Supplementary Materials

Exploratory Untargeted Plasma Lipidomics Suggests Subtype-Associated Lipid Alterations in Hereditary Hemolytic Anemias

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Abbreviations: BT, β-thalassemia; CDA, congenital dyserythropoietic anemia; FDR, false discovery rate; HHA, hereditary hemolytic anemia; HP, hemoglobinopathies (BT and SCD); HS, hereditary spherocytosis; HX, hereditary xerocytosis; IQR, interquartile range; MP, membranopathies (HS and HX); SCD, sickle cell disease. Lipid class abbreviations are defined in Table 2 of the main text.

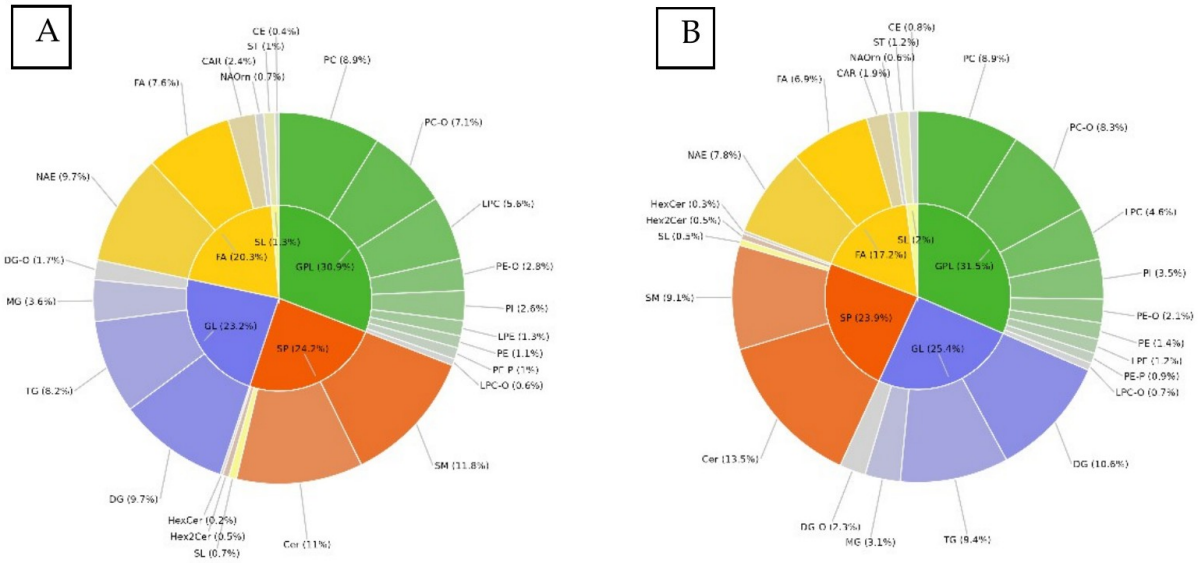


Figure S1. Lipid class distribution and differential abundance in healthy controls and patients with hereditary hemolytic anemia (HHA) (LipidOne). (A) Healthy controls; (B) HHA patients. Values are presented as relative abundance (% of total lipid content).

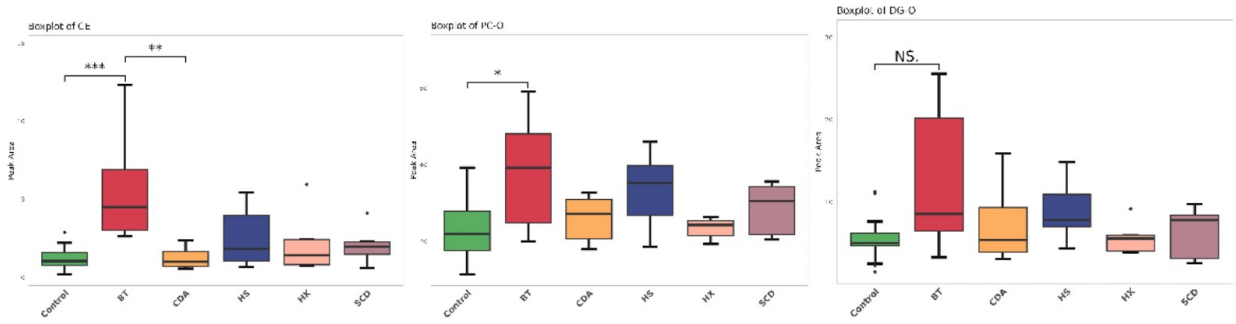


Figure S2. Pairwise comparisons of lipid classes showing nominally significant differences across study groups (CE, PC-O, and DG-O), presented as boxplots (LipidOne). *p*-values shown are unadjusted for multiple comparisons. BT, β-thalassemia; CDA, congenital dyserythropoietic anemia; HS, hereditary spherocytosis; HX, hereditary xerocytosis; SCD, sickle cell disease.

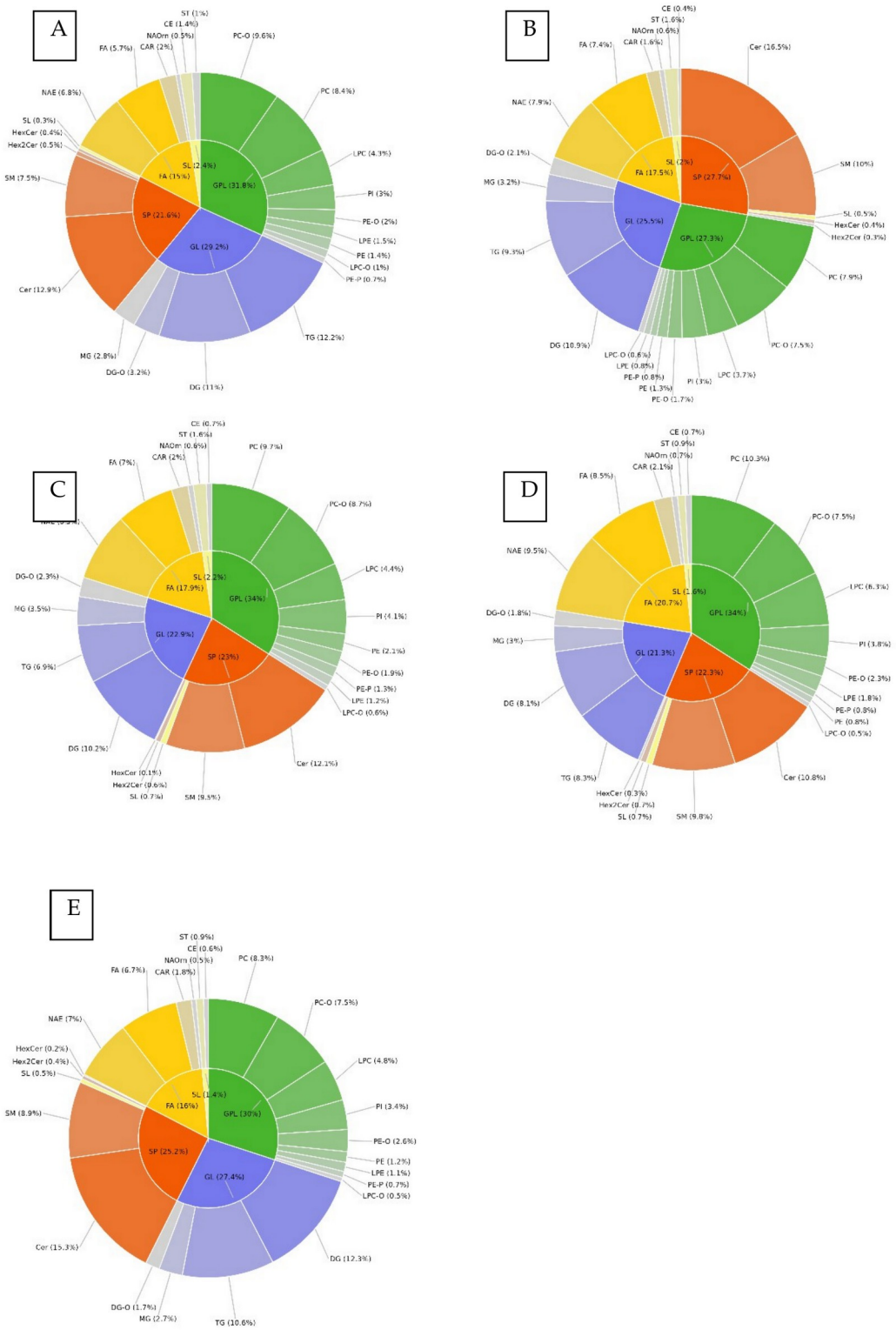
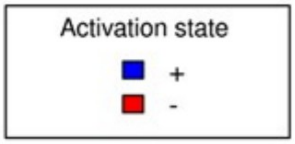
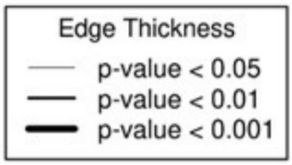


Figure S3. Lipid category and class distribution in hereditary hemolytic anemia subtypes (LipidOne), presented as relative abundance (% of total lipid content). (A) BT; (B) CDA; (C) HS; (D) HX; (E) SCD.







Interaction of lipid chains

Status of activation (+) or (-)

From	To	Homo_sapiens	Z.score	Status
24:6	22:6	ACADL	3.6	+
24:6	22:6	ECHS1	3.6	+
24:6	22:6	HADHA	3.6	+
24:6	22:6	HADH	3.6	+

Figure S4. Lipid pathway analysis based on lipid building blocks (LipidOne). Comparison between healthy controls and all HHA patients.

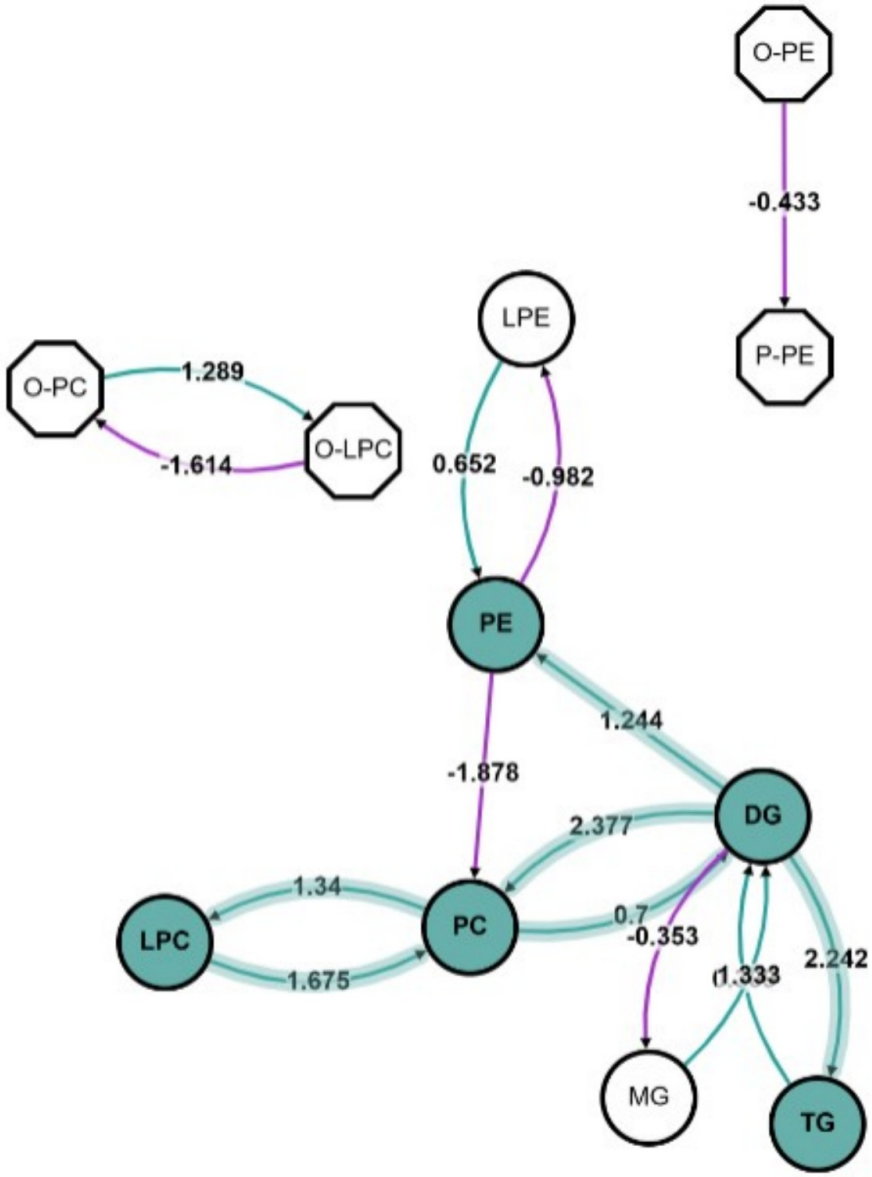


Figure S5. Lipid network graph generated using BioPAN comparing HHA patients with healthy controls. Settings: lipid type, active status, subclass level, and reaction subset; *p*-value threshold 0.05 ($|Z| > 1.645$, one-tailed); unpaired data. BioPAN does not apply correction for multiple testing; *Z*-scores are therefore unadjusted.

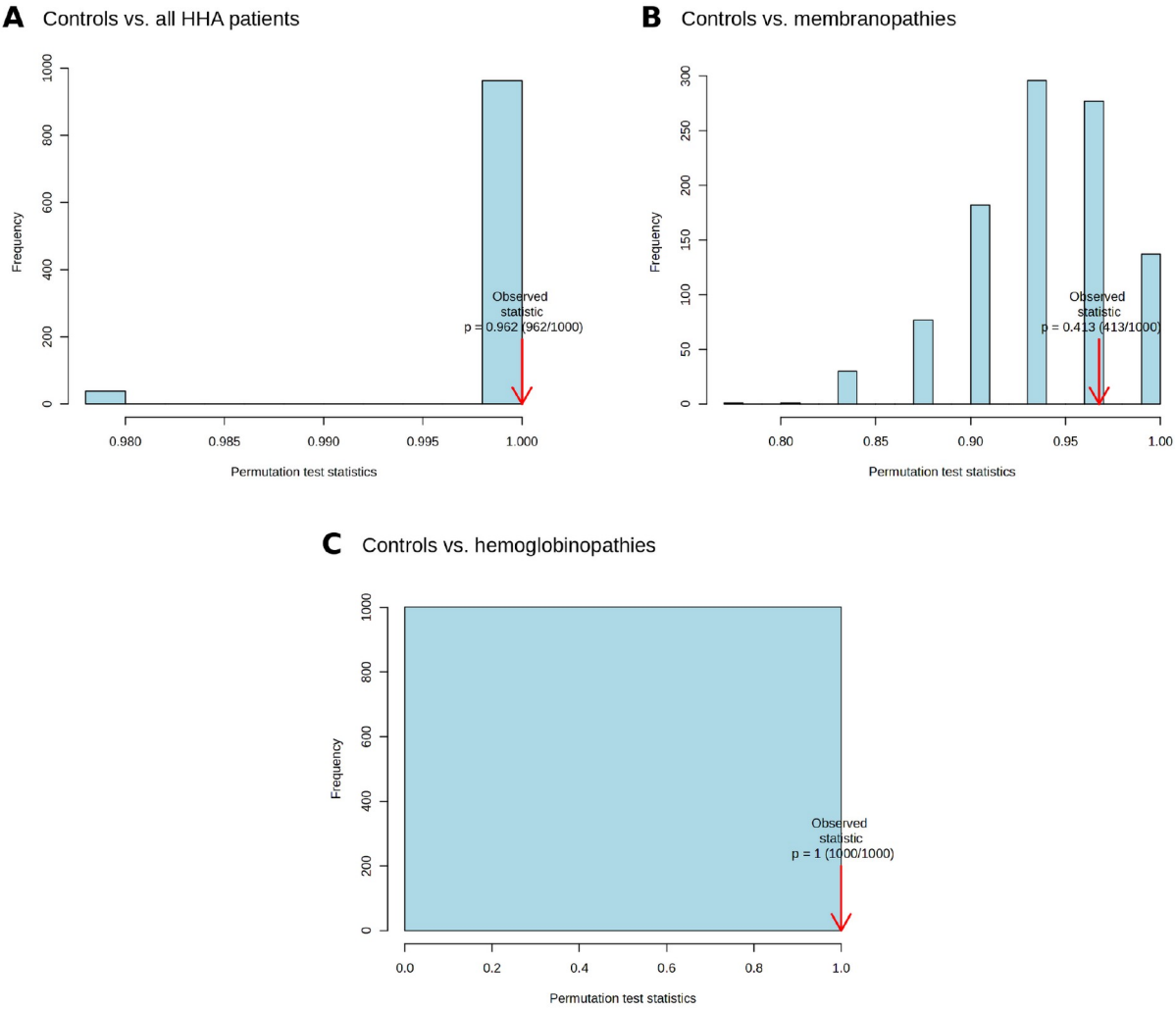


Figure S6. Permutation tests of the partial least squares–discriminant analysis (PLS-DA) models (1000 permutations; test statistic: prediction accuracy during training). (A) Controls vs. all HHA patients ($p = 0.962$); (B) controls vs. membranopathies (HS and HX; $p = 0.413$); (C) controls vs. hemoglobinopathies (BT and SCD; $p = 1.000$). The histograms show the distribution of the test statistic for models fitted to randomly permuted class labels; the red arrow indicates the observed statistic. Given the small sample size, these empirical p-values should be interpreted with caution. Because training accuracy can approach 1.0 even for permuted class labels when the number of variables greatly exceeds the number of samples, this test has limited power; the PLS-DA models therefore do not provide reliable evidence of predictive discrimination. BT, β-thalassemia; HHA, hereditary hemolytic anemia; HS, hereditary spherocytosis; HX, hereditary xerocytosis; SCD, sickle cell disease.

Table S1. Plasma lipid species showing nominal significance (raw $p < 0.05$) between healthy controls and patients with hereditary hemolytic anemia (HHA).

Lipid species	Controls (n = 19)	HHA patients (n = 29)	Raw p	FDR q
Cer 59:7;4O	0.415 (0.272-0.483)	1.182 (0.874-1.968)	<0.001	**<0.001**
PC 46:1 | PC 11:0_35:1	0.115 (0.078-0.162)	0.641 (0.358-1.569)	<0.001	**0.002**
TG 54:5;1O | TG 18:1_18:1_18:3;1O	0.324 (0.112-0.435)	0.854 (0.49-2.075)	<0.001	**0.003**
Cer 45:4;3O | Cer 13:2;2O/32:2;O	0.266 (0.097-0.419)	0.777 (0.422-1.463)	<0.001	**0.025**
PC 38:7 | PC 14:1_24:6	1.396 (1.059-1.711)	0.707 (0.374-0.942)	<0.001	**0.046**
TG 50:0 | TG 15:0_17:0_18:0	0.096 (0.008-0.219)	0.381 (0.172-1.952)	0.001	0.065
TG 53:3;1O | TG 16:0_18:1_19:2;1O	0.263 (0.109-0.375)	0.579 (0.337-2.12)	0.001	0.065
TG 53:3 | TG 16:0_18:1_19:2	0.309 (0.192-0.663)	0.823 (0.512-1.589)	0.002	0.066
Cer 48:6;3O | Cer 12:1;2O/36:5;O	0.066 (0.033-0.337)	0.98 (0.107-2.174)	0.002	0.066
PC O-43:10 | PC O-21:4_22:6	0.072 (0.018-2.072)	0.018 (0.018-0.018)	0.002	0.075
TG 40:0 | TG 12:0_14:0_14:0	1.598 (1.031-1.827)	0.685 (0.194-1.357)	0.002	0.085
PI 38:5 | PI 18:1_20:4	0.436 (0.265-0.784)	1.26 (0.748-1.948)	0.003	0.089
TG 55:6;2O | TG 18:2_18:3_19:1;2O	0.077 (0.077-0.336)	0.077 (0.077-0.077)	0.004	0.104
NAE 18:4	1.206 (0.486-2.776)	0.129 (0.039-1.266)	0.004	0.116
TG 41:0 | TG 9:0_15:0_17:0	0.208 (0.126-0.349)	0.485 (0.262-1.192)	0.005	0.118
Cer 51:5;3O | Cer 15:0;2O/36:5;O	0.095 (0.062-0.248)	0.306 (0.149-1.587)	0.006	0.120
NAE 18:5	1.323 (0.507-2.237)	0.119 (0.077-1.368)	0.006	0.120
TG 55:5;1O | TG 18:1_18:2_19:2;1O	0.584 (0.263-1.081)	0.102 (0.102-0.341)	0.006	0.120
CAR 10:0	1.162 (0.214-2.215)	0.109 (0.055-0.708)	0.007	0.130
ST 27:2;O	0.212 (0.159-0.586)	0.761 (0.26-1.461)	0.007	0.135
DG 52:3	0.113 (0.113-0.113)	0.113 (0.113-1.694)	0.009	0.138
TG 54:6;1O | TG 18:1_18:2_18:3;1O	0.252 (0.218-0.67)	0.632 (0.463-1.268)	0.009	0.138
Cer 47:4;3O | Cer 15:1;2O/32:3;O	0.152 (0.108-0.256)	0.338 (0.183-0.89)	0.009	0.138
TG 42:1 | TG 12:0_14:0_16:1	0.113 (0.062-0.228)	0.415 (0.13-1.595)	0.009	0.138
NAE 16:2	1.526 (0.598-2.021)	0.282 (0.157-1.131)	0.009	0.138
DG O-40:2 | DG O-19:2_21:0	0.106 (0.014-0.278)	0.308 (0.147-0.915)	0.011	0.147
TG 54:3 | TG 18:1_18:1_18:1	0.359 (0.112-0.731)	1.31 (0.468-2.244)	0.011	0.147
PC O-40:8 | PC O-18:3_22:5	0.309 (0.157-0.784)	1.087 (0.34-2.453)	0.011	0.147
SM 37:1;2O | SM 25:1;2O/12:0	1.508 (0.472-2.909)	0.266 (0.006-1.078)	0.012	0.147
LPC O-16:0	0.593 (0.481-1.135)	1.06 (0.747-1.337)	0.012	0.147
DG O-37:0 | DG O-17:0_20:0	0.264 (0.142-0.44)	0.816 (0.233-1.668)	0.019	0.226
DG 47:1	0.149 (0.016-0.261)	0.403 (0.065-1.052)	0.022	0.262
Cer 42:2;4O | Cer 19:1;3O/23:1;(2OH)	0.068 (0.068-0.39)	0.439 (0.068-1.349)	0.026	0.280
DG O-35:0 | DG O-15:0_20:0	0.121 (0.121-0.267)	0.372 (0.121-1.226)	0.026	0.280
SM 33:2;2O | SM 16:2;2O/17:0	0.605 (0.371-2.639)	0.435 (0.175-1.121)	0.026	0.280
SM 35:2;2O | SM 14:1;2O/21:1	0.875 (0.328-2.515)	0.171 (0.095-1.461)	0.028	0.287
DG 39:2	0.011 (0.011-0.031)	0.021 (0.011-2.761)	0.029	0.298
FA 18:0	0.224 (0.208-0.341)	0.328 (0.234-1.832)	0.031	0.303
TG 57:3 | TG 17:0_19:1_21:2	0.105 (0.045-2.435)	0.045 (0.045-0.134)	0.032	0.309
Cer 42:2;3O | Cer 19:1;2O/23:1;O	0.018 (0.018-0.431)	0.478 (0.018-1.831)	0.034	0.309
PC O-40:6 | PC O-18:1_22:5	0.64 (0.207-0.924)	1.04 (0.49-2.277)	0.034	0.309
SM 43:1;2O | SM 27:1;2O/16:0	1.368 (0.539-2.366)	0.832 (0.352-1.115)	0.036	0.320
DG 46:2 | DG 20:0_26:2	0.182 (0.182-0.498)	0.602 (0.182-1.59)	0.038	0.325
PC O-43:4 | PC O-19:0_24:4	0.607 (0.483-0.857)	1.047 (0.808-1.423)	0.039	0.325
TG 55:3 | TG 18:1_18:1_19:1	0.25 (0.145-0.66)	0.615 (0.348-1.165)	0.039	0.325
PC O-38:7 | PC O-21:4_17:3	0.214 (0.122-0.307)	0.365 (0.177-1.418)	0.040	0.325
LPC 17:0/0:0	1.066 (0.878-1.311)	0.862 (0.644-1.099)	0.042	0.334
SM 43:2;2O | SM 24:2;2O/19:0	0.186 (0.186-0.186)	0.186 (0.186-0.186)	0.045	0.341
DG 51:4	0.135 (0.135-0.135)	0.135 (0.135-0.407)	0.046	0.341
CE 22:6	0.407 (0.202-0.584)	0.625 (0.305-1.735)	0.046	0.341
PI 38:4 | PI 18:0_20:4	0.25 (0.179-0.582)	0.654 (0.283-1.105)	0.046	0.341

Values are MSTUS-normalized relative abundances presented as median (interquartile range). Raw p-values were obtained using the Mann–Whitney U test; q-values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) method. Bold values indicate FDR $q < 0.05$. Lipids are ordered by raw p.

Missing values were replaced by half of the minimum value of each lipid species; identical lower and upper IQR bounds indicate that most values in that group were imputed. Lipid annotations are putative (MSI level 2).

Table S2. Molecular characterization of patients with hereditary hemolytic anemia. Variants are described according to HGVS nomenclature on MANE Select reference transcripts (GRCh38).

Patient	Group	Gene	Transcript	cDNA (HGVS)	Protein (HGVS)	Legacy name	Zygosity	Classification	Genotype–diagnosis
P1	BT	*HBB*	NM_000518.5	c.93-21G>A	p.?	IVS-I-110 G>A	Homozygous	Pathogenic (ClinVar; expert panel)	β^{+}/β^{+}; TD
P2	BT	*HBB*	NM_000518.5	c.93-21G>A	p.?	IVS-I-110 G>A	Homozygous	Pathogenic (ClinVar; expert panel)	β^{+}/β^{+}; TD
P3	BT	—	—	Not available (tested at another center)	—	—	—	—	β-thalassemia (clinical diagnosis); TD
P4	BT	*HBB*	NM_000518.5	c.25_26del	p.(Lys9ValfsTer14)	Codon 8 (−AA)	Homozygous	Pathogenic (ClinVar)	β^{0}/β^{0}; recorded as β-thalassemia intermedia; TD
P5	BT	*HBB*	NM_000518.5	c.92+6T>C	p.?	IVS-I-6 T>C	Homozygous	Pathogenic/Likely pathogenic (ClinVar)	β^{+}/β^{+}; TD
P6	BT	*HBB*	NM_000518.5	c.118C>T	p.(Gln40Ter)	Codon 39 C>T	Homozygous	Pathogenic (ClinVar)	β^{0}/β^{0}; TD
P7 (F1)	CDA	*CDAN1*	NM_138477.4	c.3584T>C	p.(Leu1195Pro)	—	Homozygous	Conflicting interpretations (ClinVar) †	CDA type I
P8 (F1)	CDA	*CDAN1*	NM_138477.4	c.3584T>C	p.(Leu1195Pro)	—	Homozygous	Conflicting interpretations (ClinVar) †	CDA type I
P9	CDA	*SEC23B*	NM_006363.6	c.938G>A	p.(Arg313His)	—	Homozygous	Pathogenic/Likely pathogenic (ClinVar)	CDA type II
P10	CDA	*SEC23B*	NM_006363.6	c.689+106G>A	p.?	—	Homozygous	Not in ClinVar †	CDA type II
P11	CDA	*SEC23B*	NM_006363.6	No variant detected	—	—	—	—	CDA; diagnosis based on bone marrow morphology
P12	CDA	*SEC23B*, *PKLR*	—	No variant detected	—	—	—	—	CDA; diagnosis based on bone marrow morphology
P13	HS	*SPTB*	NM_001355436.2	c.1311G>A	p.(Trp437Ter)	—	Heterozygous	Not in ClinVar †	HS (β-spectrin); TD
P14 (F2)	HS	*ANK1*	NM_000037.4	c.898del ‡	p.(Ala300ProfsTer32)	—	Heterozygous	Not in ClinVar †	HS (ankyrin-1)
P15 (F2)	HS	*ANK1*	NM_000037.4	c.898del ‡	p.(Ala300ProfsTer32)	—	Heterozygous	Not in ClinVar †	HS (ankyrin-1)
P16	HS	—	—	Not performed	—	—	—	—	HS; diagnosis by osmotic fragility and EMA binding tests
P17	HS	—	—	Not performed	—	—	—	—	HS; diagnosis by osmotic fragility and EMA binding tests
P18	HS	—	—	Not performed	—	—	—	—	HS; diagnosis by osmotic fragility and EMA binding tests
P19	HS	—	—	Not performed	—	—	—	—	HS; diagnosis by osmotic fragility and EMA binding tests
P20 (F3)	HX	*PIEZO1*	NM_001142864.4	c.4274G>A	p.(Ser1425Asn)	—	Homozygous ¶	Conflicting interpretations (ClinVar) †	HX (PIEZO1-related)
P21 (F3)	HX	*PIEZO1*	NM_001142864.4	c.4274G>A	p.(Ser1425Asn)	—	Homozygous ¶	Conflicting interpretations (ClinVar) †	HX (PIEZO1-related)
P22 (F4)	HX	*PIEZO1*	NM_001142864.4	c.5389C>T	p.(Arg1797Cys)	—	Homozygous ¶	Conflicting interpretations (ClinVar) †	HX (PIEZO1-related)

Patient	Group	Gene	Transcript	cDNA (HGVS)	Protein (HGVS)	Legacy name	Zygosity	Classification	Genotype–diagnosis
P23 (F4)	HX	*PIEZO1*	NM_001142864.4	c.5389C>T	p.(Arg1797Cys)	—	Homozygous ¶	Conflicting interpretations (ClinVar) †	HX (PIEZO1-related); additional heterozygous PKLR variant (details not available)
P24	HX	*PIEZO1*	NM_001142864.4	c.7367G>A	p.(Arg2456His)	—	Heterozygous	Pathogenic (ClinVar)	HX (PIEZO1-related)
P25 (F5)	SCD	*HBB*	NM_000518.5	c.20A>T	p.(Glu7Val)	HbS (Glu6Val)	Homozygous	Pathogenic (ClinVar)	HbSS; TD
P26	SCD	*HBB*	NM_000518.5	c.20A>T	p.(Glu7Val)	HbS (Glu6Val)	Homozygous	Pathogenic (ClinVar)	HbSS; TD
P27 (F5)	SCD	*HBB*	NM_000518.5	c.20A>T	p.(Glu7Val)	HbS (Glu6Val)	Homozygous	Pathogenic (ClinVar)	HbSS; TD
P28 (F5)	SCD	*HBB*	NM_000518.5	c.20A>T	p.(Glu7Val)	HbS (Glu6Val)	Homozygous	Pathogenic (ClinVar)	HbSS; TD
P29	SCD	*HBB*	NM_000518.5	c.[20A>T];[93-21G>A]	p.[(Glu7Val)];[?]	HbS / IVS-I-110 G>A	Compound heterozygous	Pathogenic / Pathogenic (ClinVar)	HbS/β^{+}-thalassemia; TD

BT, β-thalassemia; CDA, congenital dyserythropoietic anemia; EMA, eosin-5-maleimide; HS, hereditary spherocytosis; HX, hereditary xerocytosis; SCD, sickle cell disease; TD, transfusion-dependent. P1–P29 are study codes; F1–F5 denote related patients (siblings; F2, twins). Protein positions follow HGVS numbering (initiator methionine = 1); legacy hemoglobin nomenclature is given for reference. p.?, protein effect not predictable (intronic variant). Descriptions were checked with VariantValidator; classifications are those listed in ClinVar (accessed 25 September 2026).

† Not listed in ClinVar, or listed with conflicting interpretations of pathogenicity; no laboratory ACMG/AMP classification was available.

‡ Recorded as "c.898delIA" in the patient records; interpreted as c.898del (single-nucleotide deletion).

¶ Homozygous *PIEZO1* variants were identified in two sibling pairs (F3 and F4). *PIEZO1*-related HX is usually inherited in an autosomal dominant manner; homozygosity in these families is consistent with shared parental ancestry.

Table S3. Demographic and clinical characteristics of healthy controls and the reclassified diagnostic groups (membranopathies, hemoglobinopathies, and CDA).

Variable	Control (n = 19)	MP (n = 12)	HP (n = 11)	CDA (n = 6)	Raw p	FDR q
Age (years)	18 (14-25)	11.5 (8.75-16)	16 (11-21)	18 (12.75-21)	0.074	0.074
LDH (U/L)	168 (151-186)[a,b,c]	359 (232-386)[a]	306.5 (213.25-351)[b]	286 (276-343)[c]	0.001	0.002
Total bilirubin (mg/dL)	0.7 (0.57-0.89)[a,b,c]	4.28 (2.11-6.32)[a]	3.07 (2.63-4.16)[b]	3.33 (2.72-4.74)[c]	<0.001	<0.001
Indirect bilirubin (mg/dL)	0.56 (0.46-0.74)[a,c]	3.62 (1.76-5.64)[a]	1.54 (0.62-2.58)	2.72 (2.21-4.18)[c]	<0.001	<0.001
Ferritin (ng/mL)	28.7 (13.52-126.97)[b,c]	163.6 (97.32-230.58)	1051.4 (518-1739.5)[b]	813.9 (244.55-1369)[c]	<0.001	<0.001
RBC ($\times 10^6/\mu L$)	5.24 (4.96-5.54)[a,b,c]	3.83 (3.43-4.65)[a]	3.61 (3.2-3.88)[b]	3.42 (3.06-3.8)[c]	<0.001	<0.001
Hemoglobin (g/dL)	14.8 (13.5-15.6)[b,c]	10.85 (9.8-14.37)	10.1 (9.05-11)[b]	10.4 (10.07-11.93)[c]	<0.001	<0.001
MCV (fL)	86.3 (81.2-88.1)	85.95 (81.02-91.7)	83 (80-85.55)[f]	91.75 (88.85-97.8)[f]	0.037	0.043

Values are presented as median (interquartile range). Group comparisons were performed using the Kruskal–Wallis test followed by Dunn's post hoc test with Benjamini–Hochberg adjustment. Superscript letters indicate significant pairwise differences (adjusted $p < 0.05$): a, control vs. MP; b, control vs. HP; c, control vs. CDA; d, MP vs. HP; e, MP vs. CDA; f, HP vs. CDA. HP, hemoglobinopathies (BT and SCD); MP, membranopathies (HS and HX).

Table S4. Spearman correlation between age and the 25 lipid species identified by Kruskal–Wallis analysis across the six study groups.

Lipid species	Spearman's *r*	Raw *p*	FDR *q*
TG 53:3 | TG 16:0_18:1_19:2	-0.348	0.015	0.386
Cer 59:7;4O	-0.301	0.037	0.467
TG 54:5;1O | TG 18:1_18:1_18:3;1O	-0.270	0.063	0.490
PC 46:1 | PC 11:0_35:1	-0.256	0.078	0.490
Cer 45:4;3O | Cer 13:2;2O/32:2;O	-0.232	0.113	0.564
CE 22:6	-0.203	0.167	0.588
PC O-40:7 | PC O-18:2_22:5	-0.191	0.194	0.588
SM 37:1;2O | SM 25:1;2O/12:0	0.186	0.205	0.588
PI 38:5 | PI 18:1_20:4	-0.177	0.230	0.588
TG 53:3;1O | TG 16:0_18:1_19:2;1O	-0.166	0.261	0.588
PC O-38:7 | PC O-21:4_17:3	-0.164	0.266	0.588
PC 38:7 | PC 14:1_24:6	0.158	0.282	0.588
TG 54:6;1O | TG 18:1_18:2_18:3;1O	-0.129	0.381	0.731
Cer 48:6;3O | Cer 12:1;2O/36:5;O	-0.122	0.409	0.731
TG 55:5;1O | TG 18:1_18:2_19:2;1O	0.103	0.486	0.767
TG 50:0 | TG 15:0_17:0_18:0	-0.102	0.491	0.767
Cer 42:3;2O | Cer 18:2;2O/24:1	0.094	0.523	0.769
TG 41:0 | TG 9:0_15:0_17:0	-0.063	0.670	0.836
PC 34:4 | PC 14:0_20:4	0.058	0.696	0.836
TG 54:3 | TG 18:1_18:1_18:1	0.058	0.697	0.836
DG 52:3	0.051	0.730	0.836
Cer 47:4;3O | Cer 15:1;2O/32:3;O	0.049	0.740	0.836
PC O-43:10 | PC O-21:4_22:6	0.044	0.769	0.836
ST 27:2;O	0.034	0.818	0.852
Cer 51:5;3O | Cer 15:0;2O/36:5;O	-0.022	0.883	0.883

Correlation analysis was performed in all participants (n = 48) using Spearman's rank correlation; *q*-values were adjusted using the Benjamini–Hochberg method. No lipid species was significantly correlated with age after FDR correction.

Table S5. Summary of the main findings according to the strength of statistical evidence.

Level of evidence	Comparison (test; hypotheses per FDR correction)	Findings	Interpretation
FDR-significant ($q < 0.05$)	Controls vs. all HHA patients, species level (Mann–Whitney U; 375)	Higher in HHA: Cer 59:7;4O, Cer 45:4;3O, PC 46:1, TG 54:5;1O; lower in HHA: PC 38:7 (Figure 1E; Table S1)	Most robust findings of the study (putative annotations); require confirmation in an independent cohort
FDR-significant ($q < 0.05$)	Six groups, species level (Kruskal–Wallis; 375)	Cer 59:7;4O and PC 46:1 ($q = 0.035$); higher than in controls in BT, HS, HX, and SCD, but not in CDA (Dunn's test) (Table 4; Figure 2I)	Shared alteration across most HHA subtypes
FDR-significant ($q < 0.05$)	Six groups, class level (one-way ANOVA; 27)	CE class ($q = 0.004$); nominally highest in BT (Table 3; Figure S2)	Class-level finding; pairwise differences nominal
Borderline ($0.05 \leq q < 0.10$)	Six groups, species level (Kruskal–Wallis; 375)	TG 55:5;1O and TG 54:5;1O ($q = 0.089$ for both) (Table 4)	Trend only; not considered statistically significant
Nominal only (raw $p < 0.05$; $q \geq 0.05$)	Controls vs. all HHA patients and six groups, class level (Student's t-test and one-way ANOVA; 27)	CE, DG-O, PC-O, PI, and total lipids (Table 2); DG-O and PC-O (Table 3)	Exploratory
Nominal only (Kruskal–Wallis $q > 0.20$)	Six groups, subtype-specific species (Dunn's test after a nominal Kruskal–Wallis screen; 15 per species)	e.g., PC 34:4 (higher in SCD), Cer 42:3;2O (lower in HX), PC O-38:7 and CE 22:6 (higher in BT and HS), Cer 48:6;3O and TG 53:3;1O (higher in BT), SM 37:1;2O (lower in CDA) (Table 4)	Exploratory; several differences involve values imputed at the detection limit
Computational prediction (unadjusted)	Pathway and building block analyses (LipidOne, BioPAN)	e.g., SM-to-Cer conversion (BT, SCD), DGAT2-mediated DG-to-TG synthesis, Kennedy pathway PC synthesis, C24-to-C22 chain shortening (Figure 3; Figures S4 and S5)	Hypothesis-generating; requires enzymatic or flux-based validation
Descriptive only	PCA and PLS-DA models	Partial separation with R^2–Q^2 gaps indicating overfitting; cross-validation not nested (Q^2 and accuracy likely optimistic) (Figures 1 and 2); permutation $p = 0.413$–1.000 (Figure S6)	Visualization and VIP ranking only; no reliable evidence of predictive discrimination

CE, cholesteryl ester; Cer, ceramide; DG, diacylglycerol; DG-O, ether-linked diacylglycerol; FDR, false discovery rate; HHA, hereditary hemolytic anemia; PC, phosphatidylcholine; PC-O, ether-linked phosphatidylcholine; PI, phosphatidylinositol; SM, sphingomyelin; TG, triacylglycerol; other abbreviations as in Table 4. Numbers after the test name indicate the number of hypotheses included in each Benjamini–Hochberg correction (for Dunn's test, the number of pairwise comparisons per species). Tables and figures without the prefix "S" refer to the main text.