

Age- and Islet Autoimmunity–Associated Differences in Amino Acid and Lipid Metabolites in Children at Risk for Type 1 Diabetes

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OBJECTIVE—Islet autoimmunity precedes type 1 diabetes and often initiates in childhood. Phenotypic variation in islet autoimmunity relative to the age of its development suggests heterogeneous mechanisms of autoimmune activation. To support this notion, we examined whether serum metabolite profiles differ between children with respect to islet autoantibody status and the age of islet autoantibody development.

RESEARCH DESIGN AND METHODS—The study analyzed 29 metabolites of amino acid metabolism and 511 lipids assigned to 12 lipid clusters in children, with a type 1 diabetic parent, who first developed autoantibodies at age 2 years or younger ($n = 13$), at age 8 years or older ($n = 22$), or remained autoantibody-negative, and were matched for age, date of birth, and HLA genotypes ($n = 35$). Ultraperformance liquid chromatography and mass spectroscopy were used to measure metabolites and lipids quantitatively in the first autoantibody-positive and matched autoantibody-negative serum samples and in a second sample after 1 year of follow-up.

RESULTS—Differences in the metabolite profiles were observed relative to age and islet autoantibody status. Independent of age-related differences, autoantibody-positive children had higher levels of odd-chain triglycerides and polyunsaturated fatty acid-containing phospholipids than autoantibody-negative children and independent of age at first autoantibody appearance ($P < 0.0001$). Consistent with our hypothesis, children who developed autoantibodies by age 2 years had twofold lower concentration of methionine compared with those who developed autoantibodies in late childhood or remained autoantibody-negative ($P < 0.0001$).

CONCLUSIONS—Distinct metabolic profiles are associated with age and islet autoimmunity. Pathways that use methionine are potentially relevant for developing islet autoantibodies in early infancy. *Diabetes* 60:2740–2747, 2011

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See accompanying commentary, p. 2688.

Islet autoantibodies precede the development of type 1 diabetes and can appear throughout childhood (1). In prospective studies of offspring of parents with type 1 diabetes, we have observed a peak incidence of islet autoantibody appearance at the age of ~1 year, followed by a decline through age 2 to 5 years and a subsequent rise in incidence toward puberty together with the rise in incidence of developing other autoantibodies such as thyroid peroxidase antibodies (2–4). The characteristics of the islet autoantibodies that develop in the first 2 years are not the same as those that develop later (4). Early antibodies frequently start with insulin autoantibodies (IAA), are high affinity, and spread to multiple targets, whereas children who develop islet autoantibodies late start with IAA or GAD autoantibodies (GADA) that are less likely to spread to other targets (4,5). These observations suggest age-dependent differences in the events that lead to islet autoimmunity or in the immune response to the event.

Metabolic phenotypes have been used to identify heterogeneity between subjects. The metabolomic profile has been shown to differ in a manner that is associated with genetics, environment, feeding, and disease (6–9). In type 1 diabetes, specific differences are present in islet autoantibody-positive children before islet autoantibody development (10). These could reflect early environmental exposures that influence the autoimmunization process. With respect to the heterogeneity in islet autoimmunity, we reasoned that if the age-related differences in islet autoantibody appearance reflected different immunizing events, we would observe differences in metabolomic profiles in early versus late developers of islet autoantibodies. Here we tested this by analyzing metabolomic profiles in children who developed islet autoantibodies in the first (age 1 to 2 years) and second (age ≥ 8 years) age peaks and in matched islet autoantibody-negative control subjects. We found differences that are dependent on age, islet autoantibody positivity, and the age of islet autoantibody development that support our hypothesis.

RESEARCH DESIGN AND METHODS

Serum metabolite profiles were analyzed in children from the BABYDIAB study, which examines the natural history of islet autoimmunity from birth in 1,650 children of mothers or fathers with type 1 diabetes (11). Recruitment began in 1989 and ended in 2000. Venous blood samples were obtained from children at study visits scheduled at age 9 months, and at 2, 5, 8, 11, 14, 17, and 20 years. Islet autoantibodies (IAA, GADA, IA-2A, and ZnT8A) were measured in samples taken at all scheduled visits and every 6 months when children had a positive autoantibody value. The median follow-up time from birth to the last sample was 9.5 years (maximum 20.8 years). All families gave written informed consent to participate in the BABYDIAB study. The study was approved by

the ethical committee of Bavaria, Germany (Bayerische Landesärztekammer Nr. 95357).

To date (2010), 152 offspring developed persistent islet autoantibodies (i.e., antibodies that were confirmed positive in a second serum sample) (Fig. 1). Of these, 62 children developed islet autoantibodies early (age ≤ 2 years), 36 developed antibodies at age 5 years, and 54 developed antibodies late (age ≥ 8 years). The current study included 70 children (Fig. 1 and Table 1), consisting of 35 children with islet autoantibodies and 35 children without islet autoantibodies matched for age, date of birth, sex, and HLA genotype. Autoantibody-positive children were selected on the basis of 1) the availability of samples frozen at -80°C (a requisite for the metabolomic analysis), and 2) becoming autoantibody-positive early (age ≤ 2 years; $n = 13$) or late (age ≥ 8 years; $n = 22$) (Supplementary Table 1). Seven of the 35 autoantibody-positive and none of the 35 autoantibody-negative children progressed to type 1 diabetes at a median time from first islet autoantibody development of 3.0 years (interquartile range [IQR] 2.7–6.2). Of these, 42 children had a mother with type 1 diabetes, and 28 had a nondiabetic mother but a father with type 1 diabetes.

Samples corresponding to the seroconversion time point were reported to have been taken after an overnight fast in 38 children and were not reported in the remainder. All blood glucose and insulin concentrations measured in the samples were within the normal range for children.

Metabolites of amino acid and lipid metabolism were analyzed in the first antibody-positive serum samples from children who seroconverted to islet autoantibody-positive and in the age-matched samples from children who remained islet autoantibody-negative. To assess persistency of findings, a second sample obtained 1 year later was also tested in 63 of the children. In addition, a preseroconversion sample was available from the 13 early autoantibody-positive children and their control subject counterparts. Twenty-nine metabolites of the amino acid metabolism were measured in all samples. Lipids were measured in all except the preseroconversion samples. The 511 detected molecular lipids were clustered into 12 groups or lipid clusters (LCs) (Supplementary Table 2). The measurements of the metabolites and lipids were performed on coded samples that were blinded to the operator.

Measurement of islet autoantibodies. IAA, GADA, IA-2A, and ZnT8A were measured by radiobinding assays, as previously described (11,12). The upper limits of normal were determined using Q-Q plots and corresponded to the 99th percentile of control children. Performances in the Diabetes Antibody Standardization Program are shown as laboratory 121 in published reports (13,14). Offspring were considered islet autoantibody-positive when two consecutive samples collected after birth were positive.

Measurement of lipids. Plasma samples (7 μL) were diluted with 10 μL of sodium chloride (0.9%) and spiked with 10 μL of an internal standard mixture containing 10 lipid classes (0.1 $\mu\text{g}/\text{sample}$): lysophosphatidylcholine

(LPC; 17:0/0:0), phosphatidylcholine (PC; 17:0/17:0), phosphatidylethanolamine (PE; 17:0/17:0), phosphatidylglycerol (17:0/17:0), ceramide (Cer; d18:1/17:0), phosphatidylserine (17:0/17:0), phosphatidic acid (17:0/17:0), monoacylglycerol (17:0/0:0/0:0), diacylglycerol (17:0/17:0/0:0), and triacylglycerol (TG; 17:0/17:0/17:0).

Samples were extracted with 100 μL of chloroform/methanol (2:1) by vortexing for 2 min and after 40 min extraction time were centrifuged at 10,000g for 3 min. From the lower layer, 60- μL aliquots were transferred into vial inserts and another standard mixture containing three labeled standards was added to the extracts (0.1 $\mu\text{g}/\text{sample}$): LPC (16:0/0:0-D₃), PC (16:0/16:0-D₆), and TG (16:0/16:0/16:0-¹³C₃).

Ultraperformance liquid chromatography–electrospray ionization–mass spectrometry. Lipids were analyzed on a Waters Q-ToF Premier mass spectrometer combined with a Waters Acquity UPLC system (Waters Corp., Milford, MA) equipped with a BEH C18 column (2.1 \times 100 mm with 1.7- μm particles). Column temperature was kept at 50°C , and the temperature of the sample organizer was set to 10°C . The solvent system consisted of A) water (1% 1 mol/L NH₄Ac, 0.1% HCOOH) and B) liquid chromatography–mass spectrometry grade isopropanol/acetonitrile (1:1, 1% 1 mol/L NH₄Ac, 0.1% HCOOH). The gradient started from 65% A/35% B and reached 80% B in 2 min, 100% B in 7 min, and remained at this level for the next 7 min. Total run time, including a 4-min re-equilibration step, was 18 min. The flow rate was 400 $\mu\text{L}/\text{min}$, and the injection volume was 2.0 μL .

Data were collected in centroid form by using electrospray ionization+ mode at a mass range of m/z 300–1,200 and with a scan duration of 0.2 s. The voltages of the sampling cone and capillary were 40.0 V and 3.0 kV, respectively. The source temperature was set at 120°C , and nitrogen was used as desolvation gas (795 L/h) at 270°C . Reserpine (200 $\mu\text{g}/\text{L}$) was the lock spray reference compound.

Data were processed by MZmine 2 software and the lipid identification was based on an internal spectral library including mass spectrometry and tandem mass spectrometry data. All monoacyl lipids, except cholesteryl esters, such as monoacylglycerols and monoacyl-glycerophospholipids, were normalized with PC (17:0/0:0), all diacyl lipids, except ethanolamine phospholipids, with PC (17:0/17:0), all ceramides with Cer (d18:1/17:0), all ethanolamine phospholipids with PE (17:0/17:0), and all triacylglycerols and cholesteryl esters with TG (17:0/17:0/17:0). Unidentified lipids were calibrated with LPC (17:0/0:0) for retention times of <300 s, with PC (17:0/17:0) for retention times between 300 and 410 s, and with TG (17:0/17:0/17:0) for higher retention times. The relative SD of the peak heights of the internal standards, added to all samples, was $<17\%$. For control serum samples ($n = 12$), the relative SD of the amounts of identified compounds was $<18.4\%$.

Measurement of amino acids. Proteins in serum samples were precipitated by adding an equal volume of 10% (w/v) sulfosalicylic acid containing norvaline

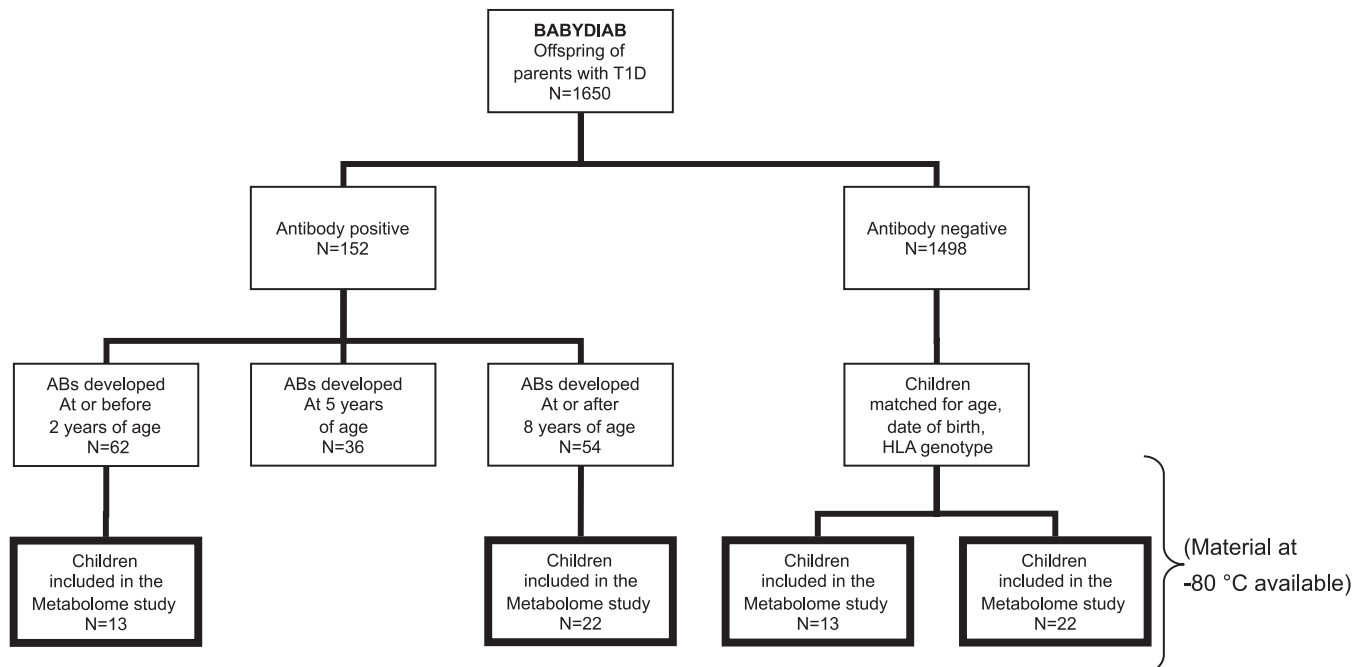


FIG. 1. Flowchart of study population for the metabolomic analysis. Heavy boxed categories ($N = 70$) were included in the analysis (material at -80°C was available). All 70 children were tested for metabolomics at seroconversion to islet autoantibodies (ABs) or at the respective age in AB- children as well as 1 year thereafter. T1D, type 1 diabetes.

TABLE 1
Characteristics of children included in the study

Variable	Children age ≤ 2 years		Children age ≥ 8 years	
	AB+	AB-	AB+	AB-
<i>n</i>	13	13	22	22
Age at sample collection (years)	1.5 (0.9–1.9)	1.2 (0.9–1.9)	9.1 (8.2–13.3)	9.7 (8.1–11.2)
<i>HLA DR4</i> positive	8	8	5	6
Proband with type 1 diabetes				
Mother	5	7	17	13
Father	8	6	5	9

Continuous data are shown as median (IQR) and categorical data as *n*. AB+, autoantibody-positive; AB-, autoantibody-negative.

(250 $\mu\text{mol/L}$) as the internal standard. The mixture was vortexed for 30 s and centrifuged at 16,000*g* for 4 min. Supernatant was drawn, and 10 μL of serum preparation and 30 μL of NaOH-borate buffer (395 μL of 0.5 N NaOH added to 2,600 μL of borate buffer from the MassTRAK amino acid kit; Waters Corp.) were added, mixture was vortexed, and 10 μL MassTRAK reagent was added to the solution, which was then analyzed by ultraperformance liquid chromatography system using tropospheric ultraviolet detector monitoring at 260 nm and column and eluents supplied by Waters Corp. The relative SD averaged 1.3% for the control samples and $<3\%$ for the amino acids. The accuracy of the method was tested in five concentration levels and averaged 99.1%.

Statistical analysis. Results for lipids and amino acid metabolites are expressed as median (IQR). Data were scaled into 0 mean and unit variance to obtain metabolite profiles comparable to each other. Bayesian model-based clustering was applied on the scaled data to group lipids with similar profiles across all samples. The analyses were performed using MCLUST method (15), implemented in R statistical language (The R Project for Statistical Computing, available at <http://www.r-project.org/>) as package “mclust.” The LCs are described in Supplementary Table 2.

The Mann-Whitney *U* test was used to compare the concentrations of metabolites and lipids between groups. Wilcoxon test was used to compare the concentrations of metabolites between the pre-seroconversion and seroconversion samples. Multivariate analyses for factors associated with individual metabolites were performed using the general linear model. The reported *P* values are not corrected for multiple comparisons. Relationships between first and second samples were analyzed by Spearman rank correlation. For all analyses, a two-tailed *P* value = 0.05 was considered significant. SPSS 18.0 software (SPSS, Chicago, IL) and GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA) were used for data analysis and visualization.

RESULTS

Islet autoimmunity-associated differences in metabolites of amino acid and lipid metabolism. Characteristic metabolite patterns were observed in relation to the appearance of islet autoantibodies. Children who were islet autoantibody-positive had significantly lower median concentrations of methionine (27.9 vs. 33.7 $\mu\text{mol/L}$, $P = 0.005$) and hydroxyproline (24.9 vs. 28.5 $\mu\text{mol/L}$, $P = 0.04$) compared with children who were islet autoantibody-negative and had higher median concentrations of lipids in the functionally diverse LC1 (0.3 vs. -0.1 , $P = 0.01$) and LC8 (0.5 vs. -0.5 , $P = 2 \times 10^{-10}$) (Fig. 2), both of which are dominated by polyunsaturated fatty acid-containing PCs and specific TGs. All measured PCs in LC8 were significantly higher in islet autoantibody-positive children (Supplementary Table 3).

For each of methionine, hydroxyproline, LC1, and LC8, a multivariate analysis was performed to determine whether autoantibody positivity remained significantly associated with metabolite differences after considering the potential confounder's proband (mother or father with type 1 diabetes), *HLA DR-DQ* genotype (The Environmental Determinants of Diabetes in the Young [TEDDY] risk genotypes or other) (16), whether the sample was known to have been collected after fasting, and the age of sample collection.

Islet autoantibody positivity remained significantly associated with methionine ($P = 0.002$) and LC8 ($P = 3 \times 10^{-11}$) concentrations. None of the confounders were significantly associated with methionine or LC8 concentrations. Islet autoantibody positivity remained associated with methionine ($P = 0.003$) and LC8 lipids ($P = 4 \times 10^{-10}$) if only children who developed multiple islet autoantibodies were considered. Differences in methionine and in LC8 concentrations could not be attributed to any particular autoantibody (IAA or GADA) or to the number of autoantibodies observed (Supplementary Table 4).

Islet autoimmunity-associated differences in metabolites in relation to age of seroconversion. Methionine and LC8 lipids were analyzed in relation to the age of antibody development (Fig. 3). Methionine concentration differed markedly in the children who developed islet autoantibodies early compared with those who developed them late ($P = 0.00009$), but not between the two control groups. The median methionine levels were low in early antibody-positive children (17.4 $\mu\text{mol/L}$) compared with late autoantibody-positive children (30.7 $\mu\text{mol/L}$), early autoantibody-negative control children (36.3 $\mu\text{mol/L}$), and late autoantibody-negative control children (30.5 $\mu\text{mol/L}$; all $P < 0.0001$). In view of the observations in the stratified analysis, a multivariate analysis was performed to test for interaction between islet autoantibody positivity and age at autoantibody appearance in their contribution to methionine concentration. The model that included potential confounders was markedly improved by the inclusion of the interaction. Methionine concentration was significantly associated with islet autoantibody positivity ($P = 5 \times 10^{-11}$), the interaction of islet autoantibody positivity, and age of appearance ($P = 7 \times 10^{-9}$). This was also observed if only the cases of multiple islet autoantibodies were considered ($P = 3 \times 10^{-7}$ for islet autoantibodies; $P = 3 \times 10^{-5}$ for interaction).

Unlike what was observed for methionine, the LC8 lipids were high in autoantibody-positive children regardless of when they developed islet autoantibodies (median 0.74 vs. 0.5, $P = 0.00003$). Consistent with the stratified analysis, inclusion of the interaction between islet autoantibody positivity and the age of seroconversion to the multivariate analysis did not improve the model, and only islet autoantibody positivity was associated with LC8 values.

The differences between methionine and LC8 lipids in relation to islet autoantibodies seroconversion age suggested a phenotype that was unique to the young seroconverters (Fig. 3). Of 13 early autoantibody-positive children, 8 had a low methionine/high LC8 lipid profile compared with only 1 of 22 late autoantibody-positive

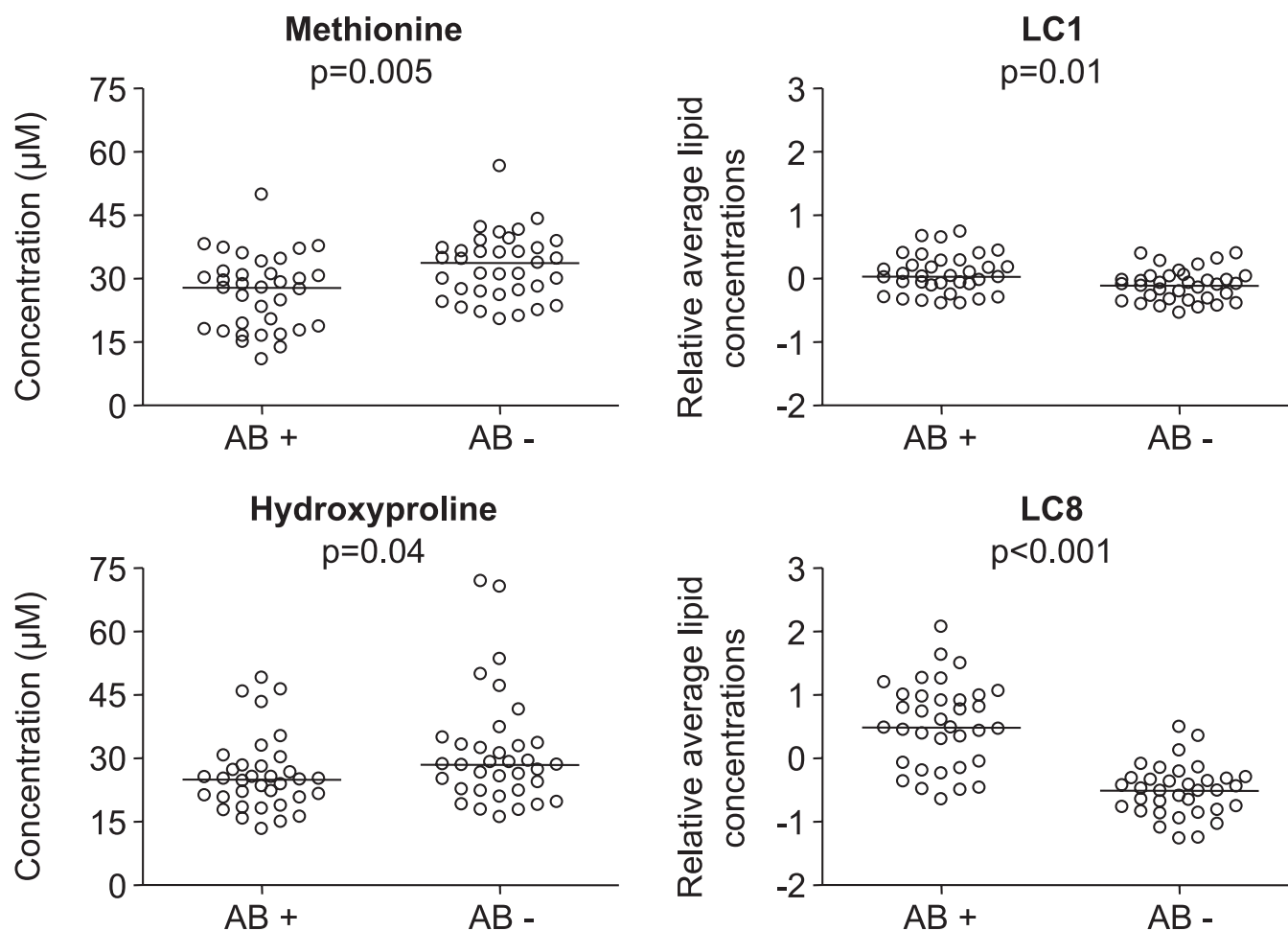


FIG. 2. Islet autoimmunity-associated differences. Concentrations of metabolites of the amino acid metabolism (left panels) and lipid metabolism (right panels) are plotted for islet autoantibody-positive (AB+) children ($n = 35$) and autoantibody-negative (AB-) children ($n = 35$). Only metabolites where significant differences were observed ($P < 0.05$) are shown. Medians are indicated.

children ($P = 0.0004$) and none of the 35 autoantibody-negative children ($P < 0.0001$).

Consistency of metabolite levels over time. Follow-up samples of 63 children, including 13 children who developed islet autoantibodies early, 22 who developed islet autoantibodies late, and 28 islet autoantibody-negative control subjects, were analyzed to determine whether the findings could be reproduced in multiple samples and were persistent or transient as follows:

- A strong correlation between the study sample and the follow-up sample was observed for most analytes (Supplementary Tables 5 and 6).
- The LC8 lipids remained significantly different between autoantibody-positive and -negative children when follow-up samples were analyzed ($P = 10^{-5}$).
- Differences in methionine with respect to islet autoantibody positivity and age of seroconversion at the seroconversion time point were also consistent in the follow-up samples (Fig. 4), with a significant contribution to methionine concentration by autoantibody positivity ($P = 4 \times 10^{-6}$) and the interaction of autoantibody positivity and age at seroconversion ($P = 4 \times 10^{-6}$).
- The low methionine/high LC8 lipid profile was again restricted to early islet autoantibody-positive children (Supplementary Fig. 1).

Amino acid metabolites preseroconversion. Samples collected before seroconversion and stored at -80°C were available from children who developed islet autoantibodies at age 2 years or younger and the matched autoantibody-negative control children. At a median age of 1.0 year (IQR 0.6–1.3), no significant differences between the two groups were observed for the concentrations of amino acid metabolites (data not shown), including methionine (Fig. 5A). Methionine concentrations decreased at seroconversion in the early autoantibody-positive children ($P = 0.002$), whereas they increased over the same period in the children who remained islet autoantibody-negative ($P = 0.0007$) (Fig. 5B). Glutamine, which the Diabetes Prediction and Prevention Project (DIPP) showed was decreased preseroconversion (10), increased in concentration from the preseroconversion sample to the seroconversion sample ($P = 0.007$), but this was also observed for the autoantibody-negative control children ($P = 0.007$).

Age-related differences in metabolites of amino acid and lipid metabolism. In addition to the differences associated with autoantibody status, significant age-related differences of metabolite levels were observed when the study sample was analyzed from all 70 children (Supplementary Fig. 2 and Supplementary Tables 7 and 8). Compared with older children, young children had lower median

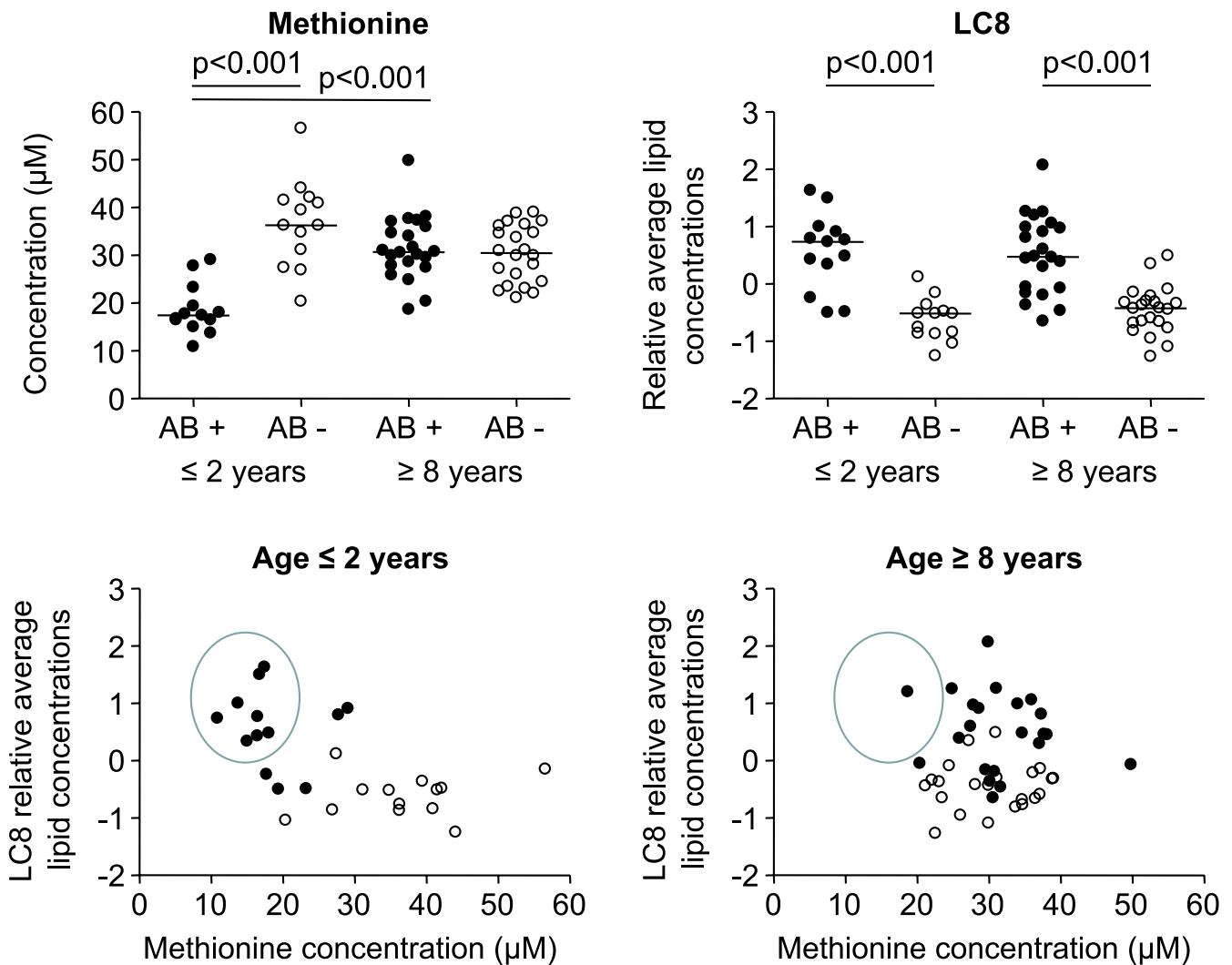


FIG. 3. Islet autoimmunity-associated differences stratified by age. *Upper panels:* Concentrations of methionine and LC8 are plotted for islet autoantibody-positive (AB+) children developing AB at age ≤ 2 or ≥ 8 years, and age-matched autoantibody-negative (AB-) children, respectively. Medians are indicated. *Lower panels:* Methionine concentrations are plotted against LC8 concentrations for children age ≤ 2 years (*lower left panel*) and children age ≥ 8 years (*lower right panel*). Concentrations are negatively correlated in the younger age-group ($r = -0.6$, $P = 0.001$) but do not correlate in the older age-group ($P = 0.9$). Islet AB+ children (●) and AB- children (○) are indicated. Circled are children with a low methionine/high LC8 profile. (A high-quality color representation of this figure is available in the online issue.)

concentrations ($\mu\text{mol/L}$) of the amino acids glutamine (627.4 vs. 717.0, $P = 0.004$), arginine (59.5 vs. 100.5, $P = 0.008$), citric acid (27.0 vs. 32.9, $P = 0.006$), and glycine (167.3 vs. 197.5, $P = 0.0004$). Younger children also had significantly lower median concentrations of lipids in LC2, which predominantly contained LPCs (-0.7 vs. -0.3 , $P = 5 \times 10^{-9}$), PC containing LC3 (-0.2 vs. 0.1 , $P = 0.001$), sphingomyelin containing LC4 (-0.3 vs. 0.1 , $P = 0.002$), and PE containing LC6 (-0.3 vs. 0.1 , $P = 0.004$), and higher concentrations of short chain fatty acid-containing TGs in LC10 (0.3 vs. -0.4 , $P = 0.00004$), and saturated fatty acid-containing TGs in LC12 (0.003 vs. -0.3 , $P = 0.005$). Many of these age-related differences were also observed if the analysis was performed in the follow-up samples available from 63 children (glutamine, $P = 0.001$; LC2, $P = 5 \times 10^{-9}$; LC6, $P = 0.02$; and LC12, $P = 0.02$).

DISCUSSION

Metabolomic endophenotype is being used to identify pathways leading to the identification of disease pathogenesis.

In type 1 diabetes, we have identified distinct endophenotypes of islet autoimmunity: one developing early, within the first 2 years of life, and one developing during puberty. As major findings of our study, we found that islet autoimmunity is associated with differences in lipid and amino acid concentrations, and in particular, children with early autoimmunity have markedly lower concentrations of methionine compared with children who develop islet antibodies late and with children who remain antibody-negative.

The study used current methods in metabolomic profiling on a unique set of samples. Profiling identified few—but consistent—differences in the panel of metabolites that were analyzed (29 amino acid metabolites and 12 LCs). Importantly, the major findings were confirmed in a second set of serum samples from the study cohort. The study was able to distinguish differences that were related to autoimmunity per se (lipids within LC8), those that were specifically associated with seroconversion at an early age (methionine), and those that were simply related to the age of the subject.

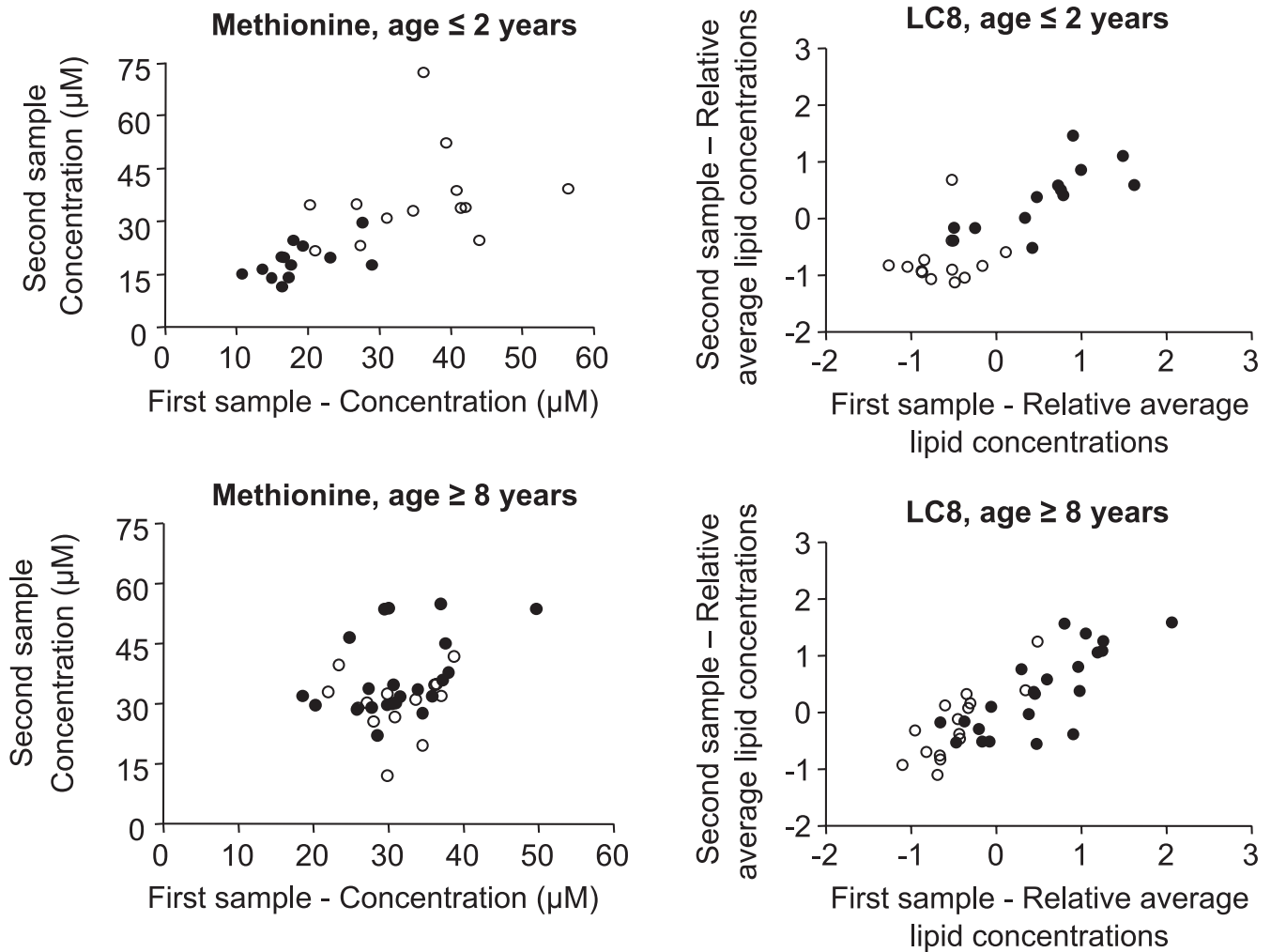


FIG. 4. Metabolite concentrations are relatively consistent over time. The concentrations of methionine (*left panels*) and LC8 (*right panels*) in the first sample at seroconversion and respectively matched samples from autoantibody-negative (AB⁻) children are plotted against the concentrations in a second sample obtained 1 year later. Correlations are shown for children age ≤ 2 years (*upper panels*) and ≥ 8 years (*lower panels*). Islet autoantibody-positive (AB⁺) children (●) and AB⁻ children (○) are indicated.

The study had some limitations. In particular, we were unable to test early samples from the late autoimmunity group and we cannot be certain that metabolite concentrations in these children were altered early in life. Also, the study design included overnight shipping of samples, which may have affected the concentrations of some of the analytes. We performed a small study to address the effect of sample processing time on stability of serum metabolites and did not observe significant differences in methionine concentration when samples were processed immediately after blood drawing or after 1, 5, and 24 h of exposure to room temperature (Supplementary Fig. 3). Furthermore, all samples were treated similarly, and the consistency of data in follow-up samples supports the integrity of the findings. Finally, variables such as fasting state of the child, the proband, and HLA genotype did not alter the major findings of the study.

Islet autoantibody-positive children had increased concentrations of odd-chain TGs and polyunsaturated fatty acid-containing phospholipids. The differences in lipids were independent of whether children developed islet autoimmunity early or late and did not define a distinct age-related autoimmunity phenotype. It is notable that these lipids contain the essential fatty acids as well as the

odd-chain fatty acids. Odd-chain fatty acids are not endogenously synthesized in the body but are abundant in some diets such as milk. In fact C15:0 and C17:0 fatty acids have been considered as biomarkers of dietary milk fat intake (17). However, the experimental setting of our study and consistency of changes with age do not favor the notion that diet alone is behind the observed increase of odd-chain TGs in the autoimmune children. Altered absorption of lipids or formation of TGs from fatty acids in enterocytes are perhaps more plausible factors that could explain the findings.

A second major finding was a reduction of methionine concentration from around seroconversion in children with early but not late autoimmunity, supporting our hypothesis that early and late autoimmunity have distinct features. Methionine was the only essential amino acid that displayed such an association with islet autoimmunity, so it is unlikely that the observed changes are due to dietary differences. Instead, changes in whole-body methionine metabolism are a more likely explanation.

Methionine is involved in various metabolic processes in the human body, including acting as 1) a substrate to protein synthesis, 2) an intermediate in transmethylation reactions, 3) an intermediate in catabolic pathway of choline,

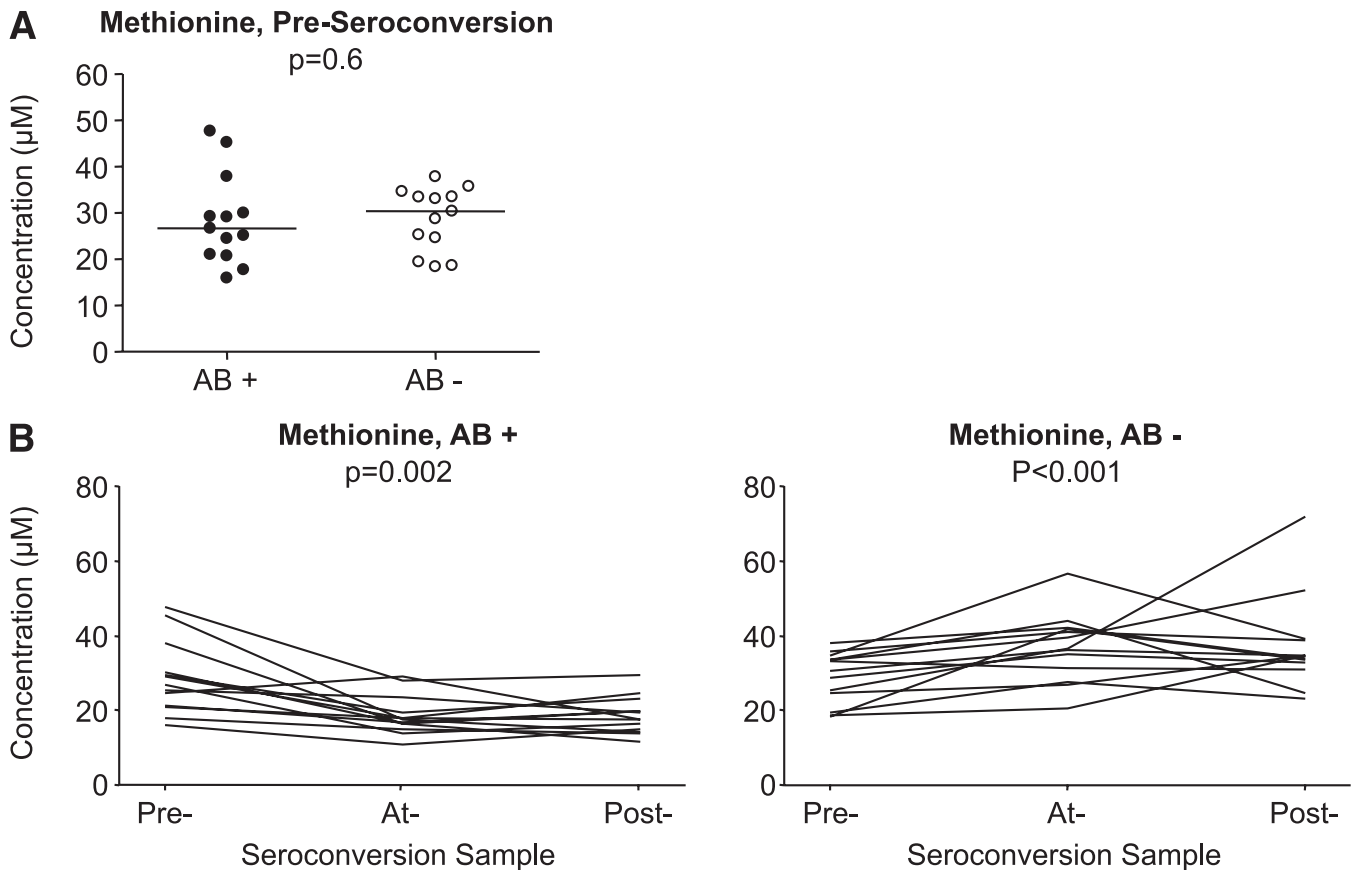


FIG. 5. Methionine concentrations pre-seroconversion. **A:** Comparison of methionine concentration in the pre-seroconversion samples from early autoantibody-positive (AB+) children and their matched control subjects. **B:** Methionine concentrations in sequential samples from pre-seroconversion to post-seroconversion in the early AB+ children ($n = 13$, left panel) and their matched control subjects ($n = 13$, right panel). AB-, autoantibody-negative.

and 4) a precursor of other sulfur amino acids, including cysteine and taurine. Methionine turnover due to transmethylation is about fourfold higher than that due to protein synthesis and breakdown (18). Specifically with respect to a potential relationship to early pathogenesis of type 1 diabetes, methionine is essential for lymphocyte proliferation (19), and the amino acid starvation response is a potent regulator of T helper 17 cell differentiation (20). In addition, methionine is a major source of methyl groups for DNA methylation, mediated by transmethylation metabolic pathways (21,22). Although methionine was not tested in the Finnish DIPP study, a reduction of choline-containing phospholipids in cord blood and prospectively in those who progressed to diabetes was observed (10). This could again point to a relevance of transmethylation pathways in type 1 diabetes because choline is also one of the major components of these metabolic pathways.

The current study also examined some of the previously reported findings associated with islet autoimmunity. The concentration of glutamine was reported to be low pre-seroconversion in children who progressed to type 1 diabetes (10). Here, we also observed low glutamine concentrations before seroconversion. However, we observed a strong relationship between glutamine concentration and age, and unlike the previous study in Finnish children, could not confirm a difference in glutamine concentration between autoantibody-positive and seronegative children. Indeed, age was an important confounder in our findings and must be considered when metabolomic data are analyzed.

With respect to previously reported lipid differences, specifically the diminishment of polyunsaturated fatty acid-containing phospholipids and ether phospholipids in children who progressed to type 1 diabetes (10), we did not observe a similar pattern in the current study. Moreover, the lipids from LC8, found elevated in autoantibody-positive children in the current study, did not differ when we compared children who progressed to diabetes and control children in the earlier study (10).

Therefore, despite reproducibility in multiple samples within cohorts, the metabolomic findings are inconsistent between cohorts. This suggests that intrinsic differences between the cohorts and samples from each cohort and/or region-specific extrinsic factors that affect the metabolite concentrations or islet autoimmunity play a role in the antibody/type 1 diabetes associations. The DIPP and BABYDIAB studies differed substantially, with DIPP recruiting genetically susceptible neonates from the general population and BABYDIAB recruiting children of parents with type 1 diabetes. The metabolomic data in the DIPP study compared islet autoantibody-negative children with children who progressed to diabetes regardless of when they became autoantibody-positive, whereas the current study examined islet autoantibody positivity at discrete ages and did not study diabetes progression. In view of the current findings, further metabolomic studies are ongoing in an expanded set of samples from the DIPP cohort to reduce selection bias and to include markers such as methionine. These studies should provide important information

about validation of the current and previous findings and whether metabolomic data can be generalized or are region/cohort specific.

In conclusion, we have observed distinct metabolic profiles associated with age and islet autoimmunity. Our data suggest changes in methionine and lipid metabolism may be altered in the development of islet autoimmunity in genetically predisposed children. Finally, the association of low methionine concentration only in children who develop islet autoimmunity early in life further supports the hypothesis that young age of islet autoimmunity is associated with endophenotypes and mechanisms of islet autoimmunity that are distinct from those observed in children developing islet autoimmunity during puberty.

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M.P. acquired and reviewed data, undertook statistical analysis, interpreted the results, and drafted the manuscript. T.S.-L. and T.S. assisted in obtaining data, undertook analysis, and critically reviewed the manuscript. T.H. established the metabolomic analyses, helped write the manuscript, and critically reviewed the manuscript for intellectual content. P.A. assisted in obtaining data, performed the statistical analysis, interpreted the results, wrote the manuscript, and critically reviewed the manuscript for intellectual content. E.B. performed the statistical analysis, interpreted the results, wrote the manuscript, and critically reviewed the manuscript for intellectual content. M.O. established the metabolomic analyses, performed the statistical analysis, interpreted the results, wrote the manuscript, and critically reviewed the manuscript for intellectual content. A.-G.Z. was the principal investigator, designed the BABYDIAB study and concept, interpreted the results, wrote the manuscript, and critically reviewed the manuscript for intellectual content.

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