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Integrated Lipidomics and Proteomics Point to Early Blood-Based Changes in Childhood Preceding Later Development of Psychotic Experiences: Evidence From the Avon Longitudinal Study of Parents and Children

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ABSTRACT

BACKGROUND: The identification of early biomarkers of psychotic experiences (PEs) is of interest because early diagnosis and treatment of those at risk of future disorder is associated with improved outcomes. The current study investigated early lipidomic and coagulation pathway protein signatures of later PEs in subjects from the Avon Longitudinal Study of Parents and Children cohort.

METHODS: Plasma of 115 children (12 years of age) who were first identified as experiencing PEs at 18 years of age (48 cases and 67 controls) were assessed through integrated and targeted lipidomics and semitargeted proteomics approaches. We assessed the lipids, lysophosphatidylcholines (n = 11) and phosphatidylcholines (n = 61), and the protein members of the coagulation pathway (n = 22) and integrated these data with complement pathway protein data already available on these subjects.

RESULTS: Twelve phosphatidylcholines, four lysophosphatidylcholines, and the coagulation protein plasminogen were altered between the control and PEs groups after correction for multiple comparisons. Lipidomic and proteomic datasets were integrated into a multivariate network displaying a strong relationship between most lipids that were significantly associated with PEs and plasminogen. Finally, an unsupervised clustering approach identified four different clusters, with one of the clusters presenting the highest case-control ratio ($p < .01$) and associated with a higher concentration of smaller low-density lipoprotein cholesterol particles.

CONCLUSIONS: Our findings indicate that the lipidome and proteome of subjects who report PEs at 18 years of age are already altered at 12 years of age, indicating that metabolic dysregulation may contribute to an early vulnerability to PEs and suggesting crosstalk between these lysophosphatidylcholines, phosphatidylcholines, and coagulation and complement proteins.

Keywords: ALSPAC, Early life, Integration, Lipidomics, Proteomics, Psychotic episode

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The early identification and treatment of subjects with psychiatric disorders, both psychotic and affective, significantly improves their clinical outcome (1). Consequently, over the last decade, there has been a shift in research focus to a high-risk paradigm for individuals at increased risk for later psychotic disorder (PD) (2–4). Research over the past 15 years has revealed that 8% to 17% of children and adolescents (5) and 7% of adults (6) in the general population report psychotic experiences (PEs). It is known that these individuals who report subclinical symptoms in early life are at increased risk of PD (7,8) as well as other disorders (9,10).

The identification of a biological signature of psychotic illnesses can provide insights into pathophysiological basis of the disorders (11,12) and also has the potential to be used as a part of biomarker signature for early detection and diagnosis (13). Recent research on schizophrenia and related psychoses has highlighted a number of metabolic perturbations such as glucoregulatory processes (14,15), lipid metabolism (16–18), mitochondrial function (19), proline (13), and tryptophan metabolism (20), with the most consistent findings involving pathways common to fatty acids and the pro-oxidant/antioxidant balance (21–23). A recent systematic review of

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metabolite biomarkers for schizophrenia by Davison *et al.* (24) revealed that although definite consistencies have been described in the literature, none of the potential biomarkers have been validated reproducibly in large cohorts. Essential polyunsaturated fatty acids, lipid-peroxidation metabolites, phosphatidylcholines (PCs) and lysophosphatidylcholines (LPCs), glutamate, 3-methoxy-4-hydroxyphenylglycol, and vitamin E emerged from this review as potential biomarkers (24), emphasizing the hypotheses of oxidative stress and inflammation (25) and membrane phospholipid alterations (26). While these studies have contributed to our understanding of the disease mechanisms, they generally focus on the adult population that has already transitioned to psychosis, with a majority being medicated. These studies are therefore limited in terms of identifying early molecular signatures of the disease.

To address this issue, we recently applied broad metabolomics, lipidomics, and shotgun and semitargeted proteomics approaches to plasma samples from children at 12 years of age who were reported to develop PD at 18 years of age, from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort (27). We observed increased PCs and LPCs, and complement and coagulation proteins among these subjects during childhood (22,23). These findings provided intriguing support for the view that psychosis is associated with a broad range of inflammatory (23,28), glucoregulatory (29), and lipid (22) dysregulation from early childhood. The interrelationship between these early lipid and protein changes has not yet been investigated. In the current investigation, we have extended our previous work by testing the hypothesis that altered LPCs and PCs and the family of coagulation proteins are associated with not only outcomes of PD, but also the milder phenotype of PEs. Specifically, lipidomic and semitargeted proteomic approaches were employed to semitarget PCs and LPCs and coagulation proteins at 12 years of age among apparently well subjects who go on to develop PEs at 18 years of age in the ALSPAC cohort. These data were then integrated with other complement protein data available of the same subjects to assess the broader functional relationships between these proteins and lipids at 12 years of age among those who later report PEs at 18 years of age.

METHODS AND MATERIALS

Study Cohort

The study comprised subjects from the ALSPAC cohort. The ALSPAC cohort is a prospective general population cohort that includes 14,062 live births from southwest England (30,31). Written informed consent was acquired before taking the plasma samples. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees (RCSI REC 1240). The study website contains details of all the data that is available through a fully searchable data dictionary (http://www.bristol.ac.uk/alspac/researchers/our-data/).

Measures of PEs and Comorbid Depression

PEs were identified at 12 and 18 years of age through the face-to-face, semistructured Psychosis-Like Symptoms interview (27), conducted by trained psychology graduates in assessment clinics, and were coded according to the definitions and rating rules for the Schedules for Clinical Assessment in Neuropsychiatry, Version 2.0 (32). Interviewers rated PEs as not present, suspected, or definitely psychotic. Patients were also assessed for the presence of depressive disorder according to the ratings on the Clinical Interview Schedule–Revised whereby Clinical Interview Schedule–Revised scores >7 are defined as fulfilling criteria for depression (28).

Study Design

We undertook a nested case-control study of the ALSPAC cohort and chose to assess all available plasma samples from 12-year-old children with outcomes of definite PEs at 18 years of age but who did not have PD (27). Available plasma samples from controls of age-matched individuals were then randomly selected. The present study consisted of a hypothesis-driven lipidomic and proteomic analysis of samples from 48 children without suspected or definite PEs at 12 years of age but with definite PEs at 18 years of age (n = 48). Control samples (n = 67) without suspected or definite PEs at 12 and 18 years of age were selected (see Table 1). Socioeconomic status and presence of depression according to Clinical Interview Schedule–Revised scores were also tested.

Plasma Sampling

Nonfasting blood samples were collected from the participants into heparin S-Monovette tubes (Sarstedt, Nümbrecht, Germany). Once collected, samples were stored on ice for a maximum of 90 minutes until processed. Postcentrifugation, the samples were stored at −80°C until further analyses.

Lipidomic Analysis and Data Preprocessing

Sample processing, data acquisition, and quantification of lipids were performed as previously described (22). Lipidomic analysis was performed using an ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry system (Agilent Technologies, Santa Clara, CA).

Lipidomic data were first processed using MZmine 2 (33), then normalized by lipid-class specific internal standards, and finally quantified using the inverse-weighted linear model (see Supplement). Analysis of lipidomics data was focused on detected PCs (n = 61) and LPCs (n = 11) based on our previous findings (22).

Table 1. Descriptive Data of the ALSPAC Individuals Included in the Study

	Cases	Controls	p
Participants, n	48	67	
Male/Female, n	22/26	39/28	.19
BMI, kg/m^2, Mean ± SD	18.16 ± 2.85	17.73 ± 2.53	.40

Descriptive information was compared between cases and controls. Statistical comparisons are from Pearson chi-square or Student's t test as appropriate.

ALSPAC, Avon Longitudinal Study of Parents and Children; BMI, body mass index.

Proteomic Analysis and Data Preprocessing

Sample analysis and data acquisition proteins were performed in the same individuals as examined in the current lipidomic analysis and using methods as previously described (23). To improve the dynamic range for proteomic analysis, 40 μL of plasma from each case in all samples was immunodepleted of the 14 most abundant proteins (34) (see Supplement).

Protein digestion and peptide purification was performed as previously described (35) and is further detailed in the Supplement. We used the semitargeted approach of data independent acquisition (DIA) to specifically target 22 members of the coagulation pathway (see Supplemental Table S1). For DIA analysis, 5 μL of each sample was injected into the Thermo Scientific Q-Exactive, connected to a Dionex Ultimate 3000 (RSLCnano; Thermo Fisher Scientific, Bremen, Germany) chromatography system, and data were acquired in DIA mode (see Supplement).

Statistical Analysis

To assess differences of demographic data among groups, Pearson chi-square test and independent Student's *t* test were used on categorical and continuous variables, respectively.

Early PEs Signatures at 12 Years of Age. Principal component analysis was used on the log-transformed, mean-centered, and scaled-to-unit-variance lipidomics dataset to acquire an overview of the data. For supervised data analysis, uni- and multivariate approaches were performed.

For univariate analysis, the Mann-Whitney *U* test was applied to the untransformed dataset to examine changes of lipids and proteins as related to PEs. Benjamini-Hochberg false discovery rate was applied to account for multiple comparisons.

Multivariate modeling of PEs was performed on the log-transformed data using a partial least squares discriminant analysis of lipidomic profiles with the KODAMA R package v 1.4 (36). Modeling was performed in a repeated double cross-validation framework (37). The goodness of fit and prediction parameters were defined using a standard description reported elsewhere (38). The features were ranked in ascending order based on the absolute loading scores (termed as loading rank) (39). Model performance was further assessed through permutation testing (R^2), considering a statistical significance at $p < .05$.

Lipidomics and Proteomics Integration. Regularized canonical correlation analysis was performed on all individuals as an integrative multivariate approach to assess correlations between both lipidomics and proteomics data using the mixOmics R package v 5.2.0 (40).

The method allows the study of the relationship of two multivariate datasets, for instance, the relationship between specific lipids and proteins within the same individuals (41). Quantitative data, derived from DIA analysis, on the broad family of complement pathway proteins were also available on these same subjects (42), and these data were available for integrative analysis. Regularization parameters were estimated by means of a leave-one-out cross-validation. Once the regularized canonical correlation analysis was acquired, the corresponding clustered heat maps, termed clustered image maps, and the integrative network were acquired (43). Data were then exported to Gephi 0.9.2 (44), and the layout algorithm Yifan Hu was used to allow the biological interpretation (45). The network graph describes connections between lipids and proteins based on a similarity score >.3 (45). To evaluate obtained multivariate correlations, a further Spearman correlation analysis was implemented for each variable individually, considering the significant correlation at a *p* value of <.05.

Identification of Metabolic Phenotypes. The unsupervised algorithm based on knowledge discovery by accuracy maximization (KODAMA) (46) was used to identify the underlying patterns representative of different metabolic phenotypes across all individuals. This learning algorithm allows an unsupervised clustering of individuals from noisy high-dimensional datasets (36). The partition around medoids method (47) along with a silhouette algorithm (48) were carried out on KODAMA scores to identify the optimal distribution of clusters (49). Further descriptions of this method are shown elsewhere (36,49). The demographic data and cholesterol profile were then tested among the identified clusters using the K-test. This method predicts an independent variable using the variance in the KODAMA scores by means of permutation testing (49,50). Thus, causality of phenotyping was explored by other variables (49) such as the cholesterol profile and demographics. Data on cholesterol profile including cholesterol esters and lipoprotein particle data of selected individuals at 7 years of age were measured and reported elsewhere (30,51). Statistical significance was considered at a false discovery rate–corrected *p* value of $< .05$.

All statistical analyses were performed in the statistical programming environment R version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). Data used for this article will be made available on request to the ALSPAC Executive Committee (alspac-exec@bristol.ac.uk).

RESULTS

The lipidomic dataset that was used to investigate potential biomarkers of PEs in children 12 years of age who reported PEs at 18 years of age included 61 PCs and 11 LPCs. PCs and LPCs were the focus because of previous results showing a potential lipidomic signature of PD with elevated levels of PCs and LPCs (22). The proteomic dataset that we assessed contained 22 members of the coagulation pathway (Supplemental Table S1) as defined by KEGG pathway analysis (http://www.genome.jp/kegg/pathway.html).

There were no significant differences between the control group and the PEs group in terms of gender, body mass index (BMI), or social class (data not shown). As expected, there was an excess of depression cases among those with PEs compared with controls, with 9 subjects in the PEs group reaching criteria for depression and no cases in the normal control group. Variance in the lipid profiles of individuals was first explored using principal component analysis. No grouping could be observed through principal component analysis when examining factors such as PEs, gender, and BMI.

Early PEs Signatures at 12 Years of Age

Univariate analysis revealed that a total of 34 molecular lipids and 3 coagulation proteins (plasminogen [PLG], coagulation factor XI, alpha2-antiplasmin) were different between PEs and healthy controls at the nominal $p < .05$ level (Table 2). After false discovery rate correction, 16 lipids and one protein (PLG) remained significantly increased. For multivariate analysis, partial least squares discriminant analysis entailed a resulting model ($R^2Y = .3$) with a permutation test $p < .05$. Interestingly, there is a strong agreement between uni- and multivariate analyses performed individually, in which the lowest p values matched the highest loading scores and, thus, lowest loading rank. Significant changes of PCs and LPCs with p value and loading rank corresponding to uni- and multivariate analyses, respectively, are also presented in Table 2.

Lipidomics and Proteomics Integration

The coagulation and complement pathway proteins are closely functionally related. For this reason, we included in our integrative analysis of lipids and proteins the levels of complement proteins in the total dataset for which there were data available (42). The regularized canonical correlation analysis revealed that 17 lipids have a positive correlation with six proteins (PLG, heparin cofactor 2, complement C2, complement factor H, clusterin, and vitronectin), which exceeded a similarity score higher than 0.3. A strong positive relationship with the 16 lipids was observed for coagulation proteins PLG, heparin cofactor 2, and the complement pathway protein vitronectin (Figure 1). A relevance network graph illustrates other minor connections observed for complement proteins clusterin, complement C2, and complement factor H (Figure 2). Interestingly, PLG had the highest number of connections, followed by vitronectin and heparin cofactor 2. Table 3 shows specific lipid connections with PLG, with 10 lipids showing a correlation exceeding a similarity score higher than 0.3.

Table 2. Differential Plasma Lipids and Proteins Between the Control and PEs Groups

Compound	Control Group	PEs Group	p	FDR	LR
Lipid					
PC(34:1)	2571.91	3013.09	.0002	.0066	1
PC(34:2)[a]	3759.47	4303.88	.0002	.0066	2
PC(32:1)	238.88	319.25	.0011	.0161	3
PC(36:4)[a]	135.46	160.55	.0023	.0241	4
PC(36:2)	2940.24	3421.47	.0003	.0067	5
LPC(16:1)	38.27	41.69	.0080	.0361	6
LPC(18:1)[a]	231.84	273.67	.0029	.0259	7
LPC(20:3)[a]	37.21	41.58	.0050	.0259	8
PC(36:1)	721.67	945.44	.0008	.0137	10
LPC(18:2)[a]	394.75	486.68	.0045	.0259	11
PC(38:2)	70.50	86.11	.0023	.0241	12
PC(O-38:6)	28.13	33.58	.0037	.0259	14
PC(38:3)	616.10	752.18	.0079	.0361	15
PC(30:0)	56.88	73.01	.0098	.0414	16
PC(32:0)	175.51	204.39	.0041	.0259	17
PC(36:3)	1753.26	2059.53	.0049	.0259	23
Protein					
PLG[b]	843,597,014.93	1,052,478,260.87	.0006	.0138	–
F11	16,925,970.15	19,053,478.26	.0304	.2379	–
SERPINF2	487,134,328.36	542,565,217.39	.0324	.2379	–

The p value of the Mann-Whitney U test and loading rank of double cross-validation partial least squares discriminant analysis are shown.

F11, coagulation factor XI; FDR, false discovery rate; LPC, lysophosphatidylcholine; LR, loading rank; PC, phosphatidylcholine; PD, psychotic disorder; PEs, psychotic experiences; PLG, plasminogen; SERPINF2, alpha2-antiplasmin.

[a]Increased compounds in agreement with O'Gorman *et al*. (22) including PD individuals.

[b]Increased compounds in agreement with English *et al*. (23) including PD individuals.

Underlying Clustering in the Data

To detect potential underlying metabolic phenotypes present in the study population, the KODAMA algorithm was applied to all individuals with available clinical data ($n = 90$). Following this, partition around medoids clustering was performed on KODAMA scores to identify underlying similar phenotypes in this study population. According to the highest silhouette median value (Supplemental Figure S1), four different clusters were identified (Figure 3), named A, B, C, and D. Interestingly, PEs occurrence was significantly different among clusters ($p = .007$). Furthermore, neither BMI nor gender was statistically significant across the clusters (Table 4). Likewise, depression status and social class were not significantly different across the clusters ($p > .05$ in both variables, data not shown). Further examination of the clusters revealed that cluster D exhibited a high probability of developing PEs. This cluster exhibited a PEs occurrence of 71%, while clusters A, B, and C showed a PEs occurrence of 42%, 29%, and 19%, respectively.

Clusters were then examined for associations between the cholesterol data with the resulting KODAMA scores. In total, nine cholesterol parameters (different parameters related to low-density lipoprotein [LDL], very low-density lipoprotein, and intermediate-density lipoprotein with specific particle sizes) were significantly associated with the clustering (Supplemental Table S3). Similarly, KODAMA score plots were performed (Supplemental Figure S3), colored by the resulted clusters, PEs occurrence, gender, and BMI. Score plots color coded by the concentration of small LDL particles and the phospholipids to total lipids ratio in small LDL particles were also performed for visualization and interpretation purposes. There was a significant difference in distribution of PEs cases across the clusters (Supplemental Figure S3B). Interestingly, the levels of certain lipoproteins across the clusters were also statistically different (Supplemental Table S3). Of particular note were differences in the small LDL particles and phospholipid to total lipid ratio in small LDL particles, with a similar distribution to PEs cases. Additional cholesterol-related parameters are shown in Supplemental Figure S4. In summary, cluster D represented a metabolic phenotype with a high probability of developing PE.

DISCUSSION

The present findings point to early dysregulation of both the lipidome and proteome several years before the development of PEs. Our findings are relevant to PD, anxiety disorder, and

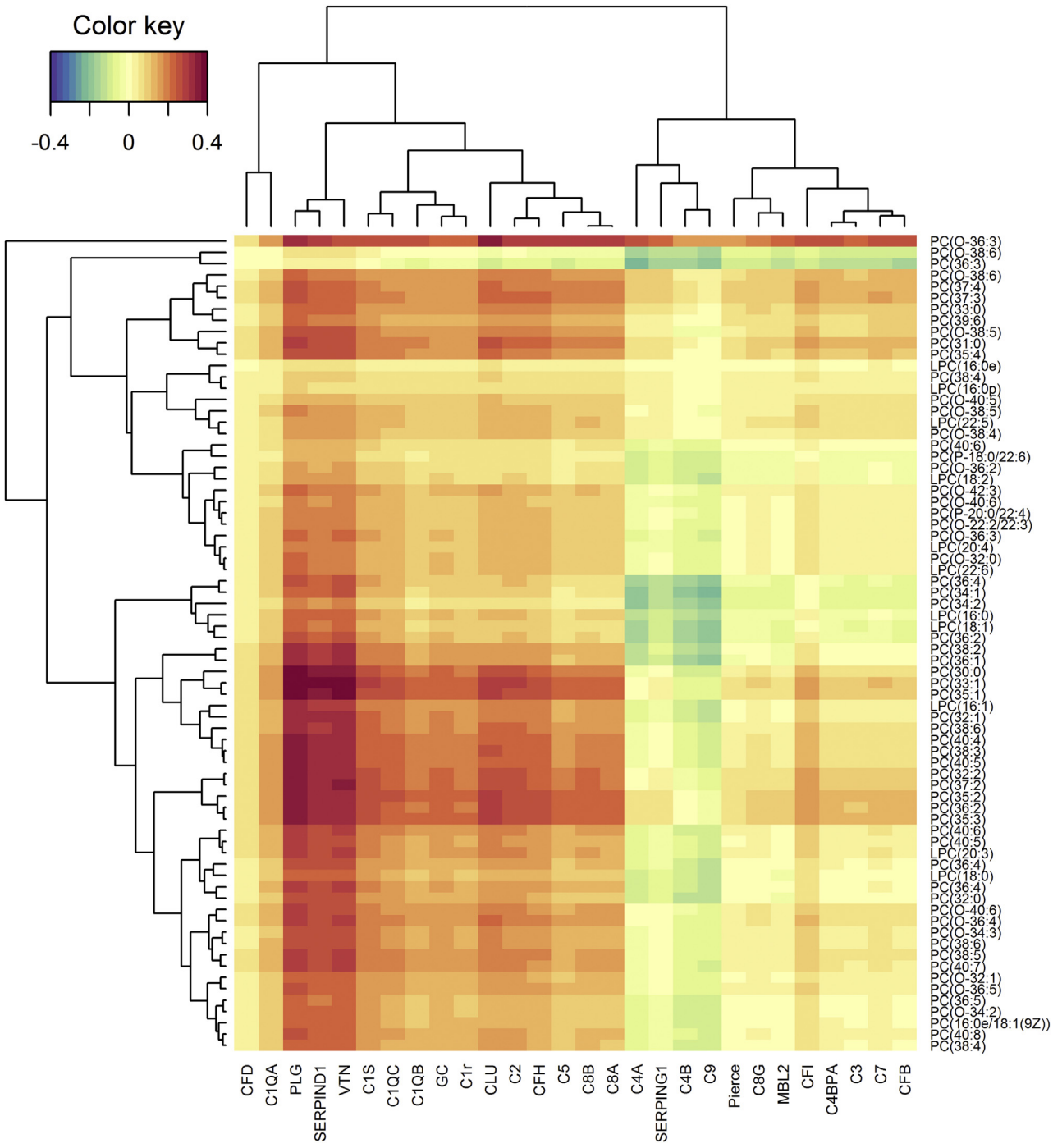

Figure 1. Heatmap analysis performed by using regularized canonical correlations analysis showing the relation between proteomic and lipidomic datasets. For proteomic data, the gene names are displayed. Correlation strengths are indicated by the color key.

depression, as approximately 20% to 30% of subjects who experience PEs go on to develop PD (52), with approximately 50% to 60% going on to develop other psychiatric comorbid disorders (2). The present findings support the literature that phospholipid metabolism and the proteins of the coagulation cascade are abnormal in schizophrenia and depression (26,53–55) and extend this literature by providing evidence for such alterations in early childhood before the development of PEs. Furthermore, the present findings are broadly in line with our findings from the previous discovery metabolomics, lipidomic, and proteomic study in the ALSPAC cohort, in which we demonstrated similar changes at 12 years of age for

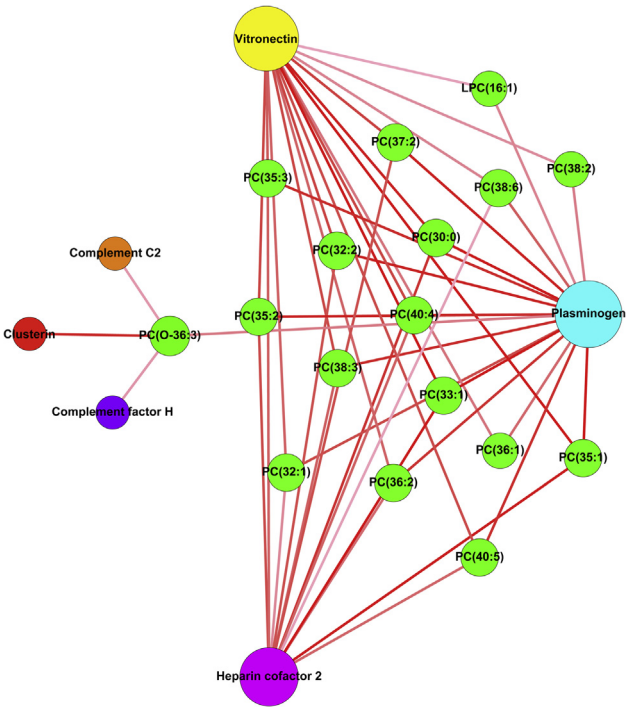

Figure 2. Relevance network graph depicting correlations derived from regularized canonical correlation analysis between lipids and proteins based on a similarity score >.3 (45). Nodes (circles) represent variables and are sized according to number of connections. Lines are colored according to association score with augmented intensity indicating higher correlation scores. LPC, lysophosphatidylcholine; PC, phosphatidylcholine.

Table 3. Significant Lipids Correlated With Plasminogen From Multi- and Univariate Approaches on the PEs Dataset

Lipid	rCCA	Spearman Correlation	*p*
PC(30:0)[a]	.38	.27	.005
PC(32:0)[a]	.26	.19	.043
PC(34:1)[a]	.24	.26	.006
PC(40:6)	.29	.19	.047
PC(32:1)[a]	.33	.28	.003
PC(38:2)[a]	.31	.20	.039
PC(38:3)[a]	.35	.22	.019
PC(36:1)[a]	.32	.22	.022
PC(35:1)	.39	.25	.007
PC(36:4)[a]	.28	.23	.014
LPC(16:1)[a]	.31	.24	.010
PC(40:5)	.35	.27	.004
PC(40:4)	.35	.26	.006
PC(33:1)	.40	.34	.001
PC(37:4)	.24	.20	.032
PC(36:3)[a]	.22	.19	.043
PC(O-36:3)	.31	.24	.013
PC(31:0)	.28	.21	.029

The *p* values of Spearman correlation analysis are shown. Results are listed for the 18 significant compounds using a *p* value < .05.

LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PEs, psychotic experiences; rCCA, regularized canonical correlation analysis.

[a]Significant lipids associated with PEs development in the present study.

subjects who later went on to develop PD (22). The findings have the potential to contribute to risk calculators for future psychotic illness and mental disorders (4,56,57) as well as to an increased understanding of psychosis and psychiatric illness as a multisystem disorder involving lipids and proteins (22,23,29). Critically, a novelty of our study lies in the integration of proteomic and lipidomic data, specifically of the PCs and LPCs and the protein members of the complement and coagulation cascades from the same subjects. In so doing, we have identified a robust yet unexpected interdependence of these biological processes that underpin psychotic disease. A tangible advance derived is that our findings highlight early lipid and protein changes associated with vulnerability to a broad range of PD and, in so doing, identify potential novel therapeutic targets.

There is no simple interpretation of the findings of early LPC and PC changes in relation to later psychiatric diseases. However, it is noteworthy that several lines of evidence implicate altered LPC and PC levels in early life and medical morbidities in later life (58). First, Hellmuth *et al.* (59) observed a positive correlation between LPCs in cord blood during pregnancy and early weight gain and later-life high BMI. Second, Rzehak *et al.* (60) showed that LPC(14:0) and PC(38:3) measured at 6 months of age positively correlated with overweight/obesity at 6 years of age. Similar to our findings, these observations suggest an early metabolic alteration that can trigger later disorder (60). Third, a cross-sectional study of early life suggested an inverse association between obesity and LPC(18:1), LPC(18:2), and LPC(20:4) in obese individuals between 6 and 15 years of age (61). These LPCs were also found at lower levels in obese children between 7 and 15 years of age in another cross-sectional study (62). Fourth, an investigation of adults sampled in the Western Australian Pregnancy Cohort showed decreased LPC(18:2) and LPC(18:1) levels in obese subjects compared with normal-weight individuals independent of LDL and high-density lipoprotein cholesterol levels, while LPC(14:0) and PC(32:2) were positively correlated with homeostatic model assessment of insulin resistance, as a measure of insulin resistance, in the same study (63). Overall, these studies suggest elevation of certain LPCs preceding later metabolic disorder and PD.

Perry *et al.* (29) recently showed an association between insulin resistance at 9 years of age and PEs at 18 years of age in the ALSPAC birth cohort. Insulin resistance was also associated with inflammation markers suggesting that inflammation and metabolic risk factors interact to increase risk of psychosis in some people (29). In relation to this, although opposite effects have also been reported (64,65), reduced levels of specific LPCs have been connected with insulin resistance (45), impaired glucose tolerance (66), and progression to diabetes (67). Furthermore, schizophrenia has been associated with a high prevalence of other comorbid disorders such as diabetes (68), metabolic syndrome (69), and cardiovascular disease (70). Therefore, the early biomarkers such as LPC(18:2), PC(34:2), and PC(32:1) found in the present study may reflect a shared vulnerability to both psychosis and cardiometabolic disorders (58,67,71). Previous lipidomic studies in

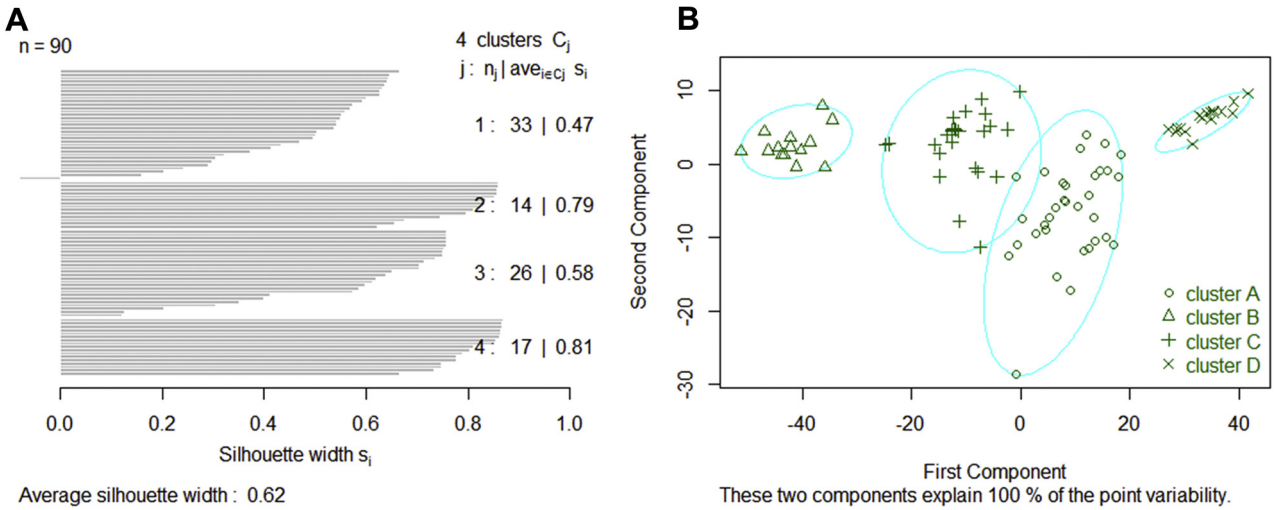

Figure 3. Partition around medoids analysis of the knowledge discovery by accuracy maximization output: **(A)** silhouette plot of partition around medoids including the optimal number of clusters (j), individuals at each cluster (n_j), and the average silhouette width by samples ($ave_{i∈Cj}$ S_i); **(B)** clustering according to the calculated silhouette mean values.

psychosis have identified elevated plasma levels of LPC(16:0), LPC(18:0), LPC(18:1), and LPC(18:2) in first-episode neuroleptic drug-naïve schizophrenia patients as compared with healthy control subjects (72). However, there are inconsistencies in the reported literature, with one study reporting diminished levels of LPCs in the serum of schizophrenia patients compared with their co-twins as well as healthy control subjects (16).

Both the coagulation and the complement pathways have recently been highlighted in schizophrenia (57,73,74). Our current study used the semitargeted proteomic method of DIA to extend these findings and show that upregulation of PLG within the coagulation pathway at 12 years of age is associated with later PEs. This more complete analysis of the coagulation pathway proteins in PEs was then combined with complement pathway protein data already available to us on the same subjects (42) to allow a unique integration of lipidomic, complement, and coagulation data. Our integrative network analysis demonstrates that PLG had the strongest connections to PCs and LPCs that were increased in the PEs group. The role of PLG as a carrier for PCs and LPCs was previously investigated by Edelstein *et al.* (75), who suggested that oxidized PCs are integral components of circulating PLG, and Leibundgut *et al.* (76), who showed that PLG covalently binds oxidized phospholipids that influence fibrinolysis, which has known roles associated with neuroinflammation and neurodegeneration (77). Therefore, increased PLG such as we described in PEs is very consistent with higher specific PC and LPC concentrations in the PEs group. Our findings of elevated levels of PLG in subjects who later report PEs are intriguing in light of recent evidence that blood-derived PLG drives brain inflammation (78) and evidence that alpha2-antiplasmin, which is the main inhibitor of PLG-derived plasmin, is upregulated in schizophrenia (79). Interestingly, proteomics studies discovered a high number of complement and coagulation proteins as lipoprotein-associated components, such as complement 4A, complement C4B, vitronectin, clusterin, complement factor H, alpha1/2-antiplasmin, and kininogen, among others (80). There is a surprisingly strong overlap with the proteins that correlate with phospholipids in this study and those that are upregulated in schizophrenia (11). Together, the data provide a link among phospholipid binding proteins, (apo)lipoproteins, complement, and coagulation, and they support growing literature implicating these processes in neuroinflammation and neurodegeneration (77,81).

Schizophrenia may represent an etiologically heterogeneous disorder, with some subjects having a largely inflammatory basis and some an autoimmune etiology (23,82,83). Similarly, it is appreciated that there are heterogeneous outcomes among subjects who experience PEs (2). This may have relevance to the results of KODAMA (36) analysis in which we identified four main clusters, of which cluster D was associated with a high probability of subjects within that cluster experiencing PEs. Interestingly, the lipoprotein particle size parameters were also significantly different across the clusters, with cluster D having

Table 4. Descriptive Data of the ALSPAC Individuals by Cluster

	Cluster A	Cluster B	Cluster C	Cluster D	*p*
PEs, Cases/Controls, *n*	14/19	4/10	5/21	12/5	.007
Male/Female, *n*	17/16	8/6	13/13	11/6	.781
BMI, kg/m^2, Mean ± SD	17.43 ± 2.29	17.95 ± 3.51	18.88 ± 2.68	17.33 ± 2.72	.170

Descriptive information was compared between clusters. Statistical comparisons are from Pearson chi-square or Student's *t* test as appropriate.
ALSPAC, Avon Longitudinal Study of Parents and Children; BMI, body mass index; PEs, psychotic experiences.

increased levels of small LDL particles. Smaller LDL particles are more susceptible to oxidation than larger particles, being more frequently associated to metabolic diseases (84–86). However, in the present study, the oxidation status and lipidomic analyses on specific LDL particle size were not included at 12 years of age, and thus the results should be interpreted with caution. Future studies evaluating different LDL subtypes might clarify these observed associations.

The present study has several strengths: the longitudinal ALSPAC cohort was used and included both longitudinal clinical assessments and biosampling. The use of samples before disease onset rules out the potential confounding from medications. Furthermore, in contrast to most other studies, our study focused on children who were well at the time of biosampling, unlike other studies, in which the subjects already had experienced a first episode of psychosis. The multiomics integration has allowed a unique insight into the existence of a functional relationship between these lipids and proteins that was unknown previously in the context of psychosis. Future work may look at the broader relationship between proteome and lipidome beyond those specific compounds that we described as discriminant for PEs prediction in this study. A number of limitations should also be acknowledged. First, the lack of validation in a similar cohort of subjects with PEs is a limitation. Second, while depletion of high-abundance proteins did not impact PLG, three of the 22 proteins had been depleted, so they were interpreted with caution. We did not covary for depression, as depression can be considered a transdiagnostic comorbidity, and thus our findings are not necessarily specific to PEs. This is reasonable, as PEs are accepted to represent a vulnerability to a broad range of psychiatric illnesses (2).

Conclusions

Our study provides evidence for protein and lipid signatures at 12 years of age in subjects who are apparently well but who report PEs at 18 years of age. These changes are not necessarily specific to PEs, as overlapping changes have been observed previously at 12 years of age in subjects who later develop PD (22) and are also observed in association with prediabetes and obesity, and before other cardiometabolic disorders (61,63,70), suggesting that these disorders share aspects of their developmental origins. Although there are inconsistences in the literature in terms of metabolic disorders and schizophrenia (24,87), the present study strongly suggests that there is early vulnerability to the development of PEs and that this involves molecular interconnections between the lipidome and the proteome.

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