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Longitudinal lipidomic profile of hypertension in American Indians: Findings from the Strong Heart Family Study

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Abstract

Background: Dyslipidemia is an important risk factor for hypertension and CVD. Standard lipid panel cannot reflect the complexity of blood lipidome. The associations of individual lipid species with hypertension remain to be determined in large-scale epidemiological studies, especially in a longitudinal setting.

Methods: Using LC-MS, we repeatedly measured 1,542 lipid species in 3,699 fasting plasma samples at two visits (1,905 at baseline, 1,794 at follow-up, ~ 5.5 years apart) from 1,905 unique American Indians in the Strong Heart Family Study. We first identified baseline lipids associated with prevalent and incident hypertension, followed by replication of top hits in Europeans. We then conducted repeated measurement analysis to examine the associations of changes in lipid species with changes in systolic blood pressure, diastolic blood pressure, and mean arterial pressure. Network analysis was performed to identify lipid networks associated with risk of hypertension.

Results: Baseline levels of multiple lipid species, e.g., glycerophospholipids, cholesterol esters, sphingomyelins, glycerolipids, and fatty acids, were significantly associated with both prevalent and incident hypertension in American Indians. Some lipids were confirmed in Europeans. Longitudinal changes in multiple lipid species, e.g., acylcarnitines, phosphatidylcholines, fatty acids, and triacylglycerols, were significantly associated with changes in blood pressure measurements. Network analysis identified distinct lipidomic patterns associated with risk of hypertension.
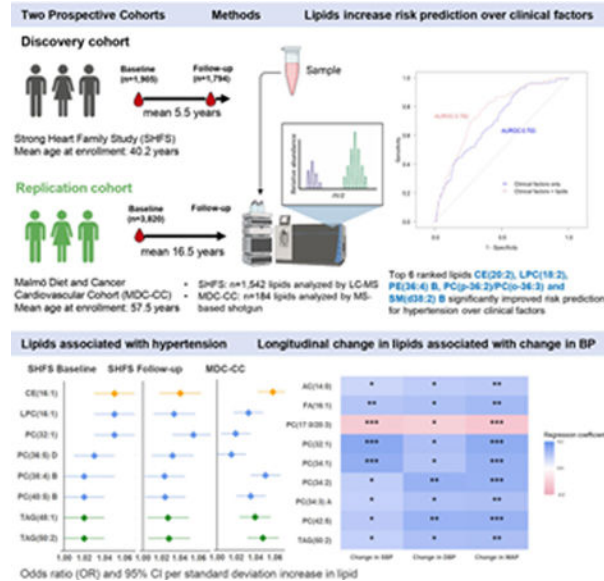
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Conclusion: Baseline plasma lipid species and their longitudinal changes are significantly associated with hypertension development in American Indians. Our findings shed light on the role of dyslipidemia in hypertension and may offer potential opportunities for risk stratification and early prediction of hypertension.

Graphical Abstract

Keywords

longitudinal lipidomics; mass spectrometry; hypertension; American Indians; Strong Heart Study

INTRODUCTION

Hypertension is a major contributor to cardiovascular diseases (CVD).[1, 2] Dyslipidemia, defined as high total cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDL-C) and/or low high-density lipoprotein cholesterol (HDL-C), is an established risk factor for both hypertension and CVD.[3–6] Deciphering the role of dyslipidemia in hypertension may lead to the discovery of novel biomarkers and therapeutic targets for prevention and treatment of CVD. However, standard lipid panel only captures a snapshot of bulk lipoproteins, which do not reflect the complexity of human plasma lipidome (i.e., all molecular lipid species in blood).[7, 8]

Lipidomic is a mass spectrometry-based omics technology that can identify and quantify hundreds to thousands of individual molecular lipid species in a biospecimen. Using lipidomics, previous studies have reported associations of altered levels of multiple individual lipid species, such as triacylglycerols (TAGs), diacylglycerols (DAGs), phosphatidylcholines (PCs), cholesterol esters (CEs) and sphingomyelins (SMs), with hypertension in Europeans,[9, 10] Asians,[11, 12] and Mexican Americans.[13] However, existing studies were largely cross-sectional and had smaller sample size and/or low coverage of the blood lipidome. To date, no large-scale longitudinal lipidomic profiling of hypertension

has been reported in any racial/ethnic group. Here, we report findings from a large-scale longitudinal profiling of plasma lipidome in 3,699 fasting blood samples from 1,905 unique American Indians attending two clinical examinations (~5.5 years apart on average) in the Strong Heart Family Study (SHFS). Putative lipid species identified in the SHFS were replicated in Europeans in the Malmö Diet and Cancer-Cardiovascular Cohort (MDC-CC). Our objectives here are to: (1) identify individual lipid species associated with prevalent and incident hypertension beyond known risk factors; and (2) examine the temporal relationship between change in plasma lipidome and changes in blood pressure measurements during an average 5.5-year follow-up.

RESEARCH DESIGN AND METHODS

The data that support the findings of this study are available from the corresponding author upon a reasonable request.

Study populations

The Strong Heart Family Study (SHFS) (2001-ongoing) is a multicenter, family-based prospective study designed to identify genetic, metabolic and behavioral factors for cardiovascular disease (CVD), diabetes and their risk factors in American Indians. Briefly, a total of 2,786 tribal members (aged 14 years and older) residing in Arizona, North Dakota, South Dakota, and Oklahoma were recruited and examined at baseline (2001–2003) and re-examined after 5-year follow-up (2006–2009). Detailed descriptions of the SHFS study design, laboratory protocols and phenotype collection have been described previously.[14–16] Participants received a personal interview and a physical examination at each visit, during which fasting blood samples were collected for laboratory tests. Laboratory methods were reported previously.[14] A total of 1,905 individuals (62% females; mean age at baseline: 40.2) who were free of overt CVD at baseline and had complete clinical data were included in the current analysis. All SHFS participants provided informed consent. The SHFS protocols were approved by the Institutional Review Boards of the participating institutions and the American Indian tribes.

The Malmö Diet and Cancer-Cardiovascular Cohort (MDC-CC) is a population-based prospective study established to investigate the epidemiology of carotid artery disease among Europeans in Malmö, Sweden.[17, 18] A total of 3,820 participants (60% females; mean age at baseline: 57.5) who were free of overt CVD at baseline and had complete clinical and lipidomic data were included in the present study. More details about the MDC-CC cohort were described in the Supplementary Materials. All participants provided written informed consent, and the study was approved by the Ethics Committee at Lund University.

Assessment of hypertension.—In both studies, blood pressure was measured by specially trained nurses on the right brachial artery using a mercury sphygmomanometer after the participant had been resting in a seated position for 5 minutes. For each participant, three measurements of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken, and the mean of the second and third measurements was used in the analysis. Mean arterial pressure (MAP) was calculated as DBP + ((SBP-DBP)/3). Hypertension was

defined as a resting SBP 140 mmHg or DBP 90 mmHg or current use of antihypertensive medication.[19]

Assessments of clinical covariates.—In the SHFS, demographic information (age and sex), lifestyle (smoking, drinking status, physical activity), medical history, and use of prescription medications were collected using structured questionnaires.[14, 15] In the MDC-CC, information on lifestyle and assessments of clinical factors was obtained as previously described.[20] More information for the assessments of covariates can be found in the Supplementary materials.

Lipidomic data acquisition, pre-processing and quality control

In the SHFS, methods for lipidomic data acquisition, processing and normalization have been described previously.[21] Briefly, relative abundance of molecular lipid species in fasting plasma samples at two time points (~5.5 years apart) was quantified by untargeted liquid chromatography-mass spectrometry (LC-MS). After pre-processing and quality control, we obtained 1,542 lipids (518 known) in 3,925 samples (1,970 at baseline, 1,955 at follow-up). After further excluding outlier samples and individuals with prevalent CVD or those with missing covariates, the final analysis included 1,542 individual lipid species in 3,699 fasting plasma samples (1,905 at baseline, 1,794 at follow-up, ~5.5 years apart) from 1,905 unique American Indians in the SHFS.

Lipidomic profiling in the MDC-CC was performed using fasting plasma samples collected at enrollment (i.e., baseline) by mass spectrometry as previously described.[22] Spectra were analyzed with in-house developed lipid identification software based on LipidXplorer.[23] Detailed methods for blood sample collection, data acquisition, processing and normalization of the lipidomic data in the MDC-CC have been described previously.[17, 24, 25] Briefly, a total of 3,820 participants (60% females) with available lipidomic data were obtained from the MDC-CC. Among these 184 lipids, 141 lipids were also available in the SHFS. The missing rates of these 141 lipids ranged from 0% to 24.2% among the 3,820 MDC-CC participants. Missing values of these lipids were imputed using the NIPALS algorithm.[26] The mean age of MDC-CC participants was 57.5 years at baseline and 72.1 years at the end of follow-up (mean 16.5 years of follow-up).

Statistical Analysis

Figure S1 illustrates the procedures for participants' selection and statistical analyses. All continuous variables including lipids were standardized to zero mean and unit variance. Multiple testing was controlled by false discovery rate (FDR) using the Storey's q-value method.[27, 28] Considering the age difference between participants in the two cohorts (mean age of participants at enrollment was 57.5 years and 40.2 years in the MDC-CC and SHFS, respectively), we first regressed out age and then used the residuals in subsequent data analyses.

Cross-sectional association analysis.—To identify lipids associated with prevalent hypertension, we constructed mixed-effects logistic model (SHFS) or logistic model (MDC-CC). In the model, status of hypertension (yes/no) was the outcome and the age-adjusted

lipid was the predictor, adjusting for sex, BMI, smoking and fasting glucose at the time blood sample was drawn. The analysis in the SHFS additionally adjusted for study center, alcohol drinking, eGFR and level of physical activity. Family relatedness in the SHFS was accounted for by including family as a random effect in the mixed model. In the SHFS, we first conducted analysis among participants at baseline (n=1,905). Top hits (lipids with P<0.05) were then confirmed among participants who attended SHFS follow-up visit (n=1,794, internal replication) and those enrolled in the MDC-CC (n=3,820, external replication). Replication was defined as lipids with P<0.05 and consistent directions of association in both cohorts. Meta-analysis was performed by inverse-variance weighted random-effect model to combine results across the two cohorts. Prevalent CVD was excluded from this analysis.

Prospective association analysis.—To identify baseline lipids predictive of risk for hypertension, we constructed mixed-effects logistic model (SHFS, n=1,430) or logistic model (MDC-CC, n=1,010), in which baseline level of each lipid was the predictor, and incident hypertension status (yes/no) was the outcome, adjusting for sex, BMI, smoking and fasting glucose at baseline. The analysis in the SHFS additionally adjusted for study center, eGFR, alcohol drinking and level of physical activity. Family relatedness in the SHFS was accounted for by including a random effect (i.e., family) in the model. Putative lipids (P<0.05) identified in the SHFS were then replicated in the MDC-CC. Results from both cohorts were combined by inverse-variance weighted random-effects meta-analysis.

To assess whether the identified lipids improve the prediction for risk of hypertension in American Indians, beyond clinical factors, we used data from two study centers (Dakota and Arizona) as training set (n=837, 152 cases), and those from another center (Oklahoma) (n=593, 112 cases) as testing set. We then compared a base model including traditional risk factors only (age, sex, BMI, smoking, alcohol drinking, fasting glucose, eGFR and physical activity) and a model containing both traditional risk factors and the top-ranked lipids identified in the SHFS. The incremental predictive value of lipids over known risk factors was assessed by area under the receiver operating characteristic curve (AUROC), net reclassification improvement (NRI) and integrated discrimination improvement (IDI).[29] Individuals with prevalent hypertension or overt CVD at baseline were excluded from this analysis.

Repeated measurement analysis.—Of 1,072 participants free of hypertension and overt CVD at baseline and follow-up, we constructed mixed-effects linear regression models to examine the temporal relationship between change in lipidome and change in blood pressure measurements, including systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP). In the model, longitudinal change in a blood pressure measurement was the outcome, and change in the relative abundance of lipid was the predictor. The model adjusted for study center, sex, smoking, alcohol drinking, and changes in BMI, eGFR, physical activity, fasting plasma glucose as well as baseline blood pressure and lipid. The analysis was performed for SBP, DBP or MAP, separately. The repeated measurement analysis was done in the SHFS only as MDC-CC only measured lipids at baseline.

Differential lipid network analysis.—To identify lipid networks (i.e., clusters of correlated lipids) associated with risk of hypertension, we performed the Weighted Correlation Network Analysis (WGCNA)[30] to construct lipid networks (or modules) based on the correlations between 1,542 baseline lipids among incident cases and non-cases, separately. Lipid species were hierarchically clustered, and those with a high topological overlap similarity were grouped into the same module. Differential modular analysis was performed to dissect intra-module difference (i.e., difference of connectivity among lipids within a module) between cases and non-cases. To quantify the intra-module difference, we calculated modular differential connectivity (MDC),[31, 32] i.e., the difference in the total connectivity of all lipid pairs for a specific lipid module between cases and non-cases. Gain of connectivity (GOC) was defined if MDC>0 and loss of connectivity (LOC) if MDC<0. Statistical significance of MDC was assessed by 1,000 permutation tests.[33, 34] We also conducted pathway enrichment analysis for lipids identified in the differential networks using the lipid ontology (LION) database.[35] More details about the differential network analysis can be found in the Supplementary Materials.

Sensitivity analysis.—To examine the potential impact of bulk lipids (e.g., HDL-C, LDL-C) and use of lipid-lowering drugs (yes/no) on the relationship between plasma lipids and hypertension, we additionally adjusted for these variables in the statistical models. This analysis focused on lipids that were significant in both SHFS and MDC-CC. Sex-specific effect of lipids on hypertension was tested by including an interaction term (lipid × sex) in the model.

RESULTS

In the SHFS, the age-standardized prevalence of hypertension was 25.7% at baseline (n=1,905, mean age: 40.2) and 31.2% at 5-year follow-up (n=1,794, mean age: 45.7). The age-standardized prevalence of hypertension was 30.5% (n=3,820, mean age: 57.5) in the MDC-CC. The age-standardized incidence rates of hypertension were 22.7% (mean follow-up: 5.5 years) in the SHFS and 29.5% (mean follow-up: 16.5 years) in the MDC-CC. Table 1 presents the characteristics of study participants in the two cohorts.

Plasma lipid species associated with prevalent hypertension.

At SHFS baseline visit, we identified 374 lipids (125 known lipids) significantly associated with prevalent hypertension at P<0.05. Of the 125 known lipids, 70 lipids were confirmed at follow-up visit with same direction at q<0.05. Specifically, 34 lipid species including 22 glycerophospholipids (e.g., phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), phosphatidylinositols (PIs)), 5 fatty acids (i.e., FA(14:1), FA(16:1), FA(20:2) A, FA(22:2), FA(22:3)), 2 acylcarnitines (i.e., AC(14:1), AC(16:0)), 3 glycerolipids (i.e., TAG(48:1), TAG(50:2), DAG(16:0/16:1)), one ceramide (i.e., CER(d40:0)), and one cholesterol ester (i.e., CE(16:1)) were positively associated with prevalent hypertension at both baseline and 5-year follow-up. In contrast, 36 lipids including 18 sphingomyelins, 14 glycerophospholipids (e.g., phosphatidylcholines, phosphatidylinositols, ether-phosphatidylcholines), 2 triacylglycerols (i.e., TAG(54:4),

TAG(54:5) A), one cholesterol ester (i.e., CE(18:2)), and one glycosylceramide (i.e., GlcCer(d34:1)) were inversely associated with prevalent hypertension at both time points.

Of the 125 known lipids identified at baseline in the SHFS, 40 lipids were also measured in the MDC-CC. Of these, 13 lipids, including 9 phosphatidylcholines, 2 triacylglycerols (i.e., TAG(48:1), TAG(50:2)), one phosphatidylethanolamine (i.e., PE(36:4) B), one cholesterol ester (i.e., CE(16:1)), were positively, whereas one lipid (i.e., SM(d33:1) B) was inversely associated with prevalent hypertension in the MDC-CC at P<0.05 (same directions of association). Of these 13 lipids, 10 known lipids (i.e., CE(16:1), LPC(16:1), PC(32:1), PC(34:1), PC(34:3) A, PC(38:4) B, PC(40:5) B, PE(36:4) B, TAG(48:1), TAG(50:2)) remained significant after multiple testing correction (q<0.05) (Table S1–2).

Of the 40 lipids measured in both cohorts, meta-analysis found that 20 lipids, including 15 glycerophospholipids (e.g., phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols, ether-phosphatidylcholines), 4 triacylglycerols, and CE(16:1), were positively whereas one lipid (i.e., SM(d33:1) B) was inversely associated with prevalent hypertension at q<0.05. Figure 1 displays plasma lipid species associated with prevalent hypertension in the SHFS and MDC-CC.

Baseline plasma lipid species predict incident hypertension beyond known clinical factors.

In the SHFS, we identified 49 baseline lipid species (out of 518 known lipids) significantly associated with incident hypertension at P<0.05. Specifically, higher baseline levels of 23 lipids, including 7 fatty acids, 7 phosphatidylethanolamines, 4 ceramides (i.e., CER(d32:1), CER(d34:1), CER(d36:1), CER(d40:0)), 3 phosphatidylinositols (i.e., PI(18:0/22:4), PI(18:0/22:5), PI(18:0/22:6)), and 2 triacylglycerols (i.e., TAG(50:2), TAG(58:6)) were significantly associated with an increased risk of hypertension. In contrast, higher baseline levels of 26 lipids, including 12 phosphatidylcholines, 8 sphingomyelins, 3 cholesterol esters (i.e., CE(18:2), CE(20:2), and CE(22:2)), 2 acylcarnitines (i.e., AC(10:1), AC(13:1)) and one glycosylceramide (i.e., GlcCer(d34:1)), were significantly associated with a decreased risk of hypertension.

Of the 49 known lipids significantly associated with risk of hypertension in the SHFS (P<0.05), 11 lipids were also measured in MDC-CC. Among them, 2 lipids, including CE(20:2) and PC(p-36:2)/PC(O-36:3), were confirmed in MDC-CC with the same direction of association.

Of the 11 lipids measured in both cohorts, meta-analysis identified 6 lipids (CE(18:2), CE(20:2), LPC(18:2), PC(p-36:2)/PC(o-36:3), SM(36:2) A, and SM(38:2) B) significantly associated with risk of hypertension at P<0.05. Of them, 5 lipids (i.e., CE(18:2), CE(20:2), LPC(18:2), PC(p-36:2)/PC(o-36:3) and SM(38:2) B) reached statistical significance at q<0.05. Figure S2 and Table S3 schematically illustrate the associations between baseline lipid species and risk of hypertension in both cohorts.

As shown in Figure 2, additional inclusion of the six lipids (i.e., CE(20:2), LPC(18:2), PE(36:4) B, PC(p-36:2)/PC(o-36:3), SM(d38:2) B and TAG(50:2)) identified in the meta-

analysis significantly improved risk prediction over clinical factors (AUROC increased from 0.703 to 0.762, P=0.002; continuous NRI = 0.398, P_{NRI} <0.001; IDI = 0.047, P_{IDI} <0.001).

Longitudinal change in lipid species associated with change in blood pressure.

After correction for covariates and multiple testing (q<0.05), longitudinal changes in 156 lipids (70 known) were significantly associated with change in SBP (Table S4). Specifically, longitudinal changes in 35 lipids, including 20 sphingomyelins, 11 phosphatidylcholines, 4 ceramides (i.e., CER(d32:1), CER(d33:1), CER(d34:2), CER(d40:2)) were inversely, whereas changes in 35 lipids, including 15 phosphatidylcholines, 7 phosphatidylethanolamines, 4 fatty acids (i.e., FA(14:1), FA(16:1), FA(22:2), FA(22:3)), 3 phosphatidylinositols (i.e., PI(16:0/16:1), PI(16:0/18:1), PI(18:0/22:4)), 2 triacylglycerols (i.e., TAG(50:1), TAG(50:2)), one diacylglycerol (i.e., DAG(16:0/16:0)), one acylcarnitine (i.e., AC(14:0)), one cholesterol ester (i.e., CE(16:1)) and one glycosylceramide (i.e., GlcCer(d42:1)), were positively associated with change in SBP. Of the 70 known lipids significantly associated with change in SBP, changes in 47 known lipids (i.e., 22 phosphatidylcholines, 8 sphingomyelins, 4 phosphatidylethanolamines, 4 fatty acids, 2 phosphatidylinositols, 2 triacylglycerols, AC(14:0), CE(16:1), CER(d33:1), DAG(16:0/16:1), GlcCer(d42:1)) were also significantly associated with changes in MAP at q<0.05. In contrast, changes in 9 (out of 70) known lipids (i.e., 6 phosphatidylcholines, AC(14:0), FA(16:1), TAG(50:2)) were also significantly associated with changes in DBP at q<0.05 (Table S5–6, Figure 3–4). Among the significant lipids identified, changes in 8 lipids, including AC(14:0), FA(16:1), PC(32:1), PC(34:1), PC(34:2), PC(34:3) A, PC(42:5) and TAG(50:2), were positively, while change in PC(17:0/20:3) was inversely associated with changes in SBP, DBP and MAP.

Differential lipid networks associated with risk of hypertension.

Network analysis in the SHFS identified 12 lipid modules among participants who developed incident hypertension (cases) and those who did not (non-cases) (Figure S3). The connectivity (i.e., corrections) between lipids in two modules (i.e., module turquoise and module blue) exhibited significant difference between cases and non-cases (Figure 5). Lipids included in the module turquoise largely include ceramides and sphingomyelins. Of these, six lipids (i.e., CE(18:0), SM(d36:2) A, SM(d36:3) A, SM(d37:1) A, SM(d38:2) A, and SM(d41:2) B)) were significantly associated with incident or prevalent hypertension. Hub lipid in the module turquoise was SM(d39:1) A. Lipids included in the module blue largely include triacylglycerols and diacylglycerols. Decreased levels of two lipids (i.e., TAG(54:4) and TAG(54:5) A)) were significantly associated with prevalent hypertension at both time points. Compared to non-cases, incident cases exhibited gain of connectivity (GOC) for lipids in the module turquoise (modular differential connectivity (MDC)=204.4, P=0.032) and the module blue (MDC=675.3, P=0.001). Hub lipids in the module blue included TAG(58:3), and TAG(51:4).

Results from sensitivity analyses.

The observed associations between plasma lipids and prevalent or incident hypertension remained largely unchanged after further adjustments for bulk lipids (e.g., HDL-C, LDL-C) and use of lipid-lowering medications (Table S7–8). Sex-specific analysis indicated that:

1) the associations between four known lipids, including CE(16:1), PC(32:1), PC(34:1), PE(36:4) B, and prevalence of hypertension significantly differ in men and women (all q<0.05); 2) longitudinal changes in three known lipids, i.e., FA(14:1), FA(16:1), and SM(d39:1) A, and their associations with change in SBP, but not DBP or MAP, significantly differ in men and women (all q<0.05). Results for sex-specific analyses are shown in Table S9.

DISCUSSION

In a large-scale lipidomic profiling comprising over 5,700 community-dwelling individuals from two prospective cohorts, we had several key findings. **First,** cross-sectional analysis showed that altered levels of glycerophospholipids (e.g., PCs, PEs, PIs), sphingolipids (e.g., SMs, CERs, GlcCers), glycerolipids (e.g., DAGs, TAGs), acylcarnitines, cholesterol esters, and fatty acids were significantly associated with prevalent hypertension in American Indians. **Second,** our prospective association analysis found that several lipid species were significantly associated with future risk for hypertension, independent of clinical factors. **Third,** our repeated measurement analysis demonstrated, for the first time, that longitudinal change in fasting plasma lipidome was significantly associated with change in blood pressure among American Indians, after adjusting for known clinical factors, baseline lipid and baseline blood pressure. **Fourth,** our network analysis identified differential lipid clusters (i.e., modules) associated with risk of hypertension. Collectively, our results revealed distinct lipidomic signatures associated with hypertension and provide novel insight into the role of dyslipidemia in hypertension pathogenesis.

We found that altered levels of plasma glycerophospholipids (e.g., PCs, PEs) were significantly associated with hypertension in American Indians. Although the specific lipid species may vary between studies (due to using different mass spectrometry platforms), our findings appeared to be consistent with previous reports showing that some species of glycerophospholipids, e.g., PC(32:1), PC(34:1), PC(36:5), PC(38:4), PC(p-36:2)/PC(o-36:3), PE(38:4), were associated with hypertension in other racial/ethnic groups.[9, 13, 36–38] In addition, many of the identified glycerophospholipids (e.g., PC(32:1), PC(34:1), PE(16:0/16:1), PE(36:4), LPC(18:2)) were also associated with risk of chronic kidney disease in American Indians[21] and CVD in Europeans.[25] Plasminogen, a subclass of membrane glycerophospholipids, is primarily present as phosphatidylcholines (PCs) or phosphatidylethanolamines (PEs) and exhibits diverse biological functions.[39] Reduced plasma plasminogen has been associated with type 2 diabetes and cardiovascular disease.[40, 41] Disturbance in plasminogen metabolism may contribute to hypertension through their roles in oxidative stress, β-oxidation and cell signaling pathways.[42]

In addition to glycerophospholipids, we also found significant associations of cholesterol esters and long-chain unsaturated sphingomyelins with prevalent or incident hypertension in both cohorts. The observed associations of cholesterol esters (e.g., CE(16:1), CE(20:2)) or long-chain unsaturated sphingomyelins (i.e., SM(d33:1), SM(d36:2), SM(d38:2) and SM(d41:2)) with hypertension have also been previously associated with hypertension in other ethnic groups.[11, 36] Moreover, longitudinal changes in some long-chain unsaturated sphingomyelins (e.g., SM(d33:1), SM(d37:1), SM(d43:1)) were inversely associated with

changes in SBP and MAP. These consistent results from different analyses herald the robustness of our findings.

Sphingolipids are major structural and functional components of cellular membranes. They play crucial roles in multiple biological processes, including growth regulation, cell migration, adhesion, apoptosis, senescence, and inflammatory responses.[43, 44] Altered sphingolipid levels have been implicated in metabolic diseases,[45, 46] neurodegenerative processes,[47] immune responses,[48] and certain cancers.[44] Interestingly, recent evidence from experimental research has shown that endothelial sphingolipid de novo production regulates blood pressure homeostasis, and decreased sphingolipids (e.g., sphingomyelins) may affect hypertension through regulating signal transduction and endothelial nitric oxide synthase via ceramides.[44]

Besides glycerophospholipids and sphingolipids, we also found that baseline levels of fatty acids (e.g., FA(17:1), FA(18:1), FA(20:1) and FA(22:3)) and their longitudinal changes were associated with both prevalent and incident hypertension in American Indians. These observed associations appeared to be consistent with previous studies reporting that hypertensive individuals had increased levels of fatty acids compared to nonhypertensive individuals.[49] Fatty acids may affect blood pressure through inhibiting endothelium-dependent vasodilatation and endothelial nitric oxide synthase.[50]

Our longitudinal lipidomic analysis revealed the temporal relationship between changes in plasma lipidome and changes in blood pressure. Specifically, after adjustment for clinical factors, baseline lipid and blood pressure, longitudinal changes in phosphatidylcholines with a lower degree of unsaturation (e.g., PC(32:1), PC(34:1), PC(34:3)) and long-chain unsaturated triacylglycerols (e.g., TAG(50:1), TAG(50:2)) were positively associated with changes in SBP or DBP. The positive associations of some species of glycerolipids (e.g., TAG(50:2), DAG(14:0/22:5), DAG(15:0/18:3), DAG(16:0/16:0)) with blood pressure have been previously reported in other populations.[11, 49] Notably, TAG(50:2) was associated with both incident and prevalent hypertension and its longitudinal change was associated with changes in SBP, DBP and MAP. The associations of triacylglycerols with hypertension are in agreement with previous studies showing that higher plasma levels of long-chain unsaturated triacylglycerols (e.g., TAG(52:2), TAG(50:4), TAG(54:2), TAG(54:3), TAG(56:3)) were associated with CVD in various human populations.[51, 52] The observed associations of diacylglycerols that contained palmitic acid (16:0) with SBP, MAP and prevalent hypertension in our study appeared to be consistent with previous studies[13] showing that multiple DAGs (e.g., DAG(16:0/22:5), DAG(16:0/22:6)) were significantly associated with SBP in Mexican Americans. It is likely that DAGs may affect blood pressure through activating protein kinase C in vascular smooth muscle response.[53]

In line with previous evidence for a sex difference in blood pressure[54] or lipid profile,[55, 56] we found that gender significantly modulates the associations of four lipids, including CE(16:1), PC(32:1), PC(34:1), PE(36:4) B, with the prevalence of hypertension in American Indians. The associations of longitudinal changes in three lipids, including FA(14:1), FA(16:1), SM(d39:1) A, with change in SBP were also significantly different between men and women. The sex-specific effect of lipids on hypertension could be attributed

to the role of sex hormones (e.g., estrogen and testosterone) in modulating vasodilator and vasoconstrictor pathways, such as the renin-angiotensin-aldosterone system (RAAS) and the vascular endothelin system,[57] both of which play pivotal roles in blood pressure regulation.[58, 59]

Our study has several limitations. Despite the large number of individual lipid species detected in our study, many were unknown compounds and we were unable to distinguish isomeric lipids. These unknown chemicals and isomers require further characterization if deemed relevant. The lack of absolute quantification may also limit the potential for clinical application. In addition, although our statistical models controlled many clinical factors associated with hypertension, we cannot exclude the possibility of residual confounding by unknown or unmeasured factors. Further, like other large-scale epidemiological studies, blood pressure was measured on the brachial artery. Despite that we measured blood pressure three times and used the average value in the data analysis, we cannot exclude the possibility of underdiagnoses of hypertension or a misdiagnosis of white coat hypertension. Finally, the observational nature of our study precludes any causal inference regarding the causal role of altered lipid metabolism in hypertension pathogenesis.

However, our study has several strengths. First, the longitudinal profiling of plasma lipidome in a community-based prospective cohort represents the major strength of this study. To our knowledge, this represents the first of its kind in the fields of lipidomic studies on hypertension in large-scale epidemiological studies. Second, the current study included over 5,700 participants in two prospective cohorts comprising individuals with diverse backgrounds in demographic (e.g., age, gender, socioeconomic status), genetic, lifestyle (e.g., smoking, diet, physical activity) and environmental exposures. Despite these differences, many identified lipids could be replicated in both cohorts, signifying the robustness of our findings. Third, we conducted comprehensive statistical analyses including both cross-sectional and prospective association analyses, repeated measurement analysis, and network analysis to identify individual lipid species and lipidomic signatures associated with hypertension in American Indians. Some of the lipids could be confirmed in an independent cohort with distinct genetic and environmental backgrounds. Fourth, our analyses adjusted for a comprehensive list of clinical variables including comorbid conditions associated with hypertension (e.g., obesity, diabetes and renal function). Moreover, we performed sensitivity analysis to additionally adjust for bulk lipids (LDL-C and HDL-C) and use of lipid-lowering medications. Thus, lipids identified in our study should be independent of these known risk factors. Notably, the newly identified lipids could significantly improve risk prediction beyond established risk factors. Finally, our high-resolution lipidomic profiling identified a larger number of lipid species. This allowed us to identify novel lipid species that might have been missed out in previous studies, and offer opportunities for future investigations.

PERSPECTIVES

Altered baseline levels of multiple individual lipid species, such as glycerophospholipids, triacylglycerols and fatty acids, and their longitudinal changes were significantly associated with hypertension in American Indians, above and over conventional risk factors. The newly

identified lipid species shed light on the role of dyslipidemia in hypertension pathogenesis and, if validated, are likely to serve as novel biomarkers for risk stratification and early detection of hypertension.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

Data Availability.

The phenotype data used in this study can be requested through the Strong Heart Study (https://strongheartstudy.org/). The lipidomic data can be obtained from the corresponding author upon a reasonable request.

Nonstandard Abbreviations and Acronyms

CVD	Cardiovascular disease
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
MAP	Mean arterial pressure
LDL-C	Low-density lipoprotein cholesterol
HDL-C	High-density lipoprotein cholesterol
TAGs	Triacylglycerols
DAGs	Diacylglycerols
PCs	Phosphatidylcholines
CEs	Cholesterol esters
SMs	Sphingomyelins

SHFS	Strong Heart Family Study
MDC-CC	Malmö Diet and Cancer-Cardiovascular Cohort
LC-MS	Liquid chromatography-mass spectrometry
eGFR	Estimated glomerular filtration rate

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NOVELTY AND RELEVANCE

What Is New?

- This is the first longitudinal lipidomic study of hypertension in a large community-based prospective cohort of American Indians, followed by replication of top hits in Europeans in an independent population study.

- Altered baseline levels of multiple individual lipid species and their longitudinal changes are significantly associated with hypertension in American Indians. Some lipid species significantly improve risk prediction for hypertension beyond traditional risk factors.

What Is Relevant?

- Identifying novel molecular lipid species not only deepens our understanding of the mechanism through which dyslipidemia contributes to hypertension, but also provides novel biomarkers for risk stratification and early detection of hypertension.

Clinical/Pathophysiological Implications?

- If validated, the identified lipid species may serve as novel biomarkers for risk stratification and early prediction of hypertension.

OR (95% CI) per SD increase in fasting plasma lipids
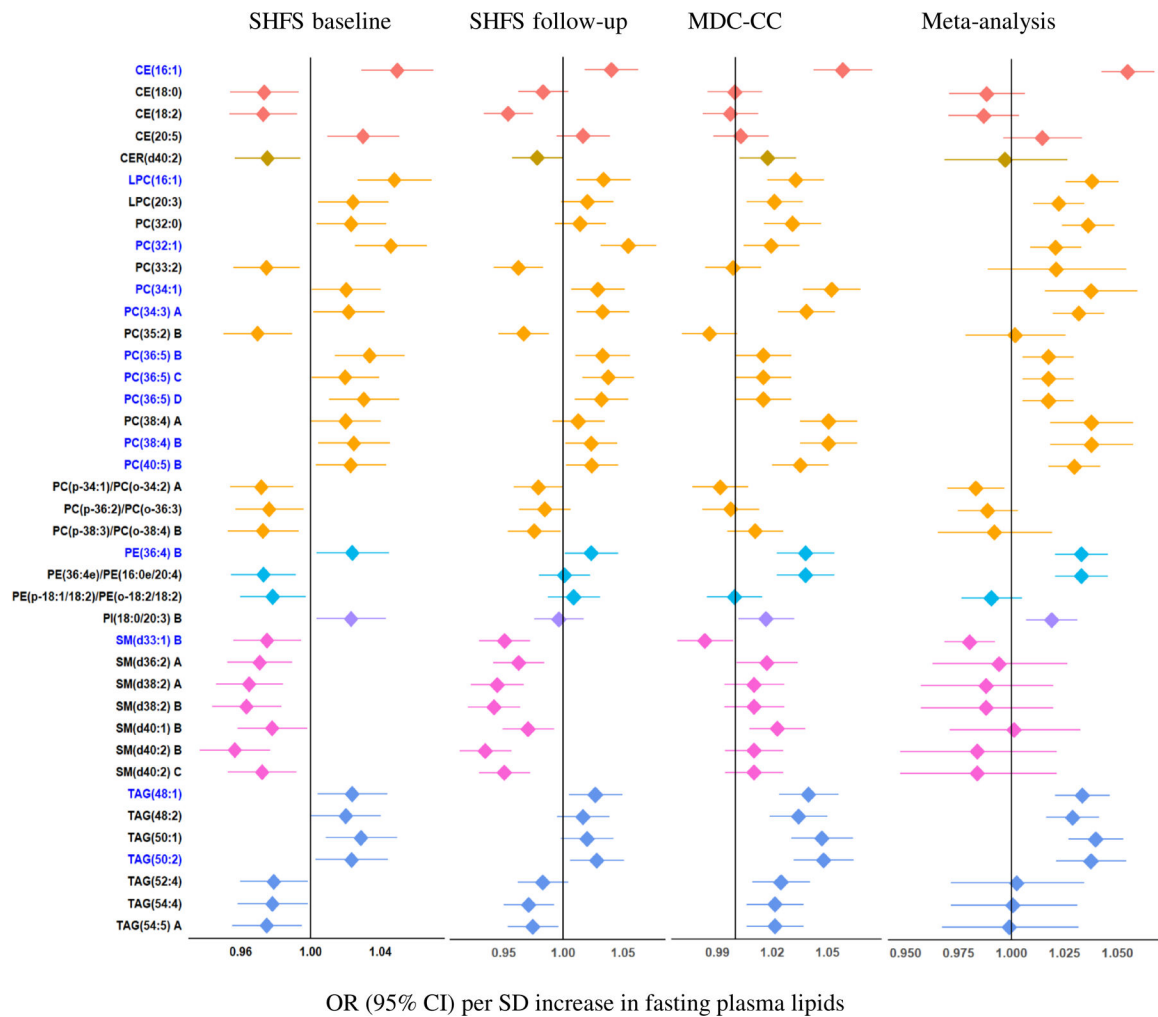
Figure 1.
Plasma lipid species associated with prevalent hypertension. Considering the age difference between participants in the SHFS and those in the MDC-CC, we first regressed out the effect of age and used the age-adjusted residuals as independent variable in the subsequent data analysis. Odds ratios (ORs) and 95% confidence intervals (CIs) in the SHFS (discovery) were obtained by mixed-effect logistic model, adjusting for center, sex, BMI, smoking, alcohol drinking, fasting glucose, eGFR and physical activity at the time blood sample was drawn. ORs in the MDC-CC (replication) were obtained by logistic regression, adjusting for sex, BMI, smoking and fasting glucose. Only known lipids with P<0.05 in the SHFS are shown. The names of lipids confirmed in the MDC-CC (q<0.05) are highlighted in blue. The letter A, B, C or D in the name of lipids represents isomers.
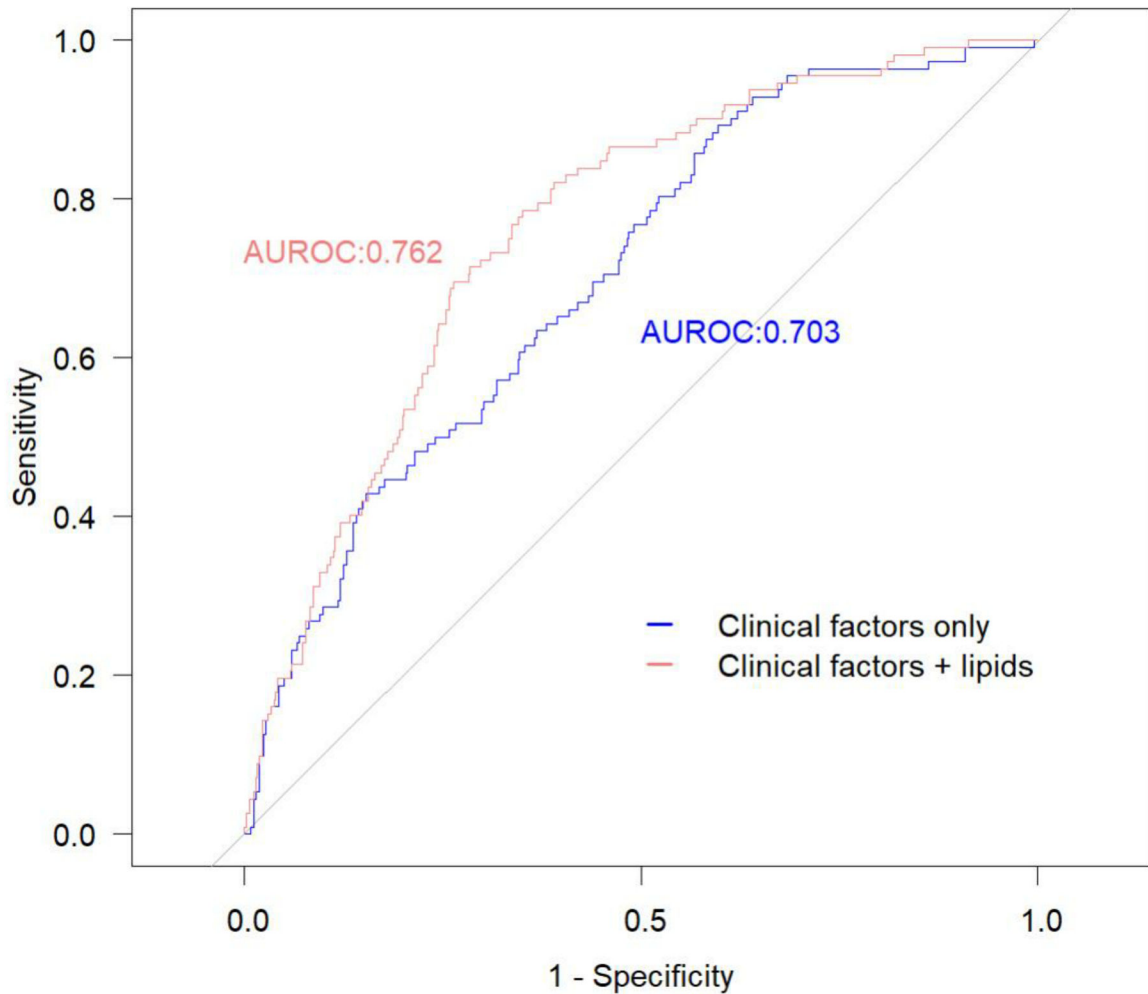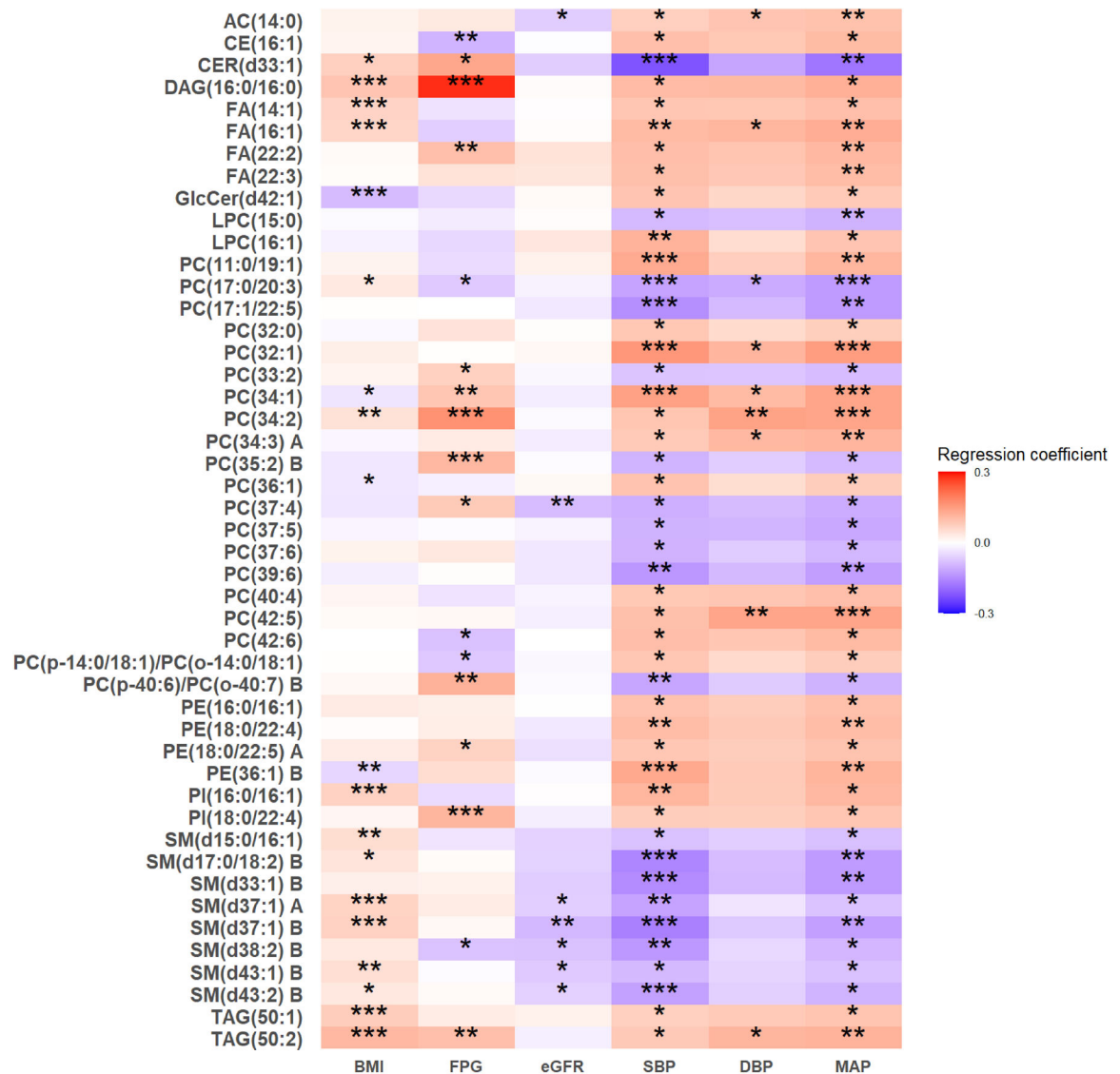
Figure 2.
Incremental value of the identified plasma lipids in risk prediction for hypertension. Model 1: clinical factors only, including center, age, sex, BMI, smoking, drinking, eGFR, fasting glucose and physical activity at baseline. Model 2: clinical factors + 6 significant lipids, including CE(20:2), LPC(18:2), PE(36:4) B, PC(p-36:2)/PC(o-36:3), SM(d38:2) B and TAG(50:2). Compared to the model that included clinical factors only (model 1), additional inclusion of plasma lipids (model 2) significantly increases risk prediction for hypertension. AUROC increases from 0.703 to 0.762 (P = 0.002); continuous NRI = 0.398, P_{NRI} <0.001; IDI = 0.047, P_{IDI} <0.001.
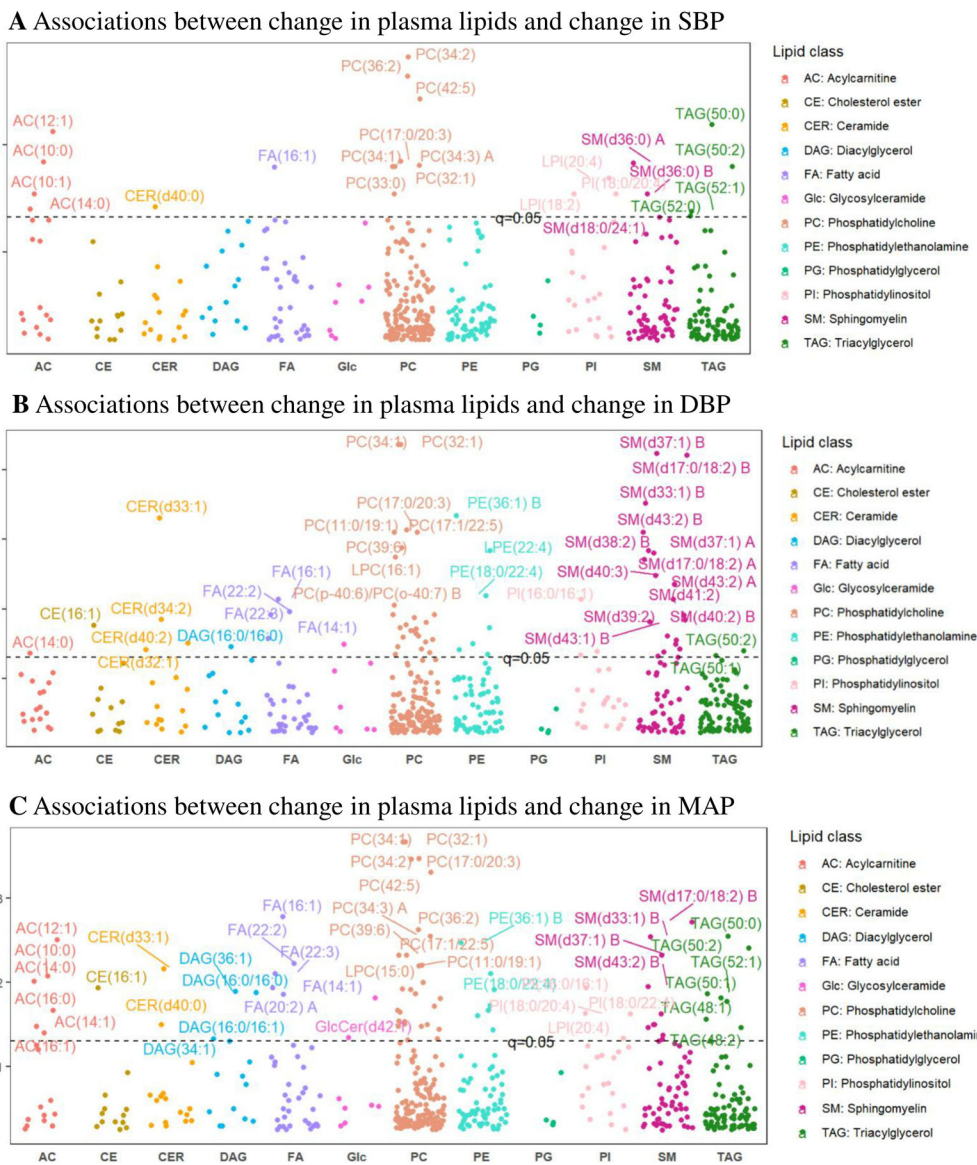
Figure 3.
Manhattan plot displaying the longitudinal associations between change in plasma lipids and change in systolic blood pressure (SBP), diastolic blood pressure (DBP) or mean arterial pressure (MAP) over an average of 5.5-year follow-up. X-axis: lipid classes; Y-axis: $-\log_{10}$ P. Different colors represent different lipid categories. The dashed line represents significance level at q = 0.05.

Figure 4.
Heatmaps showing the longitudinal associations between change in plasma lipids and changes in BMI, fasting plasma glucose (FPG), estimated glomerular filtration rate (eGFR), systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) during follow-up. Each row represents a lipid, and each column represents a phenotype. Only the top significant lipids are shown here. Color codes are based on the regression coefficients obtained from the linear mixed-effect model, adjusting for age, center, sex, BMI, smoking, drinking, physical activity, eGFR and fasting glucose at baseline as well as baseline lipid and blood pressure. *q<0.05, **q<0.01, ***q<0.001.
Abbreviations: AC: acylcarnitine, CE: cholesterol ester, CER: ceramide, DAG: diacylglycerol, FA: fatty acid, GlcCer: glycosylceramide, LPC: lysophosphatidylcholine, PC: phosphatidylcholine, PC(P)/PC(O): ether-phosphatidylcholine, PE: phosphatidylethanolamine, PI: phosphatidylinositol, SM: sphingomyelin, TAG: triacylglycerol.
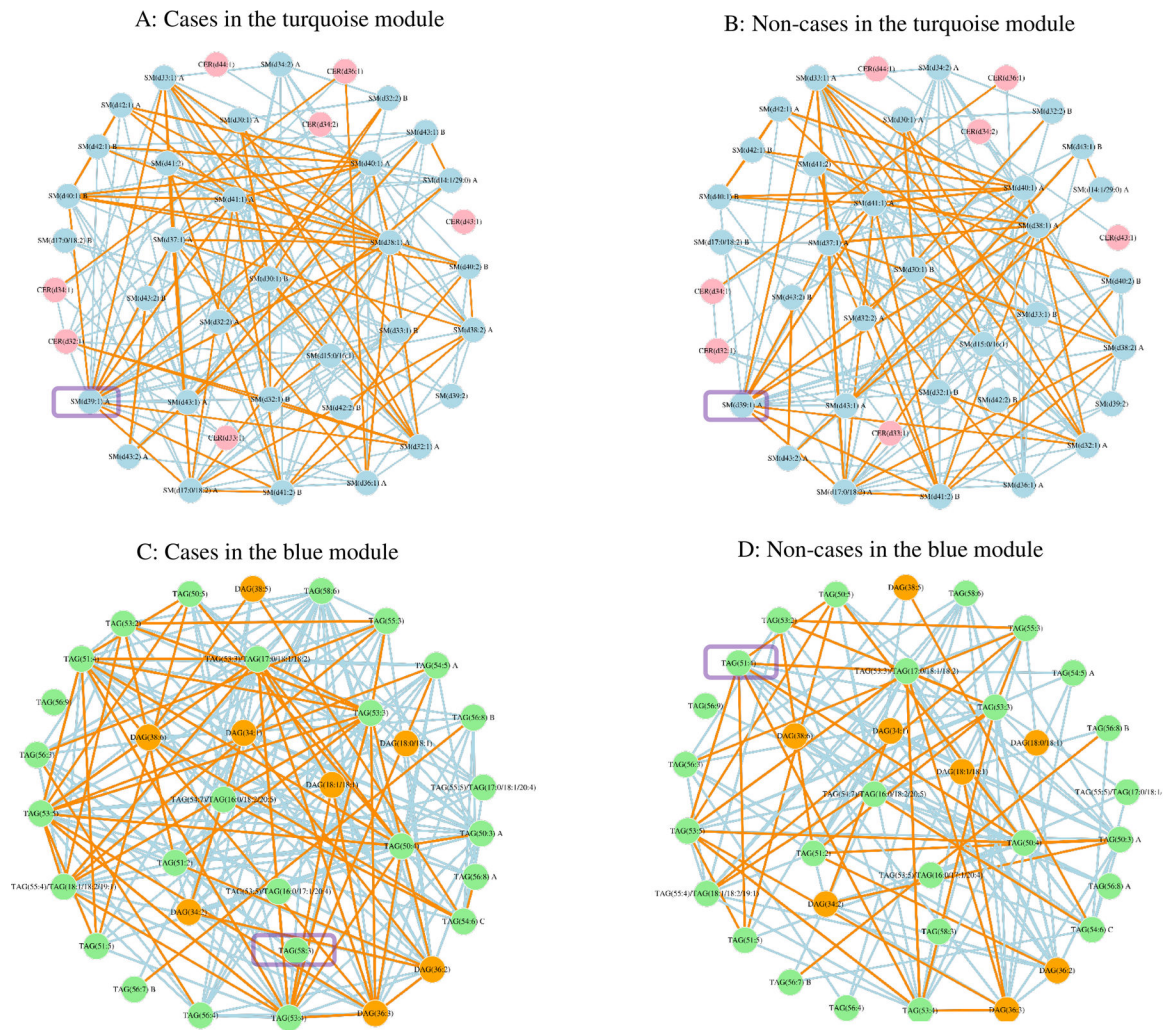
Figure 5.

Differential lipid networks associated with incident hypertension in the SHFS. Each node represents a lipid, and nodes in different colors represent different lipid classes. The edge colors reflect the strength of correlation between lipids (orange shows the strongest correlation, followed by light blue). Hub lipids were highlighted by purple squares.

Turquoise module: Gain of connectivity among participants who developed incident hypertension at follow-up (cases, **A**) compared to those who did not (non-cases, **B**). Modular differential connectivity (MDC) [cases – non-cases] = 204.4, P=0.032).

Blue module: Gain of connectivity among participants who developed incident hypertension at follow-up (cases, **C**) compared to those who did not (non-cases, **D**). Modular differential connectivity (MDC) [cases – non-cases] = 675.3, P=0.001).

Abbreviation: CER: ceramide, DAG: diacylglycerol, SM: sphingomyelin, TAG: triacylglycerol.

The letter A, B or C in the name of lipids represents isomer.

Table 1.

Clinical characteristics of study participants in the SHFS and the MDC-CC cohorts

Characteristics	SHFS baseline (n=1,905)	SHFS follow-up (n=1,794)	MDC-CC baseline (n=3,820)
Age (years)	40.2 ± 14.0	45.2 ± 13.5	57.5 ± 6.0
Female, n (%)	1,188 (62.4)	1,116 (62.2)	2,293 (60.0)
BMI (kg/m^2)	31.8 ± 7.5	32.7 ± 7.6	25.6 ± 3.9
Current smoking, n (%)	761 (39.9)	679 (37.8)	869 (22.7)
Type 2 diabetes, n (%)	346 (18.2)	437 (24.4)	334 (8.7)
Systolic blood pressure (mmHg)	122.3 ± 15.4	122.8 ± 16.3	141.6 ± 19.0
Diastolic blood pressure (mmHg)	77.2 ± 10.6	74.8 ± 11.1	86.9 ± 9.4
Mean arterial pressure (mmHg)	92.3 ± 10.9	90.8 ± 11.3	105.1 ± 11.7
Fasting glucose (mg/dL)	109.0 ± 45.4	113.5 ± 53.3	93.0 ± 23.8
HDL-C (mg/dL)	51.9 ± 14.4	50.6 ± 15.3	54.1 ± 14.3
LDL-C (mg/dL)	101.6 ± 29.9	106.1 ± 30.8	161.6 ± 38.3
Triglycerides (mg/dL)	162.9 ± 114.4	161.2 ± 135.6	99.2 ± 55.8
Total cholesterol (mg/dL)	185.0 ± 34.3	188.1 ± 38.4	238.6 ± 41.8

Continuous variables were expressed as mean ± standard deviation and qualitative variables were expressed as n (%). BMI: body mass index; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.