Metabonomic Profile of Macrosteatotic Allografts for Orthotopic Liver Transplantation in Patients With Initial Poor Function: Mechanistic Investigation and Prognostic Prediction

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Background: Our previous study revealed amplified hazardous effects of macrosteatosis (MaS) on graft failure (GF) in recipients with severe liver damage in short post-operative days, with vague mechanism inside.

Aim: We aimed to uncover the molecular mechanism of donor MaS on GF, and construct the predictive model to monitor post-transplant prognosis based on “omics” perspective.

Methods: Ultra-performance liquid chromatography coupled to mass spectrometry metabolomic analysis was performed in allograft tissues from 82 patients with initial poor function (IPF) from multi-liver transplant (LT) centers. Pathway analysis was performed by on-line toolkit Metaboanalyst (v 3.0). Predictive model was constructed based on combinative metabonomic and clinical data extracted by stepwised cox proportional analysis.

Results: Principle component analysis (PCA) analysis revealed stratification on metabolic feature in organs classified by MaS status. Differential metabolits both associated with MaS and GF were significantly enriched on pathway of glycerophospholipid metabolism ($P < 0.05$). Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) involved in glycerophospholipid metabolism was significantly decreased in cases with MaS donors and GF ($P < 0.05$). Better prediction was observed on graft survival by combinative model (area under the curve = 0.91) and confirmed by internal validation.
**INTRODUCTION**

Orthotopic liver transplantation (OLT) is one of the most effective therapeutic strategies for end-stage liver disease, hepatobiliary malignancy, and acute/chronic hepatic failure (Oleary et al., 2008). Donated allograft quality directly affects the patient prognosis (Feng et al., 2006; Flores and Asrani, 2017). Of which, macrosteatosis (MaS) was considered as an profound risk component for exceeded criteria donor (ECD), with positive impacts on inferior post-transplant survival and complications (Spitzer et al., 2016; Vodkin and Kuo, 2017; Moosburner et al., 2018). Followed with rising prevalence of non-alcoholic fatty liver disease (NAFLD) in donor pool, more steatotic allografts are put into clinical application to solve the organ shortage under the pressure of increasing demands for liver transplantation. Undoubtedly, the influence of allograft steatosis is becoming more and more prominent for increasing proportion applied in whole patient cohort (Moosburner et al., 2018).

However, more and more concerns are raised on MaS organ for their impacts on higher comorbidities and mortality for patients after LT (Croome et al., 2019, 2020). As one of prominent sign for extended criteria donor (ECD) livers, MaS was proved to be a risk predictor on adverse prognosis including severer liver injury, increased peri-operative complications, higher post-transplant mortality and graft loss (De Graaf et al., 2012; Croome et al., 2019). In spite of controversies on acceptable safety cutoff (Liu et al., 2019), donor MaS has become the major cause for organ discard and transplant cancelation in some LT centers (Moosburner et al., 2018). Steatotic allografts deteriorate the function of transplanted liver by interaction with ischemia-reperfusion injury (Gehrau et al., 2015; Dar et al., 2019). Steatotic allografts should be transplanted under restrict control on duration of cold ischemia time (7 to 8 h) for acceptable post-transplant effects (Westerkamp et al., 2015; Wong et al., 2016).

Metabolites might be served as reliable prognostic indicator and therapeutic target for patients received LT. Baseline circulating lactate and its clearance were found to be predictor for EAD occurrence and graft survival, with better performance than conventional balance of risk (BAR) score (Golse et al., 2019; Takahashi et al., 2019).

Metabolomics data provides systematic knowledge of metabolome which might be helpful for early detection of allograft quality and prediction of prognosis after liver transplantation. And these metabolomics data can be integrated to be explained for the mechanism of inferior survival caused by clinical risk covariates, and provide potent interventions for improvement of the allograft quality (Bonneau et al., 2016; Cortes et al., 2017). Online toolkit (like MetaboAnalyst) provides concise but meaningful interpretations for the metabolomic data via pathway and enrichment analysis (Xia et al., 2015). Accordingly, prospective effects of metabolome were evaluated for prediction of early allograft dysfunction (EAD) in previous studies (Cortes et al., 2014; Xia et al., 2015). Cortes et al. emphasized the clinical value of metabolomics data on functional prediction of sub-optimal organs and donor expansion (Cortes et al., 2014). Metabolic profile was also described in grafts from donors after circulatory death (DCD) (Perera et al., 2014). However, metabolic features of MaS allografts and their connections with inferior post-transplant outcomes were still lacking.

Initial poor function (IPF), usually defined with extremely higher transaminase within shorter post-operative days (PODs) (Mathe et al., 2011), plays determinant effect on post-transplant mortality and morbidity (Bolondi et al., 2016). Risk stratification was observed on post-transplant mortality and comorbidity in patients classified by IPF occurrence (Maring et al., 1997; Hao et al., 2011). Donor MaS positively affects the IPF occurrence (Hao et al., 2011). But more importantly, MaS amplified the risk of inferior prognosis with additive effect on IPF (Mccormack et al., 2011). Results from our previous study found MaS allografts had worse tolerance in patients experienced IPF. Disproportionate increment on graft failure (GF) was observed by MaS allografts in patients with severe liver damage in early PODs (44% vs. 10%) (Liu et al., 2020). However, mechanism under the mortality gap is still unclear and worthy for further elucidation.

Therefore, as continuation and sublimation of previous results, we performed a multi-center study to build the predictive model for post-transplant prognosis and investigate the mechanistic link from donor MaS to GF based on combination of clinical and metabolomic indicators in patients with IPF after LT. In accordance with development of machine perfusion (MP) for organ perservation (Nasralla et al., 2018), this study provides prospective knowledge for better assessment of MaS grafts with provision of meaningful metabolites as potential targets for further improvement of allograft quality.

**Conclusion:** Metabonomic features of allografts can be clearly distinguished by MaS status in patients with IPF. Dysfunction on glycerophospholipid metabolism was culprit to link donor MaS and final GF. Decrement on PC and PE exerted the fatal effects of MaS on organ failure. Metabonomic data might help for monitoring long-term graft survival after LT.

**Keywords:** macrosteatosis, metabonomic, mechanism, prognosis, liver transplantation

**MATERIALS AND METHODS**

**Study Flow Diagram**

Procedure of study flowchart can be shown in Supplementary Figure S1. In general, allograft metabolomic, clinical and prognostic information were collected in LT cases, respectively. Donor metabolites with difference between MaS and non-MaS
groups were collected (candidate metabolites A). Meanwhile, univariate survival analysis was performed for potential metabolic and clinical candidates (B and C). Then, the shared metabolites between candidate A and B were collected for further mechanistic investigation on link from allograft MaS to GF.

Meanwhile, risk model for prognostic prediction was fitted by multi-covariate analysis with inclusion of potential clinical and metabolomic factors after optimization by lasso regression. And details of procedure can be shown in Supplementary Figure S1B.

**Enrollement of Study Population**
Liver transplant cases were reviewed and enrolled in the period from January 1, 2015 to March 31, 2019 from two independent transplant centers (Shulan (Hangzhou) Hospital [cohort A] and The First Affiliated Hospital of Zhejiang University [cohort B]) in accordance with uniform selection criteria as follows: (1) adults recipients (age ≥ 18 years); (2) non-living donor liver transplantation (LDLT); (3) non-multi-organ transplantation (n = 1); (4) occurrence of initial poor function (IPF) with definition on consecutive ALT and AST elevation within POD3 (> 1500 IU/L) after liver transplantation; (5) availability of graft tissue samples kept during transplantation; 6. availability of survival status in the end of follow-up duration. Informed consents were obtained from enrolled participants. And this study was performed in accordance with the Declaration of Helsinki and approved by the ethical board of local hospital.

**Definition of Complication**
Early allograft dysfunction (EAD) was diagnosed in patients with severe liver damage (ALT > 3000 IU/mL or AST > 6000 IU/mL), jaundice (TB ≥ 10 mg/dL), and coagulation dysfunction (INR ≥ 1.6) simultaneously within POD7. 

**Data Collection and Follow-Up**
Clinical data related to recipients, donor, surgery and grafts was collected by experienced surgeons (ZTL and JX) respectively in local medical record system (Table 1). Graft steatosis was assessed qualitatively and quantitatively based on hematoxylin and eosin (H&E) stained sections with biopsies according to previous definition (Crowley et al., 2000). Follow-up information was collected by regular telephone call by specialized staff per month. And data on survival status, duration or death cause was provided in the end of follow-up duration.

**Sample Collection and Preparation**
Graft tissues were routinely collected from grafts for transplantation after their reperfusion in perfusates. Samples were flash frozen in liquid nitrogen once separated from allografts and kepted rountinely in ultra-low temperature freezer (−80°C) in biobank of NHC Key Laboratory of Combined Multi-organ Transplantation for long-term storage. Samples were accurately weighted and extracted in solvent ethanol/water mixture with internal reference for further metabolomic analysis. And details of the treatment can be shown in Supplementary Material.

### TABLE 1 | Summary of Clinical Information for Transplant Cases Categorized by Allograft MaS status.

| Covariates | MaS grafts | Non-MaS grafts | p-value |
|------------|------------|----------------|---------|
| Number (%) | 35 (42.7)  | 47 (57.3)      | NA      |
| **Recipient factor (R)** | | | |
| Age (R, years) | 49 (34–54) | 50 (43–56) | 0.12 |
| Gender (R, M,%) | 30 (85.7)  | 39 (83.0)   | 0.74 |
| BMI (R, kg/m²) | 23.1 ± 2.7 | 23.8 ± 3.2 | 0.34 |
| Blood Type (R) | | | 0.98 |
| A-type n (%) | 15 (42.9)  | 18 (38.3)   | |
| B-type n (%) | 4 (11.4)   | 6 (12.8)    | |
| O-type n (%) | 14 (40)    | 20 (42.6)   | |
| AB-type n (%) | 2 (5.7)    | 3 (6.4)     | |
| Diabetes (R, N,%) | 3 (8.6) | 7 (14.9) | 0.39 |
| Pre-operative AFP (R, ng/ml) | 30.4 (4.9–551.4) | 16.1 (5.6–139.0) | 0.44 |
| HBV infectors (R, N,%) | 24 (68.6) | 39 (83.0) | 0.13 |
| MELD score (R) | 33 (28–40)* | 33 (26–40) | 0.70 |
| Child–pugh score (R) | 10 (9–11)  | 11 (10–12)  | 0.11 |
| **Donor factor (D)** | | | |
| Age (D, years) | 45 (31–51) | 44 (36–53) | 0.80 |
| Gender (D, M,%) | 29 (82.9) | 40 (85.1) | 0.72 |
| BMI (D, kg/m²) | 23.8 ± 2.8 | 22.9 ± 2.5 | 0.13 |
| Blood type (D) | | | 0.60 |
| A-type n (%) | 13 (37.1)  | 14 (29.8)   | |
| B-type n (%) | 5 (14.3)   | 7 (14.9)    | |
| O-type n (%) | 13 (37.1)  | 19 (40.4)   | |
| AB-type n (%) | 4 (11.4)   | 7 (14.9)    | |
| HBV infectors (D, N,%) | 6 (17.1) | 5 (10.6) | 0.39 |
| HCV infectors (D, N,%) | 6 (17.1) | 0 (0) | NA |
| **Pre-donation blood test (D)** | | | |
| D-Potassium (D, mmol/L) | 3.7 (3.4–4.1) | 4.3 (3.7–4.6) | 0.02 |
| D-Sodium (D, mmol/L) | 145.9 (139.0–152.0) | 145.8 (138.1–153.1) | 0.79 |
| D-ALT (D, IU/L) | 44.0 (25.0–74.0) | 39.4 (25–62) | 0.55 |
| D-TB (D, µmol/L) | 14.8 (10.4–21.4) | 19.3 (11–27) | 0.15 |
| D-CR (D, µmol/L) | 87.0 (65.0–160.0) | 86.3 (61.0–151.6) | 0.82 |
| D-BUN (D, mmol/L) | 7.6 (5.5–10.9) | 8.6 (5.0–11.6) | 0.57 |
| Donation type | | | 0.25 |
| (DBD/DCD/DBCD) | | | |
| DBD (N, %) | 12 (34.2)  | 10 (21.3)   | |
| DCD (N, %) | 16 (45.7)  | 30 (63.8)   | |
| DBCD (N, %) | 7 (20.0)   | 7 (14.9)    | |
| Cause of Death | | | 0.98 |
| (TBI/Stroke/Others) | 17 (49.4) | 23 (40.3) | |
| ECMO use | 0 | 0 | NA |
| **Graft factor (G)** | | | |
| Steatosis Severity (%) | 15 (5–25) | 10 (5–18.8) | <0.01 |
| CIT (min) | 648 (542–744) | 652 (567–743) | 0.68 |
| WIT (min) | 5 (0–10)* | 9 (5–12) | 0.03 |
| **Surgery (S)** | | | 0.65 |
| Indication for LT | | | |
| Liver Cirrhosis n (%) | 13 (37.1) | 22 (46.8) | |
| Liver Failure n (%) | 10 (28.6) | 7 (14.9) | |
| PBC/PSC n (%) | 2 (5.7) | 2 (4.3) | |
| Liver Cancer n (%) | 17 (48.6) | 19 (40.4) | |
| Others n (%) | 1 (2.9) | 2 (4.3) | |
| **Post-LT Peak TB level (mg/dL)** | 205.9 (106–386) | 225 (138–387) | 0.58 |
| **Post-LT Peak ALT Level (IU/L)** | 2626 (2027–3694) | 2401 (1972–3075) | 0.35 |

(Continued)
TABLE 1 | Continued

| Covariates                  | MaS grafts | Non-MaS grafts | P-value |
|-----------------------------|------------|----------------|---------|
| Post-LT Peak AST level (IU/L) | 6576 (4673−13638) | 6049 (3665−8745) | 0.27    |
| EAD occurrence n (%)        | 22 (62.9)  | 30 (63.8)      | 0.93    |
| PNF occurrence n (%)        | 4 (11.4)   | 6 (12.8)       | 0.86    |
| Blood Transfusion during LT | 745 (630−1220)   | 775 (510−1020)  | 0.65    |
| pRBC (l)                    | 4.5 (2.0−8.0) | 5.0 (2.0−9.0)  | 0.71    |
| FFP (ml)                    | 3000 (0−3500) | 3000 (1500−4000) | 0.48    |
| PCC (l)                     | 2000 (0−3000) | 1750 (75−3000) | 0.19    |
| FIB (g)                     | 5 (7.5)     | 5 (2−10)       | 0.44    |
| ALB (g)                     | 115 (30−150) | 125 (75−150)   | 0.50    |
| Blood Loss (ml)             | 1500 (1000−2500) | 1200 (800−2003) | 0.12    |
| Surgical Duration (mins)    | 310 (275−375) | 302.4 (260−339) | 0.40    |
| ICU stay (days)             | 12.8 (7.6−17) | 13 (7.6−18)    | 0.97    |
| Length of post−LT hospitalization (d) | 29 (19−39) | 26 (12−37)    | 0.72    |
| Year of LT                  |            |                | 0.07    |
| 2015−2016 (n,%)             | 7 (20)     | 20 (42.6)      |         |
| 2017−2019 (n,%)             | 28 (80)    | 27 (57.4)      |         |
| Time from LT to the end of follow-up survey (days) | 616 (510−885) | 894 (624−1670) | 0.02 |

*p-Represented significant difference across different groups; represented by Mann−Whitney U-test for quantitative data in asymmetrical distribution; by Chi-square test for count data. D, donor; DBCD, donation after brain and cardiac death; DBD, donation after brain death; DCC, donation after cardiac death; EAD, early allograft dysfunction; ECMO, extracorporeal membrane oxygenation; FFP, fresh frozen plasma; FIB, fibrinogen; G, graft; HBV, hepatitis B virus; HCV, hepatitis C virus; ICU, intensive care unit; LT, liver transplantation; M, male; MaS, macrosteatosis; MELD, model for end-stage liver disease; PBC, primary biliary cholangitis; PCC, prothrombin complex concentrate; PNF, primary non-function; PSC, primary sclerosing cholangitis; R, recipient; RBC, red blood cell; TB, total bilirubin; TBI, traumatic brain injuries.

Ultra-Performance Liquid Chromatography Coupled to Mass Spectrometry (LC-MS) Metabolomics
Profile of metabolites were tested by Dionex Ultimate 3000 RS UHPLC system (Thermo Fisher Scientific) with heated electrospray ionization in positive and negative modules. Potential metabolites was obtained and identified by progenesis QI software (Waters Corporation), based on Human Metabolome Database (HMDB). QC samples were injected every 10 samples for accessible repeatability. Details of the parameters in sample detection and data process can be referred to Supplementary Material.

Network and Pathway Analysis
Based on SIMCA-P platform (Wu et al., 2010) (version 14.1, Umetrics, Sweden), principle component analysis (PCA) and orthogonal partial least-squares-discriminant analysis (OPLS-DA) were carried out to present metabolic alterations across MaS and non-MaS groups. Variable importance in the projection (VIP) value was calculated for each covariate, and VIP > 1 was indicative of relevance with group discrimination. Enrichment and pathway analysis on metabolomic data were conducted based on Kyoto Encyclopedia of Genes and Genomes (KEGG) database were performed and visualized by MetaboAnalyst software (version 4.0) for deeper knowledge of biological connection across potential metabolites (Xia and Wishart, 2016).

Statistical Analysis
Normally distributed data was described as mean ± standard deviation (SD) and compared by one-way ANOVA. Abnormally distributed data was presented as median (inter-quartile range, IQR) and compared by Mann-Whitney U test. Categorical data was presented as number (percentage) and compared by chi-square test.

For survival analysis, Cox proportional-hazards regression model was used for selection of prognostic factors. Lasso regression was used to select the optimal prognostic covariates by reduction of the high dimensional data (Friedman et al., 2010). Potential covariates for predictive model was filtered using multivariable cox regression model adjusted by optimized factors. Correlations across significant indicators were evaluated by spearman heatmap.

For single predictor, the Kaplan-Meier curves were plotted to show its dichotomous effect on overall survival; and two-stage random effect model was used for evaluation on its dose-response association with prognosis (Orsini et al., 2006).

Predictive nomogram was plotted based on covariates from multivariable cox regression analysis. C-statistic was used to quantitatively evaluate the discriminative performance of nomogram (Pencina and D’Agostino, 2004). And calibration curves were plotted to reflect the agreement between actual outcomes and predicted probabilities (Kramer and Zimmerman, 2007). Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the predictive performance of clinical, metabolomic and combinative clusters on GF. Diagnostic performances of these clusters were assessed using the area under the receiver operating curve (AUROC) and discriminated by z statistic analysis. Time-dependent AUROC was also plotted to evaluate the performance of these clusters in prediction of GF in different period (Hung and Chiang, 2009).

Statistic analysis was performed via R (v.3.5.1), stata (v.14.0), SPSS (v.26.0), medcalc (v.19.0.7), respectively. Details of software and algorithm were shown in Supplementary Table S1. Two-sided P-value < 0.05 was considered as statistical significance.

RESULTS
Clinical Characteristics of Enrolled Patients
Patients were included with severe post-transplant liver damage (ALT ≥ 2000 IU/L), based on cohorts of 975 LT cases. In contrast to the stable tendency on utilization MiS liver, donation of MaS

1http://www.hmdb.ca/

2https://www metaboanalyst.ca/MetaboAnalyst/home.xhtml
allografts was significantly increased from 15% in 2015 to more than one quarter in 2019 (*P* < 0.05, Supplementary Figure S2). Selection procedure can be shown in Supplementary Figure S2. Eighty-two patients with post-transplant IPF were enrolled into final analysis. As shown in Table 1, the MaS prevalence was about 42.6% in grafts received metabonomic analysis. Insignificant difference was observed in patients categorized by allograft steatosis in most dimensions (*P* > 0.05). Positive anti-HCV was only observed in MaS but not in non-MaS grafts. WIT was shorter in patients using MaS grafts for LT (*P* < 0.05). Intriguingly, the follow-up duration was nearly a third shorter in donor MaS group (616 vs. 786 days, *P* < 0.05), verifying the increasing trend on utilization of MaS allografts in the whole cohort. Insignificant difference was observed distribution of patients’ status (age, gender, blood type, etc.) and disease severity (Child-Pugh/MELD score) in groups categorized by medical centers (*P* > 0.05, Supplementary Table S2).

**Predictive Clinical Factors on Graft Failure**
Clinical factors on recipient (pre-operative child-pugh/MELD score, height, postoperative AST level), donor (pre-operative ALT), graft (macrosteatosis) and surgical (blood loss/transfusion) aspects commonly affected the graft survival in multi-covariate cox proportional hazard model (Figure 1). Noteworthy, significantly higher risk of GF was observed in patients with EAD occurrence and MaS graft utilization (HR = 2.57/2.30, *P* < 0.05).

**Metabonomic Profiles of Donor Livers**
Raw data was adjusted by QC samples according to predefined criteria (Sangster et al., 2006). A total of 3444 metabolites were detected per sample after data pretreatment by Progenesis QI (v2.3). Finally, 2155 features with identification in Human Metabolome Database (https://hmdb.ca) were enrolled for further analysis.

**Multivariate Analysis (MVA) on Donor Livers**
Multivariate analysis in OPLS-DA model revealed clear separation on metabonomic features between MaS and non-MaS grafts (Q^2^ = 0.58, R^2^ = 0.52, Figure 2A). Further validation model by permutation test also showed the specificity and reliability of the patient classification (R^2^ = 0.41, Q^2^ = −0.441, Figure 2B).

**Network Analysis on Potential Metabolites Associated With Donor MaS and Graft Failure**
Significant variation was observed across MaS and non-MaS allografts in 389 metabolites by univariate ANOVA analysis (higher in 211, but lower in 180 features for MaS grafts, Figure 3). Further functional pathway analysis revealed that the differentiated metabolites caused by MaS were mainly involved in participation of linoleic acid and glycerophospholipid metabolism (*P* < 0.05, Figure 3 and Supplementary Table S3). Compounds involved in candidate pathways for MaS allografts were reviewed in Table 2. Most of potential features can be categorized into glycerophospholipids class. Linoleic acid level was significantly higher, but phosphatidylcholine and phosphatidylethanolamine levels were decreased in MaS donors (*P* < 0.05).

Graft survival was significantly affected by 104 metabolic features using univariate cox analysis (91 hazardous and 13 protective metabolites, Figure 3). Enrichment of candidate metabolites indicated the significance of steroid biosynthesis pathway on post-transplant prognosis (*P* < 0.05, Figure 3 and Supplementary Table S4). Most involved features can be categorized into steroids class and exerted hazardous effects on post-transplant prognosis (Figure 3 and Table 2). After classification by KEGG IDs, the compounds including phosphatidylcholine (C00157), phosphatidylethanolamine (C00350), saccharopine (C00449) and glucuronide (C03033) were overlapped metabolomic clusters with both association on post-transplant prognosis and donor MaS (Figure 3). C03033 increased both risk on MaS occurrence and GF, while the C00157 and C00350 exerted protective effects on above two events (Figure 3). Network analysis revealed the overlapped metabolites were enriched significantly on pathway of glycerophospholipid metabolism (*P* < 0.01, Figure 3 and Supplementary Table S5).

**Selection of Candidates for Prognostic Analysis**
Positive clinical and metabonomic variables in prior univariate analysis were put into LASSO regression model for dimensional-reduction of the dataset. 32 factors with inclusion of 23 metabolomic and 9 clinical features were screen out for further analysis. Finally, 15 factors including 10 metabolomic and 5 clinical features were selected with most represensitivity for further predictive model for post-transplant prognosis.

**Potential Model With Cobination of Clinical and Metabonomic Signatures on Prognostic Prediction**
Fifteen factors with statistic significance in multi-covariate Cox regression were enrolled for construction of clinical-metabonomic predictive model for post-transplant prognosis (Figure 4). Prominently higher risk of GF was observed in patients with EAD occurrence or utilization of MaS donors (HR = 4.37/5.62, respectively). The panorama of enrolled susceptive metabolites was summarized in Table 3. Most of these metabolites can be clustered into to lipid and organic acid categories, respectively. Based on clinical-metabonomic model, the C00157 compound ([PC(18:4/16:0)]) exerted protective effect, while the dexamethasone (HMDB0015364) as extraneous glucocorticoid played hazardous role on inferior prognosis after LT (HR = 0.28 and 4.13, respectively). Further dose-response analysis on each potential factors revealed that the risk trend of GF was observed consistently in linear trend in 10 clinical-metabonomic factors (*P* for non-linearity > 0.05, Figure 5 and Supplementary Table S6). Hazardous effects of threoninyl-proline and PA(15:0/18:4) might
stay on plateau after their arrival on risk peak for GF. Compared to grafts in lowest quintile, the HR of Eriopside B (HMDB0038029) rose to 1.29 in Q3, but descend to 0.82 in highest quintile. As external substance, dexamethasone and N-Malonyltryptophan can’t be detected in 50% and 37% of allografts, but the GF risk was increased rapidly once tested in remaining organs.

**Nomogram for Prediction of Post-transplant Graft Failure**

Fifteen factors (10 metabonomic and 5 clinical) significantly associated with GF in cox-regression model were integrated into predictive nomogram for post-transplant graft survival in different time periods (Figure 4). The concordance index for the nomogram was 0.85 (95% CI: 0.79–0.91). Calibration plot showed good agreements between observed and predicted risks on post-transplant graft survival. All enrolled factors were relatively independent for lower intercorrelation observed in heatmap (all $r < 0.4$, Figure 4).

**Performance of Nomogram Based Algorithm on Prediction of Prognosis**

Efficiency of predictive model was estimated separately, based on clinical, metabonomic, and combinative factor clusters extracted from nomogram algorithm referred above (Figure 4 and Supplementary Table S7). Meanwhile, performance of these predictive clusters on post-transplant GF was also evaluated in subgroups divided by medical centers. The AUC for prediction of overall graft survival was 0.69 (95% CI: 0.58–0.79), 0.85 (95% CI: 0.75–0.92), and 0.91 (95% CI: 0.83–0.96) for clinical, metabonomic and combinative model (Figure 4 and Supplementary Table S7). Continuous time-dependent AUC of post-transplant GF was also evaluated in subgroups divided by medical centers. The AUC for prediction of overall graft survival was 0.69 (95% CI: 0.58–0.79), 0.85 (95% CI: 0.75–0.92), and 0.91 (95% CI: 0.83–0.96) for clinical, metabonomic and combinative model (Figure 4 and Supplementary Table S7). Continuous time-dependent AUC of post-transplant GF was also evaluated in subgroups divided by medical centers. The AUC for prediction of overall graft survival was 0.69 (95% CI: 0.58–0.79), 0.85 (95% CI: 0.75–0.92), and 0.91 (95% CI: 0.83–0.96) for clinical, metabonomic and combinative model (Figure 4 and Supplementary Table S7). Continuous time-dependent AUC of post-transplant GF was also evaluated in subgroups divided by medical centers. The AUC for prediction of overall graft survival was 0.69 (95% CI: 0.58–0.79), 0.85 (95% CI: 0.75–0.92), and 0.91 (95% CI: 0.83–0.96) for clinical, metabonomic and combinative model (Figure 4 and Supplementary Table S7). Continuous time-dependent AUC of post-transplant GF was also evaluated in subgroups divided by medical centers. The AUC for prediction of overall graft survival was 0.69 (95% CI: 0.58–0.79), 0.85 (95% CI: 0.75–0.92), and 0.91 (95% CI: 0.83–0.96) for clinical, metabonomic and combinative model (Figure 4 and Supplementary Table S7). Continuous time-dependent AUC of post-transplant GF was also evaluated in subgroups divided by medical centers. The AUC for prediction of overall graft survival was 0.69 (95% CI: 0.58–0.79), 0.85 (95% CI: 0.75–0.92), and 0.91 (95% CI: 0.83–0.96) for clinical, metabonomic and combinative model (Figure 4 and Supplementary Table S7). Continuous time-dependent AUC of post-transplant GF was also evaluated in subgroups divided by medical centers. The AUC for prediction of overall graft survival was 0.69 (95% CI: 0.58–0.79), 0.85 (95% CI: 0.75–0.92), and 0.91 (95% CI: 0.83–0.96) for clinical, metabonomic and combinative model (Figure 4 and Supplementary Table S7). Continuous time-dependent AUC of post-transplant GF was also evaluated in subgroups divided by medical centers. The AUC for prediction of overall graft survival was 0.69 (95% CI: 0.58–0.79), 0.85 (95% CI: 0.75–0.92), and 0.91 (95% CI: 0.83–0.96) for clinical, metabonomic and combinative model (Figure 4 and Supplementary Table S7).Continuous time-dependent AUC of post-transplant GF was also evaluated in subgroups divided by medical centers. The AUC for prediction of overall graft survival was 0.69 (95% CI: 0.58–0.79), 0.85 (95% CI: 0.75–0.92), and 0.91 (95% CI: 0.83–0.96) for clinical, metabonomic and combinative model (Figure 4 and Supplementary Table S7). Continuous time-dependent AUC of post-transplant GF was also evaluated in subgroups divided by medical centers. The AUC for prediction of overall graft survival was 0.69 (95% CI: 0.58–0.79), 0.85 (95% CI: 0.75–0.92), and 0.91 (95% CI: 0.83–0.96) for clinical, metabonomic and combinative model (Figure 4 and Supplementary Table S7). Continuous time-dependent AUC of post-transplant GF was also evaluated in subgroups divided by medical centers. The AUC for prediction of overall graft survival was 0.69 (95% CI: 0.58–0.79), 0.85 (95% CI: 0.75–0.92), and 0.91 (95% CI: 0.83–0.96) for clinical, metabonomic and combinative model (Figure 4 and Supplementary Table S7). Continuous time-dependent AUC of post-transplant GF was also evaluated in subgroups divided by medical centers. The AUC for prediction of overall graft survival was 0.69 (95% CI: 0.58–0.79), 0.85 (95% CI: 0.75–0.92), and 0.91 (95% CI: 0.83–0.96) for clinical, metabonomic and combinative model (Figure 4 and Supplementary Table S7). Continuous time-dependent AUC of post-transplant GF was also evaluated in subgroups divided by medical centers. The AUC for prediction of overall graft survival was 0.69 (95% CI: 0.58–0.79), 0.85 (95% CI: 0.75–0.92), and 0.91 (95% CI: 0.83–0.96) for clinical, metabonomic and combinative model (Figure 4 and Supplementary Table S7).
FIGURE 2 | Multivariate data analysis on metabolic profiles of donor livers by MaS status. (A) PCA analysis revealed clear separation on patients received MaS (blue dots) and non-MaS (green dots) allografts by OPLS-DA model; (B) Validation of OPLS-DA model by class permutation analysis for panel (A). MaS, macrosteatosis, OPLS-DA, orthogonal projection to latent structures discriminant analysis; PCA, principal component analysis.
FIGURE 3 | Pathway enrichment based on metabolites associated with donor MaS, graft failure and their intersection. (A) Bar chart discriminating the components with significant increments (red bar, n = 180) or decrement (blue bar, n = 211) in MaS grafts; (B) Volcano plot on visualization of both FC and significance for each metabolites compared between MaS and non-MaS grafts, red dots represented significantly higher metabolits (FC > 2, P < 0.05) in MaS grafts, blue dots represented significantly lower metabolits (FC < 0.5, P < 0.05) in MaS grafts; (C) Bar chart discriminating the components with significant hazardous (blue bar, (Continued)
of the results were also confirmed by internal validation tests conducted in subgroups divided by medical centers (Figure 4 and Supplementary Table S7).

**DISCUSSION**

As “last resort” for end-stage liver disease, the quality of LT is affected by multi-factors on donor, recipient, organ, and surgical aspects. More suboptimal organs are put into use to relieve the contradiction between limited organ supply and increasing demands on LT (Tullius and Rabb, 2018). As one of the commonest feature of ECDs, inferior outcomes was observed in patients received severe MaS allografts with more comorbidities, complications and graft failures (Spitzer et al., 2010). As temporary insufficient liver function in shorter PODs, the initial poor function (IPF) was considered to be influenced by donor, recipient, graft and surgical covariates (Hao et al., 2011). Usually, the IPF is reversible by intensive support within 1 month, with similar post-transplant outcomes compared to patients with immediate function (Stockmann et al., 2010). However, the GF risk was amplified in IPF patients by integration with donor MaS (Liu et al., 2020). We speculated that some metabolites as downstream products of biochemical and physiological processes might be responsible for the additional risk of GF caused by MaS donor. Based on metabolomic data from allografts with IPF after LT, we found 1. Metabolites enriched on the pathway of glycerophospholipids metabolism both affected the donor MaS and graft survival; 2. Decrement of molecules including phosphatidylcholine (PC(20:5/16:0), C00157), and phosphatidylethanolamine (PE(20:4/22:6), C00350) were found to be key regulators with responsibility on donor MaS and graft loss; 3. The combinative clinical-metabonomic model (including 10 metabolites and 5 clinical indicators) had improved performance on GF prediction in the following 3 years after LT (AUROC = 0.91). And reliability of this model on prognostic prediction was also confirmed by validation test. Highly prevalent of donor MaS (>40%) was observed in IPF patients from our study (Table 1). MaS organs suffered more HCV infection and longer time for warm ischemia (P < 0.05). Application of MaS allografts was increased over time-period (Supplementary Figure S2). Amount of metabolonomic analysis on serum, plasma, urine, liver tissue or even salivar samples from NAFLD/NASH patients in general population were performed to discriminate the suspicious objects, uncover the mechanism and evaluate the efficiency of medical treatment on hepatic steatosis (Gitto et al., 2018; Troisi et al., 2019). However, less metabolomic analysis was performed on grafts discriminated by MaS status before. As we known, the metabolomic change of MaS organs in vitro was more complex for higher stress from ischemia-reperfusion injury. Otherwise, most of deceased donors were hospitalized patients with more comorbidities and complications prior to organ donation (Merion et al., 2006). Hence, it is worthy to have metabolomic study to elucidate the metabolic signature for MaS grafts for LT.

In our study, variation on lipid metabolism played a dominant effects role in regulating the hepatic triglyceride content (HTGC) of grafts for LT. PCA analysis revealed patients can be clearly discriminated by donor MaS status (Figure 2). Key molecules was enriched on pathways that related to linoleic acid and glycerophospholipid metabolism. Linoleic acid (LC), as “omega-6 polyunsaturated fatty acid (n-6 PUFA)” is an essential fatty acid only derived from diet, with trade-off relationship to n-3 PUFA in vivo. Previous studies found lower n-6:n-3 PUFA ratio might help to ameliorate the ischemia/reperfusion injury via improvement on hepatic microcirculation with potential for clinical implication (Alwayn et al., 2005; Elbadry et al., 2007). Correspondingly, our results confirmed higher n-6 PUFA in MaS grafts on “omics” perspective. Phosphatidylcholine (PC) as antagonist of free cholesterol (FC), was down-regulated, with negative regulation on LC production. Noteworthy, PC was presented as the central hubs to connect the linoleic acid and glycerophospholipid metabolism. Meanwhile, PE was also diminished with more extents (FC = 0.31 and 0.29 vs. 0.45 for PC), with resultant increased PC/PE ratio, indicating relatively mild steatohepatitis in whole grafts (Li et al., 2006; Ling et al., 2012). In addition, increased lysophospholipids (LypoPC) as indication of oxidative stress and proinflammatory status was also involved in pathogenesis of MaS organs. Basically conformed to previous results from NAFLD patients or mice models (Puri et al., 2007; Eisinger et al., 2014). Network analysis revealed the metabolomic changes of tissues from MaS allografts were similar to biopsy tissues from NAFLD/NASH patients.

Six lipid metabolites with hazardous effects on post-transplant graft survival were enriched on the pathway of steroid biosynthesis significantly (P < 0.05, Figure 4 and Table 2).
| Metabolites | Structure ID | Category | Sub Class | FC/HR (95%CI) | P-value | Trend | Pathway | Function |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| PC(20:5/16:0) | C48H78NO8P | Lipids and lipid-like molecules | Glycero phospholipids | 0.45 | 0.041 | down | Linoleic acid/Glycerophospholipid metabolism | Known as phosphatidylcholine, consists of one chain of eicosapentaenoic acid at the C-1 position and one chain of palmitic acid at the C-2 position, involved in metabolism and signaling. |
| Linoleic acid | C18H32O2 | Lipids and lipid-like molecules | Fatty Acyls | 2.57 | 0.004 | up | Linoleic acid metabolism | Known as an essential fatty acid in human nutrition because it cannot be synthesized by humans. Used in the biosynthesis of prostaglandins and cell membranes. Associated with isovaleric acidemia, which is an inborn error of metabolism. |
| PE(20:4/22:6) | C47H74NO8P | Lipids and lipid-like molecules | Glycero phospholipids | 0.31 | 0.042 | down | Glycerophospholipid metabolism | Known as phosphatidylethanolamine |
| PE(20:5/18:2) | C43H72NO8P | Lipids and lipid-like molecules | Glycero phospholipids | 0.29 | 0.036 | down | Glycerophospholipid metabolism | Known as delta7-Avenasterol, as intermediate in the biosynthesis of steroids |
| LysoPC(20:3) | C29H54NO7P | Lipids and lipid-like molecules | Glycero phospholipids | 2.00 | 0.010 | up | Glycerophospholipid metabolism | Known as glycerophosphocholines in which the glycerol is esterified with a fatty acid at O-1 position, and linked at position 3 to a phosphocholine. |
| LysoPC(20:4) | C29H54NO7P | Lipids and lipid-like molecules | Glycero phospholipids | 2.21 | 0.008 | up | Glycerophospholipid metabolism | Known as lysosphospholipids which has a role in lipid signaling by acting on lysosphospholipid receptors. |
| LysoPC(22:4) | C30H54NO7P | Lipids and lipid-like molecules | Glycero phospholipids | 2.12 | 0.021 | up | Glycerophospholipid metabolism | Known as lysosphospholipids which has a role in lipid signaling by acting on lysosphospholipid receptors. |
| LysoPC(22:5) | C30H54NO7P | Lipids and lipid-like molecules | Glycero phospholipids | 2.03 | 0.028 | up | Glycerophospholipid metabolism | Known as lysosphospholipids which has a role in lipid signaling by acting on lysosphospholipid receptors. |
| Phosphocholine | C5H14NO4P | Organic nitrogen compounds | | | | | Glycerophospholipid metabolism | Known as choline phosphate, participates in a number of enzymatic reactions, can be converted into choline through its interaction with the enzyme phosphoethanolamine/phosphocholine phosphatase. |
| 1-Phosphatidyl-D-myo-inositol | C11H19NO13P | Lipids and lipid-like molecules | Glycero phospholipids | 0.40 | 0.046 | down | Glycerophospholipid metabolism | Unclear |
### TABLE 2 | Continued

| Metabolites         | Structure ID | Category | Statistics | Biological Involvement |
|---------------------|--------------|----------|------------|------------------------|
| Graft Survival      |              |          |            |                        |
| Calcidiol           | C27H44O2     | Lipids and lipid-like molecules | 1.67 (1.21-2.32) | Hazardous Steroid biosynthesis |
| Delta7-Avenasterol  | C29H48O      | Lipids and lipid-like molecules | 1.31 (1.03-1.66) | Hazardous Steroid biosynthesis |
| Presqualene diphosphate | C30H52O7P2 | Prenol lipids | 1.27 (1.08-1.64) | Hazardous Steroid biosynthesis |
| Episterol           | C28H48O      | Steroids and steroid derivatives | 1.08 (1.02-1.18) | Hazardous Steroid biosynthesis |
| 5-Dehydroepisterol  | C28H44O      | Steroids and steroid derivatives | 1.59 (1.05-2.66) | Hazardous Steroid biosynthesis |
| 4,4-Dimethylcholesta-8,14,24-trienol | C29H46O | Steroids and steroid derivatives | 1.98 (1.07-3.60) | Hazardous Steroid biosynthesis |
This is a novel enriched pathway associated with GF, which was never identified before. As derivatives of cholesterol, steroids were mainly regulated by liver. Steroid derangement might cause NAFLD and inflammation in liver (Charninatan et al., 2019). Consensus on benefits from early withdraw of steroid after LT also implied its potential toxicity for post-transplant prognosis.
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(Lerut et al., 2009; Kimura et al., 2018). Accordingly, our results showed concerns should also be raised on endogenous steroid dysregulation for better post-transplant prognosis.

Lipid played crucial role in determination of complications (EAD, PNF) (Cortes et al., 2014; Faitot et al., 2017) and prognosis (Xu et al., 2015; Tsai et al., 2018) after LT in previous studies. However, less study was focused on MaS related metabolites with simultaneous responsibility for GF occurrence. In our study, intersection was collected between metabolite clusters that related to MaS and GF. The intersected compounds were considered as “bridge” to connect MaS and GF. Finally, the pathway on glycerophospholipid metabolism was significant for MaS related GF (Figure 4).

As major component of cellular membrane, the glycerophospholipid includes collective species of derivative of glycerophosphoric acid (Hermansson et al., 2011). Disturbance on homeostasis of glycerophospholipid might mediate the progression of hepatic Steatosis via enhanced hepatic inflammation (Tanaka et al., 2012; Asimakopoulou et al., 2017). However, glycerophospholipid as connection from donor MaS and post-transplant GF wasn't reported before. In our study, the PC and PE as key nodes in glycerophospholipid metabolism were only two molecules with negative correlation to inferior prognosis of recipients after LT. And the standardized PC/PE ratio was decreased from 1.5 to 0.76 ($P < 0.05$, Figure 4). Our results indicated the decreased PC/PE ratio and its indicative loss of membrane integrity and severer hepatic inflammation (Li et al., 2006) might be involved in the lethal pathogenesis. The allograft quality might be improved by PC supplement, which was used for NASH patients (Buang et al., 2005).

Discrete molecules were mainly belong to lipids and organic acids classes (Table 3) by lower interaction with each other (Figure 4). Organs with extremely higher external substances like Dexamethasone or N-Malonyltryptophan (top 10%) had higher rates of graft failure (62.5%). In addition, all organs with extremely high volume of glucocorticoid residue (top 10%) were grafts from DCD donors. Previous study found inhibitory effects of dexamethasone on initial post-transplant inflammation (Tanaka et al., 2012; Asimakopoulou et al., 2017). Results in our study might refer to long-term prognosis in good accordance. Developed machine perfusion effectively preserved more organs with normal function and expanded the use of suboptimal organs (Nasralla et al., 2018). Results in our study might provide targets for further MP treatments for improvement of graft quality.

Metabonomic analysis of MaS allografts (Supplementary Figure S2) and insufficient follow-up might cause underestimation on MaS related mortality. Medication on donor and recipient per se might affect the global metabolome as confounder on association between donor MaS and GF. Otherwise, metabolome level was changed almost 1000 LT cases with IPF occurrence, which guaranteed similar post-transplant liver function for comparability on effects of metabolomic covariates on long-term prognosis. Otherwise, we found the donor MaS exerted its positive effects on GF in maximum by combination with IPF (Liu et al., 2020). And discrimination was also confirmed on metabolome of allografts by MaS status. Selective cases with IPF occurrence might help for better clarification of MaS related mechanism and its connection to GF.

As we known, LT is a systematic engineering with complicated interaction on recipient, donor, graft and surgical factors (Burra et al., 2016). Previous studies tried to build the connection between metabolites and short-term prognosis, but less was referred to long-term outcomes (Cortes et al., 2014; Xu et al., 2015; Faitot et al., 2017; Tsai et al., 2018). We firstly found the algorithm with integration of metabonomic and peri-operative factors was capable to monitor the long-term prognosis in good accordance. And new-built extended cohort is now in preparation.
| Metabolites         | Structure           | Identification       | Category                              | Biological Involvement                                                                                                                                                                                                                                                                                                                                 |
|---------------------|---------------------|----------------------|---------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                     |                     |                      | Super class                           | Main class                                           | Sub class                                      | Function                                                                                     |                                                                                                                                                                                                                                           |
| (E)-Avenanthramide D | C16H13NO4           | HMDB0038943          | Phenylpropanoids and polyketides       | Cinnamic acids and derivatives                      | Hydroxy cinnamic acids and derivatives         | Belongs to the avenanthramides. Detected outside of the human body, in, cereals and cereal products and oats, which make (e)-avenanthramide D as potential biomarker for the consumption of these foods. Also known as uridine 3'-phosphoric acid or 3'-uridylic acid, belongs to the ribonucleoside 3'-phosphates. Uridine 3'-monophosphate exists in all living organisms, ranging from bacteria to humans. Known as a basic amino. Cells synthesize it from citrulline, aspartic acid and use it as a precursor for arginine in the urea cycle or Citrulline-NO cycle. As a precursor to fumarate in the citric acid cycle via argininosuccinate lyase. Only found in individuals have used or taken this drug. It is anti-inflammatory 9-fluoro-glucocorticoid as a glucocorticoid agonist, used for its anti-inflammatory or immunosuppressive properties. Also able to penetrate the CNS, used to manage cerebral edema. Complex between Dexamethasone and cytoplasmic glucocorticoid receptors binds to DNA elements results in a modification of transcription and protein synthesis in order to achieve inhibition of leukocyte infiltration at the site of inflammation, interference in the function of mediators of inflammatory response, suppression of humoral immune responses, and reduction in edema or scar tissue. The anti-inflammatory actions of dexamethasone are thought to involve phospholipase A2 inhibitory proteins, lipocortins, which control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes. |
| 3'-UMP              | C9H13N2O9P          | C01368 HMDB000282    | Nucleosides, nucleotides, and analogs   | Ribonucleoside 3'-phosphates                       | Unclassified                                    | Belongs to the class of organic compounds, known as fatty acyl glycosides of mono- and disaccharides. Belongs to the class of organic compounds known as n-acyl-alpha amino acids. N-acyl-alpha amino acids are compounds containing an alpha amino acid which bears an acyl group at its terminal nitrogen atom. Detected outside of the human body in foods like tomato, herbs and spices, opium poppies pulses, which make it as potential biomarker for the consumption of these substance. |                                                                                                                                                                                                                                           |
| Argininosuccinic acid | C10H18N4O6          | C03406 HMDB000052    | Organic acids and derivatives          | Carboxylic acids and derivatives                    | Amino acids, peptides, and analogs             |                                                                                                                                                                                                                                           |
| Dexamethasone       | C22H29FO5           | C15643 HMDB0015364   | Lipids and lipid-like molecules        | Steroids and steroid derivatives                   | Hydroxysteroids                                 |                                                                                                                                                                                                                                           |
| Eriojaposide B      | C25H40O11           | HMDB0038029          | Lipids and lipid-like molecules        | Fatty Acyls                                         | Fatty acyl glycosides                          | Belongs to the class of organic compounds, known as fatty acyl glycosides of mono- and disaccharides. Belongs to the class of organic compounds known as n-acyl-alpha amino acids. N-acyl-alpha amino acids are compounds containing an alpha amino acid which bears an acyl group at its terminal nitrogen atom. Detected outside of the human body in foods like tomato, herbs and spices, opium poppies pulses, which make it as potential biomarker for the consumption of these substance. |                                                                                                                                                                                                                                           |
| N-Malonyltryptophan | C14H14N2O5          | HMDB0 039500         | Organic acids and derivatives          | Carboxylic acids and derivatives                    | Amino acids, peptides, and analogs             |                                                                                                                                                                                                                                           |

(Continued)
**TABLE 3 | Continued**

| Metabolites          | Structure | Identification | Category               | Biological Involvement                                                                 |
|----------------------|-----------|----------------|------------------------|----------------------------------------------------------------------------------------|
| Non-anoylcarnitine   | C16H31NO4 | HMDB00 13288   | Lipids and lipid-like  | Classified as a member of the acyl carnitines, practically insoluble in water and weak  |
|                      |           | LMFA07 070082  | molecules              | acidic. Considered as a fatty ester lipid molecule, which can be found in blood and     |
|                      |           |                | Fatty Acyls            | urine. Primarily located in the extracellular space and near the membrane.              |
| PA(15:0/18:4)        | C36H63O8P | HMDB01 14818   | Glycerophospholipids    | As glycerophospholipid in which a phosphate moiety occupies a glycerol substitution site.|
|                      |           | LMGP10 010146  | Glycerophospholipids    | PA(15:0/18:4(Z,9Z,12Z,15Z)) consists of one chain of pentadecanoic acid at the C-1 position and one chain of stearidonic acid at the C-2 position. Phosphatidic acids are quite rare but are extremely important as intermediates in the biosynthesis of triacylglycerols and phospholipids. |
| PC(18:4/16:0)        | C42H76NO8P| C00157 HMDB00 08232 | Glycerophospholipids    | Known as glycerophospholipid in which a phosphorylcholine moiety occupies a glycerol substitution site. Consists of one chain of stearidonic acid at the C-1 position and one chain of palmitic acid at the C-2 position. Ubiquitous in nature as key components of the lipid bilayer of cells, also being involved in metabolism and signaling. Stearidonic acid moiety is derived from seed oils, while the palmitic acid moiety is derived from fish oils, milk fats, vegetable oils and animal fats. |
|                      |           | LMGP01 011706  | Glycerophospholipids    | Known as glycerophospholipid in which a phosphorylcholine moiety occupies a glycerol substitution site. Consists of one chain of stearidonic acid at the C-1 position and one chain of palmitic acid at the C-2 position. Ubiquitous in nature as key components of the lipid bilayer of cells, also being involved in metabolism and signaling. Stearidonic acid moiety is derived from seed oils, while the palmitic acid moiety is derived from fish oils, milk fats, vegetable oils and animal fats. |
| Threoninyl-Proline    | C9H16N2O4 | HMDB0 029069   | Organic acids and      | Known as dipeptide composed of the threonine and proline as incomplete breakdown product of protein digestion or protein catabolism. Dipeptides are known to have physiological or cell-signaling effects although most are simply short-lived intermediates on the way to specific amino acid degradation pathways following further proteolysis. |
|                      |           |                | derivatives            |                                                                                         |
FIGURE 5 | Dose-response effects of continuous covariates on graft failure via GLS and RCS models. (A) Dose-response effects of argininosuccinic acid (HMDB0000052) on GF; (B) Dose-response effect of PC(18:4/16:0) (HMDB0008232) on GF; (C) Dose-response effect of non-anoylcarnitine (HMDB0013288) on GF; (D) Dose-response effect of dexamethasone (HMDB0015364) on GF; (E) Dose-response effect of threoninyl-proline (HMDB0029069) on GF; (F) Dose-response effect of eriojaposide B (HMDB0038029) on GF; (G) Dose-response effect of (E)-Avenanthramide D (HMDB0038943) on GF; (H) Dose-response effect of N-Malonyltryptophan (HMDB0039500) on GF; (I) Dose-response effect of 3′-UMP (HMDB0060282) on GF; (J) Dose-response effect of PA(15:0/18:4) (HMDB0014918) on GF; (K) Dose-response effect of blood loss per 1000 ml on GF; (L) Dose-response effect of pre-transplant child-pugh score of recipients on GF; (M) Dose-response effect of recipient height (cm) on GF; Linearity on effects of covariates on post-transplant GF was estimated via GLS and RCS models, respectively. GF, graft failure; GLS, generalized least squares; LT, liver transplantation; RCS, restricted cubic splines.
CONCLUSION

In conclusion, the metabonomic features can be distinguished by allograft MaS status in patients with IPF. Both endogenous steroid biosynthesis or exogenous glucocorticoid residue were responsible for post-transplant GF occurrence. Dysfunction on pathway of glycerophospholipid metabolism was the link to connect donor MaS and final GF. Decreased PC and PE were culprits to exert fatal effects of MaS on organ failure. Integrative prognostic model with combined metabonomic and peri-operative clinical data might help for monitoring the long-term GS after LT. This study uncovered the molecular pathogenic mechanism of MaS on GF based on omics data, provided accurate targets for machine perfusion which might help to improve the graft quality and expand the donor pool.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/Supplementary Material. Original anonymous omics data is available on request from the corresponding author at liuzhengtao@zju.edu.cn.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The First Affiliated Hospital of Zhejiang University and Shulan Hospital Affiliated to Zhejiang Shuren University Shulan International Medical College, respectively. The patients/participants provided their written informed consent to participate in this study.

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AUTHOR CONTRIBUTIONS

ZL and SZ conceived and designed the study. WW, JX, LZ, and JQ extracted the information. ZL, HZ, SQ, SW, and JY analyzed the data. ZL and LG wrote the manuscript. FZ, SY, HX, LinZ, and SZ reviewed the manuscript. All authors approved the final manuscript for submission.

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SUPPLEMENTARY MATERIAL

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FIGURE S1 | Study flow diagram on impact of metabonomic analysis on post-transplant outcomes.

FIGURE S2 | Trend on utilization of steatotic allografts followed with operational period and selection procedure in all LT cases. (A) Prevalence of MaS and MiS donor utilization in each year. (B) Selection procedure in all LT cases.
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