Article

Lipidomics reveals association of circulating lipids with body mass index and outcomes in IgA nephropathy patients

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IgA nephropathy (IgAN) is a leading cause of chronic kidney disease (CKD), which are commonly accompanied by dyslipidemia. Obesity is also associated with dyslipidemia and risk of CKD, but the relation of the dyslipidemia patterns with obesity and disease progression in IgAN patients remains unknown. Traditional Chinese medicine (TCM) and the combined treatment with corticosteroids and TCM have been shown to be of benefit for IgAN patients, but predictive markers for guiding these treatments are lacking. Here, we quantified 545 lipid species in the plasma from 196 participants, including 140 IgAN patients and 56 healthy volunteers, and revealed an altered plasma lipidome in IgAN patients as compared to healthy participants. Association analysis showed that a subgroup of glycerides, particularly triacylglycerols (TGs) containing docosahexaenoic acid, were positively associated with high body mass index (BMI) in under- or normal-weight IgAN patients, while several free fatty acids and sphingomyelins were positively associated with high BMI in overweight or obese IgAN patients. Further, our study suggested that elevated levels of eight lipids, mainly TG species containing linolenic acid, were independent risk factors for IgAN progression and also reported the prospective association of circulating lipids with treatment outcomes in IgAN. Taken together, our findings may not only help to achieve precision medicine but also provide a knowledge base for dietary intervention in the treatment of IgAN.

Keywords: IgA nephropathy, circulating lipids, body mass index, lipidomics, dyslipidemia, traditional Chinese medicine

Introduction

IgA nephropathy (IgAN), the most prevalent type of primary glomerular disease (PGD) worldwide (Wyatt and Julian, 2013; Cheung and Barratt, 2019), remains a leading cause of chronic kidney disease (CKD) (Rodrigues et al., 2017). Up to 40% of IgAN patients will eventually progress to end-stage kidney failure (Tam and Pusey, 2018; Yeo et al., 2019). Dyslipidemia is common in CKD and is found to contribute to the initiation and progression of CKD (Saland et al., 2010). Dyslipidemia in CKD has been characterized by high circulating triglycerides, normal or slightly reduced low-density lipoprotein (LDL) cholesterol levels, and decreased high-density lipoprotein (HDL) cholesterol levels (Trevisan et al., 2006). However, to the best of our knowledge, there was little data to show the IgAN-specific dyslipidemia patterns. Obesity is a common cause of dyslipidemia (Elkins et al., 2019), which generally consists of elevated triacylglycerols (TGs) and free fatty acids (FFAs), normal or slightly increased LDL cholesterol levels, and decreased HDL cholesterol levels (Klop et al., 2013). Obesity has been showed to be an independent risk factor for CKD (Mount et al., 2015). However, the impact of high body mass index (BMI) on clinical outcomes of IgAN patients remains controversial and uncertain (Shimamoto et al., 2015; Nagaraju et al., 2018). Moreover, much less data exist regarding the association of the dyslipidemia patterns with obesity and disease progression in patients with IgA nephropathy (IgAN).

Corticosteroids have been used in the treatment of IgA for approximately four decades, and were demonstrated to be able to reduce proteinuria, induce the improvement or stabilization of estimated glomerular filtration rate (eGFR), and improve renal outcomes in long-term follow-up (Coppo, 2017). Nevertheless, corticosteroid responsiveness is not universal in patients with IgAN. Currently, proteinuria and eGFR were used to help selecting patients likely to respond to corticosteroid therapy. The Kidney Disease: Improving Global Outcomes (KDIGO) recommendations suggest corticosteroid therapy for patients with IgAN who have an eGFR >50 ml/min/$1.73\,m^2$ and proteinuria with protein excretion >1 g/day, but recent studies have shown that corticosteroid therapy is of benefit to IgAN patients, even if their eGFR values were <50 ml/min/$1.73\,m^2$ (Tsunoda et al., 2018). However, long-term use of corticosteroids can cause a myriad of side effects, included osteoporosis, growth retardation, and altered lipid metabolism, which can result in dyslipidemia and central obesity (Lee et al., 2014; Oray et al., 2016). Our previous work has shown traditional Chinese medicine (TCM) to be a promising alternative therapy for patients with PGD including IgAN and idiopathic membranous nephropathy (Li et al., 2016; Xia et al., 2019) and suggested that TCM therapy could improve serum albumin levels (Chen et al., 2013b) and regulate Sphingosine-1-phosphate pathway, a lipid signaling that was reportedly involved in the pathogenesis of kidney disease (Zhong et al., 2015). Moreover, a combined treatment of corticosteroids and TCM (CT therapy) seemed to benefit patients with IgAN more in terms of improved eGFR and angiotensinogen level, compared to corticosteroid therapy alone (Chen et al., 2013a; Li et al., 2016). However, the clinical and molecular markers for predicting the treatment response and guiding these therapeutic strategies are lacking. Lipids are involved in variety of physiological and pathological processes, and lipid abnormalities are associated with oxidative stress, increases ROS production, and a reduction in renal function (Ruan et al., 2009; Bobulescu, 2010). Recent advances in liquid chromatography mass spectrometry (LC–MS)-based lipidomics have allowed for global identification and quantitation of lipid species alterations under normal and diseased conditions and greatly enhanced the new lipid biomarker discovery. Using LC–MS-based lipidomics, Afshinnia et al. (2016) has showed that the plasma lipid markers may improve the prediction of CKD progression. Since corticosteroids and TCM treatments are associated with lipid metabolism, we speculated that lipids may represent a useful tool not only for assessing IgAN progression but also for providing information to evaluate treatment response for IgAN patients.

Given the above, the aims of the present study are to assess the altered plasma lipidome patterns associated with IgAN and to further investigate the relationship between circulating lipids with BMI, disease progression, or treatment outcome in patients with IgAN. The study was performed on the plasma samples from 196 participants, including 140 IgAN patients and 56 healthy volunteers, and circulating lipids were quantified using LC–MS-based targeted lipidomic approach.

Results

Study participant characteristics

Demographic and clinical characteristics of the study participants are summarized in Supplementary Table S1. BMI in the IgAN patients was significantly higher compared to those in age- and sex-matched healthy controls (HCs; two-tailed t-test, $P < 0.05$). For participants in IgAN cohort, the mean follow-up periods were 12.3 months (standard deviation, SD = 2.1). There was no statistical significance in baseline clinical characteristics of patients with CT treatment and those with TCM treatment (Chi-square test for gender, two-tailed Student's t-test for others). We found that a significant increase in serum albumin levels at 12-month follow-up compared to that at baseline in patients with both CT and TCM treatments (paired two-tailed t-test, $P < 0.05$) (Figure 1A; Supplementary Table S1), while eGFR and 24-h urine protein were statistically significantly different in patients with CT treatment, but not with TCM treatment, at 12-month follow-up compared to the baseline (paired two-tailed t-test, $P < 0.05$) (Figure 1B and C; Supplementary Table S1).

Comparative analysis of the plasma lipidome profile of IgAN patients vs. healthy participants

Targeted lipidomic analysis accurately quantified 545 individual lipid species belonging to 24 lipid classes/subclasses, including cholesterol ester (CE), ceramide (Cer), diacylglycerol (DG), dihydroceramide (DhCer), fatty acid (FA), glucosylceramide (GlcCer), hexosylceramide (HexCer), lactosylceramide (LacCer), lysophosphatidylacid (LPA), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), lysophosphatidylglycerol (LPG), lysophosphatidylinositol (LPI), lysophosphatidylserine (LPS), monoacylglycerol (MG), phosphatidylacid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS), sphingomyelin (SM), TG, and cholesterol (Chol) (quantitative data presented in Supplementary Table S2). The differential clustering of IgAN patient samples and HC samples in principal component analysis (PCA) score plot for lipidomic data indicates the overall difference in lipidome profile between the two group samples (Figure 2). As shown in Figure 3A, the plasma levels of most lipid classes, except for FA, LPC, LPI, and LPS, were significantly elevated in IgAN patients compared to HCs (mean 1.46-fold; range 1.13–3.32-fold). We then investigated the plasma levels differed by the length and unsaturation level of acyl chains in each lipid class. We found a trend ($R < -0.20$) toward higher abundance of shorter carbon chain lipids within LacCer, LPE, HexCer, PC, PE, SM, and TG in IgAN patients as compared with HCs, reaching statistical significance in LacCer, PC, PE, SM, and TG ($P < 0.05$; Figure 3B), whereas an opposite trend ($R > 0.20$) toward higher abundance of longer carbon chain lipids within PA and PG in IgAN patients compared to normal controls, reaching statistical significance only in PG ($P < 0.05$). Similarly, there was a trend toward higher abundance of LPE, FA, and TG
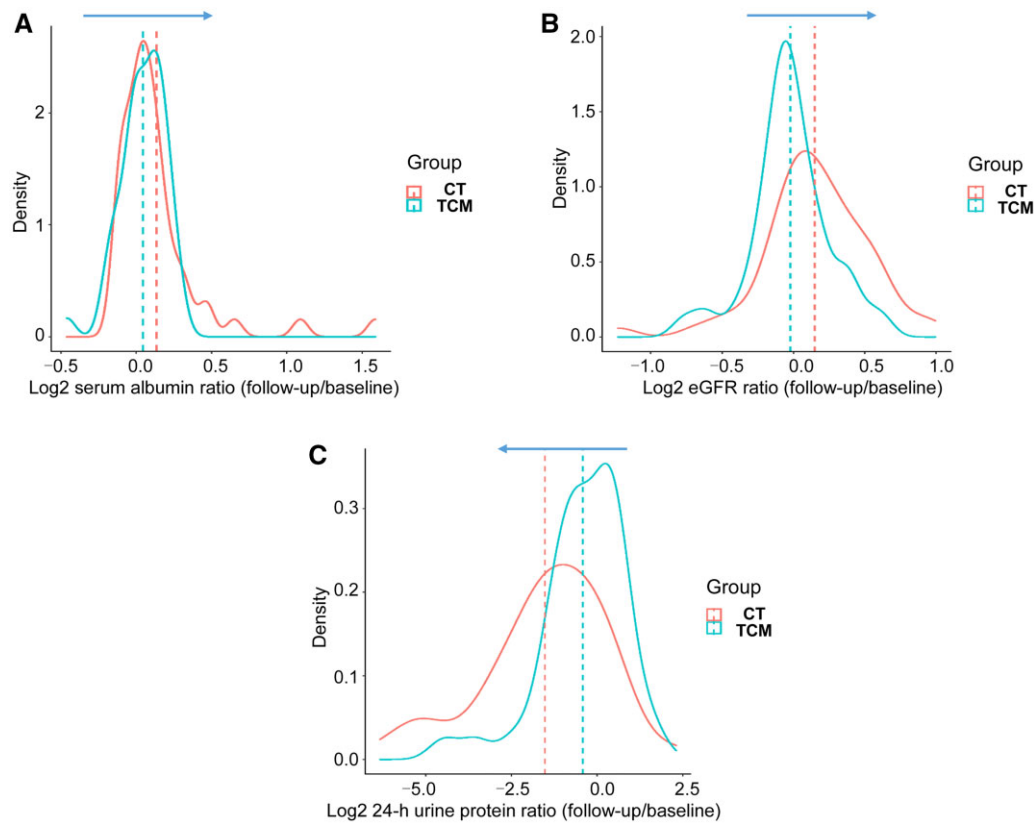
Figure 1 Density plots illustrating the distributions of changes in renal function (12-month follow-up vs. baseline) in patients with IgAN undergoing TCM (n = 50) or CT treatment (n = 54). Kernel density plots of the log$_2$ transformed ratios of serum albumin (A), eGFR (B), and 24-h urine protein (C) at 12-month follow-up against those at baseline in IgAN patients undergoing TCM (green lines) or CT (red lines) treatment. Dashed lines represent the mean of each group. Blue arrows indicate the direction of improvement.

lipids with lower number of double bonds in IgAN patients as compared with HCs, reaching statistical significance for FA and TG (P < 0.05), whereas an opposite trend only in PA (Figure 3C).

Assessment of associations between circulating lipids, BMI, and progression of IgAN

In the study, all healthy participants were measured in under- or normal-weight BMI range (17.5–22.5). Cross-sectional analysis of the association of circulating lipids and BMI in the healthy participants showed that five lipids (four positively and one negatively) were significantly associated with BMI after adjustment for age and gender (Figure 4A). To assess the relationships between BMI and circulating lipids in IgAN patients, the correlation analyses were conducted after the subjects were stratified by BMI as under- or normal-weight (BMI < 24) and overweight or obesity (BMI ≥ 24) and with controlling for age and gender. Interestingly as shown in Figure 4B, in under- or normal-weight patients, certain glycerides particularly TGs containing docosahexaenoic acid (DHA) were positively and nominally significantly associated with BMI. In overweight or obese

patients, a subset of SM and FA species were positively and nominally significantly associated with BMI (Figure 4C), with SM 34:1; 3 also approaching statistical significance (false discovery rate, FDR q-value = 0.076).

To identify highly connected lipid modules and the relevance between baseline clinical traits and each lipid module, we performed weighted correlation network analysis (WGCNA). The 490 lipids were clustered into eight modules (summarized in Supplementary Table S3). As shown in Figure 5, the pink module (22 lipid species containing the majority of SMs with the total acyl chain length from 30 to 42) negatively correlated with baseline eGFR in IgAN patients, the brown (80 lipid species), yellow (71 lipid species), and blue (85 lipid species) modules were significantly positively correlated with 24-h urine protein, and the brown module also showed a significant negative correlation with serum albumin.

The prospective associations of the baseline demographics and clinical characteristics with disease progression at 12-month follow-up were performed using the univariate logistic regression. As shown in Figure 6A, neither BMI nor other baseline clinical traits are significantly associated with disease progression of IgAN at 12-month follow-up. To explore the relationship between
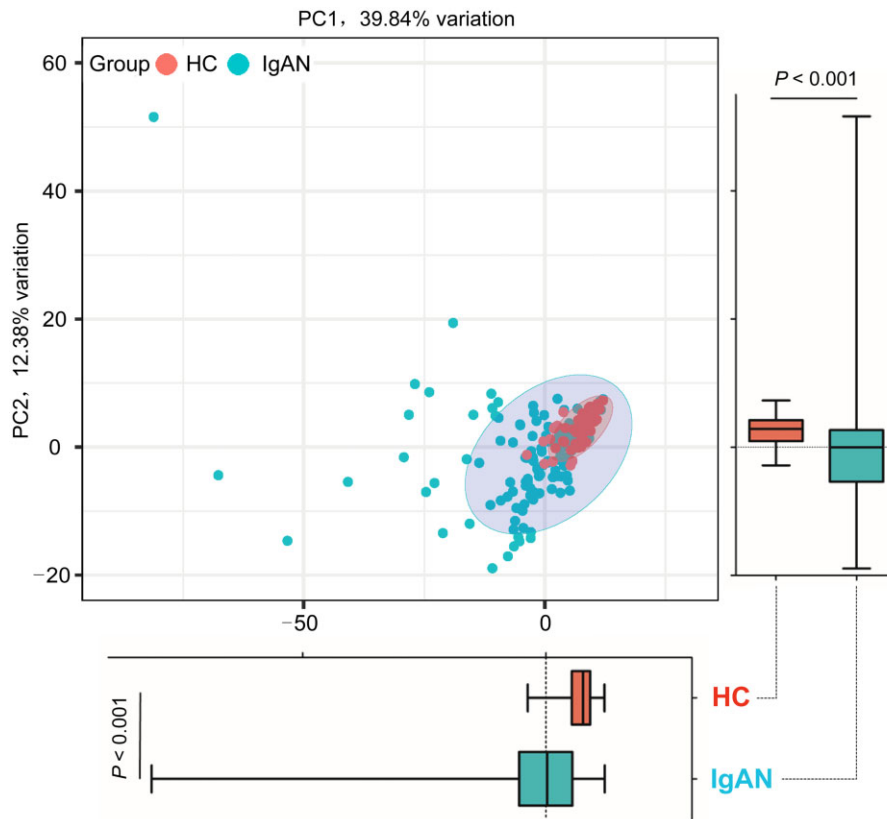
Figure 2 PCA of lipidomic data. The score plot for the first two principal components (PC1 and PC2) shows the clustering of 56 HCs and 140 IgAN patients. Ellipsoids represent a 95% confidence interval (CI) surrounding each group. Nonparametric two-sided Mann–Whitney U-test was used to analyze significant differences between HC and IgAN groups for PC1 and PC2. Box plots display first and third quartiles, and whiskers extend from each quartile to the minimum or maximum values.

circulating lipids with disease progression in IgAN, multivariate logistic regression analysis was performed. The results showed that eight lipids, including four TG species containing linolenic acid, were nominally significantly associated with IgAN progression at 12-month follow-up after adjustment for gender, age at baseline, BMI, eGFR at baseline, and treatment. The odds ratios for the associations of 1-SD higher levels of the eight lipids with the risk of disease progression are shown in Figure 6B.

Associations of circulating lipids with treatment outcomes in IgAN patients

eGFR change was used to assess the medium-term renal outcome in IgAN patients. The prospective associations of individual lipid species with eGFR changes in IgAN patients receiving TCM or CT treatment were assessed using multivariable linear regression analysis. After adjustment for age, sex, and baseline eGFR, 16 lipids, mainly glycerides (e.g. TGs and DGs), were positively and 16 lipids, mainly sphingolipids (e.g. HexCers, SMs, Cer, and GlcCer), were negatively associated with treatment outcomes in IgAN patients receiving TCM therapy (Supplementary Figure S1A), while 70 circulating lipids, including 31 TGs, 9 DGs, 9

PCs, 8 PEs, 4 LPEs, 4 LPCs, 2 SMs, 1 PI, 1 LacCer, and 1 CE, were nominally significantly ($P < 0.05$) associated with treatment outcomes in IgAN patients receiving CT therapy (Supplementary Figure S1B). Figure 7A shows significance levels for the associations of FA composition in lipid species within different lipid classes with treatment outcomes in IgAN patients receiving TCM therapy, which suggested that medium-chain triglycerides (MCTs) containing less unsaturated FAs (≤ 3 double bonds) were positively associated with better renal outcome in patients with TCM treatment. Figure 7B revealed that higher levels of circulating lipids comprising longer chain polyunsaturated fatty acids (PUFAs) in DG, LPC, LPE, PC, PE, PI, and TG classes were positively associated with better renal outcome in patients with CT treatment.

Discussion

In the present study, we explored the plasma dyslipidemia patterns as well as the associations between circulating lipids with BMI, disease progression, and treatment outcome in patients with IgAN, and reported several novel findings. (i) A subset of glycerides particularly TGs containing DHA were positively associated with BMI in under- or normal-weight
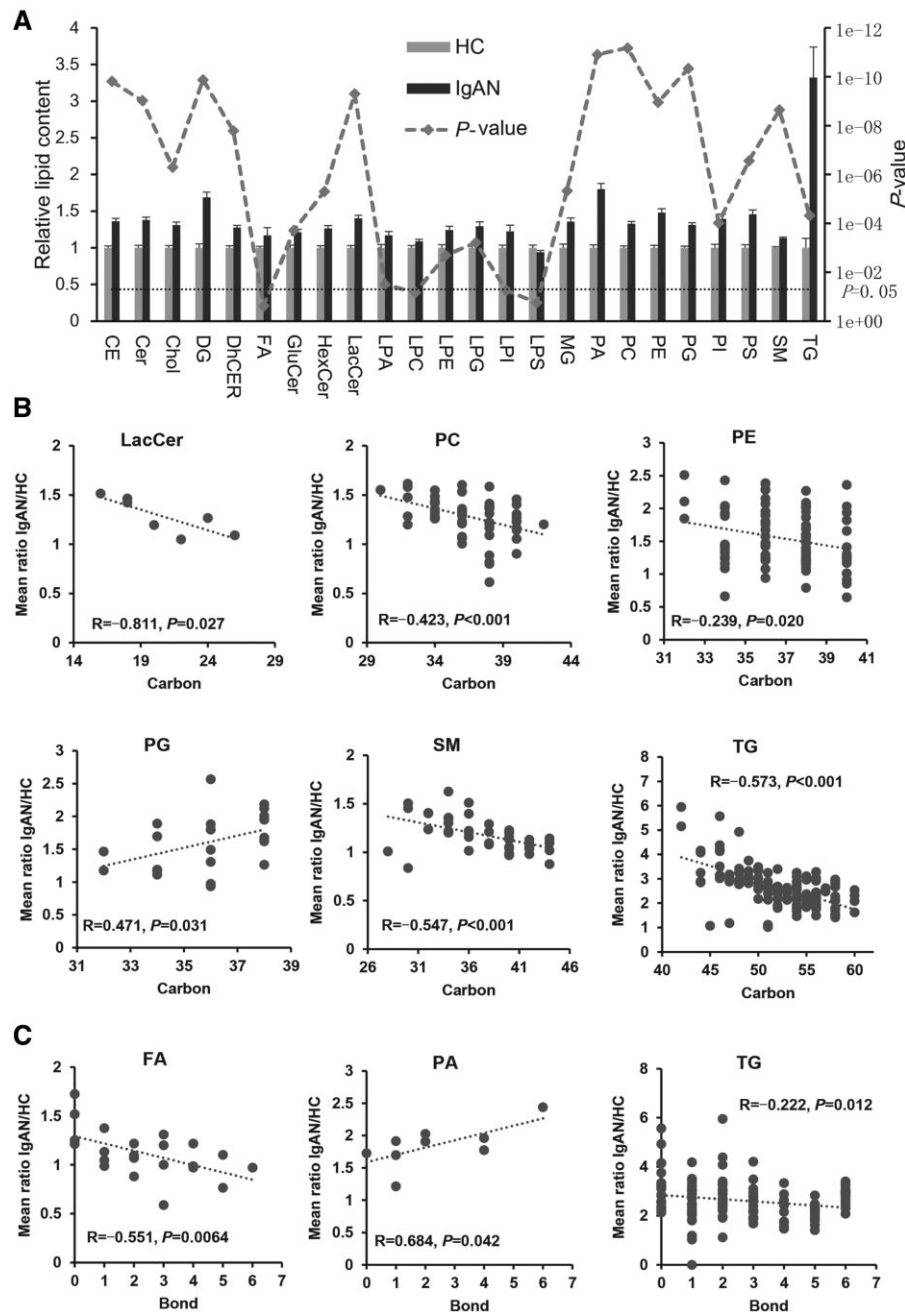
Figure 3 An overview over changes of various lipid classes in the plasma between IgAN patients (n = 140) and HCs (n = 56). (A) Relative abundance of the sum of lipid classes in IgAN samples compared to HC samples. Data represent means ± SEM. P-values were determined by two-tailed Student's t-test. (B and C) IgAN: HC mean ratio of lipid abundance by the carbon number (B) and number of bonds (C) in different lipid classes. Only the lipid classes with the Spearman's rank correlation statistically significant (P < 0.05) are shown.

IgAN patients, whereas certain FA and SM species were associated with high BMI in overweight or obese IgAN patients. (ii) Elevated levels of several TG species containing linolenic acid, including TG54:6-FA18:3, TG52:6-FA18:3, TG54:8-FA18:3, and TG54:7-FA18:3, were independent risk factors for IgAN progression. (iii) Several MCTs correlated positively, while certain sphingolipids, particularly glycosphingolipids (GSLs, e.g. HexCers and GlcCers), correlated

negatively with treatment outcome in patients with TCM treatment. (iv) The specific lipids comprising longer chain PUFAs were positively associated with treatment outcome in patients with CT therapy.

The dyslipidemia patterns in IgAN patients were characterized by comparing with that in age- and sex-matched normal participants. Our results showed that TGs, especially those with shorter chain and saturated FAs, were the most elevated lipids
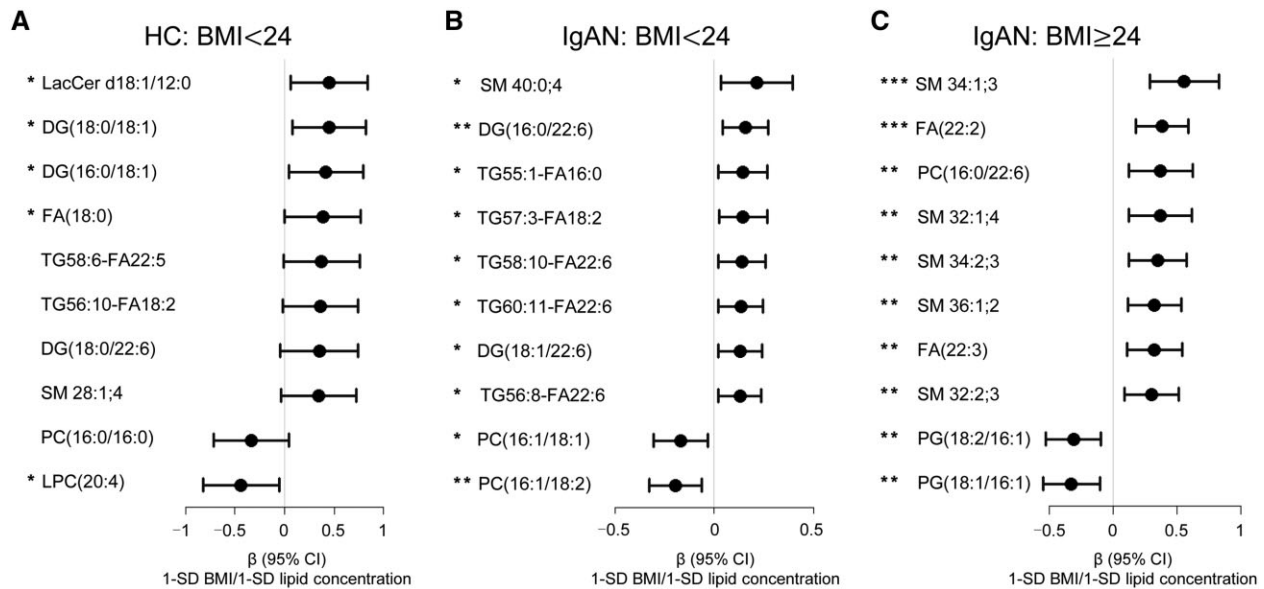
Figure 4 Associations of circulating lipids with BMI in HCs and IgAN patients. Forest plot of the estimated regression coefficients (95% CI) on the association between top 10 significant lipids and BMI in under- or normal-weight healthy participants (BMI < 24, n = 28) (A), under- or normal-weight IgAN patients (BMI < 24, n = 51) (B), and overweight or obese IgAN patients (BMI ≥ 24, n = 49) (C). Linear regression models were adjusted for gender and baseline age. Association magnitudes are in standardized units of 1-SD BMI per 1-SD lipid concentration. Error bars indicate 95% CIs. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

in the plasma of IgAN patients compared to HCs. Hypertriglyceridemia was a primary lipid abnormality among CKD patients (Mikolasevic et al., 2017). Similarly, a previous lipidomics study on CKD showed a trend toward decreased abundance of TGs with longer chains and multiple unsaturations in progressors compared to nonprogressors to end-stage kidney disease (Afshinnia et al., 2016). In IgAN, hypertriglyceridemia has been shown to be an independent risk factor for disease progression (Syrjanen et al., 2000). In addition to TGs, our results first showed that plasma levels of many lipid classes were significantly elevated in IgAN patients compared to the levels in HCs, except for FA. In FA class, we observed a trend toward lower abundance of FAs with higher number of double bonds in IgAN patients compared to HCs. PUFAs play protective effects on renal function (Taccone-Gallucci et al., 2006; Lauretani et al., 2008; Eide et al., 2016; Malhotra et al., 2016) and are highly susceptible to oxidative damage (Yang et al., 2016). Intrarenal reactive oxygen species (ROS) that increase oxidative stress play a pivotal role in the development of IgAN (Pei et al., 2016). Enhanced oxidative stress levels in the plasma and kidneys in IgAN patients could be the cause of the decreased plasma level of PUFAs (Kobori et al., 2007; Pei et al., 2016). Recent studies suggested that increasing intake of PUFAs, such as DHA and eicosapentaenoic acid (EPA), significantly decreased plasma lipid hydroperoxide, a consequence of ROS overproduction (Hassler et al., 2014) and is effective in the treatment of human IgAN (Lee et al., 2013; Hirahashi, 2017).

Cross-sectional correlation analysis showed that a subgroup of lipids, mainly TG species containing DHA, were positively associated with high BMI in under- or normal-weight IgAN

patients. The finding is in line with a study by Fisk et al. (2018) who showed a positive association between BMI and DHA in TG in healthy adults. While in overweight or obese IgAN patients, several plasma SMs and FAs were positively associated with high BMI. Previous studies have shown that obesity can cause impaired FA oxidation (Fucho et al., 2017), which may contribute to the increased plasma FA levels (Boden, 2008). FAs are the substrate and major constituents for sphingolipids (Boini et al., 2017). In obese individuals, sphingolipid metabolism is affected by increased FA levels (Torretta et al., 2019), and plasma levels of several sphingolipids, including SM species, were demonstrated to closely correlate with the parameters of obesity (Hanamatsu et al., 2014), which is in line with our findings. Sphingolipids are critical mediators of obesity-mediated inflammation (Kang et al., 2013), and altered sphingolipid metabolism contributes to the development of chronic glomerular injury associated with obesity (Boini et al., 2017). Studies have shown that high BMI was associated with poor prognosis of IgAN. However, in this study, we did not find that BMI or any other clinical trait is an independent risk factor for disease progression in IgAN patients.

To correlate the clinical traits including BMI with the lipidome patterns in IgAN patients, we performed WGCNA and correlation analysis. Our findings showed that BMI did not have a significant effect on each lipid module of IgAN patients, while 24-h urine protein has a positive and significant relationship with most of the lipid modules (brown, yellow, and blue), which is not surprising since 24-h urine protein level has been reported to be positively correlated with dyslipidemia in IgAN (Mo et al., 2018). Baseline eGFR negatively correlated with the
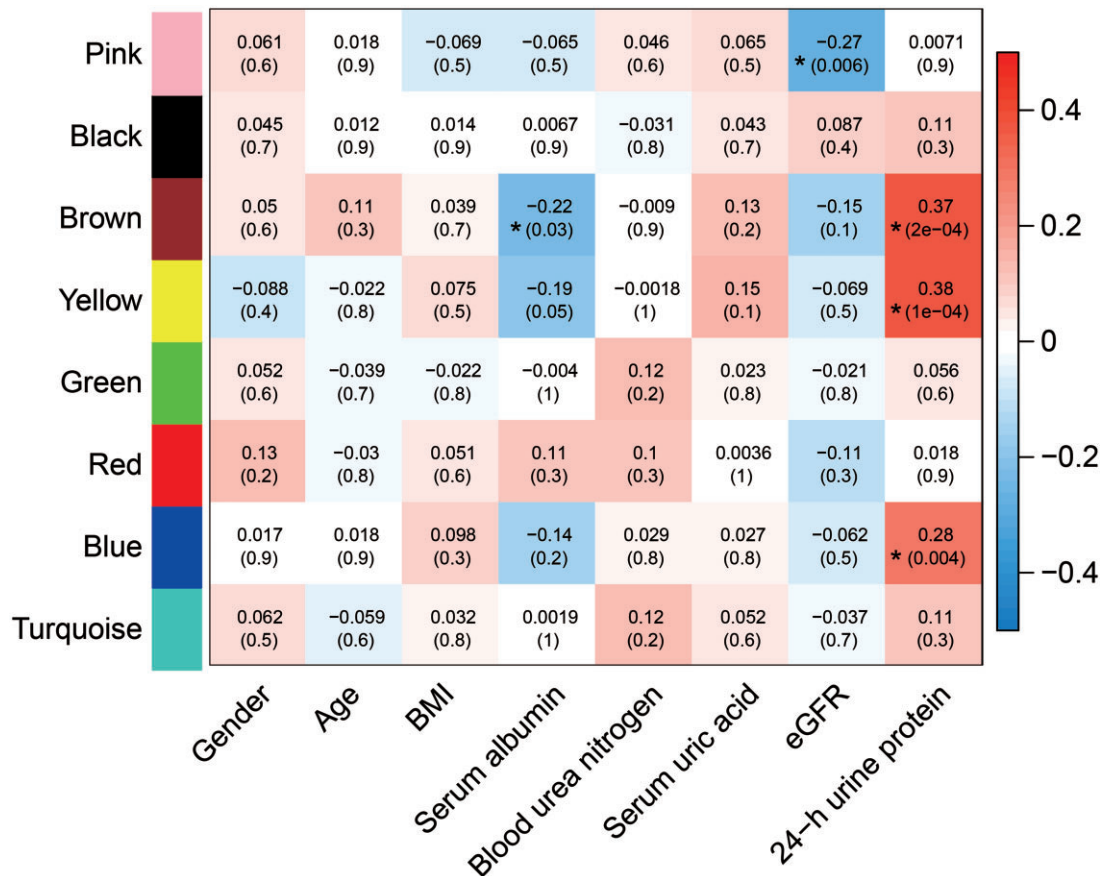
Figure 5 Association of lipid correlation network modules with demographics and clinical traits. WGCNA groups the lipid species in the plasma of IgAN patients (n = 104) into eight modules. The networks are thresholded at an adjacency of 0.02 (akin weighted correlation of 0.8). The module–trait associations are shown where the colors correspond to the correlation coefficients (red for positive correlations and blue for negative correlations). Upper values in each cell are correlation coefficients between module eigenlipids (the first principal component) and clinical traits, and lower values are the corresponding P-value (Spearman's rank correlation test). The pink, brown, yellow, and blue modules are significantly (P < 0.05) correlated with clinical traits.

pink module containing the majority of shorter chain (30–42 acyl chains) SM. This result is consistent with the findings of Makinen et al. (2012) who observed that SM negatively correlated with GFR in patients with diabetic kidney disease.

More interestingly, the prospective association analysis showed that elevated levels of eight lipids, mainly TG species containing linolenic acid, were positively associated with IgAN progression at 12-month follow-up. Due to the limitation of analytical methods, the analysis did not differentiate between the two isomers of linolenic acids, omega-3 α-linolenic acid (ALA) and omega-6 γ-linolenic acid (GLA). In humans, ALA is an essential FA that is obtained through the diet. GLA is mainly metabolized from linoleic acid, and then rapidly elongated to dihomo-γ-linolenic acid (DGLA), a precursor of a large family of anti-inflammatory eicosanoids (Kaur et al., 2014). Since GLA was found in low levels in circulating lipids (Sergeant et al., 2016), it can be inferred that ALA is the most dominant isomer of linolenic acid contained in these TG species. Although there are a great number of controversies about whether ALA has pro- or anti-inflammatory properties, our finding is in line

with a previous large population-based epidemiological study showing that a higher intake of ALA was associated with the prevalence of CKD (Gopinath et al., 2011). In IgAN, to our knowledge, this is the first study showing that higher levels of certain TG species containing linolenic acid in the plasma are the independent risk factors for disease progression, which may not only present a novel insight into the pathological roles of these lipids in renal inflammation, but also provide a rationale for future standard recommendations for dietary intervention in the treatment of IgAN patients.

Our findings showed that MCTs correlate positively, while a subgroup of sphingolipids, especially GSLs (e.g. HexCer and GlcCer), correlate negatively with treatment outcome in patients with TCM treatment. In consistent with our findings, MCTs were demonstrated to be associated with a decrease in adiposity and inflammation (Thomas et al., 2019). Meanwhile, deregulation of sphingolipid metabolism, particularly S1P signaling, which was shown to be modulated by the TCM therapy, has been implicated to participate in chronic and acute kidney injury. A recent study showed that accumulation of GSLs in
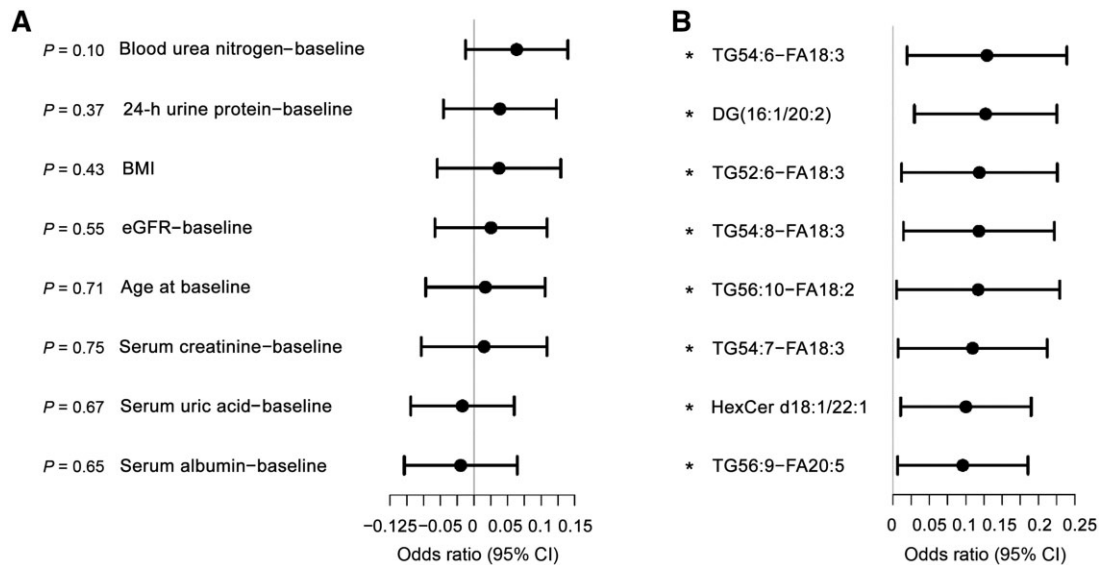
Figure 6 Odds ratios for IgAN progression at 12-**month follow-up per** 1-**SD increase in baseline demographics and clinical characteristics and lipid concentrations (n** = 140**). (A) Odds ratios of the univariate logistic regression with demographics and clinical characteristics of patients as predictors. (B) Adjusted odds ratios of the multivariable logistic regression with lipid concentrations of patients as predictors. The multivariable logistic model was adjuested for gender, age at baseline, BMI, eGFR at baseline, and treatment. Error bars indicate** 95% **CIs. *P** < 0.05**.**

mesangial cells leads to renal dysfunction in patients with type II diabetes (Subathra et al., 2015). In patients with CT therapy, the specific lipids comprising longer chain PUFAs were positively associated with treatment outcome. Corticosteroids are a powerful tool for treating patients with IgAN, but corticosteroid therapy can also induce ROS production (Flaherty et al., 2017). Recent study has shown that active cardiac sarcoidosis patients with elevated oxidative stress levels might be resistant to corticosteroid therapy (Myoren et al., 2016). Thus, the plasma lipids, especially those comprising PUFAs, may reflect systemic oxidative stress levels of patients and may serve as potential markers for assessing treatment outcomes of CT therapy. Taken together, our findings showed a promising feasibility of the lipidomic signatures in assessing treatment outcomes for IgAN patients.

Nevertheless, this study has several limitations. First, the mean BMI in IgAN patients was significant different from that in HCs. Although high level of BMI may affect the dyslipidemic profiles, the correlation analysis has revealed no significant effect of BMI on each lipid module of IgAN patients. Furthermore, the 12-month follow-up period is relative short to evaluate the long-term treatment outcomes.

In conclusion, present study characterized altered plasma lipidome profile in IgAN patients compared to HCs and showed that certain circulating lipids associated with BMI, disease progression, and treatment outcome in IgAN patients. Our findings may not only help to achieve precision medicine but also provide a knowledge base for dietary intervention in the treatment of IgAN.

Materials and methods

Study design and population

The 140 patients who were diagnosed as IgAN during February 2011 to March 2017 at Longhua Hospital were enrolled in the retrospective longitudinal cohort study. Plasma samples were collected at the time of renal biopsy from all included IgAN patients, and demographic and clinical data, such as age, gender, mean aortic pressure, serum creatinine and 24-h urine protein excretion, were recorded. The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD–EPI) equations. The BMI was calculated based on self-reported height and weight collected by means of a telephone survey. For the TCM treatment, Shentong granules made from astragalus, sealwort, eucommia, prunella, fried caltrop, epimedium, poria, fried silkworm, and salvia (Li et al., 2016) were administered through the study follow-up period. For the CT treatment, along with Shentong granules, the oral prednisone was applied to patients at a dose of 0.5–1 mg/kg of body weight per day for first 8–12 weeks, and then a reduced dosage of prednisone according to patient's renal function was administered for at least another 12 weeks during the study follow-up period. Post-treatment, 12-month clinical follow-up data, including eGFR, were collected. Finally, 104 patients (50 with TCM treatment and 54 with CT treatment) completed the 12-month follow-up assessment. The disease progression was defined as decline in eGFR ≥ 5 ml/min/1.73 m^2 over the 12-month follow-up period (Wang et al., 2014). ΔeGFR was defined as eGFR at 12-month follow-up minus eGFR at baseline. eGFR change was defined as percentage 12-month change in eGFR ($100 \times \Delta eGFR$/baseline eGFR). A total of 56 age- and
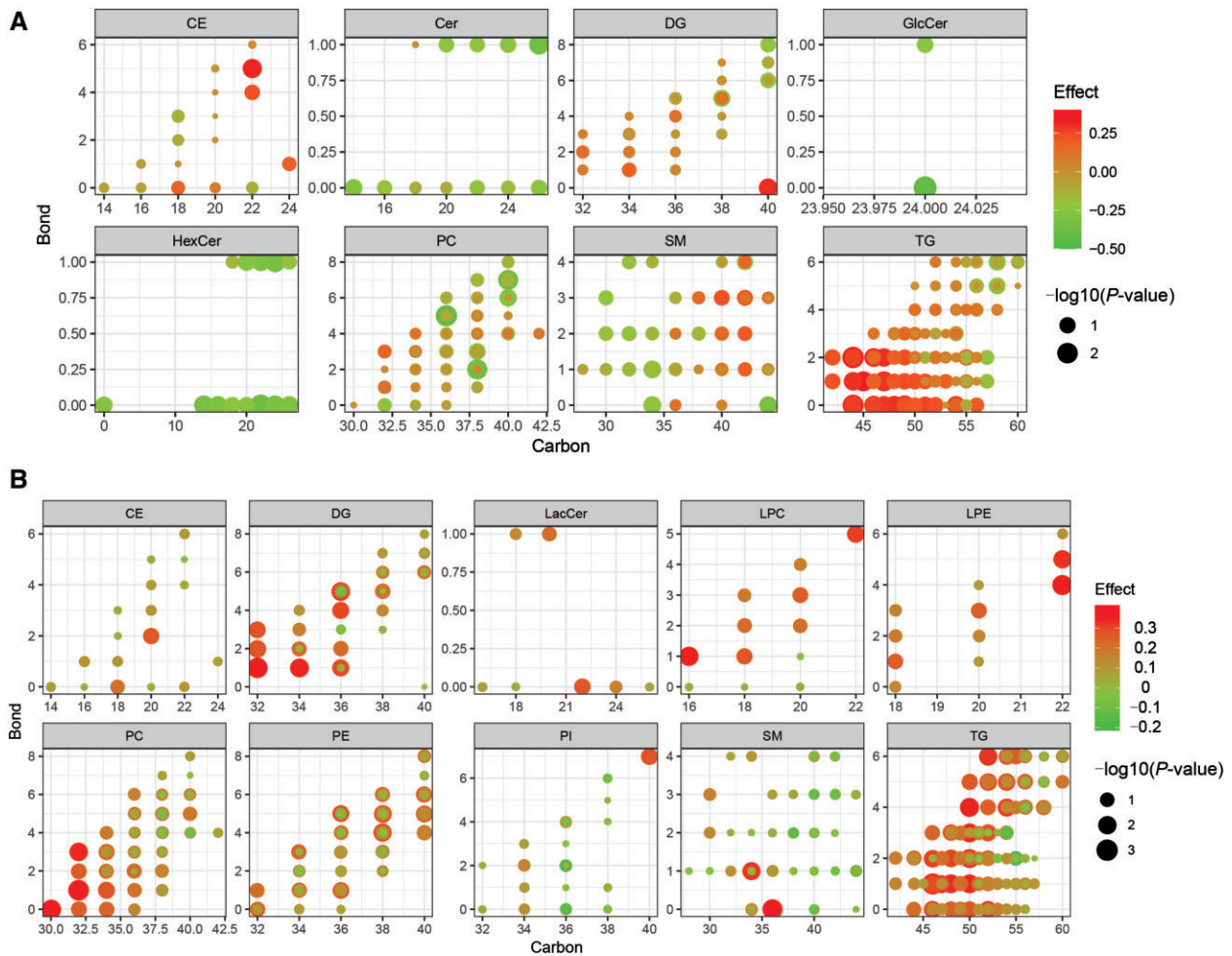
Figure 7 Associations of FA composition in different lipid classes with renal outcomes of IgAN patients receiving TCM (A, $n = 50$) or CT (B, $n = 54$) treatment. Individual lipid species are depicted as filled circles and arranged by lipid classes in panels. Within each panel, their position is determined by the total number of carbon atoms (x axes) and of double bonds (y axes) in the acyl chain. Circle size indicates the significance level, and circle color indicates the effect size per SD that was calculated using multivariable linear regression.

sex-matched healthy volunteers who had normal renal function, no nephropathy, and no other serious illness were enrolled in this study at the physical examination center of the same hospital as HCs, and plasma samples were collected. Demographics and clinical characteristics of participants at baseline and follow-up are provided in Supplementary Tables S4 and S5.

Targeted lipidomics

Lipid extraction method was based on a modified methyl-tert-butyl ether method (Matyash et al., 2008) and summarized in Supplementary material. Lipid separation was performed on an ultra-performance liquid chromatography Shimadzu Nexera X2 LC-30AD. MS data acquisition was performed on a hybrid triple quadrupole/linear ion trap mass spectrometer SCIEX 5500 QTRAP. Two separate injections were made for positive and negative ionization mode. A total of 1032 multiple reaction monitoring (MRM) transitions (631 in positive mode and 401 in negative

mode) were set up for quantitative analysis of lipids. The LC–MRM data were analyzed by the Analyst 1.6.3 software (Sciex) for manual inspection of chromatograms and for the detection of compounds. MultiQuant[TM] 3.0 Software (Sciex) was used for integration of peak areas. The signal intensity of each MRM value was normalized by an internal standard in Lipidyzer[TM] internal standard kit (Sciex) for quantitative comparisons. Further details are available in Supplementary material.

Statistical and bioinformatics analysis

Before the statistical analysis, a data cleanup procedure was performed to remove lipids with poor repeatability (high variability with coefficients of variation $>30\%$ in QCs). Multivariate linear regression was performed to examine the associations of circulating lipids with BMI and eGFR change. Univariate logistic regression was performed to examine the associations of the baseline demographics and clinical characteristics with disease progression. Multivariate logistic regression

was performed to examine the associations of circulating lipids with disease progression. Unadjusted P-values were reported for the univariate/multivariate analysis, with nominal significance set at $P < 0.05$. FDR q-values were calculated from unadjusted P-values using the Benjamini–Hochberg procedure. All the data analyses were carried out using R version 4.0.0 and using R-studio version 1.1.463 as a graphical user interface.

Supplementary material

Supplementary material is available at Journal of Molecular Cell Biology online.

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