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Authors
Zeng, Wenjie
Beyene, Habtamu
Kuokkanen, Mikko
et al.

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Lipidomic profiling in the Strong Heart Study identified American Indians at risk of chronic kidney disease

Wenjie Zeng[1], **Habtamu B. Beyene**[2], **Mikko Kuokkanen**[3], **Guanhong Miao**[1], **Dianna J. Magliano**[2], **Jason G. Umans**[4,5], **Nora Franceschini**[6], **Shelley A. Cole**[7], **George Michailidis**[8], **Elisa T. Lee**[9], **Barbara V. Howard**[4,5], **Oliver Fiehn**[10], **Joanne E. Curran**[3], **John Blangero**[3], **Peter J. Meikle**[2], **Jinying Zhao**[1]

[1]Department of Epidemiology, College of Public Health and Health Professions and College of Medicine, University of Florida, Gainesville, Florida, USA;

[2]Baker Heart and Diabetes Institute, Melbourne, Victoria, Australia;

[3]Department of Human Genetics and South Texas Diabetes and Obesity Institute, University of Texas Rio Grande Valley School of Medicine, Brownsville, Texas, USA;

[4]MedStar Health Research Institute, Hyattsville, Maryland, USA;

[5]Georgetown-Howard Universities Center for Clinical and Translational Science, Washington, District of Columbia, USA;

[6]Department of Epidemiology, University of North Carolina, Chapel Hill, North Carolina, USA;

[7]Population Health Program, Texas Biomedical Research Institute, San Antonio, Texas, USA;

[8]Department of Statistics, University of Florida, Gainesville, Florida, USA;

[9]Department of Biostatistics and Epidemiology, College of Public Health, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA;

[10]West Coast Metabolomics Center, University of California–Davis, Davis, California, USA

Abstract

Dyslipidemia associates with and usually precedes the onset of chronic kidney disease (CKD), but a comprehensive assessment of molecular lipid species associated with risk of CKD is lacking. Here, we sought to identify fasting plasma lipids associated with risk of CKD among American Indians in the Strong Heart Family Study, a large-scale community-dwelling of individuals, followed by replication in Mexican Americans from the San Antonio Family Heart Study and Caucasians from the Australian Diabetes, Obesity and Lifestyle Study. We also performed repeated measurement analysis to examine the temporal relationship between the change in the lipidome and change in kidney function between baseline and follow-up of about five years apart.

Correspondence: Jinying Zhao, Department of Epidemiology, College of Public Health and Health Professions and College of Medicine, University of Florida, 2004 Mowry Rd, Gainesville, Florida 32610, USA. jzhao66@ufl.edu.

DISCLOSURE

All the authors declared no competing interests.

SUPPLEMENTARY MATERIAL

Supplementary File (Excel)

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Network analysis was conducted to identify differential lipid classes associated with risk of CKD. In the discovery cohort, we found that higher baseline level of multiple lipid species, including glycerophospholipids, glycerolipids and sphingolipids, was significantly associated with increased risk of CKD, independent of age, sex, body mass index, diabetes and hypertension. Many lipid species were replicated in at least one external cohort at the individual lipid species and/or the class level. Longitudinal change in the plasma lipidome was significantly associated with change in the estimated glomerular filtration rate after adjusting for covariates, baseline lipids and the baseline rate. Network analysis identified distinct lipidomic signatures differentiating high from low-risk groups. Thus, our results demonstrated that disturbed lipid metabolism precedes the onset of CKD. These findings shed light on the mechanisms linking dyslipidemia to CKD and provide potential novel biomarkers for identifying individuals with early impaired kidney function at preclinical stages.

Keywords

American Indians; AusDiab; chronic kidney disease; lipidomics; Mexican Americans; San Antonio Family Heart Study; Strong Heart Study

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American Indians experience a disproportionately high burden of chronic kidney disease (CKD).[1] The prevalence of kidney failure in American Indians is >[2] times higher than that in non-Hispanic Whites.2 Epidemiologic studies have identified many risk factors for CKD, including hypertension,[3] type 2 diabetes,[3] obesity,[4,5], family history of CKD,[6] and ethnicity.[7] Dyslipidemia is a common risk factor for all these conditions. Patients with CKD often exhibit elevated triglycerides and total cholesterol, reduced high-density lipoprotein, and altered lipoprotein compositions compared with individuals with normal kidney function.[8,9] Dyslipidemia may occur many years before the recognition of overt CKD, suggesting that blood lipids may serve as prognostic and diagnostic markers. However, standard lipoproteins do not reflect the diversity and complexity of human plasma lipidome. There is an unmet need to identify novel biomarkers for early detection and risk stratification among community-dwelling individuals with high risk for CKD.

Lipidomics by mass spectrometry can simultaneously identify and quantify hundreds to thousands of individual molecular lipid species in biological samples. Altered lipid species, such as free fatty acids, triacylglycerols (TAGs), diacylglycerols (DAGs), ceramides (CERs), phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), and sphingomyelins (SMs), have previously associated with CKD or related traits in different populations.[10–18] Lipid dysregulation has also been associated with risk factors for CKD, such as obesity,[19–21] diabetes,[22–26] hypertension,[27,28] and cardiovascular disease (CVD).[29–31] However, previous studies were largely cross-sectional, were limited by small sample size, or had a relatively low coverage of plasma lipidome. No large-scale epidemiologic study has examined the relationship between a full spectrum of blood lipidome and risk of CKD in any racial/ethnic group, especially in a longitudinal setting among community-dwelling individuals. The goal of this study is to identify lipidomic markers predictive of risk for CKD in 3 large community-based prospective cohorts (American Indians, Mexican Americans, and European white populations).

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METHODS

Study participants

The discovery cohort included American Indians in the Strong Heart Family Study (SHFS), a family-based prospective study designed to identify genetic and metabolic factors for CVD and risk factors in American Indians, as previously described.[32–34] Briefly, 2780 tribal members (aged 14 years) in 3 geographic regions (Arizona, North/South Dakota, and Oklahoma) were initially examined in 2001 to 2003 and reexamined in 2006 to 2009 (mean, 5.5 years apart) using the same protocols. Information for demography, family history, medical records, and lifestyle was collected at each visit. A total of 1970 individuals (aged 18–75 years; mean age, 40 years) with complete information for clinical and lipidomic data were included in this analysis. More information for covariate assessments was described elsewhere.[24] All participants provided informed consents. The SHFS protocols were approved by the Institutional Review Boards of each participating institution and tribes.

Independent external replication was conducted in the Australian Diabetes, Obesity, and Lifestyle (AusDiab) Study and the San Antonio Family Heart Study (SAFHS). Briefly, AusDiab Study is a population-based longitudinal study established to examine the prevalence of diabetes and related risk factors in Australia.[35] A subset of 5541 individuals (aged 38–62 years; mean age, 50 years) who were free of CKD at baseline and had complete clinical and lipidomic data were used in this analysis. Of these, 228 individuals developed incident CKD over a period of 5-year follow-up. SAFHS is a family-based prospective study comprising 1431 individuals in 42 extended families at baseline and investigates the genetics and risk factors of CVD in Mexican Americans.[36–38] A subset of 632 individuals (aged 18–84 years; mean age, 42 years) who were free of CKD at baseline and had complete information for clinical phenotypes and lipidomic data were included in the analysis. Of these, 38 individuals developed incident CKD over 5-year follow-up.

Clinical measures and ascertainment of CKD

In all 3 cohorts, we calculated the estimated glomerular filtration rate (eGFR) using the CKD Epidemiology Collaboration formula.[39] CKD was defined as eGFR <60 ml/min per 1.73 m^2 or a history of dialysis or kidney transplant, as previously described.[40,41] This corresponds to stage 3 or higher CKD, according to the Kidney Disease Outcome Quality Initiative.[42] Incident CKD was identified if participants were free of CKD at baseline but develpoed CKD by end of follow-up.[40,41] Clinical covariates, including age, sex, body mass index, diabetes, hypertension, and standard lipid panels, were measured using standard protocols and are available in all 3 cohorts.

Lipidomic profiling

In the discovery cohort (SHFS), fasting plasma lipids at 2 time points (≈5 years apart) were quantified by untargeted liquid chromatography–mass spectrometry, as described previously.[24] After preprocessing and quality control, we obtained 1542 lipids (518 known; Supplementary Table S1) in 3916 plasma samples from 1958 unique individuals at 2 time points. After further excluding individuals with prevalent CKD and/or CVD and those with

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missing covariates, 1910 participants with complete clinical and lipidomic data at both time points were included in the analysis.

Lipidomic profiling in the replication cohorts (AusDiab Study and SAFHS) was performed using fasting plasma samples collected at enrollment (i.e., baseline) by liquid chromatography–electrospray ionization–tandem mass spectrometry, as previously described.[20,21,26,28,30,31,43–45] A total of 311 lipids in AusDiab Study and 288 lipids in SAFHS were also available in the SHFS and used in this analysis.

Statistical analysis

Before statistical analysis, all continuous variables, including lipids, were standardized to 0 mean and unit SD. Multiple testing was controlled using the Storey q-value method.[46]

Prospective association analysis.—To identify baseline plasma lipids predictive of risk for CKD, we performed mixed-effect logistic regression (SHFS and SAFHS) or logistic regression (AusDiab Study), in which baseline lipid was the predictor and incident CKD status was the outcome, adjusting for age, sex, body mass index, diabetes, and hypertension at baseline. The analysis in SAFHS additionally adjusted for individual-specific estimates of relative Amerindian and African admixture (as estimated from genome-wide genetic markers). Family relatedness in the SHFS was accounted for by including a random effect (i.e., family) in the model. Mixed-effect probit logistic regression was used to account for family relatedness in the SAFHS by including underlying genetic variation as a random effect in the model via a variance component that was structured by the genetic relatedness matrix. Probit regression coefficients were converted to the logit equivalents by multiplying by 1.6, as suggested by Amemiya.[47] Meta-analysis was performed by inverse-variance weighted random-effect models to combine results across cohorts. We used a stringent cutoff (i.e., $q < 0.05$) in the discovery cohort (SHFS). Lipids significantly associated with risk of CKD in the SHFS were then validated in the SAFHS and AusDiab Study, in which $P < 0.05$ was considered statistically significant.

Repeated measurement analysis.—To examine the temporal relationship between change in lipidome and change in kidney function, we conducted mixed-effect linear regression. In the model, level difference in eGFR (baseline to follow-up) was the dependent variable and difference in lipid was the independent variable, adjusting for clinical factors (age, sex, study center, changes in body mass index, systolic blood pressure, fasting glucose, total cholesterol, triglyceride, and urine albumin–creatinine ratio [UACR] at baseline) and baseline lipid and eGFR. Family relatedness was accounted for by including family as a random effect in the model. Proportion of variance in eGFR change explained by change in lipid was also calculated. Multiple testing was controlled by the Storey q-value method,46 and $q < 0.05$ was considered statistically significant. The repeated measurement analysis was done in SHFS only as AusDiab Study and SAFHS only measured lipids at baseline.

Lipid network analysis.—Given the high correlations between lipids, we performed network analysis to identify lipid classes associated with risk of CKD. Using the R package *WGCNA*,[48] we built an unsigned weighted lipid coregulation network using all 1542

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baseline lipids in the SHFS. Lipid features were hierarchically clustered, and those with a high topological overlap similarity were grouped into a same module. For each identified module, we examined its association with risk of CKD and clinical covariates. For modules significantly associated with risk of CKD, we defined hub lipids between cases (those who developed incident CKD during follow-up) and noncases (those who did not). Differential correlation was calculated using the program *DGCA*[49] by comparing the correlation patterns between cases and noncases. Gain of correlation was defined if the correlation among cases was more positive than that among noncases, and loss of correlation was defined if the correlation among cases was more negative than that among noncases.

Sensitivity analysis.—This was done by further adjusting for standard lipids (e.g., total cholesterol and triglyceride), UACR, and lipid-lowering medications in the above-described models.

RESULTS

Over an average of 5-year follow-up, a total of 324 incident CKD cases (58 in SHFS, 38 in SAFHS, and 228 in AusDiab Study) were identified. Table 1 presents the clinical characteristics of study participants in the 3 cohorts at enrollment.

Baseline plasma lipids associated with risk for CKD

After adjusting for covariates and correction for multiple testing ($q < 0.05$), 29 baseline lipids (of 518 known lipids; see Supplementary Table S1) were significantly associated with incident CKD in the SHFS (discovery cohort; Table 2). Specifically, higher baseline levels of 17 glycerophospholipids (e.g., PEs, PCs, and phosphatidylinositols [PIs]), 9 glycerolipids (e.g., TAGs and DAG), and 2 sphingolipids (e.g., SMs and CERs) were significantly associated with increased risk of CKD. One sphingomyelin (SM[d40:2]) was inversely associated with risk of CKD.

Of the 29 lipids identified in SHFS ($q < 0.05$), 24 and 25 lipids were also available in SAFHS and AusDiab Study, respectively. Of the 24 overlapping lipids in SAHFS, 7 lipids, including 6 Pes and 1 SM (SM[d32:1]), were significantly associated with risk of CKD ($P < 0.05$) with same direction. Of the 25 overlapping lipids in AusDiab Study, 6 lipids, including 3 PEs, 2 PCs, and 1 TAG (i.e., TAG[53:2]), were significantly associated with risk of CKD ($P < 0.05$) with same direction. In particular, 3 PEs (i.e., PE[34:2], PE[36:4]/PE[18:2/18:2], and PE[38:3]/[18:0/20:3]) were significantly associated with risk of CKD in all 3 cohorts. Results for replication are shown in Table 3.

Transethnic meta-analysis of 23 lipid species measured in all 3 cohorts identified 14 lipids significantly associated with risk for CKD at $P < 0.05$, of which 13 lipids reached $q < 0.05$ (Table 4). Figure 1 schematically illustrates the associations between baseline lipids and risk of CKD in the 3 cohorts and meta-analysis. Further adjustments for standard lipids (e.g., total cholesterol and triglyceride), UACR, and lipid-lowering medications slightly attenuated the associations, but most lipids (19 of 29 lipids) remained to be significant (Table 5).

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Longitudinal change in plasma lipidome associated with change in kidney function

After adjusting for covariates, including baseline lipids and eGFR, and correction for multiple testing ($q < 0.05$), longitudinal changes in 258 lipids (88 known and 170 unknown) were either positively or inversely associated with change in eGFR between baseline and follow-up (Supplementary Table S2). On average, changes in plasma lipidome explained 6.98% (range, 0.36%–13.59%) of the variability in eGFR change between baseline and 5-year follow-up. Figure 2 schematically illustrates the association patterns between change in known lipids and change in eGFR among American Indians in the SHFS.

Differential lipid networks associated with risk of CKD

Network analysis in the SHFS identified 13 distinct modules. On module (brown), enriched in TAGs, PEs, and CEs, was significantly associated with risk of CKD (Figure 3a). This module included 154 lipids (73 known), with PE(36:1), PE(36:2), and TAG(51:1) being the hub lipids (Figure 3b). Lipids in the brown module were also significantly correlated with clinical variables, especially bulk triglycerides and total cholesterol. In addition, 44 known lipids (mainly TAGs and PEs) in the module were differentially correlated with other lipids in the same module (Figure 3b). Comparing noncases with cases, several TAGs (e.g., 42:1, 42:2, and 46:3) demonstrated significant gain of connectivity, whereas other TAGs (e.g., 51:0 and 53:1) exhibited significant loss of connectivity. Moreover, the correlation patterns between module lipids (e.g., TAGs, PEs, PCs, CEs, and sphingolipids [CERs or SMs]) and clinical variables, especially bulk triglycerides and total cholesterol, appeared to be similar across the 3 cohorts (see Figure 3a for SHFS; Figure 4 for SAFHS; and Figure 5 for AusDiab Study), albeit the magnitude of correlation varies between cohorts.

DISCUSSION

In this large-scale lipidomic profiling comprising >8000 community-dwelling individuals from diverse cohorts, we had several key findings. First, we demonstrated that baseline individual lipid species, including glycerophospholipids (PEs, PCs, and PIs), glycerolipids (TAGs and DAGs), and sphingolipids (SMs and CERs), were significantly associated with future risk for CKD, independent of clinical factors. Specifically, among American Indians, higher baseline level of 17 glycerophospholipids, including 9 PEs, 5 PCs, and 3 PIs, were significantly associated with increased risk of CKD. Of these, 6 PEs and 2 PCs (i.e., PC[33:1] and PC[35:1]) were replicated (with same direction of association) in at least one external cohort (Mexican Americans in SAFHS and white populations in AusDiab Study). Three PEs (i.e., PE[34:2], PE [36:4]/PE[18:2/18:2], and PE[38:3]/PE[18:0/20:3]) were significantly associated with incident CKD in all 3 ethnic groups. We also found that altered baseline levels of 9 glycerolipids (8 TAGs and DAG[34:2]) and 3 sphingolipids (SM [d32:1], SM[d40:2], and CER[d44:1]) were significantly associated with risk of CKD in American Indians. Of these, TAG(53:2) and SM(d32:1) were replicated in AusDiab Study and SAFHS, respectively. Transethnic meta-analysis combining results of 23 lipids measured in all 3 cohorts identified 13 lipids (8 PEs, 2 TAGs, 1 DAG, 1 PC, and 1 SM) significantly associated with incident CKD at $q < 0.05$. Further adjustments for standard lipoproteins, UACR, and lipid-lowering medications did not materially change the associations. Second, our repeated measurement analysis demonstrated, for the first time, that longitudinal change

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in fasting plasma lipidome was significantly associated with change in kidney function, independent of clinical factors and baseline lipids and eGFR. Specifically, changes in 258 individual lipid species (88 known) were significantly associated with change in eGFR over 5-year follow-up in American Indians. The 88 known lipid species largely included TAGs, PCs, PEs, SMs, fatty acids, and acylcarnitines (ACs). On average, change in plasma lipidome explains ≈7% of the variance in eGFR over 5-year follow-up. Third, our network analysis identified differential lipid clusters (i.e., modules) associated with risk of CKD. The identified lipid module was enriched in TAGs and PEs, and lipids in this module showed strong correlations with clinical variables. As we built the networks based on baseline lipids, the disturbed lipid coregulations may have occurred at least 5 years before CKD onset. These findings are consistent with a previous study showing that lipid networks enriched in TAG and cardiolipins-PEs discriminated progressors and non-progressors with stage 2 or 3 CKD.[14] Together, our results revealed a distinct lipidomic signature associated with risk for CKD and provide insight into the mechanisms through which dyslipidemia may contribute to CKD. Moreover, because aberrant expression of plasma lipidome associated with CKD is clearly present at preclinical stages, before the onset of CKD, the identified lipids may provide potential biomarkers for identifying individuals with impaired kidney function at earlier stages.

We identified significant associations of multiple PEs with risk of CKD in 3 diverse cohorts comprising individuals with different genetic and environmental (or lifestyle) backgrounds. Also, these associations were consistently detected in different statistical models. Many of the identified PEs (e.g., PE[34:1], PE[34:2], PE[36:2], PE[36:4]/PE[18:2/18:2], PE [16:0/16:1], and PE[38:3]/PE[18:0/20:3]) were also significantly associated with diabetes and prediabetes in American Indians,[24] Mexican Americans, and white populations.[26] The associations of PEs with CKD are also in line with previous studies reporting that some PEs (e.g., 38:3, 38:4, 38:6, 40:4, 40:5, and 40:6) were associated with CKD in Chinese individuals[18] or blood pressure in white populations.[50] Collectively, our results suggest a potential important role of dysregulated PE metabolism in CKD.

PE is the second most abundant phospholipid, after PC, in the membranes of all mammalian cells. Besides serving as the backbone of cellular membranes and precursor for other lipids, PEs and their derived lipid mediators are involved in many biological processes, such as signal transduction, apoptosis, mitochondrial function, and modulation of cellular responses.[51–53] Dysregulation in PE metabolism has been implicated in neurodegeneration,[54,55] cancer,[56] and metabolic disorders, such as nonalcoholic liver disease, atherosclerosis, insulin resistance, and obesity.[51,57] Although the precise mechanisms linking PEs and CKD remains to be determined, it is possible that PEs may cause nephrotoxicity through their roles in insulin resistance, inflammation, oxidative stress, lipid peroxidation, and impaired β-oxidation, as well as other as yet unknown mechanisms.[9,58–63]

Besides PEs, we also identified significant associations of other glycerophospholipids (e.g., PCs and PIs), glycerolipids (e.g., TAGs and DAGs), and sphingolipids (e.g., SMs and CERs) with risk of CKD. Many lipids, such as PC(33:1), PC(35:1), TAG(53:2), TAG(52:4), DAG(34:2), and SM(d32:1), were validated in at least one external cohort

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and/or meta-analysis. The associations of SMs, ceramide, and PCs with CKD are consistent with previous studies reporting that they were associated with microalbuminuria/macroalbuminuria or kidney impairment.[16,18,64–66] The associations of glycerolipids (TAGs and DAGs) with CKD are supported by our previous studies showing that altered baseline levels of various TAGs and DAGs were significantly associated with risk of diabetes in American Indians[24] or hypertension in Mexican Americans.[28] The current study also found that higher baseline level of 3 PIs (e.g., 17:0/20:4, 18:0/20:3, and 18:0/20:4) predicted the risk of CKD in American Indians. Although these results were not replicated in external cohorts, the observed associations appear to be in line with a previous study demonstrating that altered plasma PIs were associated with diabetes in Mexican Americans and Causasians.[26]

Our repeated measurement analysis revealed, for the first time, the temporal relationship between change in plasma lipidome and change in kidney function, independent of covariates and baseline lipids and eGFR. Specifically, changes in long-chain unsaturated fatty acids, long-chain saturated acylcarnitines, and PCs with a lower degree of unsaturation were positive, whereas changes in polyunsaturated TAGs, PCs with a higher degree of unsaturation, unsaturated SMs, polyunsaturated PEs, and intermediate-chain acylcarnitines were inversely associated with change in eGFR. These processes are likely a reflection of impaired mitochondrial β-oxidation of saturated fatty acids (such as palmitate), in parallel with upregulation of elongation/desaturation processes, leading to higher abundance of unsaturated fatty acids and their incorporation into longer polyunsaturated complex lipids, such as TAGs.[11,12,67,68]

In addition, we observed differential correlation patterns between prospective association and repeated measurement analyses. Despite the fact that some lipid species (e.g., TAGs) were detected in both statistical models, lipids identified in the 2 analyses were largely not overlapped. For instance, PEs showed strong and consistent associations with risk of CKD in prospective analysis, whereas repeated measurement analysis revealed that change in other lipid species (e.g., PCs, TAGs, SMs, fatty acids, and ACs) were associated with change in eGFR. The reasons behind these differential association patterns are unclear. It is likely that baseline lipids and longitudinal changes in the lipidome reflect different aspects of disease pathophysiology. Future research should investigate the temporal relationship between plasma lipidome and disease outcomes to comprehensively understand the mechanisms underlying dyslipidemia and metabolic disorders.

In the field of lipidomics, replication of individual lipid species across populations has proved to be challenging. This is largely due to the following facts: (i) Different studies often employ different mass spectrometry platforms, which usually result in different resolution and/or coverage of the lipidome. This impedes replication of individual lipid species across studies. (ii) Blood lipidome is determined by genetic, environmental, and lifestyle factors, all of which may vary across populations. (iii) Given the high correlation between lipids, different statistical models may detect different individual lipids, even in a same population. This is especially true for complex diseases, such as CKD. Lipidomic signature of CKD progression is the net effect of differential lipolysis, lipogenesis, elongation, desaturation, and β-oxidation of fatty acids at various stages of the disease

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processes that impact many lipid classes or clusters with high degrees of similarities. As such, no single lipid can have a large enough effect to represent the variation in lipidomic signature, nor any one lipid may gain advantageous predictive power over other structurally similar significant lipids in predicting a complex disease outcome. Hence, it is not surprising that approaches based on individual lipid species often fail to be replicated in independent cohorts. Replication at the class/clusters (with similar structures or class) level may be a reasonable approach. Nevertheless, multiple individual lipids (e.g., PEs, PCs, SMs, and TAGs) identified in American Indians could be replicated in external cohorts in our study. This further highlights the robustness of our findings and signifies the potential important role of dysregulation in the metabolism of these lipids in CKD.

Several limitations of our study should be noted. First, because of our focus on participants without overt CKD and CVD at baseline, along with a relatively short period of follow-up, the number of participants with incident CKD in each cohort was relatively small, and thus our study could be underpowered in detecting lipidomic markers. Nevertheless, the current analysis included >8000 individuals without prevalent CKD at baseline, of whom 324 developed incident CKD over ≈-year follow-up. To our knowledge, this represents the largest lipidomic profiling of risk for CKD in 3 large community-based prospective cohorts of multiethnic individuals. Second, despite the large number of lipids detected in our discovery cohort, many lipids are unknowns; and we were unable to distinguish isomeric lipids either. Additional experiments are needed to characterize these unknowns if considered of interest. Also, the lack of absolute quantification does not allow clinical utility. Moreover, the static lipidomic platforms may not disclose the contribution of upstream lipid regulators with differential flux and yet steady-state concentrations. Third, as emerging evidence suggests that lipidomic signature of CKD progression may vary, depending on the etiology of CKD progression, the lipidomic alterations identified in our study may not point to specific underlying etiologies of CKD progression. Fourth, although our statistical models controlled many clinical factors known to be associated with CKD, we cannot exclude the possibility of residual confounding by unknown or unmeasured factors. Finally, as in all other observational studies, we cannot determine whether the observed associations are cause or consequence of CKD.

However, our study has several strengths. First, the current study included >8000 community-dwelling individuals of diverse cohorts, which, to our knowledge, represents the largest lipidomic profiling of risk for CKD. Second, the longitudinal profiling of plasma lipidome in a large community-based cohort represents another major strength of this study. Third, our statistical analyses in all 3 cohorts adjusted for chronic conditions associated with CKD (e.g., obesity, diabetes, and hypertension). In addition, we examined the relationship between plasma lipidome and CKD development at both individual lipid species and lipid class level. Moreover, we performed sensitivity analysis to additionally adjust for bulk lipids (total cholesterol and triglyceride), UACR, and use of lipid-lowering medications in the SHFS. Thus, lipids identified in our study should be independent of these conventional risk factors. Finally, our high-resolution lipidomic platforms in both discovery and replication cohorts identified a larger number of molecular lipid species, allowing us to identify novel lipid species associated with risk of CKD and offering unprecedented opportunities for future investigations.

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In summary, we identified a range of novel molecular lipids associated with risk of CKD at both individual lipid species and lipid class levels, independent of clinical factors. Our results demonstrated that dysregulated lipid metabolism occurs years before CKD onset. Thus, the newly identified lipids may help identify individuals with early impaired kidney function at preclinical stages. Our findings offer potential opportunities for new intervention strategies (e.g., lifestyle/drug) to prevent/attenuate CKD progression by modifying lipid metabolism. Our results further highlight the need for mechanistic studies to characterize the role of lipid species in CKD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA STATEMENT

Lipidomic data generated in the Strong Heart Family Study (SHFS) are available from the corresponding author on reasonable request. Clinical data in the SHFS can be requested through the Strong Heart Study at https://strongheartstudy.org/. Data used in the Australian Diabetes, Obesity, and Lifestyle Study can be requested via https://www.baker.edu.au/ausdiab/. Data used in the San Antonio Family Heart Study can be requested via Dr. Joanne E. Curran (joanne.curran@utrgv.edu) or Dr. John Blangero (john.blangero@utrgv.edu).

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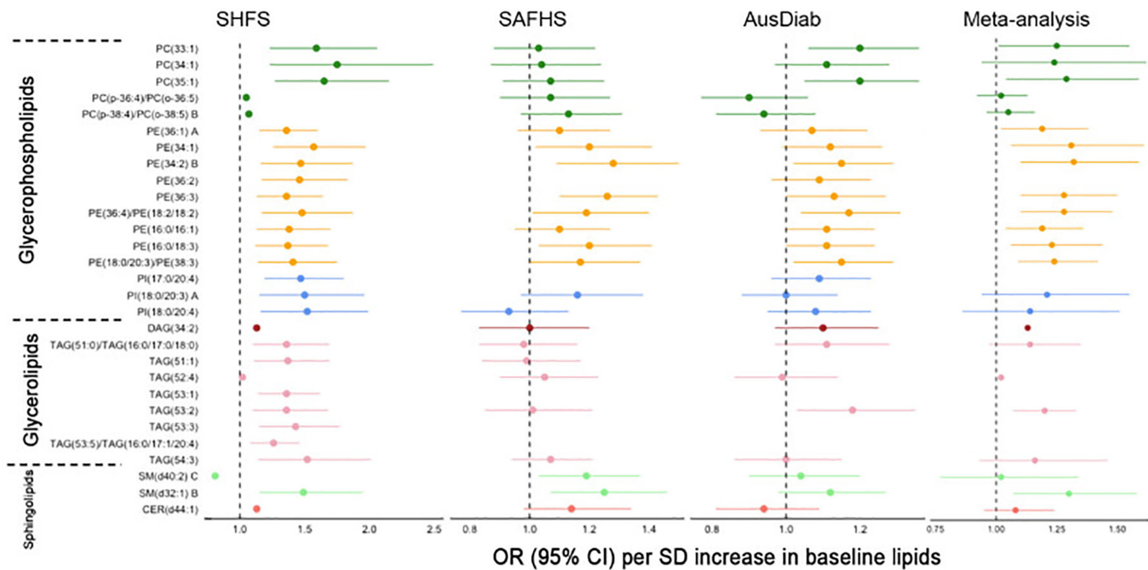

Figure 1 |. Baseline plasma lipids associated with risk of chronic kidney disease in the discovery (Strong Heart Family Study [SHFS]) and replication (San Antonio Family Heart Study [SAFHS] and Australian Diabetes, Obesity, and Lifestyle [AusDiab] Study) cohorts as well as meta-analysis.

Odds ratios (ORs) and 95% confidence intervals (CIs) were obtained by mixed-effect logistic regression (SHFS and SAFHS) or logistic regression (AusDiab Study), adjusting for age, sex, body mass index, hypertension, and diabetes at baseline. The analysis in SAFHS additionally adjusted for individual-specific estimates of relative Amerindian and African admixture (as estimated from genome-wide genetic markers). Family relatedness in the SHFS was accounted for by including a random effect (i.e., family) in the model. Family relatedness in the SAFHS was accounted for by including underlying genetic variation as a random effect in the model via a variance component that was structured by the genetic relatedness matrix. CER, ceramide; DAG, diacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; SM, sphingomyelin; TAG, triacylglycerol.

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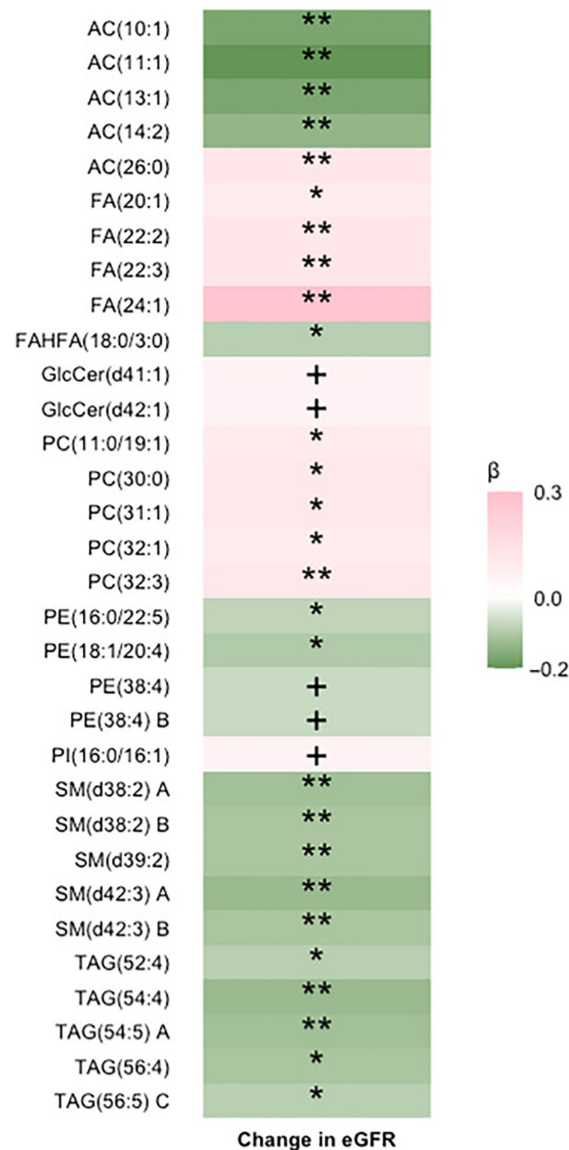

Figure 2 |. Longitudinal association between changes in plasma lipidome and change in estimated glomerular filtration rate (eGFR) over 5-year follow-up in the Strong Heart Family Study.

The heat map was arranged on the basis of lipid classes. Only known lipids (top 5 in each class) whose longitudinal changes were significantly associated with change in eGFR ($q < 0.05$) are shown. Color of the heat map was based on regression coefficients obtained from mixed-effect linear regression, adjusting for clinical factors, including baseline age, sex, study site, and changes in body mass index, systolic blood pressure, fasting plasma glucose, total cholesterol, triglyceride, and urine albumin–creatinine ratio (between baseline and follow-up) plus baseline lipids and eGFR. Family relatedness was accounted for by including a random effect (i.e., family) in the model. $^{**}P < 0.001$, $^{*}P < 0.01$, and $^{+}P < 0.05$. AC, acylcarnitine; FA, fatty acid; FAHFA, fatty acyl esters of hydroxy fatty acid; GlcCer, glycosylceramide; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; SM, sphingomyelin; TAG, triacylglycerol.

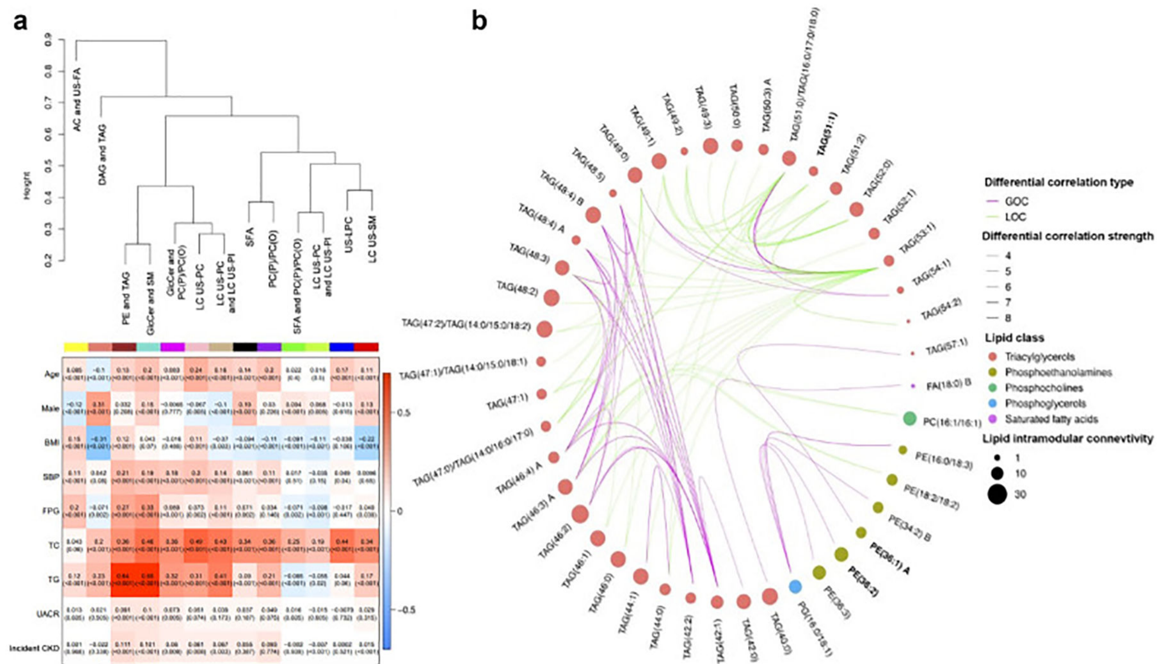

Figure 3 |. Lipid network analysis in the Strong Heart Family Study (SHFS).

(**a**) Dendrogram showing the hierarchical clustering of lipid modules (top) and heat map displaying the correlations between lipid modules and clinical traits (bottom) in the SHFS. The name of lipid modules reflects the dominating lipid class within each cluster. (**b**) Circos plot showing differential correlations between lipid pairs in the brown module. The difference in lipid correlations between incident chronic kidney disease (CKD) cases and noncases was calculated by the R package *DGCA*. Only known lipids with significant differential correlations ($P < 0.05$) were included. Edges are colored according to the differential correlation type, categorized as gain of correlation (GOC) or loss of correlation (LOC). Edge width represents differential correlation strength, defined as the absolute value of the z-score for differential correlations between cases and noncases. Node color represents lipid class. Node size is proportional to the intramodular connectivity, defined as the connectivity of a specific lipid to other lipids in the same *WGCNA* module. Hub lipids of the brown module are bolded. AC, acylcarnitine; BMI, body mass index; FA, fatty acid; FPG, fasting plasma glucose; GlcCer, glycosylceramide; LC, long chain; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; SBP, systolic blood pressure; SFA, saturated fatty acid; SM, sphingomyelin; TAG, triacylglycerol; TC, total cholesterol; TG, triglyceride; UACR, urine albumin–creatinine ratio; US, unsaturated.

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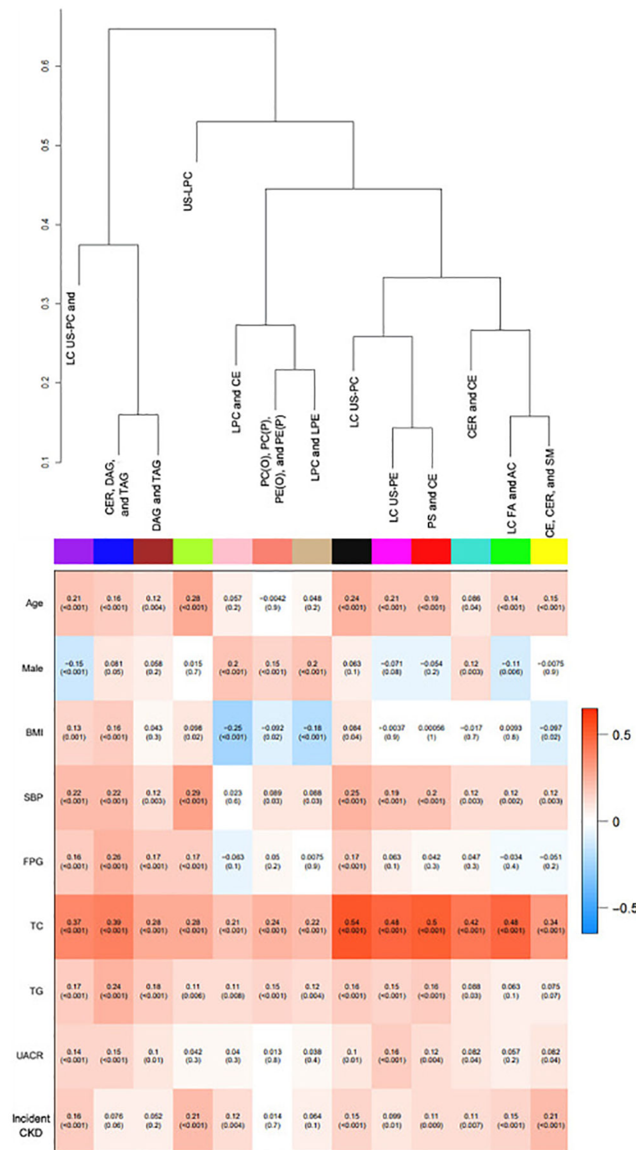

Figure 4 |. Correlations between module lipids and clinical variables in the San Antonio Family Heart Study.

Top panel: dendrogram showing the hierarchical clustering of lipid modules. Bottom panel: heat map displaying the correlations between lipid modules and clinical traits. The name of lipid modules reflects the dominating lipid class within each cluster. AC, acylcarnitine; BMI, body mass index; CE, cholesteryl ester; CER, ceramide; CKD, chronic kidney disease; DAG, diacylglycerol; FA, fatty acid; FPG, fasting plasma glucose; LC, long chain; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; SBP, systolic blood pressure; SM, sphingomyelin; TAG, triacylglycerol; TC, total cholesterol; TG, triglyceride; UACR, urine albumin–creatinine ratio; US, unsaturated.

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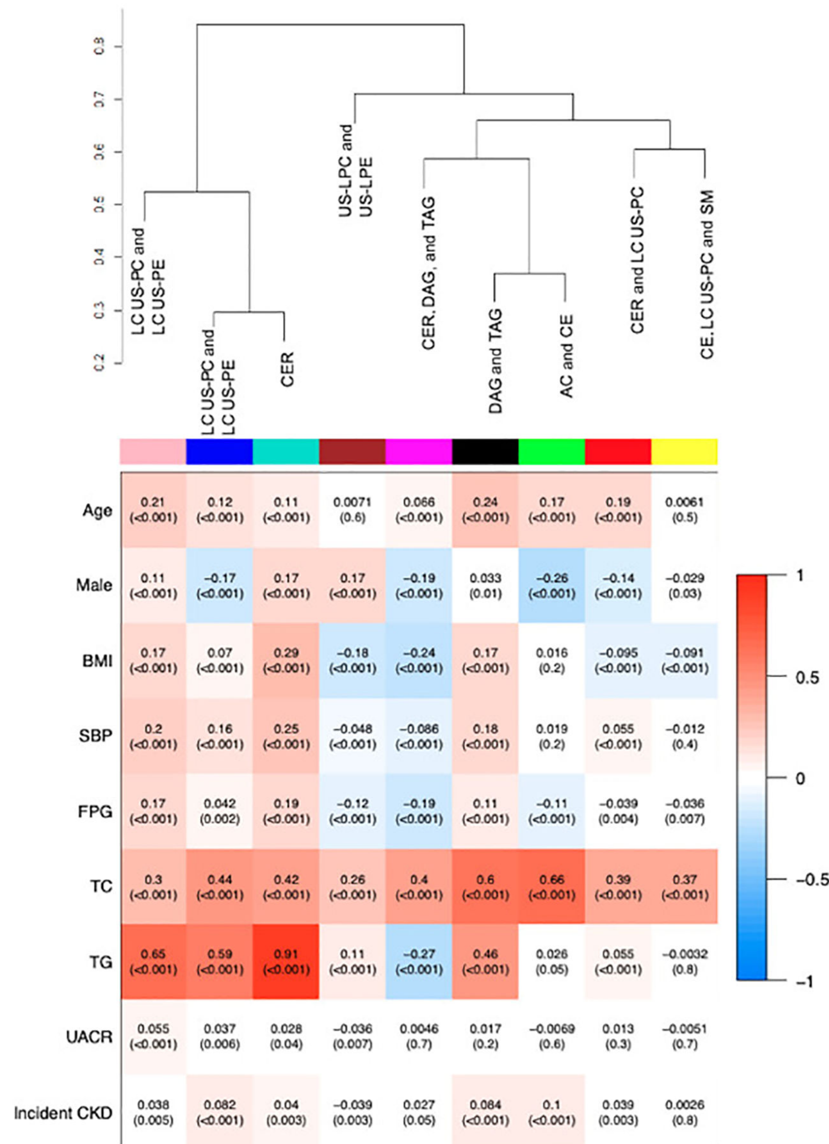

Figure 5 |. Correlations between module lipids and clinical variables in the Australian Diabetes, Obesity, and Lifestyle Study.

Top panel: dendrogram showing the hierarchical clustering of lipid modules. Bottom panel: heat map displaying the correlations between lipid modules and clinical traits. The name of lipid modules reflects the dominating lipid class within each cluster. AC, acylcarnitine; BMI, body mass index; CE, cholesteryl ester; CER, ceramide; CKD, chronic kidney disease; DAG, diacylglycerol; FPG, fasting plasma glucose; LC, long chain; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SBP, systolic blood pressure; SM, sphingomyelin; TAG, triacylglycerol; TC, total cholesterol; TG, triglyceride; UACR, urine albumin–creatinine ratio; US, unsaturated.

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Table 1.

Clinical characteristics of study participants at enrollment in the three cohorts

Characteristics	SHFS (n=1,910)	SAFHS (n=632)	AusDiab (n=5,541)
Age (years)	40.1±13.9	42.2±14.9	50.2±12.4
Female (%)	1190 (62.0)	405 (64.0)	2,944 (53.1)
BMI (kg/m^2)	31.8±7.7	31.0±6.9	26.8±4.8
SBP (mmHg)	122±15.3	124±19.1	127.9±17.2
DBP (mmHg)	77.3±10.6	71.8±10.7	70.3±11.6
Hypertension (%)	545 (28.5)	175 (28.0)	1,559 (28.2)
FPG (mg/dL)	109±46.2	106±40.2	99.0±18.0
Diabetes (%)	340 (17.8)	114 (18.0)	295 (5.3)
Total cholesterol (mg/dL)	185±36.7	178±35.9	217.7±18.7
Triglyceride (mg/dL)	171±201	154±307	107.1±85.8
HDL (mg/dL)	51.7±14.5	47.9±12.5	55.3±32.0
LDL (mg/dL)	102±29.9	102±29.3	136.5±34.8
UACR (mg/g)	47.3±281.6	69.3±431	11.5±57.9
eGFR (mL/min/1.73 m^2)	115±16.7	103±23.1	77.7±10.3

Results are expressed as mean ± SD unless otherwise noted. BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, FPG: fasting plasma glucose, HDL: high-density lipoprotein, LDL: low-density lipoprotein, UACR: urine albumin-to-creatinine ratio, eGFR: estimated glomerular filtration rate.

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Table 2.

Baseline plasma lipids associated with risk for CKD (discovery cohort, SHFS). Only lipids with q <0.05 are shown.

Lipid	Class	OR (95% CI)[≠]	q[*]
PC(33:1)	Phosphocholines	1.59 (1.23, 2.06)	0.012
PC(34:1)	Phosphocholines	1.75 (1.23, 2.49)	0.026
PC(35:1)	Phosphocholines	1.65 (1.27, 2.15)	0.006
PC(p-36:4)/PC(o-36:5)	Phosphocholines	1.05 (1.04, 1.06)	<.001
PC(p-38:4)/PC(o-38:5) B	Phosphocholines	1.07 (1.06, 1.08)	<.001
PE(36:1) A	Phosphoethanolamines	1.36 (1.15, 1.6)	0.008
PE(34:1)	Phosphoethanolamines	1.57 (1.26, 1.97)	0.003
PE(34:2) B	Phosphoethanolamines	1.47 (1.16, 1.87)	0.024
PE(36:2)	Phosphoethanolamines	1.46 (1.17, 1.83)	0.017
PE(36:3)	Phosphoethanolamines	1.36 (1.13, 1.64)	0.024
PE(36:4)/PE(18:2/18:2)	Phosphoethanolamines	1.48 (1.17, 1.87)	0.018
PE(16:0/16:1)	Phosphoethanolamines	1.38 (1.13, 1.7)	0.026
PE(16:0/18:3)	Phosphoethanolamines	1.37 (1.12, 1.68)	0.031
PE(18:0/20:3)	Phosphoethanolamines	1.41 (1.14, 1.75)	0.024
PI(17:0/20:4)	Phosphoinositols	1.47 (1.19, 1.8)	0.008
PI(18:0/20:3) A	Phosphoinositols	1.5 (1.15, 1.96)	0.033
PI(18:0/20:4)	Phosphoinositols	1.52 (1.16, 1.99)	0.031
DAG(34:2)	Diacylglycerols	1.13 (1.12, 1.14)	<.001
TAG(51:0)/TAG(16:0/17:0/18:0)	Triacylglycerols	1.36 (1.1, 1.69)	0.048
TAG(51:1)	Triacylglycerols	1.37 (1.11, 1.69)	0.038
TAG(52:4)	Triacylglycerols	1.02 (1.01, 1.03)	<.001
TAG(53:1)	Triacylglycerols	1.36 (1.14, 1.62)	0.014
TAG(53:2)	Triacylglycerols	1.36 (1.1, 1.68)	0.048
TAG(53:3)	Triacylglycerols	1.43 (1.15, 1.77)	0.024
TAG(53:5)/TAG(16:0/17:1/20:4)	Triacylglycerols	1.26 (1.08, 1.46)	0.031
TAG(54:3)	Triacylglycerols	1.52 (1.14, 2.01)	0.038
SM(d40:2) C	Sphingomyelins	0.81 (0.81, 0.82)	<.001
SM(d32:1) B	Sphingomyelins	1.49 (1.15, 1.95)	0.034
CER(d44:1)	Ceramides	1.13 (1.12, 1.14)	<.001

[≠]Obtained by the mixed-effect logistic regression, adjusting for age, sex, BMI, hypertension, and diabetes at baseline.

[*]Adjust for multiple testing using the Storey's q-value method.

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Table 3.

Replication in external cohorts (SAFHS, AusDiab). Only lipids matched to SHFS are shown. Lipids with P<0.05 are bolded

		SAFHS		AusDiab	
Lipid	**Class**	**OR (95% CI)[≠]**	**P[≠]**	**OR (95% CI)[*]**	**P[*]**
PC(33:1)	Phosphocholines	1.03 (0.88, 1.22)	0.686	**1.2 (1.06, 1.36)**	**0.004**
PC(34:1)	Phosphocholines	1.04 (0.87, 1.24)	0.676	1.11 (0.97, 1.28)	0.129
PC(35:1)	Phosphocholines	1.07 (0.91, 1.25)	0.427	**1.20 (1.05, 1.36)**	**0.006**
PC(p-36:4)/PC(o-36:5)	Phosphocholines	1.07 (0.9, 1.27)	0.469	0.90 (0.77, 1.06)	0.281
PC(p-38:4)/PC(o-38:5) B	Phosphocholines	1.13 (0.97, 1.31)	0.131	0.94 (0.81, 1.08)	0.391
PE(36:1) A	Phosphoethanolamines	1.10 (0.96, 1.27)	0.191	1.07 (0.93, 1.22)	0.338
PE(36:4)/PE(18:2/18:2)	**Phosphoethanolamines**	**1.19 (1.01, 1.4)**	**0.037**	**1.17 (1.04, 1.31)**	**0.009**
PE(34:1)	Phosphoethanolamines	**1.20 (1.02, 1.41)**	**0.030**	1.12 (0.99, 1.26)	0.079
PE(34:2) B	**Phosphoethanolamines**	**1.28 (1.09, 1.5)**	**0.003**	**1.15 (1.02, 1.29)**	**0.019**
PE(36:2)	Phosphoethanolamines	–/–	–/–	1.09 (0.96, 1.23)	0.202
PE(36:3)	Phosphoethanolamines	**1.26 (1.1, 1.43)**	**0.002**	1.13 (1, 1.27)	0.074
PE(16:0/16:1)	Phosphoethanolamines	1.10 (0.95, 1.27)	0.212	1.11 (1, 1.24)	0.055
PE(16:0/18:3)	Phosphoethanolamines	**1.20(1.03, 1.41)**	**0.024**	1.11 (1, 1.24)	0.081
PE(38:3)/PE(18:0/20:3)	**Phosphoethanolamines**	**1.17 (1, 1.37)**	**0.043**	**1.15 (1.02, 1.29)**	**0.024**
PI(17:0/20:4)	Phosphoinositols	–/–	–/–	1.09 (0.96, 1.23)	0.167
PI(18:0/20:3) A	Phosphoinositols	1.16 (0.97, 1.38)	0.113	1.00 (0.88, 1.14)	0.989
PI(18:0/20:4)	Phosphoinositols	0.93 (0.77, 1.13)	0.470	1.08 (0.95, 1.23)	0.236
DAG(34:2)	Diacylglycerols	1.00 (0.83, 1.20)	0.974	1.1 (0.97, 1.25)	0.187
TAG(51:0)/TAG(16:0/17:0/18:0)	Triacylglycerols	0.98 (0.83, 1.16)	0.849	1.11 (0.97, 1.28)	0.156
TAG(51:1)	Triacylglycerols	0.99 (0.84, 1.17)	0.924	–/–	–/–
TAG(52:4)	Triacylglycerols	1.05 (0.9, 1.23)	0.575	0.99 (0.86, 1.14)	0.462
TAG(53:2)	Triacylglycerols	1.01 (0.85, 1.21)	0.913	**1.18 (1.03, 1.35)**	**0.020**
TAG(54:3)	Triacylglycerols	1.07 (0.94, 1.21)	0.388	1.00 (0.86, 1.15)	0.799
SM(d40:2) C	Sphingomyelins	**1.19 (1.03, 1.37)**	**0.019**	1.04 (0.9, 1.2)	0.321
SM(d32:1) B	Sphingomyelins	**1.25 (1.07, 1.46)**	**0.004**	1.12 (0.98, 1.27)	0.110
CER(d44:1)	Ceramides	1.14 (0.98, 1.34)	0.097	0.94 (0.81, 1.09)	0.412

[≠]Obtained by mixed-effect logistic regression, adjusting for age, sex, BMI, ethnicity, hypertension, and diabetes at baseline.

[*]Obtained by logistic regression, adjusting for age, sex, BMI, hypertension, and diabetes at baseline.

–/– denotes lipid not measured in that cohort.

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Table 4.

Results from transethnic meta-analysis. Lipids with q<0.05 are bolded

Lipid	Class	OR (95% CI)≠	P≠	q*
CER(d44:1)	Ceramides	1.08 (0.95, 1.24)	0.249	0.302
DAG(34:2)	**Diacylglycerols**	**1.13 (1.12, 1.14)**	**<0.001**	**<0.001**
PC(33:1)	Phosphocholines	1.25 (1.01, 1.55)	0.037	0.061
PC(34:1)	Phosphocholines	1.24 (0.94, 1.62)	0.129	0.177
PC(35:1)	**Phosphocholines**	**1.29 (1.04, 1.59)**	**0.020**	**0.038**
PC(p-36:4)/PC(o-36:5)	Phosphocholines	1.02 (0.92, 1.13)	0.756	0.790
PC(p-38:4)/PC(o-38:5) B	Phosphocholines	1.05 (0.96, 1.16)	0.305	0.351
PE(16:0/16:1)	**Phosphoethanolamines**	**1.19 (1.04, 1.36)**	**0.010**	**0.023**
PE(16:0/18:3)	**Phosphoethanolamines**	**1.23 (1.06, 1.44)**	**0.007**	**0.020**
PE(38:3)/PE(18:0/20:3)	**Phosphoethanolamines**	**1.24 (1.09, 1.42)**	**0.001**	**0.006**
PE(34:1)	**Phosphoethanolamines**	**1.31 (1.06, 1.61)**	**0.012**	**0.024**
PE(34:2) B	**Phosphoethanolamines**	**1.32 (1.10, 1.59)**	**0.003**	**0.010**
PE(36:1) A	**Phosphoethanolamines**	**1.19 (1.02, 1.38)**	**0.026**	**0.046**
PE(36:3)	**Phosphoethanolamines**	**1.28 (1.10, 1.50)**	**0.002**	**0.007**
PE(36:4)/PE(18:2/18:2)	**Phosphoethanolamines**	**1.28 (1.10, 1.48)**	**0.001**	**0.006**
PI(18:0/20:3) A	Phosphoinositols	1.21 (0.94, 1.55)	0.131	0.177
PI(18:0/20:4)	Phosphoinositols	1.14 (0.86, 1.51)	0.372	0.408
SM(d32:1) B	**Sphingomyelins**	**1.30 (1.07, 1.58)**	**0.008**	**0.022**
SM(d40:2) C	Sphingomyelins	1.02 (0.77, 1.34)	0.902	0.902
TAG(51:0)/TAG(16:0/17:0/18:0)	Triacylglycerols	1.14 (0.97, 1.35)	0.117	0.177
TAG(52:4)	**Triacylglycerols**	**1.02 (1.01, 1.03)**	**<0.001**	**0.001**
TAG(53:2)	**Triacylglycerols**	**1.20 (1.07, 1.33)**	**0.001**	**0.006**
TAG(54:3)	Triacylglycerols	1.16 (0.93, 1.46)	0.197	0.252

≠Obtained by inverse-variance weighted random-effects meta-analysis combining results across all 3 cohorts. The analysis included 23 lipids measured in all 3 cohorts.

*Adjusted for multiple testing using the Storey's q-value method.

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Table 5.

Results from sensitivity analysis in the SHFS. Lipids with P<0.05 are bolded

Lipid	Class	OR (95% CI)[≠]	P[≠]
PC(33:1)	**Phosphocholines**	**1.55 (1.15, 2.09)**	**0.004**
PC(34:1)	**Phosphocholines**	**1.51 (1.02, 2.24)**	**0.039**
PC(35:1)	**Phosphocholines**	**1.51 (1.10, 2.06)**	**0.010**
PC(p-36:4)/PC(o-36:5)	Phosphocholines	1.12 (0.83, 1.52)	0.458
PC(p-38:4)/PC(o-38:5) B	Phosphocholines	1.09 (0.81, 1.47)	0.576
PE(36:1) A	**Phosphoethanolamines**	**1.71 (1.12, 2.61)**	**0.013**
PE(34:1)	**Phosphoethanolamines**	**1.52 (1.13, 2.03)**	**0.005**
PE(34:2) B	**Phosphoethanolamines**	**1.39 (1.03, 1.86)**	**0.029**
PE(36:2)	**Phosphoethanolamines**	**1.49 (1.10, 2.02)**	**0.011**
PE(36:3)	**Phosphoethanolamines**	**1.46 (1.09, 1.96)**	**0.011**
PE(36:4)/PE(18:2/18:2)	**Phosphoethanolamines**	**1.31 (1.00, 1.71)**	**0.047**
PE(16:0/16:1)	Phosphoethanolamines	1.26 (0.98, 1.61)	0.070
PE(16:0/18:3)	**Phosphoethanolamines**	**1.34 (1.03, 1.73)**	**0.028**
PE(18:0/20:3)	**Phosphoethanolamines**	**1.34 (1.03, 1.75)**	**0.029**
PI(17:0/20:4)	**Phosphoinositols**	**1.54 (1.22, 1.93)**	**<0.001**
PI(18:0/20:3) A	**Phosphoinositols**	**1.45 (1.06, 1.98)**	**0.019**
PI(18:0/20:4)	**Phosphoinositols**	**1.52 (1.10, 2.08)**	**0.010**
DAG(34:2)	Diacylglycerols	1.11 (0.83, 1.48)	0.475
TAG(51:0)/TAG(16:0/17:0/18:0)	Triacylglycerols	1.28 (0.98, 1.68)	0.071
TAG(51:1)	**Triacylglycerols**	**1.34 (1.02, 1.76)**	**0.037**
TAG(52:4)	Triacylglycerols	0.97 (0.71, 1.33)	0.841
TAG(53:1)	**Triacylglycerols**	**1.30 (1.03, 1.64)**	**0.028**
TAG(53:2)	Triacylglycerols	1.31 (0.98, 1.75)	0.073
TAG(53:3)	**Triacylglycerols**	**1.39 (1.01, 1.89)**	**0.040**
TAG(53:5)/TAG(16:0/17:1/20:4)	**Triacylglycerols**	**1.36 (1.11, 1.68)**	**0.003**
TAG(54:3)	Triacylglycerols	1.35 (0.96, 1.88)	0.083
SM(d40:2) C	Sphingomyelins	0.85 (0.60, 1.21)	0.372
SM(d32:1) B	**Sphingomyelins**	**1.47 (1.08, 2.00)**	**0.014**
CER(d44:1)	Ceramides	1.05 (0.76, 1.45)	0.763

[≠]Obtained by mixed-effect logistic regression, adjusting for age, sex, BMI, hypertension, diabetes, total cholesterol, triglyceride, UACR, and use of lipid-lowering medications at baseline.