/LPEs)], as well as triglycerides (TGs) (Table 2). First, a principal component analysis (PCA) of the whole dataset was performed, yielding an estimated predictive ability of 60% ($Q^2X=0.6$). The related loading plot

showed that the lipid class was the strongest determinant of metabolite distribution (data not shown). Consistently, the levels of total PCs (PC_{tot}), LPCs (LPC_{tot}), PEs (PE_{tot}) and SMs (SM_{tot}) were strongly inter-related ($p < 0.001$ Spearman's *rho*) in index and control children. LPC_{tot} ($p = 0.02$) and SM_{tot} ($p = 0.03$) were also related to total TGs (TG_{tot}).

Multivariate analysis of cord blood lipidome data.

Second, models were calculated to compare case-control lipidomics pattern in relation to the age of type 1 diabetes diagnosis. The best predictive model for type 1 diabetes was achieved in children less than 2 years of age at diagnosis, where PCs, PEs and TGs explained 41% of the case-control separation [$n = 18$ observations; OPLS A 1+0+0; goodness of separation (R^2_{Ycu}) 32%; goodness of prediction (Q^2_{cum}) 0.16]. The groups of children older than 4 years at diagnosis could not be modeled.

Then, S-plots were used to identify metabolic markers of age at diagnosis. Cord-blood PCs were significantly lower in children developing type 1 diabetes before 4 years of age than in matched controls (85% negative correlation between PC_{tot} and type 1 diabetes) ([Supplement Figure 1A](#)). Among children diagnosed before 2 years of age, low TGs were a better marker than PCs (0.95 negative correlation between TG_{tot} and type 1 diabetes) ([Supplement Figure 1B](#)). The 3-year olds did not differ from the 4-year olds (data not shown). A SUS-like plot comparing children diagnosed before 2 and at 2-4 years of age ([Supplement Figure 2](#)) confirmed that low PLs (PCs, PEs and SMs) marked type 1 diabetes in all children diagnosed younger than 4 years of age, though reduced TGs showed a better predictive value in children diagnosed younger than 2 years ([Supplement Figure 3](#)).

Additional analysis of cord blood lipidome data.

Pair-wise case-control analysis showed that median cord-blood levels of 50/58 PLs (PCs, PEs and SMs) but only of 7/36 TGs were reduced in children diagnosed before 4 years of age compared to their matched controls (significant PC(36:4), $p = 0.02$ and PE(36:0), $p = 0.02$). Stepwise analysis of the index children showed that median cord-blood levels of 39/44 PCs/PEs were reduced in children diagnosed before 4 years of age, compared to index diagnosed older than 4 years, and, out of these,

18 different lipids reached statistical significance (Table 3, [Supplement Figure 4](#)). Interestingly, the matched controls did not show the same trend (Table 3, [Supplement Figure 4](#)). A test on all age classes (10 months - 8 years) confirmed that several PCs and PEs were significantly decreased in the cord blood of index children with younger age at onset (Table 3). Finally, as suggested by the multivariate plots ([Supplement Figures 2 and 3](#)), children diagnosed before 4 years of age showed lower levels of 34/36 TGs than matched controls, and out of these 7 lipids reached statistical significance. However, cord-blood TGs decreased significantly with decreasing gestational age ($p=0.001$, Kruskal-Wallis test). Cord-blood TGs and gestational age appeared more strongly correlated in index ($p=0.007$) than in control children ($p=0.049$) ([Supplement Figure 5A](#)). Moreover, gestational age was found significantly related to the age at onset ($p=0.02$). The S-plot for biomarkers of gestational age ([Supplement Figure 5B](#)) confirmed that TGs strongly increased with increasing gestational age, while PLs (PCs, PEs and SMs) showed a weak, negative correlation with gestational age ([Supplement Figure 5B](#)).

Gestational infections, gestational age and gender differences.

A total of 112 mothers reported gestational infections. Only 9/54 (16.6%) index children but 21/58 (36.2%) controls had a mother reporting gestational infection in the first trimester ($p=0.02$). Among children diagnosed before 4 years of age, 5/19 (26%) had a mother reporting gestational infection during the first trimester, compared to 8/23 (34%) of matched controls ($p=0.7$). Among children diagnosed older than 4 years of age, only 3/33 (9%) had a mother reporting a gestational infection during the first trimester, compared to 13/34 (38%) controls ($p=0.009$). Lower cord blood LPCs were observed in index and control children born to mothers reporting a gestational infection ($p=0.04$ on $n=112$ children).

Males (50.9 ± 2.3 cm) were taller at birth than females (49.7 ± 1.8 cm; $p=0.03$, controlling for gestational age). Index females had shorter gestational age when compared to female controls ($p=0.01$) or index males ($p=0.005$). Index females also showed significantly higher levels of 8/10 LPCs/PEs compared to female controls or index males.

Finally, we also observed that low TGs marked neonatal jaundice (+90% positive correlation among 150 children – data not shown), as expected by the significantly shorter gestational age of children presenting with jaundice (index children $p=0.003$, controls $p<0.001$).

DISCUSSION

Our study of cord-blood lipidomics demonstrates that low levels of phosphatidylcholines and phosphatidylethanolamines increased the risk for type 1 diabetes diagnosed before 4 years of age. This finding may be of clinical relevance as epidemiological studies indicate that children diagnosed with type 1 diabetes at young age are increasing. The importance of gestational events for disease risk in the offspring has been reviewed elsewhere (9, 11, 12, 24). Altered cord-blood lipoproteins were reported in pathological conditions, as intrauterine growth restriction and eclampsia, known to confer cardiovascular disease risk for the offspring (27). It would be of general importance to identify metabolite patterns that would distinguish the risk for different diseases in the offspring.

We tested the hypothesis that lipidomic profile at birth might predict type 1 diabetes later in life. Fetal and neonatal serum lipids are markedly altered in case of maternal diabetes (28,29), but may be minimally affected by maternal lipids in normal pregnancies (28). Therefore, we excluded any kind of maternal diabetes from the study (Figure 1).

First, our study supports the recent observation of low cord-blood PC levels in 39 Finnish DIPP children who progressed to type 1 diabetes (14). We now extended this report to 75 Swedish children who developed type 1 diabetes before 8 years of age and 75 healthy and autoantibody-negative controls matched for HLA risk, gender and date of birth (Figure 1). Our major finding was that children developing type 1 diabetes before 4 years of age showed low levels of 50 different phospholipids, mainly PCs (Figures 3A, 5). Since PCs are the main source of choline, together with diet intake (30), it is possible to postulate that DiPiS children who will develop type 1 diabetes might be choline deficient at birth (14). Also an imbalance in maternal pregnancy diet might possibly

influence fetal/neonatal availability of choline (30). Low-level PCs may have a role in an imbalanced oxidative stress, as PCs are thought to have anti-inflammatory properties (31).

The second result was that cord-blood TGs were low in children diagnosed before 2 years of age ([Supplement Figure 2B](#)). The finding that TGs were strongly related to gestational age complicated this observation, since the very young children showed a significantly shorter gestational age ($p=0.02$) than children diagnosed older than 6 years of age. Thus, low-level TGs may not represent a risk factor for type 1 diabetes. In normal pregnancies, the main source of fetal TGs is *de novo* synthesis by the liver (32). However, cord-blood TG levels may partly reflect late pregnancy maternal hypertriglyceridemia (28). The observed direct correlation between cord-blood TG levels and gestational age ([Supplement Figure 5](#)), already reported in normal pregnancies (33), is probably physiological and unrelated to any gestational event conferring type 1 diabetes risk in the offspring, but of possible significance for any disorder related to short gestational age. Indeed, several clinical conditions are reported related to short gestational age, including type 1 (34) and type 2 diabetes (35). In the entire DiPiS study (18), index children have significantly shorter gestational age than controls ($p<0.001$). Our observation that age at diagnosis was related to gestational age was surprising, but may be due to the fact that all children were diagnosed younger than 8 years of age.

Taken together, short gestational age, perhaps in combination with reduced TG levels, may confer risk for type 1 diabetes at very early ages (<2 years). This subgroup of very young children may explain the significant clinical heterogeneity in relation to age at diagnosis reported in type 1 diabetes (36). Further metabolomics studies are needed (37) to disclose whether cord-blood TG levels are more important than gestational age *per se*. It should be noted in this respect that PC levels, inversely related to gestational age ([Supplement Figure 5B](#)), were still reduced in the children diagnosed before 4 years of age (Figures 3A, 4). Low PCs therefore remains the best marker for type 1 diabetes younger than 4 years of age, as TG levels were influenced by gestational age, especially among the index children.

As the samples were taken at birth, it may not be surprising that no markers for type 1 diabetes risk were found for children with later onset. Future investigation will be needed to determine at regular follow-up visits to what extent low PCs combined with serum C-peptide measurements can be used in prevention clinical studies. In the DIPP study as recently reported (17) after an analysis carried out independent of and in parallel to the present study, seven lipids were identified which predicted high risk for progression to type 1 diabetes. Reduction in choline-containing phospholipids in cord blood was found to be specifically associated with progression to type 1 diabetes. In addition, our observation that LPC/PE levels were significantly increased in female index children, regardless of age at onset, may suggest the presence of an imbalance of oxidative environment (38). Finally, the low LPCs that was found to be related to gestational infections would be consistent with the hypothesis of a decreased LPC content in patients with infective conditions (39).

We could not reinvestigate the effect of maternal age at delivery, a reported risk factor for type 1 diabetes in the offspring (40), because our case-control pairs were matched for mother's age to limit the influence of maternal metabolome.

A potential weakness of the study is the retrospective analysis of samples stored for a long time and of retrospective questionnaires collected by mothers after the delivery. The issue of metabolite stability is crucial for metabolomics evaluation, because of the potential confounding effects of non-enzymatic lipid oxidation during long storage at suboptimal temperature. In this study however, the stringent case-control matching controlled for metabolite stability and made it possible to conclude with a grade of confidence that observed metabolite variations were not due to storage. Our report on possible storage effects on metabolite concentration is of interest, since there is no data published on samples stored more than a few weeks (41).

In conclusion, cord-blood metabolic patterns, rather than single metabolites, may be a valuable measure of type 1 diabetes risk. It may be important to include essentially all phospholipidic

moieties in studies aimed at dissecting gestational events conferring disease risk in the offspring. Longitudinal studies of mothers from the first trimester until delivery will be important to disclose the character of gestational event that may lower cord-blood LPs or affect TG levels in relation to the development of islet autoimmunity and diabetes in the offspring.

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D.L.T. designed the study, analyzed and researched data, and wrote the manuscript. T.S-L., H.E.L., T.H., S.A.I. and Å.L. designed the study, researched data, contributed to discussion, and reviewed and edited the manuscript. M.O. was responsible for the lipidomics analysis, analyzed data, contributed to discussion, and reviewed and edited the manuscript. D.L.T and M.O. are the guarantors of this work and, as such, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

REFERENCES

1. DIAMOND Project Group. Incidence and trends of childhood Type 1 diabetes worldwide 1990-1999. *Diabet Med* 2006;23:857-866
2. EURODIAB ACE Study Group. Variation and trends in incidence of childhood diabetes in Europe. *Lancet* 2000;355:873-876
3. Eisenbarth GS. Prediction of type 1 diabetes: the natural history of the prediabetic period. *Adv Exp Med Biol* 2004;552:268-290
4. La Torre D, Lernmark A. Immunology of beta-cell destruction. *Adv Exp Med Biol* 2010;654:537-583
5. Torn C, Mueller PW, Schlosser M, Bonifacio E, Bingley PJ. Diabetes Antibody Standardization Program: evaluation of assays for autoantibodies to glutamic acid decarboxylase and islet antigen-2. *Diabetologia* 2008;51:846-852
6. DPT-1 Study Group The Diabetes Prevention Trial-Type 1 diabetes (DPT-1): implementation of screening and staging of relatives. *Transplant Proc* 1995;27:3377
7. Cucca F, Lampis R, Congia M, et al. A correlation between the relative predisposition of MHC class II alleles to type 1 diabetes and the structure of their proteins. *Hum Mol Genet* 2001;10:2025-37
8. TEDDY Study Group, The Environmental Determinants of Diabetes in the Young (TEDDY) Study. *Ann N Y Acad Sci* 2008;1150:1-13
9. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. *N Engl J Med* 2008;359:61-73
10. Dahlquist GG, Patterson C, Soltesz G. Perinatal risk factors for childhood type 1 diabetes in Europe. The EURODIAB Substudy 2 Study Group. *Diabetes Care* 1999;22:1698-1702
11. Elfving M, Svensson J, Oikarinen S, et al. Maternal enterovirus infection during pregnancy as a risk factor in offspring diagnosed with type 1 diabetes between 15 and 30 years of age. *Exp Diabetes Res* 2008;2008:271958
12. Lindehammer SR, Bjorck S, Lynch K, et al. Early human pregnancy serum cytokine levels predict autoimmunity in offspring. *Autoimmunity* 2011;44:445-452
13. Bain JR, Stevens RD, Wenner BR, et al. Metabolomics applied to diabetes research: moving from information to knowledge. *Diabetes* 2009;58:2429-2443
14. Oresic M, Simell S, Sysi-Aho M, et al. Dysregulation of lipid and amino acid metabolism precedes islet autoimmunity in children who later progress to type 1 diabetes. *J Exp Med* 2008;205:2975-2984
15. Pflueger M, Seppanen-Laakso T, Suortti T, et al. Age- and islet autoimmunity-associated differences in amino acid and lipid metabolites in children at risk for type 1 diabetes. *Diabetes* 2011;60:2740-2747
16. Knip M, Veijola R, Virtanen SM, et al. Environmental triggers and determinants of type 1 diabetes. *Diabetes* 2005;54 Suppl 2:S125-136
17. Oresic M, Gopalacharyulu P, Mykkänen J, et al. Cord Serum Lipidome in Prediction of Islet Autoimmunity and Type 1 Diabetes. *Diabetes* 2013 Apr 29. [Epub ahead of print]
18. Lynch KF, Lernmark B, Merlo J, et al. Cord blood islet autoantibodies and seasonal association with the type 1 diabetes high-risk genotype. *J Perinatol* 2008;28:211-217
19. Lindehammer SR, Hansson I, Midberg B, et al. Seroconversion to islet autoantibodies between early pregnancy and delivery in non-diabetic mothers. *J Reprod Immunol* 2011;88:72-79
20. Nygren H, Seppanen-Laakso T, Castillo S, et al. Liquid chromatography-mass spectrometry (LC-MS)-based lipidomics for studies of body fluids and tissues. *Methods Mol Biol* 2011;708:247-257
21. Quehenberger O, Dennis EA. The human plasma lipidome. *N Engl J Med* 2011;365:1812-1823

22. Larsson HE, Lynch K, Lernmark B, et al. Diabetes-associated HLA genotypes affect birthweight in the general population. *Diabetologia* 2005;48:1484-1491
23. Larsson HE, Hansson G, Carlsson A, et al. Children developing type 1 diabetes before 6 years of age have increased linear growth independent of HLA genotypes. *Diabetologia* 2008;51:1623-1630
24. Larsson K, Elding-Larsson H, Cederwall E, et al. Genetic and perinatal factors as risk for childhood type 1 diabetes. *Diabetes Metab Res Rev* 2004;20:429-437
25. Wiklund S, Johansson E, Sjostrom L, et al. Visualization of GC/TOF-MS-based metabolomics data for identification of biochemically interesting compounds using OPLS class models. *Anal Chem* 2008;80:115-122
26. Kall L, Storey JD, MacCoss MJ, et al. Posterior error probabilities and false discovery rates: two sides of the same coin. *J Proteome Res* 2008;7:40-44
27. Rodie VA, Caslake MJ, Stewart F, et al. Fetal cord plasma lipoprotein status in uncomplicated human pregnancies and in pregnancies complicated by pre-eclampsia and intrauterine growth restriction. *Atherosclerosis* 2004;176:181-187
28. Herrera E. Lipid metabolism in pregnancy and its consequences in the fetus and newborn. *Endocrine* 2002;19:43-55
29. Kilby MD, Neary RH, Mackness MI, et al. Fetal and maternal lipoprotein metabolism in human pregnancy complicated by type I diabetes mellitus. *J Clin Endocrinol Metab* 1998;83:1736-1741
30. Li Z, Vance DE. Phosphatidylcholine and choline homeostasis. *J Lipid Res* 2008;49:1187-1194
31. Treede I, Braun A, Sparla R, et al. Anti-inflammatory effects of phosphatidylcholine. *J Biol Chem* 2007;282:27155-27164
32. Chow SN, Chen HY, Ouyang PC, et al. Serum triglyceride levels in newborns and mothers at parturition. *Int J Biol Res Pregnancy* 1982;3:30-32
33. Christensen NC. Concentrations of triglycerides, free fatty acids and glycerol in cord blood of newborn infants with a birth weight of less than or equal to 2700 grams. *Acta Paediatr Scand* 1977;66:43-48
34. Svensson J, Carstensen B, Mortensen HB, et al. Early childhood risk factors associated with type 1 diabetes--is gender important? *Eur J Epidemiol* 2005;20:429-434
35. Kaijser M, Bonamy AK, Akre O, et al. Perinatal risk factors for diabetes in later life. *Diabetes* 2009;58:523-526
36. Andersson C, Larsson K, Vaziri-Sani F, et al. The three ZNT8 autoantibody variants together improve the diagnostic sensitivity of childhood and adolescent type 1 diabetes. *Autoimmunity* 2011;44:394-405.
37. Varmus H. Ten years on--the human genome and medicine. *N Engl J Med* 2010;362:2028-2029.
38. Fuchs B, Muller K, Paasch U, et al. Lysophospholipids: potential markers of diseases and infertility? *Mini Rev Med Chem*;12:74-86
39. Lissauer E, Johnson B, et al. Decreased lysophosphatidylcholine levels are associated with sepsis compared to uninfected inflammation prior to onset of sepsis. *Journal of Surgical Research* 2007;137:206-206.
40. Cardwell CR, Stene LC, Joner G, et al. Maternal age at birth and childhood type 1 diabetes: a pooled analysis of 30 observational studies. *Diabetes*;59:486-494
41. Zivkovic AM, Wiest MM, Nguyen UT, et al. Effects of sample handling and storage on quantitative lipid analysis in human serum. *Metabolomics* 2009;5:507-516.

TABLES**Table 1.** Baseline characteristics of index and control children.

| Children | Index | Controls | p-value |
|--------------------------|---------------------|--------------------|---------|
| n | 76 (40 males) | 76 (40 males) | |
| Birth weight (g) | 3581 \pm 536 | 3592 \pm 439 | n.s. |
| Birth length (cm) | 50.2 \pm 2.4 | 50.3 \pm 2.0 | n.s. |
| Gestational age (weeks) | 39.0 \pm 1.7 | 39.2 \pm 1.3 | 0.046 |
| Age at diagnosis (years) | 10 months – 8 years | (median 4.3 years) | n.a. |

n.a. is not applicable.

Table 2. Metabolites identified in cord blood samples.

Lysophosphatidylcholines/ethanolamines (n=12)

LysoPC(14:0), LysoPC(16:0), LysoPC(16:1), LysoPC(18:0), LysoPC(18:1), LysoPC(18:2), LysoPC(20:3), LysoPC(20:4), LysoPC(20:5), LysoPC(22:6), LysoPE(18:2), LysoPE(20:1)

Phosphatidylcholines (n=35)

PC(31:1)/PE(34:1), PC(32:0), PC(32:1), PC(32:2), PC(33:1)/PE(36:1), PC(33:2)/PE(36:2), PC(34:1), PC(34:2), PC(34:2e), PC(34:3), PC(34:3e)/PE(37:3e), PC(35:2), PC(36:1), PC(36:1e), PC(36:2), PC(36:2e), PC(36:3), PC(36:3e), PC(36:4), PC(36:5), PC(36:5e)/PE(38:5e), PC(37:4)/PE(40:4), PC(38:2), PC(38:3), PC(38:3e), PC(38:4), PC(38:5), PC(38:5e), PC(38:6), PC(40:4), PC(40:5), PC(40:5e), PC(40:6), PC(40:7), PC(40:8)

Phosphatidylethanolamines (n=9)

PE(34:0), PE(34:3e), PE(36:0), PE(36:1), PE(38:1), PE(38:4), PE(38:4e), PE(40:4), PE(40:7e)

Sphingomyelins (n=14)

SM(d18:1/14:0), SM(d18:1/16:0), SM(d18:1/16:1), SM(d18:1/18:0), SM(d18:1/18:2), SM(d18:1/18:3), SM(d18:1/20:0), SM(18:1/20:1), SM(d18:1/21:0), SM(d18:1/22:0), SM(d18:1/22:1), SM(d18:1/22:3), SM(18:1/23:1), SM(d18:1/24:1)

Triglycerides (n=36)

TG(18:1/16:1/18:2)/TG(18:2/18:2/16:0), TG(18:1/18:2/18:1), TG(46:0), TG(46:1), TG(46:2), TG(47:1), TG(48:0), TG(48:1), TG(48:2), TG(48:3), TG(49:1), TG(49:2), TG(50:0), TG(50:1), TG(50:2), TG(50:3), TG(51:1), TG(51:2), TG(51:3), TG(52:1), TG(52:2), TG(52:3), TG(52:4), TG(52:5), TG(53:3), TG(53:8), TG(54:1), TG(54:2), TG(54:3), TG(54:4), TG(54:5), TG(54:6), TG(54:9), TG(56:5), TG(56:6), TG(56:7)

Table 3. Phospholipids differing between children who developed diabetes before and after 4 years of age.

| METABOLITES | Children developing type 1 diabetes (n=75) | | | | | P value† | Controls (n=75) Median <4/>4 yrs ratio and p value§ |
|-------------------|--|------------------------|-------|----------|-----------------------------------|----------|---|
| | Median index <4yrs | Median index >4 yrs | Ratio | P value* | Q value <i>FDR (Pi_0)=0.84</i> | | |
| PC(38:5) | 3.01 | 4.21 | 0.71 | 0.001 | 0.1 | | 0.92 (p=ns) |
| PC(36:4) | 79.11 | 96.45 | 0.82 | 0.003 | 0.1 | 0.01 | 1.02 (p=ns) |
| PC(36:5+PE385:e) | 2.56 | 3.21 | 0.78 | 0.004 | 0.1 | 0.002 | 0.93 (p=ns) |
| PC(38:5e) | 0.33 | 0.39 | 0.84 | 0.004 | 0.1 | 0.02 | 0.91 (p=ns) |
| PC(40:4) | 1.21 | 1.57 | 0.77 | 0.007 | 0.12 | | 0.98 (p=ns) |
| PE(38:4) | 13.84 | 16.85 | 0.82 | 0.007 | 0.12 | 0.03 | 0.95 (p=ns) |
| PC(37:4+PE40:4) | 1.76 | 2.14 | 0.82 | 0.009 | 0.13 | 0.046 | 0.94 (p=ns) |
| PC(38:6) | 28.82 | 33.19 | 0.87 | 0.01 | 0.13 | | 0.96 (p=ns) |
| PE(40:4) | 2.46 | 3.12 | 0.79 | 0.01 | 0.14 | | 1.01 (p=ns) |
| PC(40:5) | 1.26 | 1.84 | 0.68 | 0.02 | 0.14 | | 0.97 (p=ns) |
| PC(40:5e) | 0.26 | 0.31 | 0.85 | 0.02 | 0.14 | | 0.3 (p=ns) |
| PC(38:4) | 2.54 | 3.14 | 0.81 | 0.02 | 0.14 | 0.049 | 0.88 (p=ns) |
| PC(32:1) | 5.77 | 8.02 | 0.72 | 0.02 | 0.15 | | 1.14 (p=ns) |
| PC(40:8) | 0.39 | 0.32 | 1.21 | 0.03 | 0.16 | | 1.02 (p=ns) |
| PE(38:4e) | 60.65 | 56.02 | 1.08 | 0.03 | 0.16 | | 1.10 (p=0.021) |
| PC(31:1+PE34:1) | 1.26 | 1.48 | 0.85 | 0.03 | 0.16 | | 0.96 (p=ns) |
| Pctot | 447.65 | 502.83 | 0.89 | 0.03 | 0.17 | | 0.8 (p=ns) |
| PC(34:3e+PE37:3e) | 0.56 | 0.69 | 0.81 | 0.03 | 0.17 | | 0.6 (p=ns) |
| PC(40:7) | 0.45 | 0.51 | 0.88 | 0.047 | 0.23 | | 1.08 (p=ns) |

*Mann-Whitney test between the two classes of age at diagnosis (index children); † Kruskal-Wallis non-parametric ANOVA test on all age classes (index children); § Mann-Whitney test between control children matched to the two classes of age at diagnosis

Q-value estimates the false discovery rate (FDR) for a given p-value cut-off in multiple hypothesis testing (see text reference 26). In these series, the estimated q-value is reported for each test. Pi_0 is the maximum estimated q-value among all p-values < 0.05 and estimates the false discovery rate (FDR) when considering all p-values < 0.05 significant. In these series, FDR was 0.84 for Mann-Whitney and 0.5 for Kruskal-Wallis test.

All Phosphatidylcholines (PCs) except PC (40:8) and phosphatidylethanolamines (PEs) except PE(38:4e) were significantly reduced in children who developed diabetes younger than 4 years of age. The same analysis performed on all controls matched to the two age classes of index children did not show the same trend.

FIGURE LEGENDS

Figure 1. Flow chart of study design. Index and control children were selected from a population-based newborn screening study (DiPiS study) between 2000 and 2004. Ab is the abbreviation for autoantibody.

LEGENDS TO SUPPLEMENT FIGURES

Supplement Figure 1. S-plot showing the correlation of all metabolites with sample storage duration. The y axis (p(corr)) reports marker reliability expressed as correlation with predicted event (range -1 +1), in this case storage duration; on the x axis is reported the abundance of the biomarkers in the samples. Metabolites in lower left quadrant (phosphatidylcholines, PCs) have the strongest negative correlation with storage duration (p (corr) on negative y-axis is 70-80%). The color codes are: GREEN triglycerides (TG), BLUE Phosphatidylcholines (PC), ORANGE Phosphatidylethanolamines (PE), LIGHT GREEN LysoPC and lysoPE, PURPLE Sphingomyelins (SM), GREY total metabolites.

Supplement Figure 2, panels A, B. S-plots showing the best biomarkers of type 1 diabetes onset at different ages. The y axis (p(corr)) reports marker reliability expressed as correlation with predicted event (range -1 +1), in this case development of diabetes. Metabolites in the lower left quadrant have a strong inverse correlation with the predicted variable, as they are significantly decreased in the cord blood from children who will develop type 1 diabetes. **Panel A.** Children diagnosed before 4 years of age show low levels of phosphatidylcholines (PCs, p(corr)= -0.85) and triglycerides (TGs, less significant). **Panel B.** In children diagnosed before the age of 2 years the strongest negative correlation is for TGs (-100%). The color codes are: GREEN triglycerides (TG), BLUE Phosphatidylcholines (PC), ORANGE Phosphatidylethanolamines (PE), LIGHT GREEN LysoPC and lysoPE, PURPLE Sphingomyelins (SM), GREY total metabolites.

Supplement Figure 3. SUS-like plot comparing diabetes-related metabolic pattern among children diagnosed before 2 (x axis) and at 2-3.9 years of age (y axis). All triglycerides (TGs) are significantly negatively related to the diagnosis of type 1 diabetes only among children younger than 2 years at diagnosis (correlation $p(\text{corr}) = -70$ to -95% on the x axis and $=0$ on the y axis), as they are significantly decreased only in the cord blood of children developing diabetes before the age of 2. Phospholipids (phosphatidylcholines PCs, phosphatidylethanolamines PEs and sphingomyelins SMs) are significantly homogeneously decreased in children diagnosed before the age of 4 years (< 2 and at 2-3.9 years of age). The color codes are: GREEN triglycerides (TG), BLUE Phosphatidylcholines (PC), ORANGE Phosphatidylethanolamines (PE), LIGHT GREEN LysoPC and lysoPE, PURPLE Sphingomyelins (SM), GREY total metabolites.

Supplement Figure 4. Phospholipids differing between children who developed diabetes before and after 4 years of age. The levels of 16/18 phospholipids (phosphatidylcholines PCs and phosphatidylethanolamines PEs) are significantly reduced in children diagnosed before 4 years of age compared to children diagnosed after 4 years (violet vs purple bars); the matched children do not show the same trend (white vs aquamarine bars). PC(40:8) and PE(38:4e) are significantly reduced in children who develop diabetes younger than 4 years of age. The color codes are VIOLET bars: median PC in index children < 4 years at diagnosis, PURPLE bars: median PC in index children > 4 years at diagnosis, YELLOW bars: median PC in controls matched to index < 4 years at diagnosis; AQUAMARINE bars: median PC in controls matched to index > 4 years at diagnosis.

Supplement Figure 5. Correlation between metabolites and gestational age. **Panel A.** Box plot showing changes in Triglycerides (TGs) levels according to increasing gestational age in cases (left)

and controls (right); TGs were found significantly directly related to gestational age in both index ($p=0.007$, R^2 linear 0,094) and control children ($p=0.049$ R^2 linear 0,054). The correlation did not change after excluding one case born at the 32nd week of gestation. **Panel B.** SUS-like plot comparing the metabolite pattern related to gestational age in index (x axis) and controls (y axis). TGs are homogeneously increased in both groups (upper right quadrant, strong positive correlation on both axes). Phosphatidylcholines (PCs) levels are more weakly, indirectly related to gestational age ($p(\text{corr})=-0.2$ to -0.4). There was no clear pattern for PEs (LPCs). The color codes are: GREEN triglycerides (TG), BLUE Phosphatidylcholines (PC), ORANGE Phosphatidylethanolamines (PE), LIGHT GREEN LysoPC and lysoPE, PURPLE Sphingomyelins (SM), GREY total metabolites.