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PLASMA LIPIDOMIC PROFILE SIGNATURE OF HYPERTENSION IN MEXICAN AMERICAN FAMILIES: SPECIFIC ROLE OF DIACYLGLYCEROLS

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Abstract

Both as a component of metabolic syndrome and as an independent entity, hypertension poses a continued challenge with regard to its diagnosis, pathogenesis and treatment. Previous studies have documented connections between hypertension and indicators of lipid metabolism. Novel technologies like plasma lipidomic profiling promise a better understanding of disorders in which there is a derangement of the lipid metabolism. However, association of plasma lipidomic profiles with hypertension in a high-risk population, like Mexican Americans, has not been evaluated before. Using the rich data and sample resource from the ongoing San Antonio Family Heart Study, we conducted plasma lipidomic profiling by combining high performance liquid chromatography with tandem mass spectroscopy to characterize 319 lipid species in 1192 individuals from 42 large and extended Mexican American families. Robust statistical analyses employing polygenic regression models, liability threshold models and bivariate trait analyses implemented in the SOLAR software were conducted after accounting for obesity, insulin resistance and relative abundance of various lipoprotein fractions. Diacylglycerols in general and the DG 16:0/22:5 and DG 16:0/22:6 lipid species in particular were significantly associated with systolic, diastolic and mean arterial pressures as well as liability of incident hypertension measured during 7767.42 person-years of follow-up. Four lipid species, including the DG 16:0/22:5 and DG 16:0/22:6 species, showed significant genetic correlations with the liability of hypertension in bivariate trait analyses. Our results demonstrate the value of plasma lipidomic profiling in the context of hypertension and identify disturbance of diacyglycerol metabolism as an independent biomarker of hypertension.

Keywords

lipidomics; hypertension; blood pressure; lipid species; Mexican Americans

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CONFLICT OF INTEREST DISCLOSURES

None

INTRODUCTION

Hypertension, an important component of metabolic syndrome and one of the most important contributors to cardiovascular disease,[1–5] strongly correlates with measures of lipid metabolism.[6–10] Traditionally, total serum cholesterol, triglyceride, high-density lipoprotein and low-density lipoprotein concentrations in plasma are considered important and clinically useful biomarkers of hypertension. The next level of resolution is offered by the lipoprotein fractions such that differential relative abundance of some HDL or LDL fractions can provide a more informative picture of hypertension pathophysiology.[11–13] Dyslipidemia is a characteristic feature of hypertension. Subsequent and consequent visceral fat deposition[14] and endothelial dysfunction are hallmarks of early essential hypertension.[15, 16] In this context, a deeper understanding of the perturbed lipid homeostasis in the pathophysiology of hypertension is needed.

More recently, Graessler et al[17] demonstrated that the rapidly evolving technology of plasma lipidomic profiling can be used effectively to characterize the lipid metabolism changes associated with hypertension and other components of metabolic syndrome. For example, hypertension was independently associated with the ether phosphatidylcholine, ether phosphatidylethanolamine and triacylglycerol classes more strongly as compared to the routinely used clinical measures of lipid metabolism derangement.[17] The combination of liquid chromatography with mass spectroscopy has proved to be extremely valuable in the identification and quantification of a plethora of lipid species and their associations with complex diseases.[18–22] The advantages of this method are high resolution, accuracy and associative information at the level of lipid species.[23, 24] It is noteworthy that serum lipidomic studies have already offered critical insights in the pathogenesis of complex diseases like obesity and insulin resistance.[22, 25, 26] Initial studies in relation to hypertension have also been very encouraging although generally limited by small sample sizes and lower resolution of lipidomic profiling.[17, 27]

Mexican Americans account for 66% of the United States Hispanic population, and are at a high risk of dyslipidemia and insulin resistance.[28] In spite of this, to our knowledge, currently there are no studies available that characterize the serum lipidomic profile and associate it with the risk of hypertension in this population. The San Antonio Family Heart Study (SAFHS) is an ongoing study of large extended Mexican American families.[29, 30] Using the rich data and sample resource from this study, we conducted an investigation into the association of plasma lipid profile with blood pressure and hypertension. We hypothesized that specific lipid species associate with the risk of hypertension in the study subjects independently of lipoprotein fractions. Here, we present the results that demonstrate a strong and independent association of some lipid species with hypertension-related traits.

METHODS

Study participants

The San Antonio Family Heart Study (SAFHS) focuses on 1,431 individuals of 42 Mexican American families in San Antonio. Details of this collaborative study have been described elsewhere.[29, 30] Briefly, the aim of SAFHS is to quantify the relative contributions of genetic and environmental factors to the risk of developing cardiovascular diseases and metabolic syndrome. Extensive phenotypic assessment for a number of traits related to metabolic syndrome has been performed in these individuals. This project involving the Texas Biomedical Research Institute and the University of Texas Health Science Center at San Antonio was initiated in 1991. Informed consent was obtained from all participants before collection of samples. The Institutional Review Board of the University of Texas Health Sciences Center at San Antonio approved the study. For this study, we included data

on participants who were followed-up for up to two additional visits spaced approximately 5 years apart in addition to the enrolment visit. The details of data collection are described elsewhere.[29, 30] We included a total of 1,192 subjects for whom blood pressure data at the time of enrolment was available. The mean age of the study subjects at the first clinic visit was 39.42 years (SD 16.89) and 63% were female.

Outcomes and predictors

We studied the association of a total of 319 plasma lipid species with the following four hypertension-related traits: systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and liability of incident hypertension. Of these, SBP and DBP were cross-sectionally measured on the same day on which the blood sample for lipidomic profiling was drawn and MAP was defined as DBP + ((SBP-DBP)/3). Measurement procedures for SBP and DBP have been described previously.[31, 32] Briefly, these measurements were conducted using a random-zero sphygmomanometer on the left arm. To account for the potential variability in the blood pressure measurements, we measured the blood pressure thrice with 5-minute intervals but used the average of the last two readings as the phenotypic trait value. In this study the mean (SD) SBP, DBP and MAP were 120.44 (18.73), 70.65 (10.33) and 87.25 (11.59) mmHg, respectively. Incident hypertension was defined as SBP>140 mmHg and/or DBP>90 mmHg or history of anti-hypertensive treatment during the follow-up visits in subjects who were normotensive at enrolment. For the outcome of incident hypertension, we therefore excluded subjects on whom data for all three visits were not available or who were detected to have hypertension at enrolment or who were already receiving antihypertensive treatment. This data was available on 736 subjects and represented a total follow-up of 7140.17 person-years. Of these 736 subjects, 250 (34.0%) developed hypertension during follow-up with an incidence rate of 35.0 new cases of hypertension per 1000 study subjects per year.

To examine the independence of the association of lipid species with the abovementioned outcomes, we adjusted for the following variables in regression models: age, age^2, sex, age x sex interaction, age^2 x sex interaction, eight HDL fractions (HDL1, HDL2b1, HDL2b2, HDL2a, HDL3a, HDL3b, HDL3c1 and HDL3c2), five LDL fractions (LDL1, LDL2, LDL3, LDL4a and LDL4b), body mass index (BMI as a measure of obesity), homeostasis model of assessment-insulin resistance (HOMA-IR as a measure of insulin resistance) and use of lipid lowering drugs. For the outcomes of SBP, DBP and MAP, we additionally included use of antihypertensive drugs as a covariate. Methods of assessment of the relative abundance of lipoprotein fractions in SAFHS have been described elsewhere.[33] BMI was estimated as weight (Kg)/height^2 (m) while HOMA-IR was estimated as fasting glucose (mmol/L)*fasting insulin (μU/ml)/22.5.[34]

Lipidomic studies

Samples were analysed in the Metabolomics Laboratory, Baker IDI Heart and Diabetes Institute. A 10 μL aliquot of plasma was combined with 200 μL $CHCl_3$/MeOH (2:1) and 15μL of internal standard mix and then briefly vortexed. Samples were mixed (rotary mixer, 10 min), sonicated (water bath, 30 min) then allowed to stand (20 min) at room temperature. Samples were centrifuged (16,000×g, 10 min) and the supernatant was dried under a stream of nitrogen at 40°C. The extracted lipids were resuspended in 50μL H_2O saturated BuOH with sonication (10 min), followed by 50μL of 10 mM NH_4CHOO in MeOH. Extracts were centrifuged (3,350×g, 5 min) and the supernatant transferred into 0.2 mL glass inserts in vials with teflon lined caps. Mass spectrometric analysis was performed using 5 μL and 1 μL (for TG and DG species) injections of the lipid extracts.

Identification and quantitation of lipid species was performed by liquid chromatography electrospray ionisation-tandem mass spectrometry using an Applied Biosystems 4000 QTRAP. Liquid chromatography was performed on a Zorbax C18, 1.8 μm, 50 × 2.1 mm column at 300 μL/min using the following gradient conditions; 0% B to 100% B over 8.0 min, 2.5 min at 100% B, a return to 0% B over 0.5 min then 3.0 min at 0% B prior to the next injection. DGs and TGs were separated using the same solvent system with an isocratic flow (100μL/min) of 85% B over six minutes. Solvents A and B consisted of tetrahydrofuran:methanol:water in the ratios (30:20:50) and (75:20:5) respectively, both containing 10 mM NH_4CHOO. Quantification of individual lipid species was then performed using scheduled multiple-reaction monitoring (MRM) in positive ion mode.[35, 36] Lipid concentrations were calculated by relating the peak area of each species to the peak area of the corresponding internal standard. Cholesterol ester species were corrected for response factors determined for each species. Total measured lipids of each class were calculated by summing the individual lipid species.

Statistical analysis

We examined the association of each lipid species with hypertension-related traits. For this, we ran polygenic models with the given lipid species as an independent variable along with the covariates mentioned earlier. For the outcome of incident hypertension, we used a liability threshold model in which an individual was considered to belong to the hypertensive class if the latent, multivariate normal liability of that individual (based on the kinship structure) exceeded a threshold of 0. Relative risk of incident hypertension was determined as $e^{-\sqrt{\pi}\beta}$ where, β is the polygenic regression coefficient estimated from the polygenic liability threshold model since the SOLAR software returns a negative regression coefficient from a probit model for a positively associated covariate. We tested the statistical significance of the association of lipids with the hypertensions-related traits by constraining the estimated regression coefficient to zero and then estimating χ^2(1 degree of freedom) as $-2(LL_{\text{unconstrained model}} - LL_{\text{constrained model}})$, where LL represents the log-likelihood. The significance values were corrected for multiple comparisons using the false discovery rate method of Benjamini and Hochberg. Additionally, we also estimated the phenotypic variability in SBP, DBP and MAP explained by each lipid species using variance components approach.[37] For the dichotomous trait of incident hypertension, we estimated the Kullback-Liebler R^2 based on the information distance [38] as a measure of the explained variance.

Next, we conducted bivariate trait analyses in which we used each lipid species in a separate bivariate trait polygenic model along with liability of incident hypertension as the two traits. We thus estimated the genetic correlation (ρ_g) and the environmental correlation (ρ_e) coefficients. The statistical significance of these correlation coefficients was tested by constraining the respective parameters to zero and estimating the χ^2 statistics as mentioned above. All genetic analyses were conducted using the SOLAR software.[37] Statistical significance was assessed at a global type I error rate of 0.05.

RESULTS

We first examined the association of each lipid species with SBP, DBP and MAP after adjusting for age, age^2, sex, age x sex interaction, age^2 x sex interaction, HDL and LDL fractions, BMI, HOMA-IR, lipid lowering medication use and antihypertensive drug use. Table 1 shows the statistically significant results for at least one of the three outcomes (full results for all lipid species are shown in Table S1). We found that after accounting for the aforementioned covariates, six lipid species (phosphatidylinositol 36:2 and five diacylglycerols – DG 16:0/18:0, DG 16:0/20:3, DG 16:0/22:5, DG 16:0/22:6 and DG

18:0/22:4) were significantly associated with a high SBP. Similarly, 8 lipid species (three monohexosylceramide species – MHC 22:0, MHC 24:0 and 24:1, a phosphatidylcholine species – PC 34:4, a phosphatidylinositol species – PI 40:6 and three diacylglycerol species – DG 16:0/22:5, DG 16:1/18:1 and DG 18:0/18:1) were significantly associated with an increased diastolic blood pressure. MAP (which conceptually captures the associations of both SBP and DBP with lipid species) was significantly associated with following 10 lipid species: MHC 24:0, MHC 24:1, PC 34:4, PI 34:1, PI 36:2, DG 16:0/18:0, DG 16:0/22:5, DG16:0/22:6, DG 16:1/18:1 and DG 18:0/20:4. Thus, the only lipid species that was consistently associated with SBP, DBP and MAP was DG 16:0/22:5 – a 1 standard deviation (SD) increase in this lipid species was associated with approximately 0.14, 0.15 and 0.16 SD increase in SBP, DBP and MAP, respectively. Further, this species explained 1.27, 1.61 and 1.69% variance of SBP, DBP and MAP, respectively.

We next considered the association of individual lipid species with the genetic liability of incident hypertension in the subset of initially normotensive subjects (n=736). Using polygenic regression models in the context of discrete trait analysis (implying a liability threshold model), we observed that there were a total of 100 lipid species that were nominally significantly associated with the liability of incident hypertension. However, when we adjusted these associations for multiple comparisons using FDR method, we found that only 3 lipid species retained their statistical significance at an FDR-corrected p-value of 0.05. These were: one phosphatidylethanolamine species - PE 40:6 and two diacylglycerol species DG 16:0/22:5 and DG 16:0/226. The polygenic regression coefficients (FDR-corrected p) were −0.3668 (0.01582), −0.3934 (0.0141) and −0.3984 (0.0089), respectively. These regression coefficients translated to relative risks of incident hypertension of 1.92, 2.01 and 2.03, respectively. The Kullback-Leibler R^2 values for these three species were 7.4%, 6.0% and 5.7%, respectively. Full results for all the 319 lipid species are provided in Table S 2. In the regression models presented thus far, we had used the HDL and LDL subfractions as covariates. We reasoned that the subfractions would be more informative than the commonly used clinical characteristics like total serum cholesterol, triglycerides and HDL levels. Therefore, we conducted all these analyses by adjusting for these clinically used indexes and found that the interpretations were similar and there was an approximately 80% concordance in the results obtained by the two different adjustments in multivariate regressions (Table S 3 and Figure S 1). Additionally, our interpretations remained robust even after adjusting for baseline systolic and diastolic blood pressures (Table S 4).

We then examined whether the liability of incident hypertension and the 14 lipid species that showed significant association with blood pressure shared common genetic determinants. For this we conducted a series of bivariate analyses after correction for all the aforementioned covariates as well as for multiple comparisons. As shown in Table 2 we found that four lipid species demonstrated statistically significant genetic correlations with hypertension, all of which were diacylglycerols that contained palmitic acid (16:0). All of these four lipid species also demonstrated statistically significant environmental correlation with liability of incident hypertension, however the point estimates of the environmental correlation coefficients were very small as compared to the genetic correlation coefficients indicating that the phenotypic correlation between these diacyglycerols and liability of incident hypertension was mainly due to the genetic component.

DISCUSSION

Derangement of lipid metabolism is one of the key characteristics of hypertension.[6–10] Currently, to our knowledge there exists only one case-control study in men that has attempted to uncover the underpinnings of the lipid-blood pressure nexus.[17] Our study is the largest such study, includes substantially higher number of detected lipid species and is the

first study in Mexican Americans. While our results partly agree with those of Graessler et al[17] they do offer additional insights into the pathophysiology of hypertension.

In our study the most significant class of lipids associated with the risk of hypertension was diacylglycerols. In this context it is interesting that vasopressins are known to bind G-protein coupled receptors and activate the intracellular phospholipase-C.[39] Diacylglycerol is an end product of this reaction and, along with the second messenger inositol-1,4,5-triphosphate, it regulates the cytosolic calcium ion concentration and protein kinase C activity. Therefore, there is a strong biological plausibility for the role of diacylglycerols in hypertension pathophysiology. The involvement of these lipid classes suggests a distinct and specific upregulation of the diacylglycerol axis in the pathogenesis of hypertension. It should be noted that evidence demonstrating a correlation between plasma diacylglycerol concentrations and intracellular signaling involving diacylglycerols is currently lacking. However, the “lipid metabolite hypothesis” states that an increase in the plasma free fatty acid levels is associated with intramyocytic and intrahepatic generation and congregation of several reesterified metabolites that include acyl-coenzyme A and diacylglycerol.[40] Although the reasons for increase in intracellular diacyglycerols in response to lipid infusions are currently not understood, it is conceivable that the plasma levels of some diacylglycerol species may track the myocytic potential to generate intracellular secondary messengers like diacylglycerol and thereby serve as biomarkers of hypertension, especially if this mechanism is operational in the vascular smooth muscle cells. It is noteworthy in this context that the TRPC6 channel expressed widely in vascular smooth muscles is a potent regulator of the Ca^{2+} fluxes and is activated by diacylglycerol.[41, 42] The other class of interest observed in our study was that of monohexosylceramides. Jhang et al[43] recently demonstrated that ceramides can mediate vascular dysfunction by inhibiting the eNOS/Akt/Hsp90 signaling complex. Thus, ceramides and their analogs can be expected to contribute to hypertension pathophysiology by way of endothelial dysfunction as also observed by Spijkers et al.[27]

The DG 16:0/22:5 lipid species was i) consistently associated with increased SBP, DBP and MAP; ii) strongly associated with liability of incident hypertension; and iii) strongly genetically correlated with the liability of incident hypertension. With the exception of a marginally significant association with DBP, the DG 16:0/22:5 lipid species also closely followed pattern of associations demonstrated by the DG 16:0/22:5 lipid species. These observations combined with the finding that all 16:0 fatty acid containing diacylglycerols were strongly genetically correlated with incident hypertension point towards the potential association of palmitic acid with hypertension. Previous studies[44–47] have demonstrated this relationship and our results afford additional credence to it. Of more interest, however, is our finding that not all diacyglycerol species can be equally implicated in the pathophysiology of hypertension. For example, the genetic correlations of the 16:1 or 18:0 containting diacylglycerols with the liability of incident hypertension were considerably smaller as compared to the 16:0 containing diacylglycerols. These findings are important since they underscore the precision and resolution of lipidomic studies. Our results indicate that the DG 16:0/22:5 and DG 16:0/22:6 lipid species may be important drug targets or modifiable factors which can be advantageously used in early detection, risk stratification or prevention of hypertension.

There are five important limitations of this study. First, there exists a tight association between hypertension and other components of metabolic disease and this makes it difficult to differentiate between independent and intermediate associations. Boden[40] has reviewed the mechanisms that lead to insulin resistance consequent to inflammation induced by fatty acids. Plasma free fatty acids lead to accumulation of diacylglycerols and triacylglycerols in the liver and muscles and activate some serine/threonine kinases. These mechanisms are also

common to the pathogenesis of hypertension and cardiovascular conditions. Our analytical approach was similar to that employed by Graessler et al[17] in which all statistical models were adjusted for BMI and HOMA-IR. Therefore, the associations reported here are less likely to have been influenced by other traits implicit in metabolic syndrome. Second, our definition of hypertension was based on cross-sectionally as well as longitudinally detected rise in blood pressure. The actual predictability of the lipid species in the context of future hypertension is unknown. Nevertheless, the consistency of associations of the lipid species with SBP, DBP, MAP and hypertension indicates that our definition of hypertension is unlikely to have confounded the results or interpretations. Third, the plasma lipidomic profile itself has been shown to vary across males and females[17, 48] as do several key pathways involved in hypertension. However, our primary aim was to investigate the association between lipid species and hypertension regardless of gender therefore in our analytical protocol we adjusted all the models for age, gender and their interactions. Fourth, observational studies of this nature can be biased by the inadequacy of information related to treatment effects. With special reference to incident hypertension it is conceivable that the use of lipid lowering drugs may influence both the abundance of the lipid species in plasma as well as the risk of hypertension. However, only 1.8% of the study subjects were receiving lipid-lowering agents and we therefore believe that our results are unlikely to be significantly affected by drug use. Lastly, we did not have data on kidney function tests during the first visit of the study participants. However, when we correlated the SBP, DBP and MAP measured during the third visit with the estimated glomerular filtration rate (eGFR, also measured in the third visit samples), we found no association among the blood pressure measurements and kidney function (data not shown). Moreover, none of the 319 lipid species were significantly associated with eGFR. These findings indirectly imply that the association of DG species with blood pressure observed in this study is unlikely to be due to an association with kidney function.

PERSPECTIVES

Our study demonstrates the added and independent association of plasma lipidomic profiles with blood pressure and incident hypertension in Mexican American families. The technology of lipidomic profiling is constantly evolving and its use in detection of complex diseases like hypertension and metabolic syndrome can also be envisioned in the near future. In that context and to that end our study shows novel and important lipidomic signatures of blood pressure and hypertension. More studies that replicate these findings are warranted. Future studies also need to intensively investigate the possibilities related to the putative role of diacylglycerols (specifically the DG 16:0/22:5 and DG 16:0/22:6 species) in blood pressure regulation and hypertension. Since hypertension is itself a risk factor for numerous cardiovascular disorders, it is conceivable that these two lipid species found to be significantly associated with a risk of incident hypertension may be useful in prevention of cardiovascular diseases especially since increased plasma concentrations of these two lipid species may be harbingers of impending derangements ultimately culminating in cardiovascular morbidity. Our results therefore open several potential leads into the characterization of the role of lipidomic studies in hypertension.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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NOVELTY AND SIGNIFICANCE

What is new?

- Plasma lipidomic studies can identify potential biomarkers of hypertension
- Whether plasma lipidomic signatures can independently predict risk of hypertension is unknown

What is relevant?

- Consistency and independence of the genetic and environmental contributions of plasma lipid species to systolic, diastolic and mean arterial pressures and to the risk of incident hypertension

Summary

- Study of large and extended Mexican American families
- Disturbances in the diacylglycerol axis independently influence the risk of incident hypertension
- DG 16:0/22:5 and DG 16:0/22:6 lipid species are promising candidates that deserve investigation in future studies

Table 1

Multivariate association of plasma lipid species with blood pressure

Shown here are statistically significant results for at least one of the following three traits – systolic blood pressure, diastolic blood pressure and mean arterial pressure. Full results for all 319 lipid species are shown in Table S 1. Statistically significant results are shown in bold.

Lipid species	Systolic blood pressure			Diastolic blood pressure			Mean arterial pressure		
	β	FDR p	Ve	β	FDR p	Ve	β	FDR p	Ve
MHC 22:0	0.0693	1.0000	0.85	0.1093	**0.0375**	1.02	0.0958	0.0930	1.03
MHC 24:0	0.0902	0.0871	1.39	0.1287	**0.0027**	1.44	0.1188	**0.0037**	1.61
MHC 24:1	0.0894	0.1327	1.36	0.1235	**0.0096**	1.23	0.1175	**0.0071**	1.50
PC 34:4	0.0948	0.0800	0.98	0.1159	**0.0390**	1.20	0.1126	**0.0213**	1.24
PI 34:1	0.0935	0.0634	1.16	0.1007	0.1842	1.15	0.1044	**0.0452**	1.32
PI 36:2	0.0948	**0.0482**	0.84	0.1094	0.0552	1.12	0.1111	**0.0150**	1.15
PI 40:6	0.0812	0.2479	0.73	0.1082	**0.0391**	1.27	0.0993	0.0535	1.06
DG 16:0/18:0	0.1283	**0.0133**	1.50	0.1362	0.0637	1.29	0.1357	**0.0230**	1.42
DG 16:0/20:3	0.1219	**0.0461**	1.23	0.1264	0.2243	1.13	0.1307	0.0594	1.30
DG 16:0/22:5	0.1386	**0.0054**	1.27	0.1540	**0.0129**	1.61	0.1577	**0.0022**	1.69
DG 16:0/22:6	0.1198	**0.0300**	1.22	0.1339	0.0565	1.38	0.1337	**0.0204**	1.43
DG 16:1/18:1	0.0946	0.5719	0.88	0.1389	**0.0319**	1.43	0.1278	**0.0396**	1.34
DG 18:0/18:1	0.1001	0.7322	0.86	0.1495	**0.0364**	1.49	0.1317	0.0830	1.28
DG 18:0/20:4	0.1202	**0.0224**	1.11	0.1276	0.0915	1.20	0.1322	**0.0194**	1.31

MHC, monohexosylceramide; PC, phosphatidylcholine; PI, phosphatidylinositol; DG, diacylglycerol; β, regression coefficient; FDR, false discovery rate; Ve, explained variance (%)

Table 2

Bivariate trait analyses of plasma lipid species with liability of incident hypertension

Statistically significant results are shown in bold.

Lipid species	Genetic correlation		Environmental correlation	
	ρ_g	FDR p	ρ_e	FDR p
MHC 22:0	0.3028	0.3160	−0.3918	0.8560
MHC 24:0	0.2808	0.3480	−0.3233	0.8560
MHC 24:1	0.1365	0.3480	−0.0588	0.8560
PC 34:4	0.2926	0.3480	0.1071	**0.0491**
PI 34:1	0.2286	0.3480	0.2540	**0.0024**
PI 36:2	0.3280	0.3480	0.0223	0.0932
PI 40:6	0.2503	0.3480	0.2284	**0.0031**
DG 16:0/18:0	0.6649	**0.0082**	0.0363	**0.0006**
DG 16:0/20:3	0.6963	**0.0036**	0.0370	**0.0004**
DG 16:0/22:5	0.6161	**0.0054**	0.0392	**0.0002**
DG 16:0/22:6	0.6309	**0.0054**	0.0800	**<0.0001**
DG 16:1/18:1	0.3583	0.3480	0.1564	**0.0021**
DG 18:0/18:1	0.5238	0.0615	0.0490	**0.0014**
DG 18:0/20:4	0.4546	0.1323	0.1452	**0.0005**

MHC, monohexosylceramide; PC, phosphatidylcholine; PI, phosphatidylinositol; DG, diacylglycerol; ρ, correlation coefficient; FDR, false discovery rate