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Dysregulated Lipid Metabolism Precedes Onset of Psychosis

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ABSTRACT

BACKGROUND: A key clinical challenge in the management of individuals at clinical high risk for psychosis (CHR) is that it is difficult to predict their future clinical outcomes. Here, we investigated if the levels of circulating molecular lipids are related to adverse clinical outcomes in this group.

METHODS: Serum lipidomic analysis was performed in 263 CHR individuals and 51 healthy control subjects, who were then clinically monitored for up to 5 years. Machine learning was used to identify lipid profiles that discriminated between CHR and control subjects, and between subgroups of CHR subjects with distinct clinical outcomes.

RESULTS: At baseline, compared with control subjects, CHR subjects (independent of outcome) had higher levels of triacylglycerols with a low acyl carbon number and a double bond count, as well as higher levels of lipids in general. CHR subjects who subsequently developed psychosis ($n = 50$) were distinguished from those that did not ($n = 213$) on the basis of lipid profile at baseline using a model with an area under the receiver operating curve of 0.81 (95% confidence interval = 0.69–0.93). CHR subjects who became psychotic had lower levels of ether phospholipids than CHR individuals who did not ($p < .01$).

CONCLUSIONS: Collectively, these data suggest that lipidomic abnormalities predate the onset of psychosis and that blood lipidomic measures may be useful in predicting which CHR individuals are most likely to develop psychosis.

Keywords: At-risk mental state, Clinical high risk for psychosis, Lipid metabolism, Lipidomics, Mass spectrometry, Schizophrenia

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A key clinical challenge in the management of individuals at clinical high risk for psychosis (CHR) is that it is difficult to predict their clinical outcomes (1). Identifying biomarkers that could be used to stratify CHR subjects according to these different outcomes would facilitate more personalized clinical intervention in this group.

Recent studies have shown that metabolic comorbidities such as weight gain, insulin resistance, altered glucose metabolism, and dyslipidemias are common in patients with psychotic disorders (2–4). Although these can arise as a result of unhealthy lifestyles and treatment with antipsychotic medication (5), there is growing evidence that they are already present at the onset of psychosis in patients who are not obese and are medication naïve (6,7).

Changes in the concentrations of specific groups of metabolites, including lipids, are sensitive and specific to several factors that can affect the risk of psychosis, such as genetic variation, environmental exposure, neurodevelopment, age, immune system function, and stress (8–11). Metabolomics has therefore emerged as a powerful tool for the characterization of host-environment interactions and complex phenotypes like psychosis (11). In patients who have recently developed psychosis, rapid weight gain is associated with alterations in circulating lipids that are linked with nonalcoholic fatty liver disease (NAFLD) and insulin resistance (10,12,13). However, the extent to which metabolic abnormalities are evident in CHR individuals before the onset of illness has not been investigated.

We addressed this issue by measuring the levels of circulating molecular lipids in a large sample of CHR individuals and examining their relationship to the onset of psychosis and other clinical outcomes in this group. We performed comprehensive mass spectrometry (MS)–based lipidomics in serum samples that were collected at baseline from a cohort of CHR individuals. This cohort was then followed up for at least 2 years to determine their clinical outcomes. We first tested the hypothesis that the CHR group (independent of clinical outcome) would show alterations in lipidomic measures compared with a healthy

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control (HC) group. Our second hypothesis was that within the CHR sample, the levels of molecular lipid measures at baseline would be associated with 3 clinical outcomes at follow-up, specifically, persistence of symptoms, transition to psychosis, and a low level of functioning.

METHODS AND MATERIALS

Study Population

The EU-GEI (European Network of National Schizophrenia Networks Studying Gene-Environment Interactions) High Risk study is a multicenter longitudinal observation study of CHR individuals. A total of 344 CHR individuals identified according to Comprehensive Assessment of At-Risk Mental States (CAARMS) criteria (14) were recruited at 11 sites (Amsterdam, Barcelona, Basel, Cologne, Copenhagen, London, Melbourne, Paris, São Paulo, The Hague, and Vienna). Sixty-seven HC subjects were recruited at 4 of these sites (Amsterdam, London, Melbourne, and The Hague). Each site obtained ethical permission for the study, and written informed consent was obtained from all participants. CHR and HC participants were interviewed at baseline, had repeated assessments at 12 and 24 months, and then further clinical follow-up for up to 5 years. Within the CHR group, 65 (18.9%) developed psychosis during the study, 57 within 2 years and 8 after 2 years.

Inclusion and Exclusion Criteria. Presence of the inclusion criteria for the CHR state were determined with the CAARMS. Exclusion criteria for CHR and HC were past or present diagnosis of a psychotic disorder, inadequate understanding of the language local to the site, and not able or willing to provide a blood or saliva sample. Previous exposure to antipsychotic medication was not an exclusion criterion for CHR subjects, but it was for HC subjects.

Demographic and Clinical Measures. Data on age, sex, ethnicity, height, and weight were obtained using the modified Medical Research Council Sociodemographic Schedule (15). At baseline and follow-up, trained raters assessed participants using the CAARMS and the Global Assessment of Functioning (GAF) split scale (16). Inter-rater reliability was assessed using online CAARMS and GAF training videos. The results of that assessment have been previously published (17). In brief, GAF = 0.83, CAARMS (Positive Items, intensity scores) = 0.78, CAARMS (Positive Items, frequency scores) = 0.90. CHR participants were determined to be in remission if they no longer met the criteria for the CHR state (assessed using the CAARMS) at follow-up. Demographic characteristics of the study population are presented in Table 1.

Lipidomic Analysis

Nonfasting serum samples, stored at −80°C, were analyzed using an established global lipid profiling protocol, based on ultra-high-pressure liquid chromatography (UHPLC) coupled with high-resolution quadrupole time-of-flight MS (18), with the data processed using the MZmine (version 2.18) open source software package (19). The protocol is described in detail in the Supplemental Methods.

Statistical Methods

Statistical Design. At the baseline, the lipidomics dataset was divided into 2 study groups, CHR (n = 263) and HC (n = 51). Subsequently, during the follow-up period, some CHR subjects underwent symptomatic remission, such that they no longer met clinical criteria for the CHR state (CHRr), some had persistent symptoms of the CHR state (CHRp), and others developed psychosis (CHRt) (Figure 1).

Data Transformation, Quality Control, and Visualization. The data were $\log_2$ transformed. Homogeneity of the samples were assessed by principal component analysis (20), and no outliers were detected (Figure S1). The R statistical programming language (version 3.6.0) (21) was used for data analysis. Principal component analysis was performed using the *prcomp* function included in the *stats* package. *Heatmap.2*, *boxplot*, *beanplot*, *gplot*, and *ggplot2* libraries and packages were used for data visualization.

Quantifying the Effect of Factors on the Lipidome. The effect of different factors such as age, sex, body mass index (BMI), site, subject (study group) status, ethnicity, use of antipsychotics, and transition status on the lipidomics dataset were evaluated. The data were centered to zero mean and unit variance (autoscaled). The relative contribution of each factor to the total variance in the dataset was estimated by fitting a linear regression model where the normalized intensities of metabolites were regressed to the factor of interest, and thereby median marginal coefficients (R^2) were estimated. This analysis was performed using the *Scater* package.

Model-Based Clustering. Clustering of the lipidomic data was applied by using the *mclust* R package (version 5.4.5). *mlust* is a model-based clustering method where the model performances are evaluated by the Bayesian information criterion (Figure S2). Generally, the model with the highest Bayesian information criterion is chosen. At first, lipidomic clusters (LCs) were generated for the HC group, and the mean value of the lipids in each cluster was estimated. Similarly, clustering was performed on the lipidome from the total CHR sample, and the mean value of the lipids in each cluster was estimated. A 2-sample t test was performed to assess the significance of the mean difference in the intensities of the lipids between the HC and CHR groups within each cluster. Differences in the intensities of the lipids between the CHR subgroups and HC were assessed using 1-way analysis of variance, followed by Tukey's honestly significant difference at a significance threshold of $p < .05$.

Sparse Partial Least Squares Discriminant Analysis. Pairwise sparse partial least squares discriminant analysis (sPLS-DA) (22) models, comparing the lipid intensities of CHR versus HC at baseline and follow-up, were developed, and variable importance in projection scores (23) were calculated. sPLS-DA modeling was performed using the *splsda* function coded in the *mixOmics* package (version 6.3.2). Moreover, the sPLS-DA models were cross-validated by 7-fold cross-validation and model diagnostics were generated using the *perf* function.

Table 1. Demographic Characteristics of the Study Population

Variable	Control	Clinical High Risk
No. of Subjects	51	263
Age, Years	22.76 ± 4.13	22.47 ± 4.84
Sex		
Male	29	141
Female	22	122
Body Mass Index, kg/m^2	22.69 ± 3.45	24.18 ± 4.96
Ethnicity[a]		
White	33	184
Black	9	23
Mixed	4	25
Asian	5	9
North African	0	11
Other	0	10
Baseline GAF Disability Score	85.82 ± 10.51	54.89 ± 11.43
No. of Transitions	–	50
CAARMS Positive Symptoms (≥1)		
Disorganized speech	–	164
Perceptual abnormalities	–	218
Unusual thought content	–	197
Use of Antipsychotic Medication	–	28

Values are presented as mean ± SD or *n*.

CAARMS, Comprehensive Assessment of At-Risk Mental States; GAF, Global Assessment of Functioning.

[a]Ethnicity could not be determined for 1 CHR participant.

A Consensus Multiunivariate Approach for Variable Selection. The multivariate analysis was complemented by univariate analysis (2-sample *t* test) using the *t.test* function, in order to identify differences in the concentration of individual lipids between CHR and HC groups at baseline and follow-up. The consensus approach enabled us to select the altered lipid species with higher confidence. All lipids that passed the alpha criterion of variable (lipid) selection from both multi- and univariate approaches, that is, with sPLS-DA model areas under the receiver operating curve (AUCs) ≥ 0.6, regression coefficients > 0.05, variable importance in projection scores > 1, and $p_{adjusted}$ (*t* test) $<.1$, were listed as significant at baseline; *p* values were subjected to false discovery rate adjustment using *p-adjust*.

However, the mean differences in the lipid intensities between the CHR subgroups and/or HC at follow-up did not pass the false discovery rate ($p_{adjusted} < .1$), and therefore a nominal *p* value threshold ($p < .01$) was used for the selection of the lipids based on the univariate analysis. These lipids also exhibited regression coefficients > 0.05 and variable importance on projection scores > 1, as estimated by the multivariate (sPLS-DA) models (AUCs ≥ 0.6).

Twenty-one common lipids that were significantly altered between CHR and HC at baseline or at follow-up had fold changes (FCs) >1.2, which were then considered for predictive logistic ridge regression (LR) modeling.

Partial Correlation Analysis. The *qpgraph* R package (version 2.18.0) was used for partial correlation network analysis. The *qpNrr* function was used to estimate the nonrejection rates of the correlation between the lipids and/or the clinical variables. About 10,000 tests for nonrejection were performed for each pair of variables included in the correlation matrix. This analysis was performed separately for the HC, CHRp, CHRt, and CHRr groups. All the spurious correlations/associations (nonrejection rates ≤ 0.5) were removed. Spearman's rank correlation coefficient was estimated using the *rcorr* function coded in the *Hmisc* package (version 4.2-0).

Penalized LR and Variable Importance. In order to understand the relative importance of LCs and clinical and demographic variables in stratifying HC and CHR subjects at baseline, we performed LR modeling (24). Here, 70% of the lipidome data was used to train the model and 30% as test data. LR modeling was performed using the *cv.glmnet* function in the R package *glmnet* (version 2.0-18). The dataset was sampled for 10,000 times. All of the LR models with AUC > 0.60 (with 10-fold cross-validation) were considered. The mean AUC, mean model coefficient, and standard error for each variable were estimated. Positive or negative coefficient of a variable denotes direct or inverse relationship of that particular variable with the outcome (e.g., classification of CHR vs. HC groups at baseline). A coefficient of zero suggests that no relationship exists between the variable of interest and the outcome.

Predictive LR Modeling and Selection of the Optimal Lipids. LR models were developed to stratify CHR versus HC, CHRt versus CHRp + CHRr, and CHRr versus CHRp + CHRt. Twenty-one significantly changed lipids between HC, CHRp, CHRt, CHRr were used either singly or in combination for LR modeling. A recursive feature elimination scheme was implemented for the optimal selection of the lipids. The lipids in LR models were either incorporated or removed in an iterative manner, starting with all 21 selected lipids. The models were adjusted for age, sex, and BMI. Accuracy of prediction was determined by AUCs. The mean AUC of a model was estimated by bootstrapping, that is, resampling 1000 times with replacements and partitioning (70% training and 30% test sets) of the lipidomic dataset using the *createDataPartition* function coded in the *caret* package (version 6.0.84). The model with the highest mean AUC was considered to be the best model, which was assessed by their receiver operating characteristic curves using the *pROC* package (version 1.15.3). Regularized ridge models in *cv.glmnet* requires a hyperparameter λ. Here, $\lambda_{minimum}$, which corresponds to the minimum cross-validation error, was determined by 10-fold cross-validation. Maximization of sensitivity and specificity of an LR model at an optimal threshold was determined by the Youden index.

Differential Analysis of GAF Scores. To identify differences in the serum lipid levels between the CHR individuals with high or low GAF scores at follow-up, we divided the CHR group into 2 subgroups with either high GAF (>65) or low GAF (≤ 65) at follow-up, corresponding to relatively good or poor levels of functioning, respectively. Welch's *t* test was used to assess the differences in the mean lipid levels.

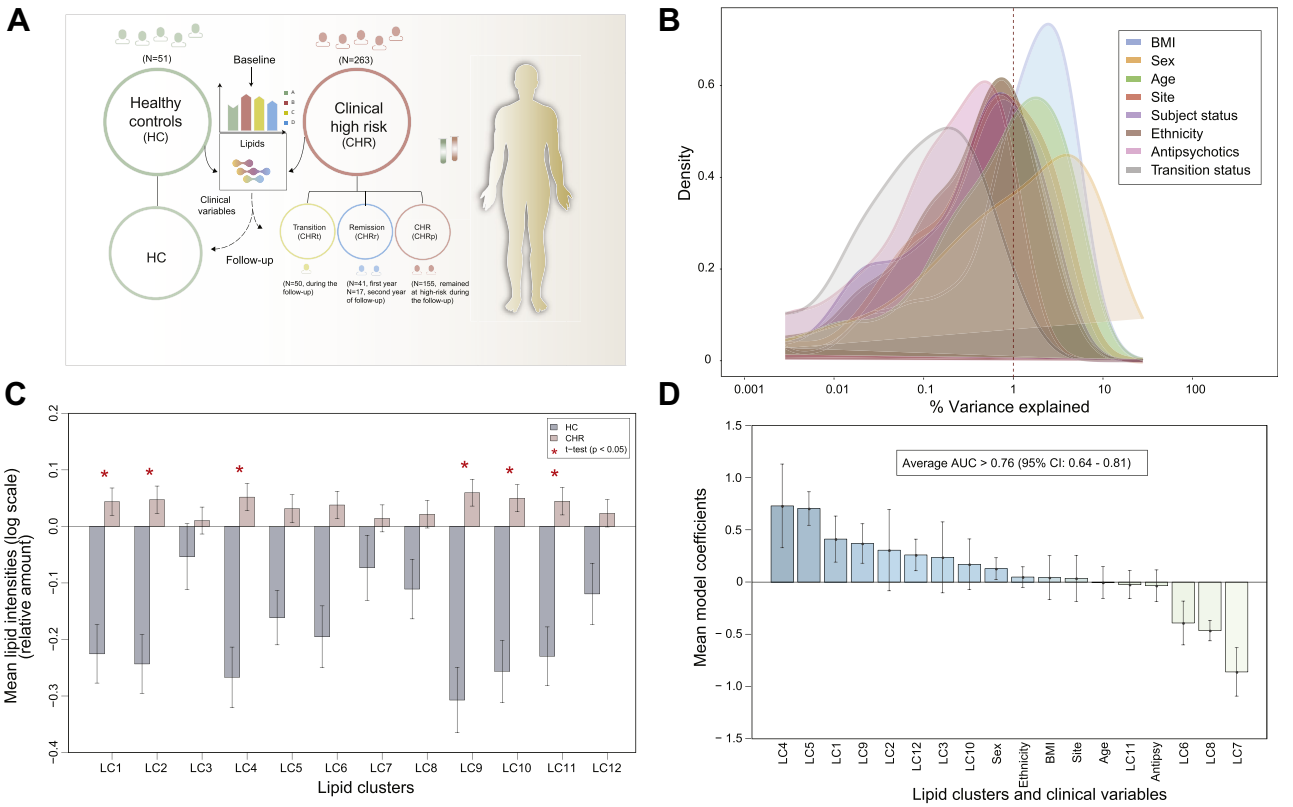

Figure 1. Study overview. **(A)** Study design and description. **(B)** Contributing factors affecting the variance of the lipidome. % of explained variances of all the factors across the lipid samples are shown. **(C)** Lipid clusters and their mean differences between clinical high risk (CHR) and healthy control (HC) groups. *Statistically significant difference (2-sample *t* test: $p < .05$) of group means. Lipid types within each cluster are presented in Table 2. **(D)** Model coefficients of different lipid clusters and demographic variables for classifying CHR vs. HC. Antipsy, antipsychotics; AUC, area under the receiver operating curve; BMI, body mass index; CI, confidence interval.

RESULTS

Lipid Signature of CHR Group

Lipidomic analysis, using UHPLC-MS, was performed on baseline serum samples from CHR individuals (n = 263) and HC subjects (n = 51) (Figure 1A; Table 1). A total of 173 identified lipids (based on matched UHPLC-MS/MS spectra) were included in the final lipidomic dataset (Table S1). Among these lipids, the following classes were represented: cholesterol esters (CEs), ceramides (Cers), lysophosphatidylcholines

Table 2. Lipid Types in Each Cluster at Baseline

Cluster	Size	Lipid Type(s)	Representative Lipids in the Cluster
LC1	24	CE, Cer, PC, PI	CE(20:3), PC(40:5), PI(18:0/20:4)
LC2	10	TG	TG(16:0/18:2/22:6), TG(54:7), TG(56:8)
LC3	9	Cer, TG	Cer(d18:1/18:1), TG(50:2), TG(56:6)
LC4	18	PCs, SM	PC(16:0/16:0), SM(d34:1), SM(d41:1)
LC5	11	LPC, LPE	LPC(20:3), LPC(16:1), LPE(18:1)
LC6	22	PCs, SM	PC(16:0/18:1), SM(d18:2/14:0), SM(d32:1)
LC7	22	PC, PE	PC(O-22:2/22:3), PC(O-40:5), PE(P18:0/22:6)
LC8	5	PE	PE(16:0/18:1), PE(34:2), PE(38:6)
LC9	16	TG	TG(14:0/16:0/18:1), TG(14:0/18:1/18:1), TG(48:3)
LC10	23	TG	TG(18:1/18:1/16:0), TG(54:2), TG(56:2)
LC11	9	TG	TG(18:0/18:0/18:0), TG(51:2), TG(55:5)
LC12	4	TG	TG(O-50:1) or TG(P-50:0), TG(O-52:1) or TG(P-52:0), TG(O-52:2) or TG(P-52:1)

CE, cholesterol ester; Cer, ceramide; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholines; PE, phosphatidylethanolamine; PI, phosphatidylinositol; SM, sphingomyelin; TG, triacylglycerol.

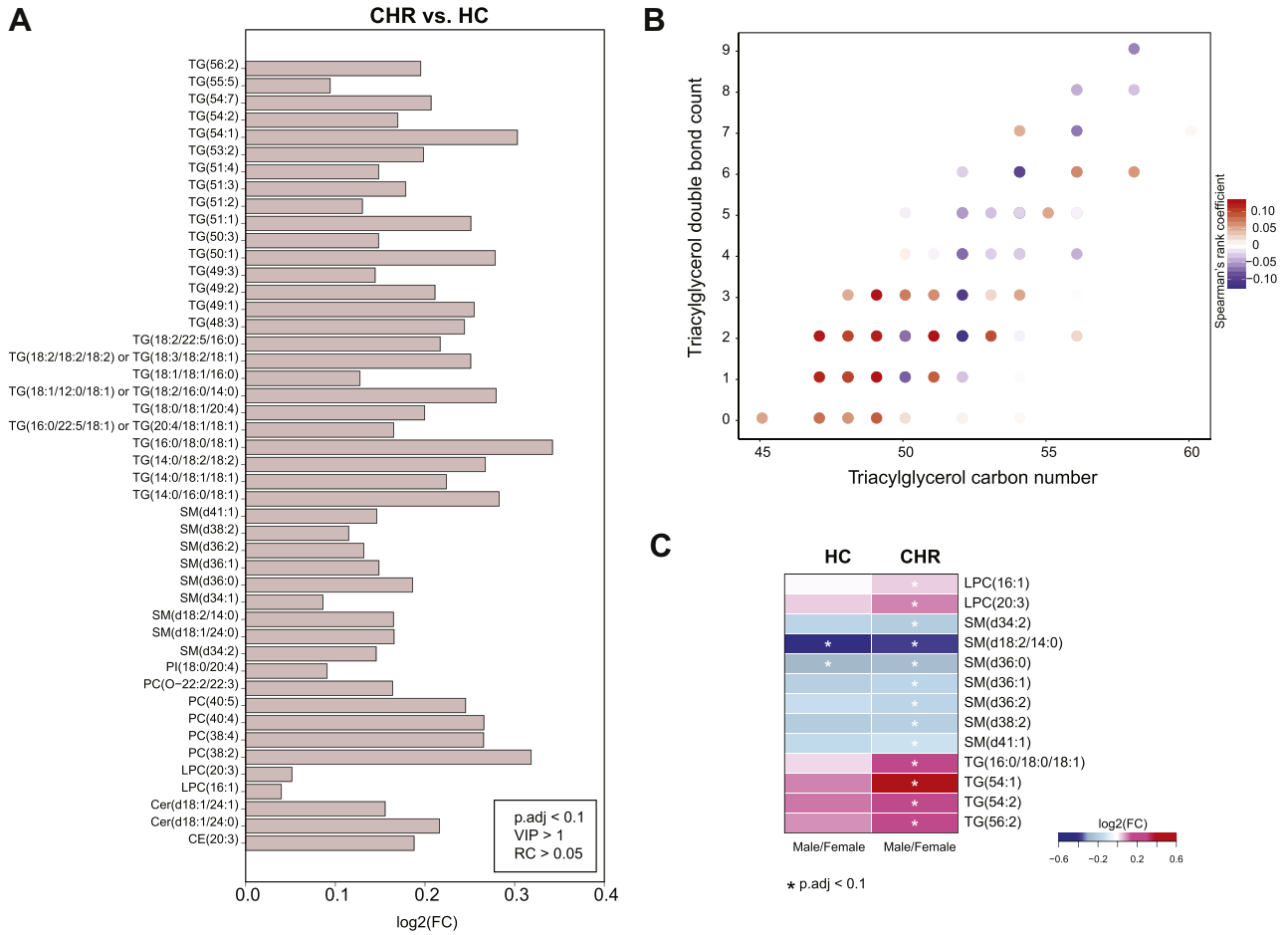

Figure 2. Molecular lipids in clinical high risk for psychosis (CHR). **(A)** Lipid species that are altered between CHR vs. healthy control (HC) groups at baseline, as detected by both multivariate (sparse partial least squares discriminant analysis [sPLS-DA]: areas under the receiver operating curve [AUCs] = 0.60, regression coefficients [RCs] $>\pm0.05$, and variable importance in projection [VIP] scores >1) and univariate (2-sample *t* test: $p_{adjusted}<.1$) analyses. **(B)** Scatter plot showing the number of acyl carbons and double bonds in individual triacylglycerols. Spearman's coefficient (δ) is color coded with red as positive and blue as negative correlation between the triacylglycerol levels in CHR vs. HC. **(C)** List of lipids that are altered between males and females within the CHR and HC groups at baseline, as identified by both multivariate (sPLS-DA: AUCs = 0.60, RCs $>\pm0.05$, and VIP scores >1) and univariate (2-sample *t* test: $p_{adjusted}<.1$) analyses. CE, cholesterol ester; Cer, ceramide; FC, fold change; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; SM, sphingomyelin; TG, triacylglycerol.

(LPCs), lysophosphatidylethanolamines (LPEs), phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), phosphatidylinositols (PIs), sphingomyelins (SMs), and triacylglycerols (TGs).

As expected, the variables that explained most of the lipid variance were BMI, sex, and age (Figure 1B). Therefore, the confounding effects of these variables were taken into account in the regression analyses. The effect of antipsychotics on the baseline lipids levels was, however, minimal, contributing to <0.5% of explained variance.

Owing to the high degree of coregulation between lipids within the same structural class, we analyzed the lipidomics data using model-based clustering, which generated 12 LCs (Table 2; Table S2). At baseline, the levels of LCs 1, 2, 4, 9, 10, and 11 were significantly higher in the CHR group than in the HC group (Figure 1C). LCs 2, 9, 10, and 11 comprised TGs, while LC1 contained a mixture of CEs, ceramides, PCs, and a PI, and LC4 included PCs and some SMs.

Combining the LC data and the clinical variables (Figure 1D), an LR model was built which discriminated between CHR and HC at baseline with AUC = 0.76 (95% CI 0.64–0.82). Based on the mean model coefficients, LC4 and LC5 had the greatest impact on the separation of CHR and HC groups, whereas demographic variables such as ethnicity, BMI, study site, antipsychotics, and age had relatively little effect. Sex was the only demographic factor that had a positive impact on the regression model (Figure 1D). A partial correlation analysis between various demographic variables and lipid clusters in CHR and HC subgroups is shown in Figure S3.

At the individual molecular lipid level, in line with the findings at the LC level, the lipids that passed the threshold test for significance (see Statistical Methods) in both the multivariate and univariate tests were higher in the CHR group than in the HC group (Figure 2A), with more than half being TGs. Most of the TGs that were at higher levels in the CHR group had a low

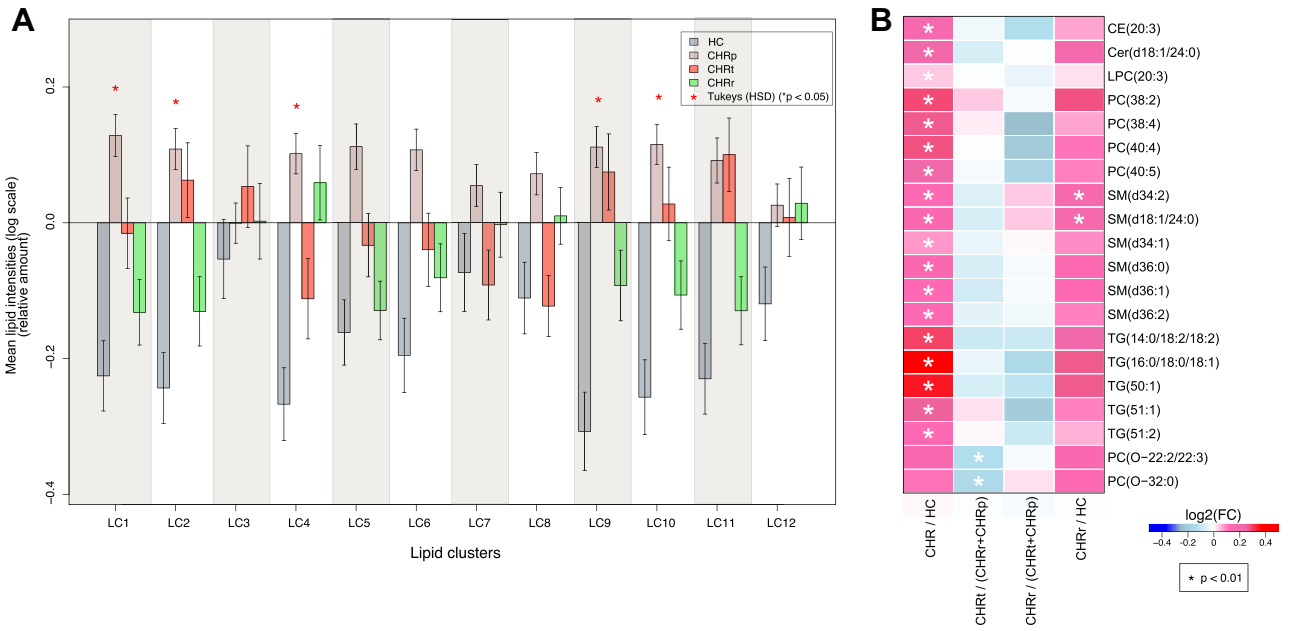

Figure 3. Clinical high risk for psychosis (CHR) and health outcomes in follow-up. **(A)** Lipid clusters at baseline for 4 study groups: CHRp (remained CHR in follow-up), CHRt (transition from CHR to psychosis), and CHRr (remission from CHR), and healthy control (HC). *Statistically significant difference of the group means as determined by analysis of variance and post hoc Tukey's honestly significant difference (HSD). **(B)** List of lipids that were altered between CHR vs. HC, CHRt vs. CHRr + CHRp, CHRr vs. CHRt+CHRp, and CHRr vs. HC groups at baseline and in follow-up (fold changes [FCs] > 1.2) in at least one condition, as identified by both multivariate (sparse partial least squares discriminant analysis: area under the receiver operating curve > 0.60, regression coefficient > ±0.05, variable importance in projection score >1) and univariate (2-sample *t* test: $p_{adjusted} < .01$) analyses. *$p_{adjusted} < .01$.

carbon number and double bond count. In contrast, levels of several TGs containing longer and polyunsaturated fatty acyl chains were lower in CHR subjects than in HC subjects (Figure 2B).

As there was a strong effect of sex in the LR model, we also examined differences in relation to sex. There were 13 lipids that differed by both sex and by group (CHR vs. HC) (Figure 2C, Figure S4). The majority of these were SMs, all of which were at lower levels in male compared with female CHR and HC individuals (Figure 2C; and Figure S4, D–F). Conversely, levels of 4 TGs were higher in male compared with female CHR and HC individuals (Figure 2C; Figure S4, G–I).

Lipid Signatures of Clinical Outcomes in CHR Subjects

The same 12 LCs described above for the CHR versus HC comparisons were used to compare the CHR outcome subgroups and the HC group. Five LCs significantly differed between these subgroups at baseline. For 4 of these clusters (LC1, LC2, LC9, and LC10), the subgroups that contained subjects who had persistent symptoms or were psychotic at follow-up (CHRp and CHRt) had higher levels than the subgroups that contained subjects who were in remission (CHRr) or the HC group (Table 2; Table S2; Figure 3A).

In terms of individual lipids, there was a trend for lower levels in the CHRt subgroup than in the CHR subgroups containing subjects who did not become psychotic (CHRs + CHRr) (Figure 3B). Among these lipids, the levels of 2 ether phospholipids, PC(O-22:2/22:3) and PC(O-32:0), were significantly lower in the CHRt than in the CHRr and CHRp subgroups (Figure 3B). Two lipids, SM(d34:2) and SM(d18:1/24:0), had higher levels in the CHRr subgroup than in the HC group.

Lipids as a Predictor in CHR for Psychosis

Next, we examined whether the lipids that significantly differed ($p < .05$) between the CHRp, CHRt, and CHRr subgroups could be used to stratify individuals within the total CHR sample (Figure 4). These lipids were subjected to LR modeling in an iterative manner, and optimal sets of lipids were identified. Cer(d18:1/24:0), LPC(22:5), PC(38:4), PC(40:5), PC(O-32:0), SM(d18:1/24:0), SM(d36:0), and SM(d36:1) were able to distinguish the total sample of CHR individuals from the HC group with a good level of accuracy (mean AUC = 0.83, 95% CI = 0.71–0.95) (Figure 4A). Cer(d18:1/24:0), LPC(22:5), PC(38:4), PC(40:5), and PC(O-32:0) were able to differentiate CHRt from CHR subjects who did not develop psychosis (mean AUC = 0.81, 95% CI = 0.69–0.93) (Figure 4B). Ether phospholipid PC(O-32:0) was diminished in CHRt compared with CHR individuals who did not develop psychosis (Figure 4E). CHRr were distinguished from CHR subjects who did not achieve remission by Cer(d18:1/24:0), LPC(22:5), PC(38:4), PC(40:5), PC(O-32:0), and SM(d18:1/24:0) (mean AUC = 0.83, 95% CI = 0.72–0.95) (Figure 4C).

Association Between Lipids and Functional Outcomes

When comparing baseline serum lipids between CHR subjects with high and low GAF disability scores at follow-up, no

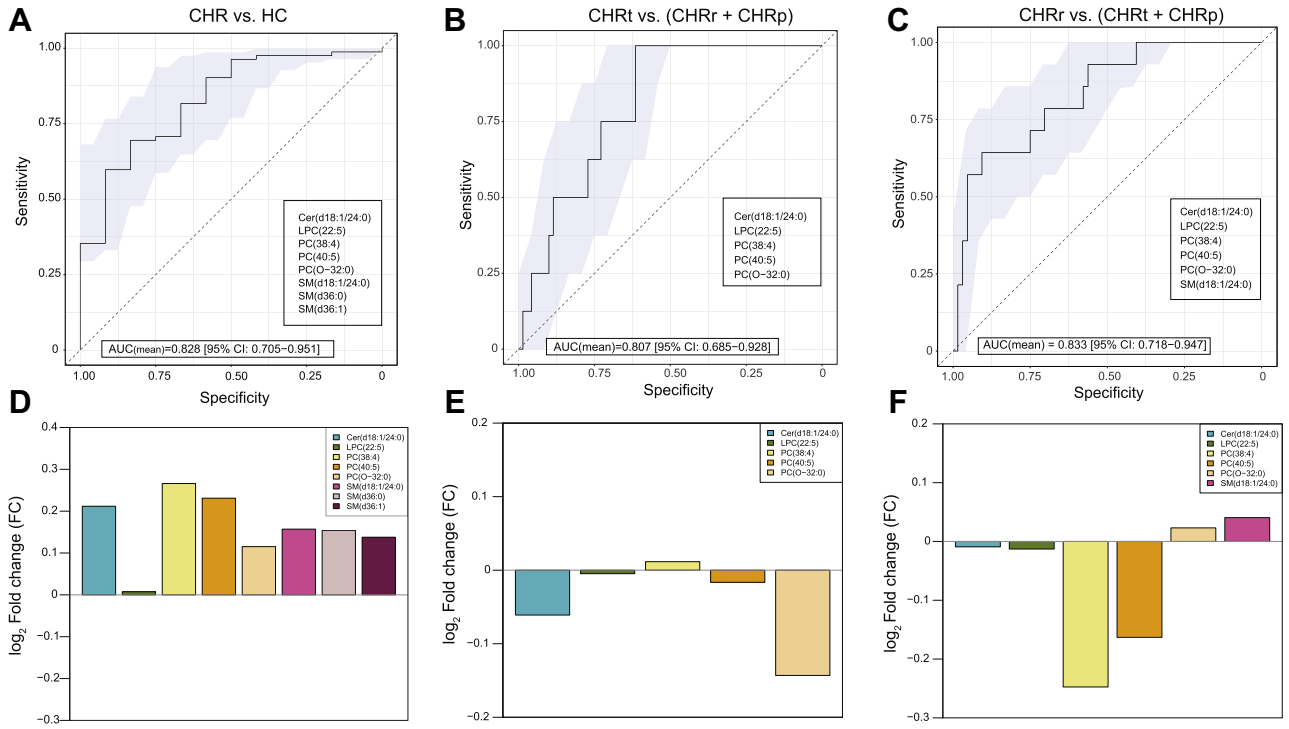

Figure 4. Predictive models of clinical high risk (CHR) conditions. Logistic ridge regression (LR) models showing lipids as predictive markers to stratify patient groups of healthy control (HC) subjects and/or CHR subjects (divided into CHRp [remained CHR in follow-up], CHRt [transition from CHR to psychosis], and CHRr [remission from CHR]). **(A–C)** Receiver-operating characteristic curves showing the performance of the LR models (CHR vs. HC, CHRt vs. CHRp + CHRr, and CHRr vs. CHRp + CHRt) with highest mean areas under the curve (AUCs). The light blue shaded area denotes the 95% confidence intervals (CIs) as calculated using bootstrapping. Specific lipids that compose each of these models are shown. Sensitivity and specificity of the LR models CHR vs. HC, CHRt vs. CHRp + CHRr, and CHRr vs. CHRp + CHRt were, respectively, 0.69 and 0.83, 0.52 and 0.88, and 0.64 and 0.90. Maximization of sensitivity and specificity of an LR model at an optimal threshold was determined by the Youden index. **(D–F)** Log_2 fold change in the intensities of the lipids corresponding to the LR models. CE, cholesterol ester; Cer, ceramide; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; SM, sphingomyelin; TG, triacylglycerol.

significant differences were found after correction for multiple comparisons. Four TGs passed the nominal p value threshold of $p < .05$ (Welch's t test): levels of TG(52:2), TG(52:3), TG(52:4), and TG(56:6) were higher in the subjects with low GAF disability scores (Figure S5). Iterative LR modeling of these TGs identified TG(52:3), TG(52:4), and TG(56:6) as an optimal set for distinguishing between subjects with high and low GAF disability scores at follow-up (mean AUC = 0.80, 95% CI = 0.68–0.90) (Figure S6).

DISCUSSION

Our first major finding was that the blood levels of many lipids were higher in individuals at CHR than in HC subjects. The types of lipid that were different were similar to those previously seen in comparisons of patients with psychosis and HC subjects (25,26). The identification of lipidomic differences before the onset of psychosis is consistent with similar findings in healthy children who subsequently reported psychotic experiences in later life (9) and in siblings of psychotic patients (27), and suggests that altered serum lipid levels are related to an increased vulnerability to psychosis and not simply a secondary consequence of the disorder or its treatment.

The higher levels of TGs in the CHR group were primarily TGs with low acyl carbon number and double bond count (i.e., those containing shorter and more saturated fatty acyl chains). A similar TG lipid signature was previously observed in a subgroup of first-episode psychosis patients who rapidly gained weight after presentation (10). Serum TGs with low acyl carbon number and double bond count are also increased in NAFLD (13) and precede clinical type 2 diabetes (28). In NAFLD, these TGs characterize the subtype of NAFLD associated with obesity and insulin resistance (12). At least in part, changes in these TGs may be related to increased de novo hepatic lipogenesis, a hallmark of NAFLD (29,30).

When comparing the 3 CHR subgroups at the LC level (Figure 3), individuals whose symptoms remitted had a lipid profile similar to HC subjects. Although the lipids tended to be higher in CHRr than in HC, the only significant difference at the individual lipid levels was in 2 SMs, SM(d34:2) and SM(d18:1/24:0), with these 2 lipids being elevated also in the total CHR sample compared with the HC group. These SMs were previously found to be associated with obesity and insulin resistance (31), and SM is considered to be a risk factor of coronary artery disease (32). Altered SM levels compared with HC in CHRr may thus reflect impaired metabolic profile in CHR individuals.

Using a machine learning approach, we developed diagnostic signatures that discriminated subgroups within the CHR sample with distinct clinical outcomes. The relatively high

accuracy of the model suggests that measures of blood lipids may help clinicians predict outcomes in this population. CHR individuals who later developed psychosis were discriminated from those who did not by lower levels of ether phospholipids. Ether phospholipids are highly enriched in brain (33) and have many structural and functional roles (34). Plasmalogens are a structural subgroup of ether phospholipids that are supplied to the brain by the liver (35). They are thought to be scavengers of free radicals and may act as endogenous antioxidants (36,37). Our data thus raise the possibility that CHR individuals who develop psychosis may have an increased vulnerability to oxidative stress. This is of particular interest, as an independent body of work has implicated oxidative stress in the pathophysiology of psychosis (38). We have also collected measures of oxidative stress from the present sample and will assess the relationship between these and ether phospholipids in a forthcoming study.

An unexpected finding was that within the total CHR sample, there were marked sex differences in lipid profile. Although the trend of lipid changes, when comparing CHR and HC subjects, was the same for both sexes, sex differences were much more pronounced in the CHR group than in HCs. While TGs and LPCs were increased, SMs were decreased in male relative to female CHR subjects. Plasma SMs are known to be elevated in females (39), which reflects the effect of 17β-estradiol on serum SM levels (40). This finding suggests that sex may be an important confounder that needs to be considered in studies of lipid metabolism in psychosis. Another potential cofounder in the present study was the fact that the samples were not collected in a fasted state. The CHR population is difficult to recruit to studies in general, and requiring fasting would have been a major limitation to recruitment.

In conclusion, although the mechanisms linking dysregulation of lipid metabolism with the pathophysiology of psychosis is unclear, our findings suggest that metabolic abnormalities are evident in people who are vulnerable to psychosis but do not have the disorder and have not been treated. A similar lipid profile is observed in patients with NAFLD and in prediabetes, as well as in nonobese first-episode psychosis patients who later gain weight. In addition, our data suggest that assessment of the circulating lipidome may assist in the identification of CHR individuals who are at the highest risk for transition to psychosis. Our AUC of 0.83 for predicting transition is promising, but the model must be validated in similar independent CHR datasets. The predictive power of lipidomic data may be enhanced by combining these with measures of other factors that may influence clinical outcomes in CHR individuals, such as neuroimaging data, psychopathology, oxidative stress, proteomic, and inflammatory markers (41).

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