Altered platelet lipidome in bleeding patients with unexplained platelet function defects

Bianca de Jonckheere,[1,2*] Dino Mehic,[3,4*] Dominik Kopczynski,[1] Anita Pirabe,[3] Waltraud Schrottmaier,[3] Anna Schmuckenschlager,[3] Cristina Coman,[1] Tim Dreier,[4] Helmuth Haslacher,[5] Alexander Tolios,[6] Cihan Ay,[4] Ingrid Pabinger,[4] Johanna Gebhart,[4] Robert Ahrends[1#] and Alice Assinger[3#]

[1]Institute of Analytical Chemistry, University of Vienna; [2]Vienna Doctoral School in Chemistry, University of Vienna; [3]Institute of Vascular Biology and Thrombosis Research, Center of Physiology and Pharmacology, Medical University of Vienna; [4]Clinical Division of Hematology and Hemostaseology, Department of Medicine I, Medical University of Vienna; [5]Department of Laboratory Medicine, Medical University of Vienna and [6]Department of Transfusion Medicine and Cell Therapy, Medical University of Vienna, Vienna, Austria

BdJ and DM contributed equally as first authors.

#RA and AA contributed equally as senior authors.

Correspondence: R. Ahrends
robert.ahrends@univie.ac.at

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Bianca de Jonckheere[1,2], Dino Mehic[3,4], Dominik Kopczynski[1], Anita Pirabe[3], Waltraud Schrottmaier[3], Anna Schmuckeschlager[3], Cristina Coman[1], Tim Dreier[4], Helmuth Haslacher[5], Alexander Tolios[6], Cihan Ay[4], Ingrid Pabinger[4], Johanna Gebhart[4], Robert Ahrends[1], Alice Assinger[3]

1 Institute of Analytical Chemistry, University of Vienna, Vienna, Austria; 2 Vienna Doctoral School in Chemistry, University of Vienna, Vienna, Austria; 3 Institute of Vascular Biology and Thrombosis Research, Centre of Physiology and Pharmacology, Medical University of Vienna, Vienna, Austria; 4 Clinical Division of Hematology and Hemostaseology, Department of Medicine I, Medical University of Vienna, Vienna, Austria; 5 Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria; 6 Department of Transfusion Medicine and Cell Therapy, Medical University of Vienna, Vienna, Austria

B.D.J and D.M. contributed equally and share the first authorship.
R.A. and A.A. contributed equally and share the last authorship.

Correspondence: Robert Ahrends, Department of Analytical Chemistry, University of Vienna, 1090 Vienna, Austria; e-mail: robert.ahrends@univie.ac.at

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- Supplemental Methods
- Supplemental Figure 1-6
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- ILS Reporting Summary – Separation Workflow

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- Supplemental Table 1-4

Supplemental Methods

Study inclusion and blood sampling

Upon study inclusion[1], patients and controls underwent a structured interview on the general medical and bleeding history using a standardized questionnaire, and blood sampling. All study participants were instructed to withhold NSAIDs and other medications known to affect platelet function for at least 10 days before the visit, to have had no surgery within the preceding 6 weeks, and no acute infections in the prior 2 weeks.

Flow cytometry

To obtain platelet-rich plasma (PRP), whole blood anticoagulated with 3.2% sodium citrate was centrifuged for 20 min at 120 g at room temperature without brake. PRP was then activated for 15 minutes using one of the following agonists: the PAR-1 receptor agonist TRAP-6 (10 µM; AnaSpec), adenosine diphosphate (ADP, 10 µM; Sigma-Aldrich), or the GPVI agonist cross-linked collagen-related peptide (CRP-XL, 12 ng/mL; CambCol Laboratories). Platelet activation was assessed by measuring surface P-selectin expression with anti-CD62P BV605 (1:100, clone AK4; BioLegend) and activated GPIIb/IIIa binding with PAC-1-FITC (1:40; BioLegend). After 20 minutes of staining, platelets were fixed with 1% formaldehyde and analyzed using a CytoFLEX flow cytometer with CytExpert 2.4 software (both Beckman Coulter).

Light transmission aggregometry

Platelet-poor plasma (PPP) was generated by centrifuging PRP in the presence of prostacyclin (0.1 µg/mL) for 1.5 min at 1000 g. PRP was stirred in an optical 4-channel aggregometer (PAP-8, MöLab) at 37°C for 1 min, thereafter the indicated agonists, ADP (14 µM, Sigma) and PAR-1 receptor agonist TRAP-6 (15 µM, AnaSpec), were added and changes in light transmission recorded over 10 min. Light transmission was determined between 0% (PRP) and 100% (PPP). Results are expressed as the area under the aggregometry curve (AUC), measured as arbitrary units/min (AU/min).

Platelet isolation for lipidomics analysis

To 9 mL of whole blood in an acid-citrate dextrose (ACD) tube, 1 mL of ACD stock (22 g/L citric acid, trisodium salt, dihydrate; 7.30 g/L citric acid, anhydrous and 24.50 g/L D-(+) glucose; pH:5.5, Sigma) was added, and the mixture was centrifuged at 120 g for 20 min at room temperature without brake. Then, 1 mL aliquots of platelet-rich plasma (PRP) were transferred to new tubes, and diluted with 1 mL of Thyrode-HEPES (TH) buffer (140 mM NaCl, 3mM KCl, 1mM $MgCl_2$, 16.63 mM $NaHCO_3$, 10 mM HEPES; pH7.4) supplemented with 15%

ACD before centrifuging at 1000 g for 1.5 min. The platelet pellets were washed in 1 mL of TH buffer with 15% ACD and centrifuged again at 1000 g for 1.5 min. The platelet pellets were resuspended and pooled in 400-800 µL TH buffer. The suspension was diluted 1:10 or 1:20, and the platelet count was measured immediately on a blood counter (Sysmex). Basal platelets were frozen immediately at -80°C. For activation experiments, platelets were stimulated with TRAP-6 (20 µM), incubated for 5 min, and then frozen immediately at -80°C. For the initial experiment (Figure 1-3), platelet samples were collected over three months and stored for a maximum of six months before lipid extraction and analysis. Platelet samples from the follow-up investigations (Figure 4-6) were collected and measured within one week. Per sample, 5×10^7 (initial experiment) or 10^8 (follow-up investigation) platelets were transferred into clean polypropylene Eppendorf tubes prior to lipid extraction.

Lipid standards

Mouse SPLASH Lipidomix ® Mass Spec Standard (Avanti Polar Lipids 330710X-1EA) consisting of phosphatidylcholine (PC) 15:0-18:1(d7), phosphatidylethanolamine (PE) 15:0-18:1(d7), phosphatidylserine (PS) 15:0-18:1(d7), phosphatidylglycerol (PG) 15:0-18:1(d7), phosphatidylinositol (PI) 15:0-18:1(d7), phosphatidic acid (PA) 15:0-18:1(d7), lyso-PC 18:1(d7), lyso-PE 18:1(d7), cholesterol ester (SE) 18:1(d7), phosphatidylcholine-ether (PC O-a) 18:0-18:1(d9), phosphatidylethanolamine-ether(PE O-a) 18:0-18:1(d9), diacylglycerol (DG) 15:0-18:1(d7), triacylglycerol 15:0-18:1(d7)-15:0, and sphingomyelin d18:1-18:1(d9). Cholesterol-d7 (Avanti Polar Lipids 700041P) and CAR 12:0-d9 (Sigma 870326P) were dissolved in 1:1 MeOH/$CHCL_3$ (v/v).

Lipid extraction

Platelet samples were processed using the SIMPLEX extraction procedure described elsewhere[2]. Briefly, lipid standards were spiked into sample suspensions before adding 225 µl of cold ULC/MS grade methanol (MeOH, -20°C) (Biosolve 13684102). Samples were then homogenized by 20 s of vortex mixing and 5 min sonication in a 4°C water bath. Then, 750 µl of cold methyl tert-butyl ether (MTBE,-20°C) (VWR 34875-1L) was added, and samples were incubated at 4°C for 1 h at 950 rpm. To achieve phase partitioning, 188 µl cold ULC/MS-grade water (4°C) (Biosolve 23214102) was added, and samples were incubated for 5 min on ice, followed by 10 min centrifugation at 10.000 g. The upper lipid-containing phase was collected and dried under nitrogen flow. Lipids were then reconstituted in 100 µl isopropanol (Sigma Aldrich 1010402500)/MeOH/chloroform (Sigma Aldrich 650498-1L) (4:2:1, v/v/v) containing 7.5 mM ammonium acetate (Merck 73594-25G-F). Lipid samples were stored at -80°C until further analysis. To the remaining sample, 527 µl cold MeOH was added to precipitate proteins for 2 h at -20°C. Proteins were pelleted via 30 min centrifugation at 4 °C, 12000 g, dried,

resuspended in SDS lysis buffer (1% SDS, 50 mM Tris, 150 mM NaCl, pH 7.8), and stored at -80°C. Protein quantification was performed using the Pierce™ BCA protein assay kit (Thermo Scientific).

Shotgun lipidomics

Lipids were measured with direct infusion using a TriVersa Nanomate ion source (Advion Biosciences) coupled to an Orbitrap mass spectrometer (Q Exactive HF or Exploris 240, Thermo Fisher Scientific). A 12 µl sample was delivered over 14 min with a backpressure of 0.95 psi. After 6 min, polarity switching from +1.30 kV to -1.30 kV was applied to acquire mass spectra in both positive and negative ion mode in a single run. Full MS spectra were acquired over a mass range of 350 – 1200 m/z in both polarities, with a resolution of 240.000, an AGC target of 1e6, and a maximum injection time (IT) of 105 ms. MS1 acquisition was followed by data-independent acquisition (DIA) of precursor masses at an interval of 1.001 Da. The precursor isolation window was 1 Da, and normalized collision energy (nCE) was 21 % and 26 % for positive and negative mode, respectively. MS2 spectra were acquired with a resolution of 60.000, an AGC target of 1e5, and a maximum IT of 105 ms, covering a 150-1500 m/z range.

Lipid Identification and quantification

All spectra from shotgun experiments were converted to centroid mode using MSConvert (v3.0.20186-dd907d757) and analyzed using LipidXplorer (v1.2.8.1)[3] under the following parameters: mass tolerance of 10 ppm with an intensity threshold of 8e3 for MS1 and a mass tolerance of 20 ppm with an intensity threshold of 4e3 for MS2. Minimum occupation and frequency filters were set to zero. Lipid identification was performed using modified molecular fragmentation query language (MFQL) queries[4] to match precursor and fragment ions for accurate lipid species identification. The quantification of GLs (DG, TG), SM, STs (SE, ST), and fatty acyls (CAR) was conducted in positive ion mode. GPs (CL, LPI, LPG, LPC, LPE, LPA, LPS, PA, PG, PC, PCO, PE, PEO, PI, PS) were quantified in negative ion mode. All signal intensities were normalized to the corresponding deuterated internal standard (Mouse SPLASH® LIPIDOMIX® Mass Spec Standard) and their respective pmol concentrations. TGs, CLs, and SMs were quantified based on precursor intensities. Relative lipid concentrations were calculated as mol% of the sample.

Identification of shared features by LipidSpace

LipidSpace[5] was used for the structural comparison of lipidomes within each sample. The structural similarity of all lipid pairs builds a unique profile (lipid space) for each sample. By combining these spaces using the Hausdorff distance, a dendrogram is created, visualizing the relations of all samples to each other. For the selection of significant lipids (features) based on the separation of the cohorts, a forward feature selection approach was utilized, as described previously[5]. Here, the target variable for each sample is the summed z-scores of all lipid concentrations provided by the feature selection. A regular principal component analysis (PCA) was performed on the concentration matrix of the selected lipids. To determine the accuracy, a modified Kolmogorov-Smirnov method was used to find the biggest separation spot within the overlap of the two cohort distributions. Below that spot, all control z-scores are considered correctly annotated, while the patient's z-scores are incorrectly annotated. Above that spot, the annotations are vice versa. The accuracy is the ratio of correct annotations to all samples.

Plasma acylcarnitine analysis via targeted reversed-phase liquid chromatography (RPLC)-MS/MS

Citrated whole blood was centrifuged at 1000 g for 10 min at 4°C, followed by a second centrifugation of the supernatant at 10.000 g for 10 min at 4°C[6] to yield purified plasma. Per sample, 20 µl plasma was extracted using the Bligh & Dyer lipid extraction[7] with few modifications. In brief, CAR 12:0-d9 internal standard was added to samples prior to extraction, and blanks and a pooled plasma sample were processed in parallel as quality control. Cold 600 µl chloroform/MeOH (1:2, v/v) and 140 µl ULC/MS-grade water was added, and samples were incubated for 1 h at 950 rpm at 4°C. Afterwards, chloroform and water were added to a final solvent ratio of chloroform/MeOH/water of 2:2:1.8 (v/v/v). Samples were further centrifuged for 10 min at 10.000 g, 4°C, before transferring the lower organic phase to a new Eppendorf tube. Samples were dried under N_2 flow and resolubilized in 1-butanol (Sigma Aldrich 1019881000)/isopropanol/water (8:23:69, v/v/v) + 5 mM phosphoric acid for subsequent LC-MS analysis.

Analysis of acylcarnitines was adapted from Zhang et al.[8] into an optimized targeted method previously described by Peng et al.[9]. RPLC was performed using a Vanquish Flex UHPLC system (Thermo Fisher Scientific, Germering, Germany) equipped with an Ascentis Express C18 column (150 mm × 2.1 mm, 2.7 µm, Supelco) fitted with a guard cartridge (50 mm × 2.1 mm, 2.7 µm, Supelco), at 60 °C oven temperature. The mobile phase consisted of solvent A (acetonitrile/water, 3:2, v/v) and solvent B (isopropanol/acetonitrile, 9:1, v/v), both supplemented with 0.1% formic acid, 10 mM ammonium formate, and 5 µM phosphoric acid.

Chromatographic separation was carried out at a flow rate of 0.5 ml/min using a 25-min gradient: 0–2 min, 30% B, 2–3 min, increase to 56.1% B, 3–4 min, to 58.3% B, 4–5.5 min, to 60.2% B, 5.5–7 min, to 60.6% B, 7–8.5 min, to 62.3% B, 8.5–10 min, to 64% B, 10–11.5 min, to 64.5% B, 11.5–13 min, to 66.2% B, 13–14.5 min, to 66.9% B, 14.5–15 min, to 100% B, 15–19 min, held at 100% B, 19 min, drop to 5% B, 19–22 min, held at 5% B, 22–25 min, re-equilibrated at 30% B. The injector needle was rinsed with 30% B containing 0.1% phosphoric acid, and 5 µl per sample was injected.

The LC system was coupled to a QTRAP 6500+ mass spectrometer (Applied Biosystems Sciex, Darmstadt, Germany) equipped with a Turbo V ESI source. Data acquisition was performed in positive ion mode with the following source parameters: curtain gas, 30 AU; source temperature, 250 °C; ion source gas 1, 40 AU; ion source gas 2, 65 AU; collision gas, medium; ion spray voltage, +5500 V; declustering potential, +145 V; entrance potential, +10 V; and exit potential, +13 V. For the scheduled SRM, Q1 and Q3 were set to unit resolution and the cycle time was set to 0.5 s. Data was acquired using the Analyst software version 1.7.2 (Applied Biosystems Sciex, Concord, ON, Canada) and peaks were manually integrated in Skyline (v 24.1.0.414). Peak areas were quantified relative to the internal standard and normalized to the extracted plasma volume to calculate lipid species' abundance.

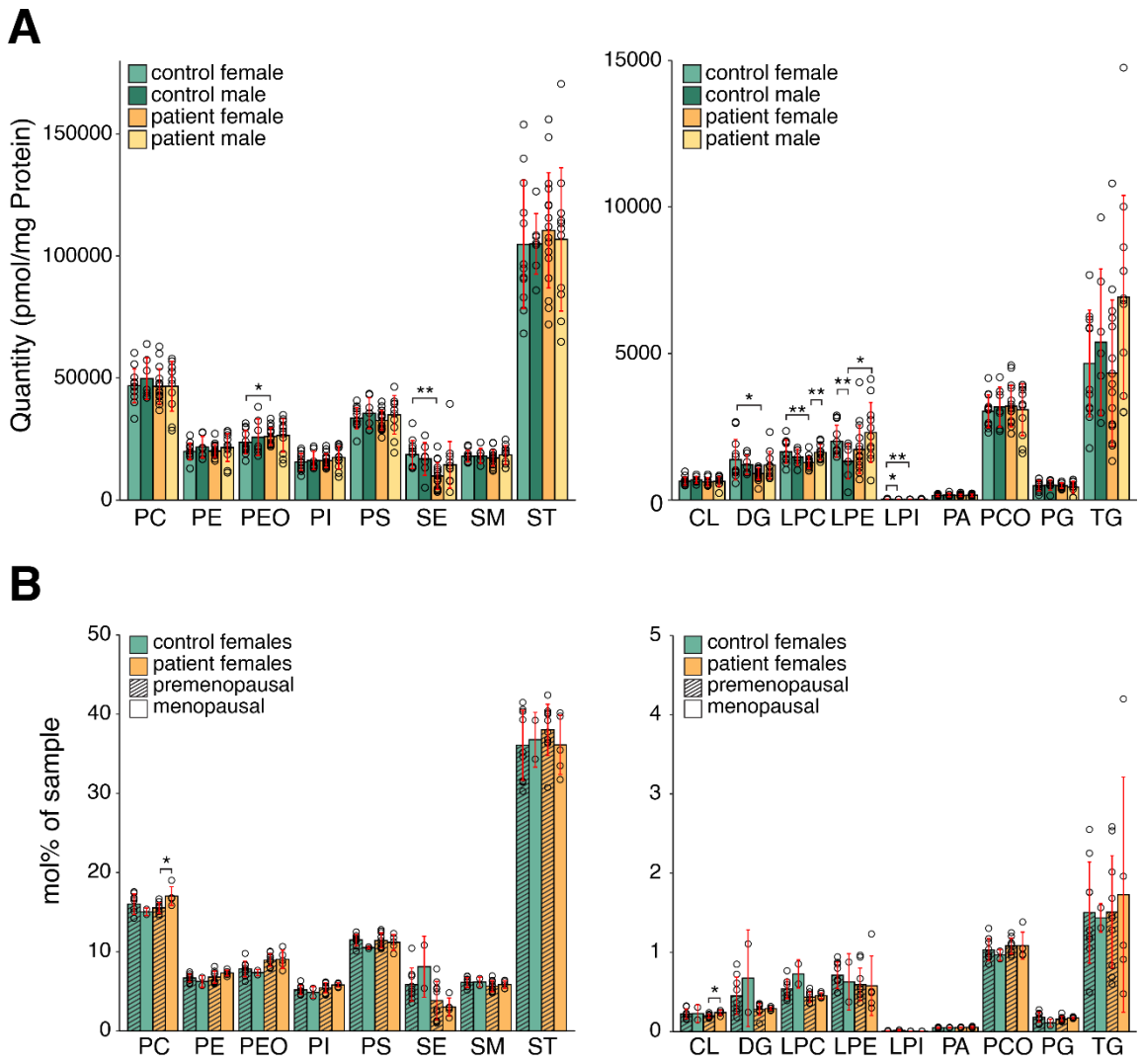
Phospholipase C (PLC) activity assay

PLC activity was determined in washed platelets by a colorimetric PLC activity assay (BioCat, Heidelberg, Germany) according to the instructions of the manufacturer.

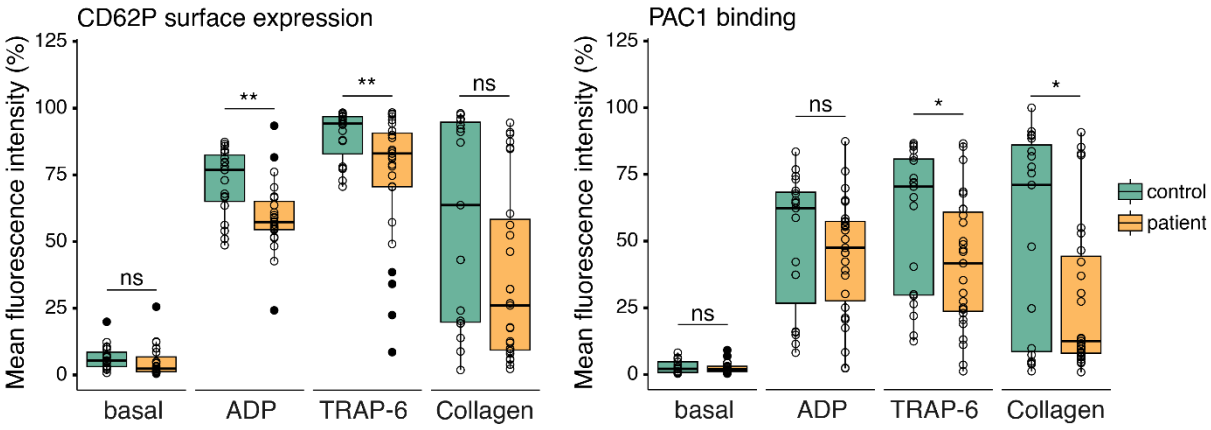
References for Supplemental Methods

1. Mehic D, Gebhart J, Pabinger I. Bleeding Disorder of Unknown Cause: A Diagnosis of Exclusion. Hamostaseologie. 2024;44(4):287-297.
2. Coman C, Solari FA, Hentschel A, Sickmann A, Zahedi RP, Ahrends R. Simultaneous Metabolite, Protein, Lipid Extraction (SIMPLEX): A Combinatorial Multimolecular Omics Approach for Systems Biology. Mol Cell Proteomics. 2016;15(4):1453-1466.
3. Herzog R, Schuhmann K, Schwudke D, et al. LipidXplorer: a software for consensual cross-platform lipidomics. PLoS One. 2012;7(1):e29851.
4. Herzog R, Schwudke D, Schuhmann K, et al. A novel informatics concept for high-throughput shotgun lipidomics based on the molecular fragmentation query language. Genome Biol. 2011;12(1):R8.
5. Kopczynski D, Hoffmann N, Troppmair N, et al. LipidSpace: Simple Exploration, Reanalysis, and Quality Control of Large-Scale Lipidomics Studies. Analytical Chemistry. 2023;95(41):15236-15244.
6. Mussbacher M, Schrottmaier WC, Salzmann M, et al. Optimized plasma preparation is essential to monitor platelet-stored molecules in humans. PLOS ONE. 2017;12(12):e0188921.
7. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol. 1959;37(8):911-917.
8. Zhang J, Chen Q, Zhang L, et al. Simultaneously quantifying hundreds of acylcarnitines in multiple biological matrices within ten minutes using ultrahigh-performance liquid-chromatography and tandem mass spectrometry. Journal of Pharmaceutical Analysis. 2024;14(1):140-148.
9. Peng B, Weintraub ST, Coman C, et al. A Comprehensive High-Resolution Targeted Workflow for the Deep Profiling of Sphingolipids. Analytical Chemistry. 2017;89(22):12480-12487.

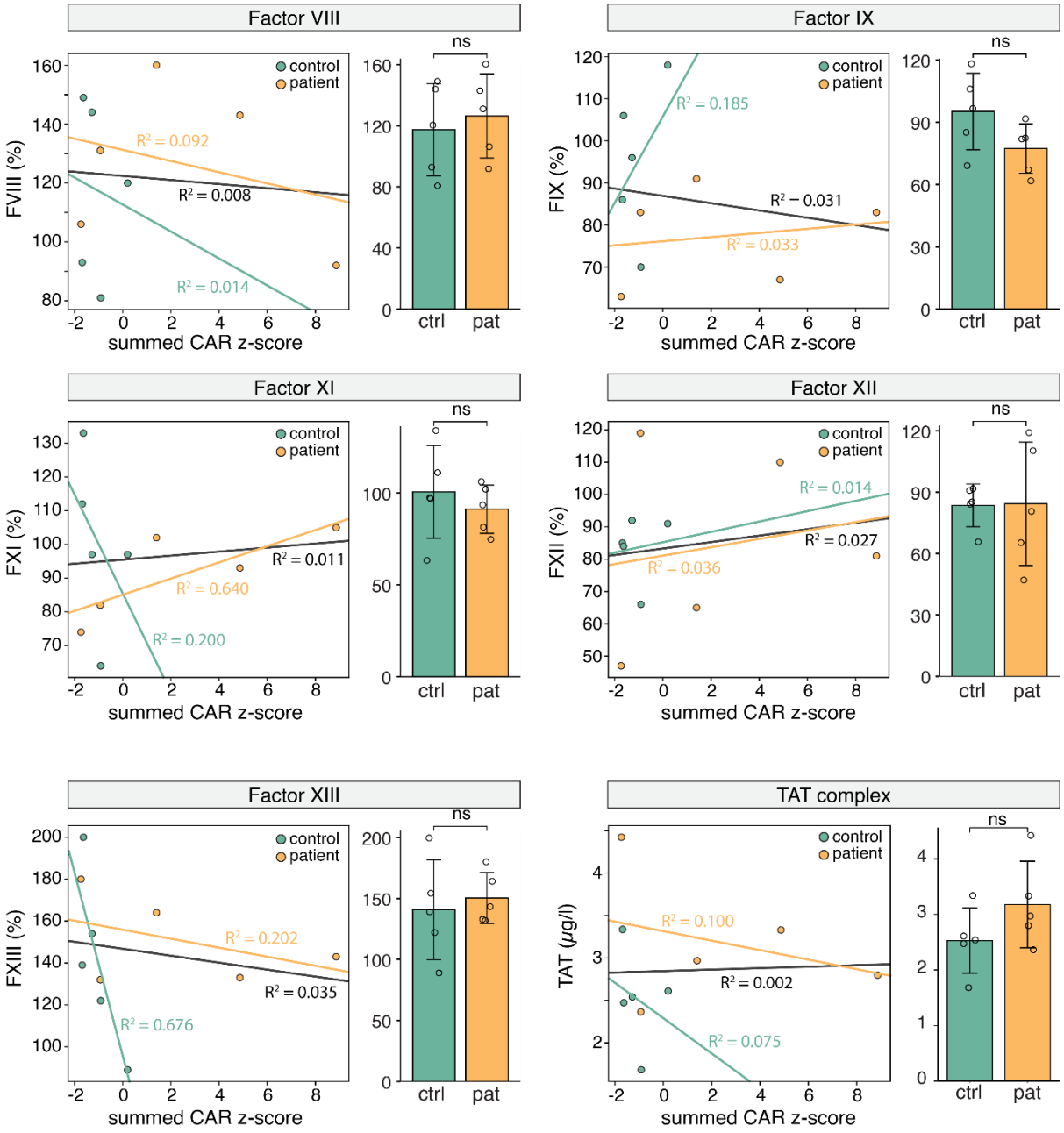
Supplemental Figures



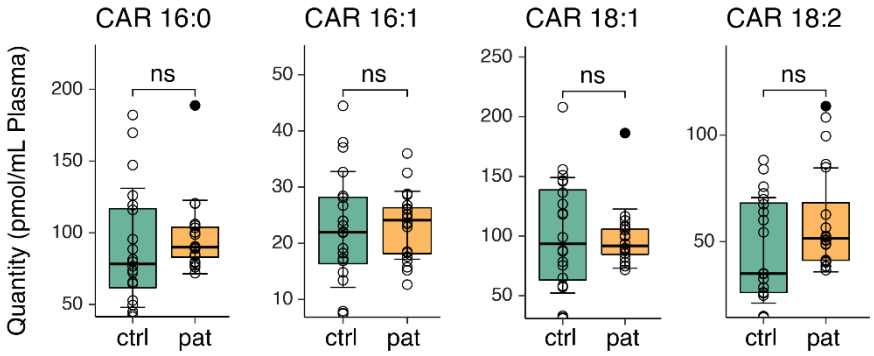
Supplemental Figure 1. Platelet lipid class distribution in control and patient groups. (A) Absolute quantification of high (left) and low (right) abundant lipid classes. A two-sided t-test was performed to statistically compare the control subgroups and sex-matched groups. Comparison of patient subgroups was not considered. Each data point represents one donor (control female (n=12), control male (n=7), patient female (n=17), patient male (n=10)). (B) Mean relative abundance of high- (left) and low-abundance (right) lipid classes from female study participants, separated by menopausal status. A Welch's t-test was performed to compare groups with unequal sample sizes. Each data point represents one donor (healthy females: premenopausal (n=9), menopausal (n=2); patient females: premenopausal (n=10), menopausal (n=5); not available: (n=2)). PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEO, PE-ether; PI, phosphatidylinositol; PS, phosphatidylserine; SE, cholesterol ester; SM, sphingomyelin; ST, cholesterol; CL, cardiolipin; DG, diacylglycerol; LPC, lyso-PC; LPE, lyso-PE; LPI, lyso-PI; PA, phosphatidic acid; PCO, PC-ether; PG, phosphatidylglycerol; TG, triacylglycerol. ($p \leq 0.05$ (*), $p \leq 0.01$ (**)). Associated to Figure 1.



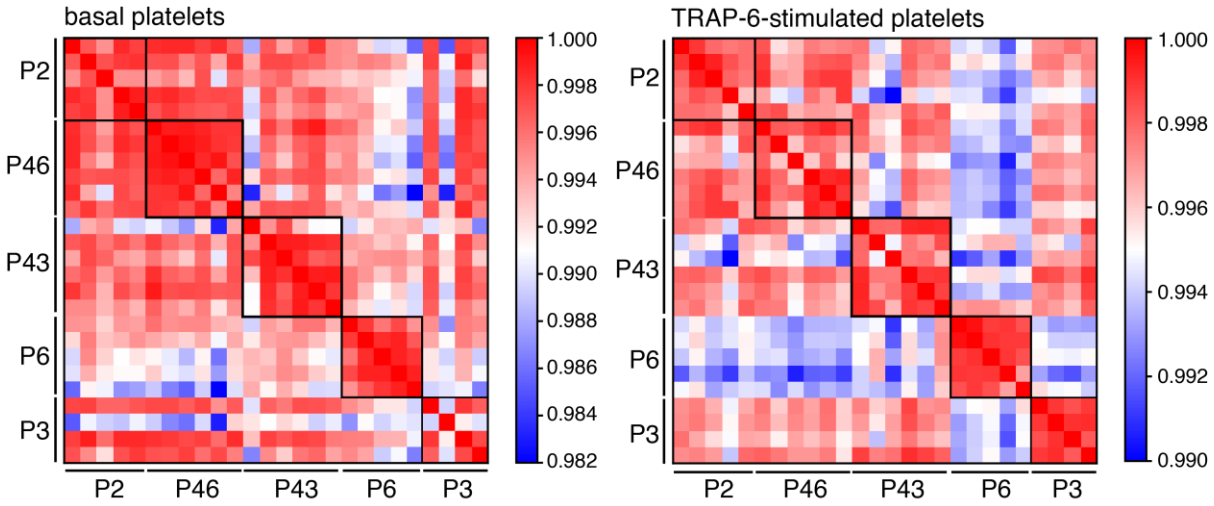
Supplemental Figure 2. Flow cytometry staining of CD62P expression and PAC1 binding after platelet activation. Mean fluorescence intensity (MFI) was measured in platelets under basal and activated conditions with different stimuli. MFI was calculated as percentage (%). ($p \leq 0.05$ (*), $p \leq 0.01$ (**))



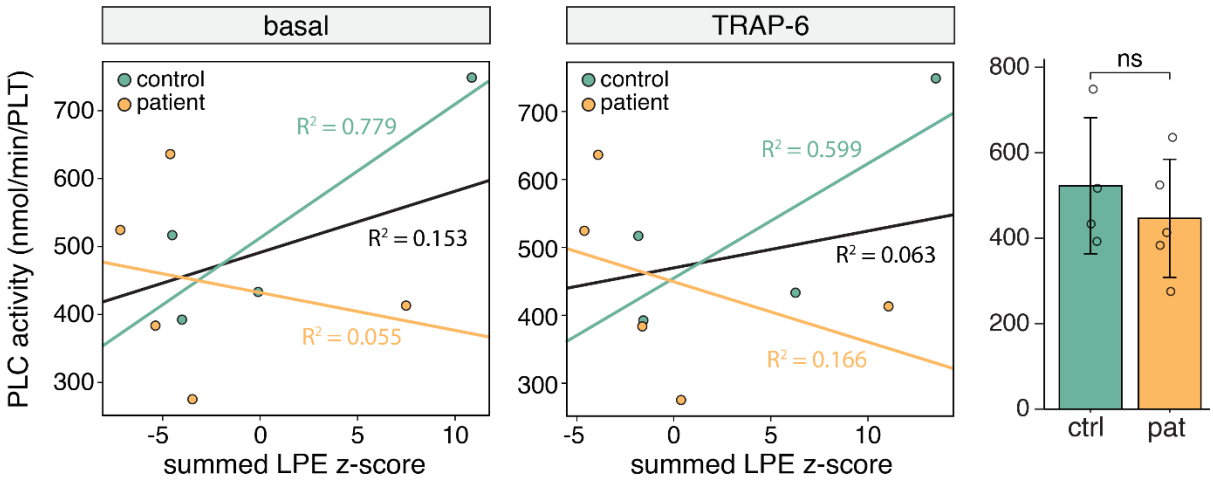
Supplemental Figure 3. Correlation analysis of coagulation parameters with acylcarnitine levels in PFD patients and healthy controls. Scatter plots display the correlation between the summed z-score of significantly upregulated CAR species (CAR 16:0, CAR 16:1, CAR 18:1 and CAR 18:2) and the activity levels (expressed as % relative to reference pooled plasma) of coagulation factors VIII, IX, XI, XII, XIII, and the thrombin-antithrombin (TAT) complex. Linear regression lines and R^2 values are shown separately for patients (orange) and controls (green), black indicates combined groups. Corresponding bar graphs indicate no statistically significant differences in coagulation parameters between groups. Data represent individual donors. (ns, not significant)



Supplemental Figure 4. Plasma levels of long-chain acylcarnitines in patients with PFD and healthy controls. Boxplots show acylcarnitine (CAR) pmol concentrations per mL plasma from follow-up study participants. (ns, not significant)



Supplemental Figure 5. Correlation matrix of all 26 platelet lipidomes from 5 PFD patients in basal or TRAP6-activated conditions. Correlation is based on Pearson correlation coefficients, indicated by the color scale. Patient IDs are labeled, and lines represent rows and columns of 4-6 replicates per patient. Associated to Figure 6A.



Supplemental Figure 6. Correlation analysis of phospholipase C (PLC) activity and LPE levels in PFD patients and healthy controls. Scatter plots display the correlation between the summed z-score of significantly upregulated LPE species (LPE 16:1, LPE 18:1, LPE 18:3, LPE 20:1, LPE 20:3, LPE 20:5, LPE 22:1, LPE 22:3, and LPE 22:4) and the PLC activity levels (nmol/min per platelet). Linear regression lines and R^2 values are shown separately for patients (orange, n=5) and controls (green, n=4), black indicates combined groups. Corresponding bar graphs indicate no statistically significant differences between groups. Data represent individual donors. (ns, not significant)

Contents of Report

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Direct Infusion Workflow

Overall study design

Title of the study	Altered platelet lipidome in bleeding patients with abnormal platelet function		
Document creation date	01/09/2025	Corresponding Email	robert.ahrends@univie.ac.at
Principal investigator	Robert Ahrends	Is the workflow targeted or untargeted?	Untargeted
Institution	University of Vienna, Faculty of Chemistry, Institute of Analytical Chemistry	Clinical	Yes

Lipid extraction

Extraction method	2-phase system	Were internal standards added prior extraction?	Yes
pH adjustment	None	Special conditions	-
2-phase system	MTBE	Derivatization	-

Analytical platform

Ionizaton additives	Ammonium acetate	Mass accuracy in ppm at MS1	10
Detector	Mass spectrometer	Recording mode of raw data at MS1	Centroid mode
MS type	Orbitrap	Mass window for precursor ion isolation (in Da total isolation window)	1
MS vendor	Thermo	Mass resolution for detected ion at MS2	High resolution
Direct type	Chip	Resolution at m/z 200 at MS2	60000
MS Level	MS1, MS2	Mass accuracy in ppm at MS2	20
Mass resolution for detected ion at MS1	High resolution	Recording mode of raw data at MS2	Centroid mode
Resolution at m/z 200 at MS1	240000	Was/Were additional dimension/techniques used	No

Quality control

Blanks	Yes	Quality control	Yes
Type of Blanks	Extraction blank, Solvent blank, Internal standard blank	Type of QC sample	Sample pool

Method qualification and validation

Method validation	Yes	Precision	Yes
Lipid recovery	Yes	Accuracy	Yes
Dynamic quantification range	Yes	Guidelines followed	None
Limit of quantitation (LOQ)/Limit of detection (LOD)	Yes		

Reporting

Are reported raw data uploaded into repository?	Available on request	Raw data upload	Available on request
Are metadata available?	Available on request	Additional comments	-

Sample Descriptions

platelets in basal conditions / Human / Cells

Storage and collection conditions	Available	Storage temperature	-80 °C
Provided preanalytical information	Time to freeze (min), Storage time (month)	Storage time (month)	6
Temperature handling original sample	Room temperature	Additives	None
Instant sample preparation	No	Were samples stored under inert gas?	No
Time to freeze (min)	60	Additional preservation methods	No
Snap freezing in liquid N2	No	Biobank samples	No

platelets basal and activated / Human / Cells

Storage and collection conditions	Available	Storage temperature	-80 °C
Provided preanalytical information	Time to freeze (min), Storage time (month)	Storage time (month)	0
Temperature handling original sample	Room temperature	Additives	None
Instant sample preparation	Yes	Were samples stored under inert gas?	No
Time to freeze (min)	60	Additional preservation methods	No
Snap freezing in liquid N2	No	Biobank samples	No

Lipid Class Descriptions

1) CL[M-H]- / Lipid identification

Lipid class	CL	Did you presume assumptions for identification?	No
MS Level for identification	MS1	Check on:	Isomeric overlap
Identification level	Species level	Limit of detection	No
Polarity mode	Negative	Additional dimension/techniques	-
Type of negative (precursor)ion	[M-H]-	Lipid Identification Software	LipidXplorer
Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
MS1 verified by standard	Yes	Nomenclature for intact lipid molecule	Yes
Background check at MS1	Yes	Further identification remarks	-

1) CL[M-H]- / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS1	Normalization to reference	No
Internal lipid standard(s) MS1		Lipid Quantification Software	LipidXplorer
Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

Internal standard	Endogenous subclass
PG 15:0-18:1 (d7)	CL XX:X

2) DG[M+NH4]+ / Lipid identification

Lipid class	DG	Background check at MS2	Yes
MS Level for identification	MS1, MS2	Did you presume assumptions for identification?	No
Identification level	Molecular species level	Check on:	Isomeric overlap
Polarity mode	Positive	Limit of detection	No
Type of positive (precursor)ion	[M+NH4]+	Additional dimension/techniques	-
Fragments for identification		Lipid Identification Software	LipidXplorer
Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS2	Type 2	Nomenclature for intact lipid molecule	Yes
MS1 verified by standard	Yes	Nomenclature for fragment ions	Yes
MS2 verified by standard	Yes	Further identification remarks	-
Background check at MS1	Yes		

Fragment name
-FA1(-H)-(H2O+NH3)
-FA2(-H)-(H2O+NH3)

2) DG[M+NH4]+ / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS2	Normalization to reference	No
Internal lipid standard(s) MS2		Lipid Quantification Software	LipidXplorer
Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

Internal standard	Fragment(s)	Endogenous subclass
DG 15:0-18:1 (d7)	-FA1(-H)-(H2O+NH3); -FA2(-H)-(H2O+NH3)	DG XX:X_XX:X

3) LPA[M-H]- / Lipid identification

Lipid class	LPA	Background check at MS2	Yes
MS Level for identification	MS1, MS2	Did you presume assumptions for identification?	No
Identification level	Molecular species level	Check on:	Isomeric overlap
Polarity mode	Negative	Limit of detection	No
Type of negative (precursor)ion	[M-H]-	Additional dimension/techniques	-
Fragments for identification		Lipid Identification Software	LipidXplorer
Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS2	Type 2	Nomenclature for intact lipid molecule	Yes
MS1 verified by standard	Yes	Nomenclature for fragment ions	Yes
MS2 verified by standard	Yes	Further identification remarks	-
Background check at MS1	Yes		

Fragment name
FA1 (+O)

3) LPA[M-H]- / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS2	Normalization to reference	No
Internal lipid standard(s) MS2		Lipid Quantification Software	LipidXplorer
Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

Internal standard	Fragment(s)	Endogenous subclass
LPE 18:1 (d7)	FA1 (+O)	LPA XX:X

4) LPC[M+CH3COO]- / Lipid identification

Lipid class	LPC	Background check at MS2	Yes
MS Level for identification	MS1, MS2	Did you presume assumptions for identification?	No
Identification level	Molecular species level	Check on:	Isomeric overlap
Polarity mode	Negative	Limit of detection	No
Type of negative (precursor)ion	[M+CH3COO]-	Additional dimension/techniques	-
Fragments for identification		Lipid Identification Software	LipidXplorer

Fragment name
FA1 (+O)
-(CH3+CH3COO)

Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS2	Type 2	Nomenclature for intact lipid molecule	Yes
MS1 verified by standard	Yes	Nomenclature for fragment ions	Yes
MS2 verified by standard	Yes	Further identification remarks	-
Background check at MS1	Yes		

4) LPC[M+CH3COO]- / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS2	Normalization to reference	No
Internal lipid standard(s) MS2		Lipid Quantification Software	LipidXplorer

Internal standard	Fragment(s)	Endogenous subclass
LPC 18:1 (d7)	FA1 (+O)	LPC XX:X

Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

5) LPE[M-H]- / Lipid identification

Lipid class	LPE	Background check at MS2	Yes
MS Level for identification	MS1, MS2	Did you presume assumptions for identification?	No
Identification level	Molecular species level	Check on:	Isomeric overlap
Polarity mode	Negative	Limit of detection	No
Type of negative (precursor)ion	[M-H]-	Additional dimension/techniques	-
Fragments for identification		Lipid Identification Software	LipidXplorer
Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS2	Type 2	Nomenclature for intact lipid molecule	Yes
MS1 verified by standard	Yes	Nomenclature for fragment ions	Yes
MS2 verified by standard	Yes	Further identification remarks	-
Background check at MS1	Yes		

Fragment name
FA1 (+O)

5) LPE[M-H]- / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS2	Normalization to reference	No
Internal lipid standard(s) MS2		Lipid Quantification Software	LipidXplorer
Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

Internal standard	Fragment(s)	Endogenous subclass
LPE 18:1 (d7)	FA1 (+O)	LPE XX:X

6) LPG[M-H]- / Lipid identification

Lipid class	LPG	Background check at MS2	Yes
MS Level for identification	MS1, MS2	Did you presume assumptions for identification?	No
Identification level	Molecular species level	Check on:	Isomeric overlap
Polarity mode	Negative	Limit of detection	No
Type of negative (precursor)ion	[M-H]-	Additional dimension/techniques	-
Fragments for identification		Lipid Identification Software	LipidXplorer
Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS2	Type 2	Nomenclature for intact lipid molecule	Yes
MS1 verified by standard	Yes	Nomenclature for fragment ions	Yes
MS2 verified by standard	Yes	Further identification remarks	-
Background check at MS1	Yes		

Fragment name
FA1 (+O)

6) LPG[M-H]- / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS2	Normalization to reference	No
Internal lipid standard(s) MS2		Lipid Quantification Software	LipidXplorer
Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

Internal standard	Fragment(s)	Endogenous subclass
LPE 18:1 (d7)	FA1 (+O)	LPG XX:X

7) LPI[M-H]- / Lipid identification

Lipid class	LPI	Background check at MS2	Yes
MS Level for identification	MS1, MS2	Did you presume assumptions for identification?	No
Identification level	Molecular species level	Check on:	Isomeric overlap
Polarity mode	Negative	Limit of detection	No
Type of negative (precursor)ion	[M-H]-	Additional dimension/techniques	-
Fragments for identification		Lipid Identification Software	LipidXplorer
Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS2	Type 2	Nomenclature for intact lipid molecule	Yes
MS1 verified by standard	Yes	Nomenclature for fragment ions	Yes
MS2 verified by standard	Yes	Further identification remarks	-
Background check at MS1	Yes		

Fragment name
FA1 (+O)
HG(PI,241)

7) LPI[M-H]- / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS2	Normalization to reference	No
Internal lipid standard(s) MS2		Lipid Quantification Software	LipidXplorer
Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

Internal standard	Fragment(s)	Endogenous subclass
LPE 18:1 (d7)	FA1 (+O)	LPI XX:X

8) LPS[M-H]- / Lipid identification

Lipid class	LPS	Background check at MS2	Yes
MS Level for identification	MS1, MS2	Did you presume assumptions for identification?	No
Identification level	Molecular species level	Check on:	Isomeric overlap
Polarity mode	Negative	Limit of detection	No
Type of negative (precursor)ion	[M-H]-	Additional dimension/techniques	-
Fragments for identification		Lipid Identification Software	LipidXplorer
Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS2	Type 2	Nomenclature for intact lipid molecule	Yes
MS1 verified by standard	Yes	Nomenclature for fragment ions	Yes
MS2 verified by standard	Yes	Further identification remarks	-
Background check at MS1	Yes		

Fragment name
FA1 (+O)
-(C3H5NO2,87)

8) LPS[M-H]- / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS2	Normalization to reference	No
Internal lipid standard(s) MS2		Lipid Quantification Software	LipidXplorer
Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

Internal standard	Fragment(s)	Endogenous subclass
LPE 18:1 (d7)	FA1 (+O)	LPS XX:X

9) PA[M-H]- / Lipid identification

Lipid class	PA	Background check at MS2	Yes
MS Level for identification	MS1, MS2	Did you presume assumptions for identification?	No
Identification level	Molecular species level	Check on:	Isomeric overlap
Polarity mode	Negative	Limit of detection	No
Type of negative (precursor)ion	[M-H]-	Additional dimension/techniques	-
Fragments for identification		Lipid Identification Software	LipidXplorer
Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS2	Type 2	Nomenclature for intact lipid molecule	Yes
MS1 verified by standard	Yes	Nomenclature for fragment ions	Yes
MS2 verified by standard	Yes	Further identification remarks	-
Background check at MS1	Yes		

Fragment name
FA1(+O)
FA2(+O)

9) PA[M-H]- / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS2	Normalization to reference	No
Internal lipid standard(s) MS2		Lipid Quantification Software	LipidXplorer
Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

Internal standard	Fragment(s)	Endogenous subclass
PA 15:0-18:1 (d7)	FA1(+O); FA2(+O)	PA XX:X_XX:X

10) PC[M+CH3COO]- / Lipid identification

Lipid class	PC	Background check at MS2	Yes
MS Level for identification	MS1, MS2	Did you presume assumptions for identification?	No
Identification level	Molecular species level	Check on:	Isomeric overlap
Polarity mode	Negative	Limit of detection	No
Type of negative (precursor)ion	[M+CH3COO]-	Additional dimension/techniques	-
Fragments for identification		Lipid Identification Software	LipidXplorer

Fragment name
FA1(+O)
FA2(+O)
-(CH3+CH3COO)

Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS2	Type 2	Nomenclature for intact lipid molecule	Yes
MS1 verified by standard	Yes	Nomenclature for fragment ions	Yes
MS2 verified by standard	Yes	Further identification remarks	-
Background check at MS1	Yes		

10) PC[M+CH3COO]- / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS2	Normalization to reference	No
Internal lipid standard(s) MS2		Lipid Quantification Software	LipidXplorer

Internal standard	Fragment(s)	Endogenous subclass
PC 15:0-18:1 (d7)	FA1(+O); FA2(+O)	PC XX:X_XX:X

Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

11) PC O[M+CH3COO]- / Lipid identification

Lipid class	PC O	Background check at MS2	Yes
MS Level for identification	MS1, MS2	Did you presume assumptions for identification?	No
Identification level	Molecular species level	Check on:	Isomeric overlap
Polarity mode	Negative	Limit of detection	No
Type of negative (precursor)ion	[M+CH3COO]-	Additional dimension/techniques	-
Fragments for identification		Lipid Identification Software	LipidXplorer

Fragment name
-FA2(-H)
FA2(+O)
-(CH3+CH3COO)

Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS2	Type 2	Nomenclature for intact lipid molecule	Yes
MS1 verified by standard	Yes	Nomenclature for fragment ions	Yes
MS2 verified by standard	Yes	Further identification remarks	-
Background check at MS1	Yes		

11) PC O[M+CH3COO]- / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS2	Normalization to reference	No
Internal lipid standard(s) MS2		Lipid Quantification Software	LipidXplorer

Internal standard	Fragment(s)	Endogenous subclass
PC 15:0/18:1 (d7)	FA1(+O); FA2(+O)	PC O-XX:X_XX:X

Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

12) PE[M-H]- / Lipid identification

Lipid class	PE	Background check at MS2	Yes
MS Level for identification	MS1, MS2	Did you presume assumptions for identification?	No
Identification level	Molecular species level	Check on:	Isomeric overlap
Polarity mode	Negative	Limit of detection	No
Type of negative (precursor)ion	[M-H]-	Additional dimension/techniques	-
Fragments for identification		Lipid Identification Software	LipidXplorer
Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS2	Type 2	Nomenclature for intact lipid molecule	Yes
MS1 verified by standard	Yes	Nomenclature for fragment ions	Yes
MS2 verified by standard	Yes	Further identification remarks	-
Background check at MS1	Yes		

Fragment name
FA1(+O)
FA2(+O)

12) PE[M-H]- / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS2	Normalization to reference	No
Internal lipid standard(s) MS2		Lipid Quantification Software	LipidXplorer
Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

Internal standard	Fragment(s)	Endogenous subclass
PE 15:0-18:1 (d7)	FA1(+O); FA2(+O)	PE XX:X_XX:X

13) PE O[M-H]- / Lipid identification

Lipid class	PE O	Background check at MS2	Yes
MS Level for identification	MS1, MS2	Did you presume assumptions for identification?	No
Identification level	Molecular species level	Check on:	Isomeric overlap
Polarity mode	Negative	Limit of detection	No
Type of negative (precursor)ion	[M-H]-	Additional dimension/techniques	-
Fragments for identification		Lipid Identification Software	LipidXplorer
Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS2	Type 2	Nomenclature for intact lipid molecule	Yes
MS1 verified by standard	Yes	Nomenclature for fragment ions	Yes
MS2 verified by standard	Yes	Further identification remarks	-
Background check at MS1	Yes		

Fragment name
-FA2(-H)
FA2(+O)

13) PE O[M-H]- / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS2	Normalization to reference	No
Internal lipid standard(s) MS2		Lipid Quantification Software	LipidXplorer
Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

Internal standard	Fragment(s)	Endogenous subclass
PE 15:0/18:1 (d7)	FA1(+O); FA2(+O)	PE O-XX:X_XX:X

14) PG[M-H]- / Lipid identification

Lipid class	PG	Background check at MS2	Yes
MS Level for identification	MS1, MS2	Did you presume assumptions for identification?	No
Identification level	Molecular species level	Check on:	Isomeric overlap
Polarity mode	Negative	Limit of detection	No
Type of negative (precursor)ion	[M-H]-	Additional dimension/techniques	-
Fragments for identification		Lipid Identification Software	LipidXplorer
Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS2	Type 2	Nomenclature for intact lipid molecule	Yes
MS1 verified by standard	Yes	Nomenclature for fragment ions	Yes
MS2 verified by standard	Yes	Further identification remarks	-
Background check at MS1	Yes		

Fragment name
FA1(+O)
FA2(+O)

14) PG[M-H]- / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS2	Normalization to reference	No
Internal lipid standard(s) MS2		Lipid Quantification Software	LipidXplorer
Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

Internal standard	Fragment(s)	Endogenous subclass
PG 15:0-18:1 (d7)	FA1(+O); FA2(+O)	PG XX:X_XX:X

15) PI[M-H]- / Lipid identification

Lipid class	PI	Background check at MS2	Yes
MS Level for identification	MS1, MS2	Did you presume assumptions for identification?	No
Identification level	Molecular species level	Check on:	Isomeric overlap
Polarity mode	Negative	Limit of detection	No
Type of negative (precursor)ion	[M-H]-	Additional dimension/techniques	-
Fragments for identification		Lipid Identification Software	LipidXplorer

Fragment name
FA1(+O)
FA2(+O)
HG(PI,241)

Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS2	Type 2	Nomenclature for intact lipid molecule	Yes
MS1 verified by standard	Yes	Nomenclature for fragment ions	Yes
MS2 verified by standard	Yes	Further identification remarks	-
Background check at MS1	Yes		

15) PI[M-H]- / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS2	Normalization to reference	No
Internal lipid standard(s) MS2		Lipid Quantification Software	LipidXplorer

Internal standard	Fragment(s)	Endogenous subclass
PI 15:0-18:1 (d7)	FA1(+O); FA2(+O)	PI XX:X_XX:X

Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

16) PS[M-H]- / Lipid identification

Lipid class	PS	Background check at MS2	Yes
MS Level for identification	MS1, MS2	Did you presume assumptions for identification?	No
Identification level	Molecular species level	Check on:	Isomeric overlap
Polarity mode	Negative	Limit of detection	No
Type of negative (precursor)ion	[M-H]-	Additional dimension/techniques	-
Fragments for identification		Lipid Identification Software	LipidXplorer

Fragment name
FA1(+O)
FA2(+O)
-(C3H5NO2,87)

Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS2	Type 2	Nomenclature for intact lipid molecule	Yes
MS1 verified by standard	Yes	Nomenclature for fragment ions	Yes
MS2 verified by standard	Yes	Further identification remarks	-
Background check at MS1	Yes		

16) PS[M-H]- / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS2	Normalization to reference	No
Internal lipid standard(s) MS2		Lipid Quantification Software	LipidXplorer

Internal standard	Fragment(s)	Endogenous subclass
PS 15:0-18:1 (d7)	FA1(+O); FA2(+O)	PS XX:X_XX:X

Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

17) TG[M+NH4]+ / Lipid identification

Lipid class	TG	Background check at MS2	Yes
MS Level for identification	MS1, MS2	Did you presume assumptions for identification?	No
Identification level	Molecular species level	Check on:	Isomeric overlap
Polarity mode	Positive	Limit of detection	No
Type of positive (precursor)ion	[M+NH4]+	Additional dimension/techniques	-
Fragments for identification		Lipid Identification Software	LipidXplorer

Fragment name
-FA1(+HO)-(NH3)
-FA2(+HO)-(NH3)
-FA3(+HO)-(NH3)

Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS2	Type 2	Nomenclature for intact lipid molecule	Yes
MS1 verified by standard	Yes	Nomenclature for fragment ions	Yes
MS2 verified by standard	Yes	Further identification remarks	-
Background check at MS1	Yes		

17) TG[M+NH4]+ / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS1	Normalization to reference	No
Internal lipid standard(s) MS1		Lipid Quantification Software	LipidXplorer

Internal standard	Endogenous subclass
TG 15:0-18:1(d7)-15:0	TG XX:X

Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

18) LCL[M-H]- / Lipid identification

Lipid class	LCL	Did you presume assumptions for identification?	No
MS Level for identification	MS1	Check on:	Isomeric overlap
Identification level	Species level	Limit of detection	No
Polarity mode	Negative	Additional dimension/techniques	-
Type of negative (precursor)ion	[M-H]-	Lipid Identification Software	LipidXplorer
Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
MS1 verified by standard	Yes	Nomenclature for intact lipid molecule	Yes
Background check at MS1	Yes	Further identification remarks	-

18) LCL[M-H]- / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS1	Normalization to reference	No
Internal lipid standard(s) MS1		Lipid Quantification Software	LipidXplorer

Internal standard	Endogenous subclass
PG 15:0-18:1 (d7)	LCL XX:X

Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

19) CAR[M+H]+ / Lipid identification

Lipid class	CAR	Background check at MS2	Yes
MS Level for identification	MS1, MS2	Did you presume assumptions for identification?	No
Identification level	Molecular species level	Check on:	Isomeric overlap
Polarity mode	Positive	Limit of detection	No
Type of positive (precursor)ion	[M+H]+	Additional dimension/techniques	-
Fragments for identification		Lipid Identification Software	LipidXplorer

Fragment name
(**C4H5O2,**85)

Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS2	Type 2	Nomenclature for intact lipid molecule	Yes
MS1 verified by standard	Yes	Nomenclature for fragment ions	Yes
MS2 verified by standard	Yes	Further identification remarks	-
Background check at MS1	Yes		

19) CAR[M+H]+ / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS1	Normalization to reference	No
Internal lipid standard(s) MS1		Lipid Quantification Software	LipidXplorer

Internal standard	Endogenous subclass
LPC 18:1 (d7)	CAR XX:X

Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

20) ST[M+NH4]+ / Lipid identi ication

Lipid class	ST	Background check at MS2	Yes
MS Level for identification	MS1, MS2	Did you presume assumptions for identification?	No
Identification level	Molecular species level	Check on:	Isomeric overlap
Polarity mode	Positive	Limit of detection	No
Type of positive (precursor)ion	[M+NH4]+	Additional dimension/techniques	-
Fragments for identification		Lipid Identification Software	LipidXplorer

Fragment name
-(ST,35)

Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS2	Type 2	Nomenclature for intact lipid molecule	Yes
MS1 verified by standard	Yes	Nomenclature for fragment ions	Yes
MS2 verified by standard	Yes	Further identification remarks	-
Background check at MS1	Yes		

20) ST[M+NH4]+ / Lipid quanti ication

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS1	Normalization to reference	No
Internal lipid standard(s) MS1		Lipid Quantification Software	LipidXplorer

Internal standard	Endogenous subclass
ST 27:1;O (d7)	ST 27:1;O

Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

21) SE[M+NH4]+ / Lipid identi ication

Lipid class	SE	Background check at MS2	Yes
MS Level for identification	MS1, MS2	Did you presume assumptions for identification?	No
Identification level	Molecular species level	Check on:	Isomeric overlap
Polarity mode	Positive	Limit of detection	No
Type of positive (precursor)ion	[M+NH4]+	Additional dimension/techniques	-
Fragments for identification		Lipid Identification Software	LipidXplorer
Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS2	Type 2	Nomenclature for intact lipid molecule	Yes
MS1 verified by standard	Yes	Nomenclature for fragment ions	Yes
MS2 verified by standard	Yes	Further identification remarks	-
Background check at MS1	Yes		

Fragment name
-FA(+HO)-(ST,35)

21) CE[M+NH4]+ / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS1	Normalization to reference	No
Internal lipid standard(s) MS1		Lipid Quantification Software	LipidXplorer
Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

Internal standard	Endogenous subclass
SE 27:1/18:1 (d7)	SE 27:1/XX:X

22) SM[M+H]+ / Lipid identification

Lipid class	SM	Did you presume assumptions for identification?	No
MS Level for identification	MS1	Check on:	Isomeric overlap
Identification level	Species level	Limit of detection	No
Polarity mode	Positive	Additional dimension/techniques	-
Type of positive (precursor)ion	[M+H]+	Lipid Identification Software	LipidXplorer
Isotope correction at MS1	Type 2	Data manipulation	Centroiding, Lock mass correction
MS1 verified by standard	Yes	Nomenclature for intact lipid molecule	Yes
Background check at MS1	Yes	Further identification remarks	-

22) SM[M+H]+ / Lipid quantification

Quantitative	Yes	Limit of quantification	Signal theshold
MS Level for quantification	MS1	Normalization to reference	No
Internal lipid standard(s) MS1		Lipid Quantification Software	LipidXplorer

Internal standard	Endogenous subclass
SM 18:1;O2/18:1 (d9)	SM XX:X;O2

Type of quantification	Internal standard amount	Batch correction	No
Response correction	No	Further quantification remarks	-
Type I isotope correction	Yes		

Contents of Report

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Separation Workflow

Overall study design

Title of the study	Altered platelet lipidome in bleeding patients with unexplained platelet function defects		
Document creation date	06/03/2025	Corresponding Email	robert.ahrends@univie.ac.at
Principal investigator	Robert Ahrends	Is the workflow targeted or untargeted?	Targeted
Institution	University of Vienna, Faculty of Chemistry, Institute of Analytical Chemistry	Clinical	Yes

Lipid extraction

Extraction method	2-phase system	Were internal standards used?	Yes
pH adjustment	None	Internal standards used	CAR 12:0-d9 (Sigma 870326P)
2-phase system	Bligh&Dyer		

Analytical platform

Ionizaton additives	Ammonium formate	Ion source	ESI
Number of separation dimensions	One dimension	MS Level	MS^2
Separation type 1	LC	Mass window for precursor ion isolation (in Da total isolation window)	unit
Separation mode 1 (liquid)	RP	Mass resolution for detected ion at MS^2	Low resolution
Detector	Mass spectrometer	Resolution at MS^2	Low
MS type	QTrap		
MS vendor	SCIEX		

Quality control

Blanks	Yes	Quality control	Yes
Type of Blanks	Extraction blank, Solvent blank, Internal standard blank	Type of QC sample	Sample pool

Method qualification and validation

Method validation	Yes	Precision	No
Lipid recovery	Yes	Accuracy	No
Dynamic quantification range	Yes	Guidelines followed	None
Limit of quantitation (LOQ)/Limit of detection (LOD)	Yes		

Reporting

Are reported raw data uploaded into repository?	No	Raw data upload	Available on request
Are metadata available?	No		

Sample Descriptions

Plasma / Human / Plasma

Storage and collection conditions	Available	Storage temperature	-80 °C
Provided preanalytical information	Time to freeze	Additives	None
Temperature handling original sample	Room temperature	Were samples stored under inert gas?	No
Instant sample preparation	No	Additional preservation methods	No
Time to freeze	between 0 and 60 min	Biobank samples	Yes
Snap freezing in liquid N2	Yes		

Lipid Class Descriptions

1) CAR / Lipid identification

Lipid class	CAR	Check on:	Isomeric overlap
MS Level for identification	MS^2	Limit of detection	S/N ratio $>$ 3
Identification level	Molecular species level	RT verified by standard	Yes
Fragments for identification		Separation of isobaric/isomeric interferece confirmed	Yes
Isotope correction at MS^2	No	Model for separation prediction	No
MS^2 verified by standard	Yes	Lipid Identification Software	Skyline
Background check at MS^2	Yes	Nomenclature for intact lipid molecule	Yes
Did you presume assumptions for identification?	No	Nomenclature for fragment ions	N/A

Fragment name
CAR m/z 85

1) CAR / Lipid quantification

Quantitative	Yes	Type I isotope correction	No
MS Level for quantification	MS^2	Limit of quantification	S/N ratio $>$ 10
Internal lipid standard(s) MS^2		Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	Skyline
Response correction	No	Batch correction	No

Internal standard	Fragment(s)	Endogenous subclass
CAR 12:0-d9	CAR m/z 85	CAR XX:X